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Biomarkers of oxidative damage and inflammation in Alzheimer's disease

Douglas Galasko[1,†] and **Thomas J Montine**[2]

[1]Department of Neurosciences, UCSD, 9500 Gilman Drive, M0624, La Jolla, CA 92093, USA

[2]Division of Neuropathology, University of Washington, 300 9th Ave, Room 703B, Seattle, WA 98104, USA Tel.: +1 206 744 4106 Fax: +1 206 897 5249 tmontine@u.washington.edu

Abstract

Oxidative damage and inflammation are important features of the brain pathology of Alzheimer's disease (AD). Oxidative damage can be found in membranes (lipid peroxidation), proteins (nitrosylation and other post-translational changes) and nucleic acids. Inflammatory changes include activation of microglia and astrocytes, with increased levels of proinflammatory cytokines. Not all of these changes are specific to AD, and occur in other neurodegenerative disorders. Both oxidative stress and inflammation are potential therapeutic targets in AD, and biomarkers could help to identify and monitor key pathways in patients with AD. This article summarizes progress in developing cerebrospinal fluid biomarkers related to oxidative stress and inflammation, problems and pitfalls related to systemic (blood- or urine-based) biomarkers in this area, and future research directions and applications.

Keywords

Alzheimer's disease; biomarker; cytokines; F_2-isoprostanes; oxidative stress

The importance of oxidative damage and inflammation in Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. It is associated with two defining pathological lesions:

- Senile plaques that are comprised of aggregates of the amyloid-β (Aβ) protein, surrounded by damaged neuronal processes and reactive glia;
- Intraneuronal aggregates of the microtubule-associated protein tau, forming paired helical filaments, and evident in dystrophic neurites and neurofibrillary tangles.

However, the pathologic changes of AD include many other biological alterations. Although oxidative stress and inflammation occur in other neurodegenerative disorders, and are not specific to AD, they are particularly prominent features in studies of AD; data from experimental models indicate that these processes may be causally related to dysfunction and death of neurons in AD [1-5]. These findings have spurred the development of assays for biomarkers to examine these processes in human studies related to aging and dementia.

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†Author for correspondence: Tel.: +1 858 534 6224 Fax: +1 858 534 1993 dgalasko@ucsd.edu .

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Oxidative stress & oxidative damage

Normal metabolism generates oxygen free radicals and other reactive oxygen species (ROS) that are part of several physiologic processes including signal transduction pathways (e.g., related to some growth factors, cytokines and calcium signaling). Examples of ROS include superoxide and hydrogen peroxide. There are elaborate mechanisms to terminate these reactive molecules, which are highly conserved throughout evolution, such as superoxide dismutases and peroxidases. Oxidative stress refers to the pathological states in which ROS production is increased; the best studied of these is inflammation but there are many others. When oxidative stress exceeds the capacity to terminate ROS, then oxidative damage ensues. These can damage cell or organelle membranes directly (e.g., through peroxidation), and can react with metals, nitrogen or carbon to form intermediates that react with proteins (e.g., through nitration, carbonylation and nitrosylation). ROS may also damage DNA or RNA, including mitochondrial DNA. It is important to stress that because of the reactive nature of ROS, oxidative damage is a complex array of biochemical reactions that occur simultaneously. Moreover, just as there are extensive metabolic pathways to limit ROS, there are even more extensive enzymatic pathways to limit or repair oxidative damage. When these antioxidant defense mechanisms, which include enzymes and scavenger molecules, are overwhelmed, oxidative damage may cause dysfunction of many organelles within cells. This damage may be cumulative and not amenable to repair, particularly in postmitotic cells such as neurons.

In AD, a number of factors may account for increased vulnerability to oxidative stress and damage [1,2]. Aging in general is accompanied by a generalized increase in oxidative damage, perhaps because of waning antioxidant defenses [3]. This may represent a special vulnerability for the longest-lived cells, CNS neurons, leading to impaired reserve and function. The brain is a major user of glucose and oxygen for energy and, therefore, generates ROS in abundance. The brain contains a relatively high concentration of polyunsaturated fatty acids, the macromolecules most vulnerable to oxidative damage. Aβ, which is suggested to be a likely initiating factor in AD owing to a large amount of evidence, has been found to increase oxidative stress in model systems. Furthermore, oxidative stress may interact with inflammation; for example, reactive microglia produce ROS. Oxidative stress is an early feature of AD pathology. In studies of the brains of patients who died at the stage of the earliest recognizable clinical syndrome related to AD, mild cognitive impairment, there is extensive evidence of oxidative damage [4].

