Role of Cerebrospinal Fluid and Plasma Biomarkers in the Diagnosis of Neurodegenerative Disorders and Mild Cognitive Impairment

Luis F. Gonzalez-Cuyar, **Joshua A. Sonnen**, **Kathleen S. Montine**, **C. Dirk Keene**, and **Thomas J. Montine**
Department of Pathology, University of Washington, Seattle WA 98104

Abstract

Biomarkers are one type of laboratory testing being developed in response to the therapeutic imperative for diseases that cause cognitive impairment and dementia. The role of biomarkers is already transforming the organization and conduct of clinical trials, and if successful will likely contribute in the future to the medical management of patients with these diseases. Despite the obvious utility of practicality of blood- or urine-based biomarkers, so far results from these fluid compartments have not been reproducible. In contrast, substantial progress has been made in cerebrospinal fluid biomarkers. Here we review the stages of cerebrospinal fluid biomarker development for several common and unusual diseases that cause cognitive impairment and dementia, stressing the distinction between diagnostic and mechanistic biomarkers. Future applications will likely focus on diagnosis of latent or early-stage disease, assessment of disease progression, mechanism of injury, and response to experimental therapeutics.

Keywords

Alzheimer's disease; Parkinson's disease; vascular brain injury; biomarkers; cerebrospinal fluid; neurodegenerative disorders; mild cognitive impairment

Introduction

Cognitive impairment and dementia already are major public health problems for older individuals and are poised to amplify tragically with our increasingly aged population. Epidemiologic studies estimate that prevalence rates of dementia double every 5 years after age 65, and the prevalence of cognitive impairment is even higher [1]. These facts compel a therapeutic imperative that is pursued currently by many laboratories around the world in search of etiologies, key pathogenic steps, and effective interventions that will at least treat and hopefully cure diseases causing cognitive impairment and dementia.

Community- and population-based studies of brain aging with autopsy end points from across the United States have repeatedly identified three disease processes that commonly contribute to cognitive impairment and dementia in the elderly: Alzheimer's disease (AD), defined by moderate-tohigh levels of neuritic plaques and isocortical neurofibrillary tangles (NFTs); vascular brain injury (VBI), especially the form that results in microinfarcts (μVBI); and isocortical Lewy body disease (LBD). Other diseases that also can cause dementia include frontotemporal lobar degenerations (FTLDs) or prion disease, neither of

Address correspondence to: Thomas J. Montine, MD, PhD, tmontine@uw.edu, ph. 206-543-1140, fax: 206-897-5249.

which is well represented in community- and population-based studies of incident dementia. Part of this may be due to excluding individuals who developed dementia at a younger age from such studies, and part may be due to the lower incidence and prevalence of these diseases compared with AD, μVBI, and LBD. It is also worth considering the role of μVBI, seemingly caused by disease of small caliber cerebrovasculature, versus VBI from large caliber vessels. Large and small vessel cerebrovascular disease and their consequences to brain commonly occur together [2], most likely due to overlapping pathogenic mechanisms; for this reason they are difficult to separate completely. In individuals with cerebrovascular disease that results predominantly in large vessel VBI, the clinical consequences are more readily recognized as strokes rather than as the dementia syndrome. In contrast, individuals with progressive accumulation of μVBI are less likely to be recognized as having had a stroke but can—and commonly do—present with the dementia syndrome.

Although estimates of the burden of these diseases commonly contributing to dementia vary among different cohorts, results from the ACT (Adult Changes in Thought) study of men and women in the Seattle area represent typical values and point estimate the population-attributable risk for dementia as 45% from AD, 33% from μVBI, and 10% from isocortical LBD [3]. It is interesting to speculate why approximately 12% of the population-attributable risk of dementia remains unexplained in ACT. A similar degree of unexplained dementia also has been observed in other population-based studies [4]. It is important to realize that because these are autopsy-based data, it is unlikely that some other known disease process, such as FTLD or prion disease, is contributing significantly to these cases of unexplained dementia, since the tools exist for detecting these disease processes in autopsy specimens. It seems more likely that the cutoff values for "high" pathologic change sufficient to explain dementia do not capture all patients who actually had dementia. For example, in ACT we define Braak stage V or VI for NFTs as sufficient to explain dementia from AD, whereas it is entirely possible that some individuals are more vulnerable to clinical expression of dementia with stage IV (or lower) AD pathologic changes.