Inflammation

Features of inflammation in AD have been studied over many years, and this topic has been reviewed extensively [5,6]. There are differences in the cellular components and the ways in which inflammation is mediated in the brain (neuro-inflammation) compared with the periphery. A complex network of cells, signaling molecules and molecular mediators of inflammatory responses interact within the brain. Astrocytes undergo activation in the areas surrounding senile plaque formation in AD, and may release a variety of signaling molecules. Microglia also show evidence of activation in AD, again in proximity to Aβ deposits. Activated microglia can take part in many cellular processes, which, like chronic inflammation in other organs, can be both beneficial and damaging. Key pathways include cellular proliferation, migration in response to signaling molecules (e.g., chemokines) and release of cytotoxic and inflammatory mediators. Microglia can also act as cytotoxic effector cells by releasing substances such as proteases, ROS, nitric oxide and proinflammatory cytokines. Activated microglia may take on properties of phagocytic cells. Although they can engulf Aβ and some aggregates of Aβ, it is unclear whether they can successfully degrade and clear larger aggregates and fibrils.

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Components of the innate immune system may play a role in AD. For example, microglia carry numerous receptors that function in recognition of phagocytosis, which can be activated in response to foreign proteins, pathogens or aggregates of Aβ, such as Toll-like receptors, receptor for advanced glycation endproducts, various cytokine and chemokine receptors, and receptors for the Fc fragment of antibodies and for complement [7]. Activation of these receptors can lead to increased production of free radicals as well as secretion of signaling molecules by the microglia, which, in turn, may set off a complex chain of cellular responses.

A host of secreted molecules enable the communication between astrocytes, microglia and neurons. Many of these have been implicated as inflammatory mediators in AD. Examples include: s100B and α1-anti-chymotrypsin (α1-ACT), which are produced by astrocytes; cytokines such as TNF-α, IL-1β and IL-6; and chemokines such as macrophage colony-stimulating factor and macrophage inflammatory proteins, which can promote proliferation and trophic support of specific types of inflammatory cells. Astrocytes, microglia and neurons are all able to produce complement proteins, which may act as direct mediators of inflammation and have been detected in neuropathologic studies of AD. In concert, these multiple cells, and the array of factors they elaborate, produce a local ecology that may either be trophic or protective, or stressful or damaging. The key to determining the outcome in a local environment is likely to be the relative concentrations of the ensemble of secreted factors. For example, low levels of TNF-α may promote neuroprotective signaling pathways, whereas high levels may trigger cellular damage. The overall balance and interactions among networks of inflammatory cells, cytokines and chemokines undergoes many changes in the brain in AD. Although activated glial cells and evidence of proinflammatory changes can be found in pathologic studies of AD brain, it is unclear whether changes in the production of these signaling molecules are early events in AD or are obligatory for tissue damage.

Direct genetic evidence that inflammatory mediators contribute to AD risk has been sought, by examining polymorphisms in DNA coding for candidate cytokines. To date, regarding the contribution to AD risk have been inconsistent across many candidate genes in this category. Polymorphisms in α*1-ACT*, *IL-1*β, *IL-2*, *IL-6*, *TNF*-α and others were reviewed by Bertram *et al*., in a detailed meta-analysis of candidate gene studies in AD [8]. After data were pooled across published studies, *TNF*-α was the only inflammatory mediator studied that showed consistent genetic evidence for a potential polymorphism that could modulate AD risk. Two recent genome-wide association studies (GWAS), comparing patients with late-onset AD and controls, found a significant association for single-nucleotide polymorphisms (SNPs) related to the gene for clusterin, a secreted molecule that can bind Aβ and interacts with innate immunity pathways [9,10]. These are similar to roles for apoE isoforms, which show isoform-specific trafficking of Aβ peptides [11] and isoform-specific modulation of innate immune response by microglia and astrocytes. Neither of the GWAS identified significant associations between polymorphisms in the gene for TNF-α and AD. One of these GWAS did observe a significant association between AD and a complement receptor SNP [9].