It is critical to realize that although populationattributable risk is a statistical estimate of the public health burden of disease, it does not reflect the common comorbidity among these three diseases. Cognitive impairment and dementia in the elderly are syndromes that derive most commonly from an idiosyncratic convergence of AD, μVBI, and LBD [3–5]. Because AD, μVBI, and LBD are chronic diseases, this means that each, or some combination, has a clinical stage of full expression that is called dementia; a prodromal stage with clinically detectable cognitive impairments that do not reach the diagnostic threshold for dementia and that go by several names, including mild cognitive impairment (MCI) or cognitive impairment not dementia; and latent stage, during which the disease has started but it is clinically undetectable. Individuals who were enrolled in ACT, who were examined within 1 year of death, and who at that time had normal cognitive function show a mix of AD, μVBI, and LBD at autopsy, similar to patients with dementia but with a lower burden of disease [6]. Recently, we and others have shown that this observation is widely replicated across many community- and population-based studies [7, 8]. From these cross-sectional data we infer that if these individuals with clinically silent AD, μVBI, and/or LBD had lived longer some may have progressed to MCI or even dementia; however, this is impossible to know from autopsy studies. Thus, although the autopsy record strongly suggests the existence of latent forms of AD, μVBI, and LBD, proof of this concept will require biomarkers, rather than autopsy data, to demonstrate the presence of latent disease in asymptomatic individuals who are then followed in longitudinal studies of clinical progression to prodrome or dementia stage.

Although we have pathologic tools to identify AD, μVBI, and LBD in autopsy studies, as well as less common causes of cognitive impairment and dementia, there is a clear need to

develop validated methods to detect and quantify each in living patients. A key component of the response to the therapeutic imperative for neurodegenerative diseases that cause cognitive impairment and dementia is the development of different types of laboratory testing for these common diseases. The two major—and complementary— approaches are neuroimaging and biomarkers. In this review we focus on biomarkers: dynamic quantitative in vivo measures of ongoing disease, stress, injury, or response to injury (Table 1). Biomarkers stand in sharp distinction to risk assessment, commonly done in the laboratory setting by DNA sequencing, because genetic risk factors are immutable and are used to predict the likelihood of future disease.

Two major roles for biomarkers in neurodegenerative disease have the potential to be transformative. ***Disease-specific biomarkers*** have multiple applications. (1) Detect latent disease and thereby provide an opportunity for early intervention. An example is detection of hypercholesterolemia and intervention with statins prior to onset of angina or first myocardial infarction. (2) Aid in differential diagnosis, especially determining what disease or combination of diseases is contributing to a patient's cognitive impairment or dementia syndrome. This disease-specific information will be enormously helpful in designing and assembling subjects for clinical trials to test disease-specific interventions and may also help harmonize cohorts, thereby yielding reduced variance and smaller required cohort size [9, 10]. (3) Provide robust quantitation of disease progression that may be used to reduce the time to primary outcome in clinical trials. ***Mechanism-specific biomarkers*** will have multiple complementary applications. (1) Once the disease diagnosis is made, biomarkers of a particular type of stress, injury, or response to injury may also be useful in following disease progression. (2) More importantly, biomarkers of specific mechanisms will help discern the biochemical or cellular actions by which experimental therapeutics actually achieve beneficial effects in people and thereby accelerate rational treatment development. Ultimately, once clinical investigations have yielded effective disease-modifying interventions, some ensemble of validated biomarkers will assist health care providers in the medical management of patients with these common diseases.

Before embarking on discussions of biomarkers for specific diseases or mechanisms of injury, it is important to stress that the level of scientific evidence in support of different biomarker candidates varies widely. Several schemes have been proposed to categorize the evidence in support of biomarker candidates. My colleagues and I have devised a simple and practical five-level ranking for the development of biomarkers [11] (Table 2).

Alzheimer's Disease

AD, the most prevalent cause of cognitive impairment and dementia, is characterized pathologically by the accumulation of modified proteins in two abnormal structures: plaques and tangles. In the first case amyloid β (Aβ) proteins, which are endoproteolytic products of the amyloid precursor protein (APP), accumulate in structures called "plaques" that may be senile, diffuse, or neuritic. C terminal cleavage of APP to generate the Aβ fragment is promiscuous and leads to the production of a number of closely related peptides, the two most common being 40 or 42 amino acids in length.