Detecting biomarkers of oxidative stress & inflammation in CSF & plasma in AD: methodological issues

There are a number of important conceptual and methodological issues in identifying and measuring biomarkers of oxidative damage, and inflammation in patients with AD. A fundamental question is whether to measure biomarkers in the cerebrospinal fluid (CSF) or in blood components such as plasma or serum or even in urine. As CSF bathes the brain and exchanges with the extracellular fluid of the brain, it contains molecules produced by neurons, astrocytes and microglia, and therefore can provide a biochemical window indicating how these cells are altered in AD. Blood or urine, while more convenient to sample, largely reflect systemic production of oxidative or inflammatory molecules; biomarkers originating in the

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brain would be extensively diluted during their passage to the bloodstream, making their detection challenging and potentially confounding their interpretation. Biomarkers can gain access from the bloodstream to CSF, and the process of developing biomarkers for oxidative stress and inflammation should include simultaneous measurement in both the CSF and periphery to understand whether and to what extent the biomarker originated in the brain rather than systemically.

Biomarkers of oxidative stress are often short-lived and labile. To measure indices of oxidative stress in bodily fluids, chemically and metabolically stable markers are needed. These include some products of lipid peroxidation (F_2-isoprostanes [IsoPs]), or oxidatively modified DNA [12]. While these two products of oxidative damage have been validated as accurate *in vivo* biomarkers, this does not mean that the many other products of oxidative damage are not important pathophysiologically, but that F_2-IsoPs and protein carbonyls have superior performance characteristics as quantitative *in vivo* biomarkers.

Inflammatory markers include secreted and diffusible proteins, which are potentially detectable in serum and CSF. Levels of these inflammatory molecules in serum are likely to reflect systemic changes and may not be specific for neuroinflammation. Factors such as weight loss, which is commonly seen in AD, or the general response to having a chronic illness, may alter serum inflammatory markers without providing insight into specific processes occurring in the brain. Comorbid illnesses, medications or other factors may alter peripheral biomarkers of oxidative stress (e.g., smoking or obesity) or of inflammation (e.g., arthritis, anti-inflammatory medications, recent infectious or inflammatory diseases). Indeed, the important issue of drug modulation of AD biomarkers is only beginning to be investigated systematically. Variables such as the time of day samples are drawn and whether they are drawn fasting or not is also potentially important, but have not always been rigorously analyzed in research studies.

The handling of biological samples from accession to assay measurement are important; attention needs to be paid to the types of collection and storage tubes, sample preparation and storage conditions. Recommendations have been developed for biofluid handling and preparation by the AD Neuroimaging Initative (ADNI) [101] and for biomarker analysis of Aβ in CSF and plasma [13], and these are good starting points when considering studies of oxidative damage or inflammation.

Cytokines and other secreted signaling molecules are often present at low levels. Their detection in body fluids requires sensitive assay methodology. Sandwich ELISAs, using high-affinity capture and reporter antibody pairs, have been developed for a host of cytokines and other secreted signaling proteins, and the most sensitive of these can detect low picomolar concentrations (the range often reported in CSF or plasma). Inflammatory signaling molecules may diffuse for short distances between networks of cells, and contribute locally to inflammation without necessarily diffusing into CSF in detectable amounts. Therefore, the failure to detect a cytokine or similar molecule in CSF does not rule out a potential role for that molecule in the pathogenesis of AD.