With respect to their usefulness as biomarkers, both types of Aβ peptides are generated in the brain but also by other organs. Aβ_{40} is more abundant in cerebrospinal fluid (CSF) and plasma than Aβ_{42}. The relevance of plasma Aβ peptides to AD is yet to be fully clarified; this is an area of intense investigation. A lower concentration of CSF Aβ_{42} is correlated repeatedly with AD [12]. In the second case, the microtubule-associated protein tau accumulates in structures called NFTs. The tau in these structures is extensively post-translationally modified and described as paired helical filament (PHF) tau. One

characteristic of PHF-tau is extensive phosphorylation. With respect to its usefulness as a biomarker, increased concentration of tau and some phosphorylated tau isoforms have been observed in AD, as well as several other neurodegenerative diseases and ischemic injury. Tau isoforms have yet to be detected in peripheral body fluids.

There is a large effort underway to develop biomarkers for all stages of AD, and there has been considerable progress for CSF biomarkers (Table 3). Three recent publications on consensus clinical criteria for the diagnosis or evaluation of different stages of AD have stressed the role of biomarkers [13–15]. Although several plasma- or urine-based assays have been proposed at Level I or Level II, we are unaware of any that have withstood validation.

Following the work of many laboratories, the AD Neuroimaging Initiative (ADNI) has taken the arduous step of moving to Level IV for reduced CSF $A\beta_{42}$ plus elevated CSF tau concentrations in individuals with AD at dementia and prodromal stages, and likely soon in latency as well [16••]. Moreover, ADNI now provides an international platform on which to cross-compare a variety of laboratory and functional tests for the diagnosis of different stages of AD [17]. Although this may sound straightforward, it is a herculean and necessary step to move biomarkers from the research setting to general medical practice. Furthermore, elegant imaging studies, a few buttressed by subsequent postmortem examination, indicate that decreased CSF $A\beta_{42}$ in individuals with AD is associated with increased $A\beta_{42}$ accumulation in brain [18]. The basis for increased CSF tau concentration in AD is more speculative but appears in several degenerative and destructive diseases of brain and may be a consequence of neuronal injury. Although still speculative, one possibility is that reduced CSF $A\beta_{42}$ will be an early diagnostic biomarker of AD and elevated CSF tau a biomarker of disease progression. The application of mechanism-specific biomarkers, including F_2-isoprostanes, to AD diagnosis is discussed in the "Inflammation and Free Radical Injury" section below.

Parkinson's Disease and Other "Synucleinopathies"

Given the potential for biomarkers and the successes achieved so far in AD, many investigators are pursuing biomarkers for other neurodegenerative diseases. An area of major focus is Parkinson's disease (PD), evidenced by the Parkinson's Progression Markers Initiative (PPMI) sponsored by the Michael J. Fox Foundation, and the Parkinson's Disease Biomarkers Identification Network (PD-BIN) being established by the National Institute of Neurological Disorders and Stroke (Table 4).

PD is one of a group of neurodegenerative diseases called "synucleinopathies" because all share the pathologic feature of α-synuclein (SNCA)-containing inclusions. In PD and dementia with Lewy bodies (DLB), SNCA inclusions are contained within a subset of neurons and are called Lewy bodies. For this reason, PD and DLB are sometimes grouped together as LBD. The regional distribution of LB in PD and DLB broadly overlaps, and as methods to detect SNCA-immunoreactive inclusions have become more sensitive, it has become more difficult to distinguish clearly between PD and DLB at autopsy. The clinical distinction between PD and DLB is somewhat arbitrary and related to the relative timing of onset of cognitive impairments versus motor impairments. This issue is further complicated by the recently recognized cognitive impairments that commonly occur in patients with PD, even at the time of initial diagnosis. Nevertheless, it is important to realize that with the current consensus criteria, DLB very commonly is associated with comorbid changes of AD; thus, one would expect that biomarkers of AD more commonly will be "positive" in patients with DLB than patients with PD. The other "synucleinopathy" that is commonly investigated along with PD is multiple system atrophy (MSA), which can be difficult to

distinguish clinically from PD especially in early stages of disease, but is characteristically less responsive to dopamine replacement therapy. MSA is distinguished pathologically from PD and DLB because SNCA-immunoreactive inclusions occur prominently in glia rather than neurons.