Results of clinical studies of oxidative stress & inflammation as AD biomarkers

Candidate CSF biomarkers of oxidative stress

The range of biochemical reactions that occur under conditions of oxidative stress and damage is large and so is the number of potential biomarker candidates. The Biomarker of Oxidative Stress Study (BOSS) was coordinated by the NIH to determine which among 16 commonly studied biochemical products (determined by 19 different assays) of oxidative damage had

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acceptable performance characteristics as quantitative *in vivo* biomarkers in an experimental model of oxidative injury to rat liver [12]. It is important to note that good performance as a biomarker does not indicate supremacy as a pathogenic agent and, conversely, that poor performance as a biomarker does not mean the processes that underlie its production are not critical to disease. Products of oxidative damage measured in plasma were: lipid hydroperoxides (two assays), thiobarbituric acid reactive substances, malondialdehyde (MDA; three assays), free F_2-IsoPs (comigrating with deuterated 8-*iso*-$PGF_{2\alpha}$), free and esterified F_2-IsoPs (comigrating with deuterated 8-*iso*-$PGF_{2\alpha}$), protein carbonyls, methionine sulfoxidation, 3-nitrotyrosine, 3-hydroxytyrosine, DNA damage by the Comet assay and leukocyte M1G DNA adduct. Products of oxidative damage measured in urine were: MDA, free F_2-IsoPs (comigrating with deuterated 8-*iso*-$PGF_{2\alpha}$), free F_2-IsoPs (comigrating with deuterated 8,12-*iso*-$iPF_{2\alpha}$-VI), dityrosine and 8-hydroxy-2′-deoxyguanosine (8-OHdG). Of these, MDA, F_2-IsoPs and 8-OHdG were acceptable quantitative *in vivo* biomarkers of oxidative damage. Of these experimentally validated biomarkers of oxidative damage, F_2-IsoPs have been the most extensively investigated in CSF from patients with AD (see later), although 8-OHdG has been reported in one study to be increased in lumbar CSF in AD versus controls [14]. We have observed that CSF levels of protein carbonyls do not differ between AD and controls [Galasko D, Unpublished Data].

The IsoPs are a series of prostaglandin-like compounds that are produced largely if not exclusively by nonenzymatically catalyzed oxidation of arachidonic acid while still esterified to lipid [15]. Esterified F_2-IsoPs can be hydrolyzed and released from tissue into the extracellular fluid. In tissue subjected to oxidative damage, esterified F_2-IsoPs exceed free F_2-IsoPs by several orders of magnitude. F_2-IsoPs are a group of four regioisomers that have a cyclopentanedione structure similar to $PGF_{2\alpha}$. Several classes of IsoPs and other isoprostanoids exist; however, among the isoprostanoids, F_2-IsoPs are the only experimentally validated quantitative *in vivo* biomarkers of oxidative damage. As noted earlier, F_2-IsoPs can be measured by several methods, the most precise and sensitive (picograms) being stable isotope dilution assays that use chromatographic separation followed by mass spectrometry. A number of methods have been developed to detect subsets of the ensemble of F_2-IsoPs, which vary in the type of chromatography and deuterated internal standard. Antibody-based assays for F_2-IsoPs are also available, but these have not been validated as quantitative *in vivo* biomarker assays.

In some transgenic mouse models of AD that deposit Aβ in plaques, cerebral or hippocampal levels of esterified F_2-IsoPs increase early in the course of pathology, and continue to increase further as the mice age [16]. In human brain tissue, esterified F_2-IsoPs levels are increased in patients with mild cognitive impairment (MCI) or AD [17]. Free F_2-IsoPs are detectable in human CSF in pg/ml abundance; esterified F_2-IsoPs are not detectable in CSF.

Clinical studies of AD versus controls have consistently shown an increase in CSF F_2-IsoPs measured with stable isotope dilution assays that used either deuterated 8-*iso*-$PGF_{2\alpha}$ or 8,12-*iso*-$iPF_{2\alpha}$-VI [18-20]. CSF F_2-IsoPs levels do not correlate strongly with the severity of dementia, and are increased early in the symptomatic course of AD [21,22]. Longitudinal studies with sequential lumbar punctures have found that the concentration of F_2-IsoPs in CSF increases during the progression of the disease [22]. In presymptomatic carriers of familial AD mutations, increased CSF F_2-IsoPs accompanies decreased levels of $A\beta_{42}$ [23]. A few small studies have indicated that CSF F_2-IsoPs levels may improve diagnostic classification of AD relative to controls when combined with other CSF biomarkers such as $A\beta_{42}$ and tau [20,21].

Plasma levels of free F_2-IsoP were reported to be increased in AD and MCI by one research group [21]. However, levels were not altered in AD compared with the controls and were, in fact, lower in a study by another group [24]. A recent study of plasma analyzed by the first

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laboratory failed to replicate a difference between AD and controls [25]. It therefore appears that systemic increases in F_2-IsoPs are not a consistent feature that results from AD. Plasma levels of F_2-IsoPs need to be interpreted with caution. Experimental studies that induced high levels of oxidative injury to rat brain, as measured by esterified F_2-IsoPs, did not yield detectable increases in plasma or urine free F_2-IsoPs. Moreover, several systemic factors, such as weight loss, chronic ill health, recent exercise and smoking, have all been shown to increase peripheral concentrations of F_2-IsoPs.

A few studies have examined the potential utility of CSF F_2-IsoPs to assess the effects of antioxidant treatment for AD. In a naturalistic study, a series of patients with AD had CSF measured at baseline and again 12 months later, and were analyzed according to their use of antioxidant supplements [26]. Those subjects who took vitamin E (α-tocopherol) and vitamin C showed no changes in CSF F_2-IsoP levels, whereas patients who did not take any supplements showed an increase at 12 months. In a recent clinical trial, patients with AD were randomized to receive coenzyme Q, a combination of 'cytosolic' antioxidants (α-tocopherol), vitamin C and α-lipoic acid or placebo for 16 weeks. CSF was obtained at baseline and at the end of the 16-week treatment period. The group who received the cytosolic antioxidants showed a significant decrease in CSF F_2-IsoPs relative to placebo [Galasko D *et al.*, Manuscript in Preparation]. This suggests that F_2-IsoPs may be used to evaluate suppression of oxidative damage to CNS by treatment interventions. The clinical significance of these interventions will require long-term evaluation with clinical end points.

Candidate CSF biomarkers of inflammation

α1-anti-chymotrypsin is secreted by astroglial cells and can colocalize with Aβ in plaques in AD. It has been studied for many years in serum and plasma as a potential AD biomarker, with mixed results – some studies showed no changes in levels in AD, while others showed a slight increase, but with considerable overlap [27,28]. A recent large-scale study provided definitive data on this biomarker [29]. Plasma α1-ACT was measured in over 500 subjects, who spanned a wide range of clinical severity of AD, and in a group of age-appropriate controls, and a subset of these subjects had CSF sampled. Plasma α1-ACT was significantly increased in AD overall relative to controls, but the increase was small and was highly influenced by those subjects with greater severity of dementia. α1-ACT was increased in AD CSF versus controls, and levels again correlated with the degree of dementia in AD. There was less clear separation of mild AD and controls. Therefore, α1-ACT is unlikely to be useful as an early diagnostic marker.

s100B is secreted by astrocytes, and levels are increased in response to many different types of injury and inflammation. Evidence supports both trophic and cytotoxic roles for s100B, depending on its levels [30]. Increased levels in CSF (and even in plasma) have been reported after acute stroke or CNS trauma. Postmortem studies have shown an increase in immunostaining for s100B, particularly in the vicinity of neuritic plaques; the extent of staining increased with the progression of dementia. A study comparing levels in CSF in AD and healthy controls found increased CSF levels in mild-to-moderate AD, but not in severe AD [31]. This raises the possibility that s100B is an index of astrocytic processes involved in the earlier stages of AD, possibly accompanying plaque maturation. In another study, CSF s100B was increased to a similar extent in AD and fronto–temporal dementia relative to controls [32]. In the AD patients, higher CSF levels of s100B correlated with greater degrees of brain atrophy, as shown by MRI. Although it is not a specific marker for AD, s100B could potentially be used to monitor the effects of treatment aimed at decreasing astrocyte activation in the disease. Glial fibrillary acidic protein is a protein whose expression increases when astroglia are activated. Levels are detectable in CSF, and show an increase with aging [33]. A recent study found that CSF levels of glial fibrillary acidic protein were increased in AD compared with controls, and were higher in AD than in Creutzfeld–Jakob disease (CJD); however, in the same patients, CSF levels of

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s100B were slightly increased in AD but markedly increased in CJD [34], suggesting that there may be different patterns of astrocytic activation in AD and CJD.

Many studies have tried to measure cytokines and chemokines in CSF. These molecules occur at low levels, and require extremely sensitive assays for detection in CSF and plasma. Results of studies with well-described groups of patients and controls are summarized in Table 1. For many of these inflammatory biomarkers, findings have been inconsistent. For example, levels of TNF-α were found to be increased in AD, even at the stage of MCI, by one research team [35,36], while other studies reported much lower levels, and failed to show differences between AD and controls [37,38].

Several cytokines or chemokines have been found to be increased in CSF in MCI, which provides supportive evidence that activation of their signaling pathways occurs relatively early in the clinical expression of AD. Examples include monocyte chemotactic protein-1, IL-8 [39], IL-1 receptor type II and IL-18 [40] (for more examples see Table 1). Many of these studies have been relatively small and not all have followed the MCI patients to determine whether they progressed to develop clinical dementia or not. Thus, these findings of changes in inflammatory molecules in CSF in MCI require larger-scale replication in cohorts of patients followed longitudinally to determine their predictive value. Panels of inflammatory molecules have not been systematically studied in MCI. Measuring a large number of these signaling molecules simultaneously could provide a more detailed picture of how a network of aberrant signaling could arise in the brain and contribute to AD.

Panels of assays for cytokines, chemokines and other secreted molecules are becoming increasingly available as multiplex assays that allow the simultaneous measurement of many markers in a small sample of biofluid. Recently, studies have begun to apply these panels to plasma from AD patients [41]; however, to date, there are few published studies using multiplex analyses of inflammatory biomarkers in AD CSF. Findings have not been consistent, and reports have noted that many individual cytokines or chemokines are undetectable [42-44]. Analyses of large panels of secreted signaling molecules are currently in progress (e.g., as part of the ADNI [101]), and these will help to establish whether a set of inflammatory and other secreted proteins are consistently altered in MCI and AD.

As mentioned earlier, recent GWAS found significant association of SNPs related to clusterin in AD. Clusterin is secreted in native and glycosylated forms, presumably in response to different stimuli, and interacts with Aβ and innate immunity pathways. Levels of glycosylated forms were found to be increased in CSF in AD relative to controls [45]. To determine whether this change is specific to AD or has diagnostic utility will require further investigation.

There have been limited efforts to examine how therapeutic interventions targeting inflammatory pathways affect CSF biomarkers. In one recent study, patients with AD were randomized to a high dietary intake of omega-3 fatty acids or to placebo. After 6 months of treatment, subjects in both groups underwent lumbar punctures. There were no differences between groups regarding CSF levels of the core AD biomarkers Aβ and tau, and also no differences in levels of IL-6, TNF-α and soluble IL-1 receptor type II [46].

Plasma markers of inflammation

To date, no plasma inflammatory biomarkers have been shown to distinguish AD from controls, particularly when AD patients were studied at mild stages of dementia. The relationship between plasma and CSF levels of these biomarkers has rarely been studied. For some markers (e.g., α1-ACT, IL-6 and monocyte chemotactic protein-1), a significant correlation between levels of biomarkers in CSF and plasma was found [42]. Levels of many cytokines are lower

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in CSF than in plasma, which raises the possibility that passage from plasma to the CSF, rather than intrathecal synthesis, may be a major route of access of the biomarker into CSF [42,43].

A number of studies have examined whether levels of plasma inflammatory biomarkers may predict the development of AD. Studies in this area have been relatively recent, and consensus has not yet emerged regarding which biomarkers are likely to be most helpful. Epidemiological studies have provided the most extensive data to address these questions. For example, in the Rotterdam study, elderly subjects with higher plasma levels of α1-ACT and IL-6 had an increased risk of incident dementia, which remained significant in analyses restricted to incident cases of AD [47]. In the Framingham study, release of cytokines by peripheral blood mononuclear cells was analyzed in elderly community-dwelling subjects. Subjects with the highest extent of peripheral blood mononuclear cell production of IL-1β and TNF-α had an increased risk of developing incident AD [48]. In a population-based study, levels of C-reactive protein in plasma were increased in people with MCI relative to controls [49].

The effects of intermittent systemic inflammation were carefully monitored in a recent study in which patients with AD had blood drawn for levels of TNF-α at baseline and 2-month intervals over the next 6 months, and caregivers were interviewed to document incident systemic inflammatory events. Higher levels of TNF-α at baseline, and intermittent increases in plasma TNF-α, triggered by factors such as acute infections, were found to be associated with more rapid progression of cognitive decline [50]. This intriguing observation, that intermittent inflammatory illnesses may accelerate the clinical progression of AD, will require further study to confirm these findings and uncover the mechanisms that may be responsible.

Future perspective

Table 2 lists a number of potential uses of biomarkers related to oxidative damage and inflammation. They carry promise in being able to assist in the evaluation of mechanisms of disease, preclinical animal models and treatment effects, diagnosis (including antecedent diagnosis at the stage of MCI) and prediction or risk analysis. Specific recommendations for clinical applications are limited by the lack of replication studies and, for many biomarkers in this area, by the lack of well-validated assays. An important need is to carry out larger studies looking at the timing of appearance of signatures of inflammatory or oxidative damage in CSF. Natural history studies of the transition from intact cognition to the earliest deficits associated with AD, including detailed studies of people at risk of AD by virtue of age or genetic risk factors, are receiving widespread attention, and many ongoing and proposed studies are collecting biomarkers, including CSF and imaging. These studies will help to determine whether incorporating oxidative or inflammatory biomarkers into multianalyte panels will improve diagnostic or prognostic ability.

More extensive profiling of CSF analytes, particularly for complex processes such as inflammation, is likely to be more informative than measuring a small number of analytes. For example, a recent study screened over 100 signaling molecules in plasma using a filter-based assay, and used data mining to reduce these to a set of 14 markers that distinguished AD from controls reasonably well, and in a separate series of plasma samples from patients with MCI, had predictive value in determining which patients progressed to develop AD on follow-up and which patients did not [51].

Treatment studies for AD are increasingly focusing on novel approaches to diseasemodifying therapy. Owing to the complex pathophysiology of AD, the consequences of interfering with a particular pathogenic pathway in AD are uncertain. In animal models of amyloid deposition related to AD, inflammation and oxidative stress can be used as outcome measures to assess interventions. In early-phase human studies of novel drugs, biomarkers can serve in principle as tools to provide information about how well drugs hit their targets and influence pathways

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such as oxidative damage and inflammation. With the exception of F_2-IsoPs, which have shown utility in relation to clinical trials of antioxidants, data to guide specific choices of biomarkers of inflammation or oxidative damage to evaluate treatment are extremely limited. Stored biosamples from ongoing and future clinical trials will allow wide profiling of biomarkers, which will clarify how inflammation and oxidative stress change in response to treatment. An unrealized hope is for predictive or antecedent biomarkers, which could assist in selecting subjects for prevention trials.

Executive summary

Alzheimer's disease

- Alzheimer's disease (AD) is the most common form of dementia; early and accurate diagnosis could benefit from biomarkers.
- The pathophysiology of AD is complex.
- Amyloid-β protein may initiate a chain of pathological events, which include oxidative stress and inflammation.

Oxidative stress & inflammation in AD

- Oxidative stress and inflammation in the brain lead to the release of many biomarkers.
- Biomarkers reflecting brain metabolism are more likely to be detected in the cerebrospinal fluid (CSF) than in blood.
- Many oxidative stress markers have been proposed; F_2-isoprostanes are validated quantitative *in vivo* biomarkers of oxidative damage that have been extensively studied in human CSF.
- A host of secreted inflammatory molecules exist, a relatively small number of which are detectable in the CSF.

CSF F_2-isoprostanes in AD

- At present, CSF F_2-isoprostanes are the most consistent index of oxidative damage in AD.
- They are increased in early stages of mild cognitive impairment, in diseased regions of AD brain and in CSF from patients with very early signs of dementia.
- Preliminary studies suggest that they may increase diagnostic accuracy when combined with AD lesion biomarkers.

Inflammatory biomarkers for AD

- Inflammatory biomarkers are altered in CSF in established AD, but less clearly in mild cognitive impairment.
- s100B, α1-anti-chymotrypsin, IL-8, IL-1β, macrophage inflammatory protein-1α, macrophage migration inhibitory factor, granulocyte-macrophage colony-stimulating factor and TNF-α appear to be increased in mild-to-moderate AD. This is consistent with activation of microglia and astrocytes in AD.
- At present, none of these biomarkers has diagnostic or predictive value.

Oxidative damage biomarkers may be responsive to antioxidant therapy

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- CSF biomarker studies of patients given antioxidant supplements support this possibility, but this area requires further study.

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Table 1

Inflammatory biomarkers reported in cerebrospinal fluid in relation to Alzheimer's disease.

Inflammatory biomarker	CSF changes in AD, MCI and other disorders relative to controls	Ref.
IL-1β	↔ in AD	[27]
	↔ in AD, ↔ in MCI	[37]
	↑ in AD	[47]
IL-1 R2	↑ AD	[37]
	↔ in MCI, ↔ in AD	[39]
IL-6	↑ in AD	[52-54]
	↔ in AD	[55]
	↔ in AD, ↑ in MCI	[38]
IL-8	↑ in AD , ↑ in MCI	[43]
IL-11	↑ in AD , ↑ in FTD	[53]
TNF-α	↑ in AD, ↑ in VaD	[35]
	↑ in MCI	[36]
	↔ in AD, ↔ in MCI	[37,38]
Granulocyte-macrophage colony stimulating factor	↑ in AD, ↑ in VaD	[56]
TGF-β	↑ in AD	[57,58]
	↑ in AD , ↑ in VaD	[59]
	↓ in MCI, ↔ in AD	[38]
VEGF	↑ in AD, ↑ in VaD	[54]
Monocyte chemotactic protein-1	↑ in AD	[38,43]
Macrophage migration inhibitory factor-1α	↔ in AD	[38]
Macrophage migration inhibitory factor	↑ in AD ↑ in MCI	[40]
IFN-γ	↔ in AD, ↔ in MCI	[57]
Interferon γ-inducible protein 10	↑ in AD , ↑ in MCI	[43]
α-1-antichymotrypsin	↔ in AD	[27]
	↑ in AD	[28,29]
s100B	↑ in AD	[31]
	↑ in AD , ↑ in FTD,	[32]
	↑↑ in CJD	[34]
Glial fibrillary acidic protein	↑ in AD, slight ↑ in CJD	[33,34]

↓Decreased levels in AD or MCI compared with controls

↑Increased levels in AD or MCI compared with controls

↑↑Markedly increased levels in AD or MCI compared with controls

↔No difference in AD or MCI compared with controls.

AD: Alzheimer's disease; CJD: Creutzfeld–Jakob Disease; CSF: Cerebrospinal fluid; FTD: Fronto–temporal dementia; MCI: Mild cognitive impairment; VaD: Vascular dementia.

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Table 2

Potential applications of biomarkers of oxidative damage and inflammation for Alzheimer's disease.

Potential uses	Oxidative biomarkers	Inflammation biomarkers
Animal models	Explore disease mechanisms and pathways Assess responses to interventions, especially antioxidants	Explore disease mechanisms and pathways Assess responses to interventions, especially anti-inflammatory drugs
AD diagnosis	CSF F_2-IsoP, in combination with lesion-specific markers such as $A\beta_{42}$, may help with AD diagnosis	Several cytokines and chemokines are altered in CSF in AD versus controls The degree of separation appears weaker than for established biomarkers ($A\beta_{42}$ and tau) Larger studies are required to establish whether these may add to diagnostic accuracy
Antecedent marker for AD	Unclear for CSF Initial reports for plasma and urine F_2-IsoPs were not reproducible	Unknown for CSF Plasma levels of some cytokines and of CRP are relatively weak risk factors for AD
Clinical trials for AD	Assess response to antioxidants	Assessment of inflammatory responses will require a panel of markers because of complex interactions Choice of biomarker panels for specific studies will depend on the nature of the therapeutic target

Aβ: Amyloid-β; AD: Alzheimer's disease; CRP: C-reactive protein; CSF: Cerebrospinal fluid; F_2-IsoP: F_2-isoprostanes.