Decreased CSF SNCA concentration in patients with PD relative to controls has been observed by several groups of investigators; however, not all groups have been able to reproduce this finding [19]. Two groups recently contributed excellent, large cross-sectional studies of CSF SNCA concentration in control individuals without neurologic disease, patients with AD, and patients with PD or other "synucleinopathies." Using two different assays for CSF SNCA, they both concluded that CSF SNCA concentration is significantly reduced in all three "synucleinopathies" [20•, 21•] (highlighted in Nature Reviews Neurology [22]), solidly achieving Level III for decreased CSF SNCA as a biomarker for "synucleinopathies." The performance of standardized CSF SNCA assay as a clinical laboratory assay for "synucleinopathy" (Level IV) awaits the outcome of large multicenter studies such as PPMI and PD-BIN.

In addition to CSF SNCA, some groups have investigated CSF DJ-1 as a biomarker of PD and related diseases. DJ-1 is a multifunctional redox-sensitive protein involved in mitochondrial function [23]. Importantly, loss-of-function mutations in the gene that encodes DJ-1 is a cause of inherited PD. The first group to investigate CSF DJ-1 in patients with sporadic (not caused by known mutations) PD used an immunoblotting approach, and concluded that CSF DJ-1 is increased in patients with PD, especially at an early symptomatic stage [24]. Subsequently, much larger cross-sectional studies using X-MAP–based quantification of CSF DJ-1 concluded that concentration of this protein was decreased in the CSF of patients with PD compared to controls and patients with AD, but did not correlate with PD severity [21•, 25•]. Critically, one of these studies highlighted the importance of controlling for both blood contamination of CSF and age when interpreting CSF concentration of DJ-1 and SNCA [25•]. DJ-1 and SNCA are detectable in plasma and serum; however, levels in these biofluids are not correlated with PD [25•, 26]. There is Level I evidence for salivary SNCA and DJ-1 in patients with PD [27].

It is interesting that results from these studies cited above have yet to identify abnormal CSF SNCA or DJ-1 concentrations in a subset of asymptomatic controls, as has been observed with CSF $A\beta_{42}$ and tau in elderly controls, perhaps because of lower prevalence of latent "synucleinopathies." Moreover, the results from these studies indicate that the performance characteristics of CSF SNCA or DJ-1 concentration as a clinical laboratory test are insufficient as a single measure, and that there is a clear need for improved laboratory testing for individuals with PD or related diseases [21•].

Although these results focused on diagnosing PD without considering cognitive status, cognitive impairment in patients with PD is an area that has received much recent attention. One hypothesis tested by several groups is that the biomarkers of AD, (e.g., CSF $A\beta_{42}$ and tau) might be useful in evaluating at least a subset of patients with PD and cognitive impairment or PD and dementia (PD-D). Several groups of investigators have observed reproducibly reduced CSF $A\beta_{42}$ levels, but not increasing concentrations of CSF tau species, in patients with PD-D [28–30] but no significant change in either CSF protein concentration in patients with PD without cognitive impairment [20•]. These intriguing observations suggest an incomplete overlap in the pathophysiologic processes of PD-D and AD, a relationship that requires further investigation by other modalities including neuroimaging approaches. One hypothesis arising from these data is that the dopaminergic neurodegeneration that occurs in PD may clinically unmask AD at an earlier stage, while

$A\beta_{42}$ is being deposited in parenchyma (and decreasing in CSF) but before the occurrence of large-scale neuron death resulting in elevated CSF tau concentration.

Vascular Brain Injury

In addition to a very large scientific literature on risk factors for large vessel VBI, there also are many reports about CSF—and in some instances plasma or serum— biomarkers for VBI from large vessel disease [31, 32]. In contrast, although there is also a substantial literature on the risks for μVBI, prominently including diabetes mellitus and hypertension, there are no studies reporting CSF or blood-based biomarkers of cerebral μVBI beyond Level I. Several groups have explored the intersection of VBI and AD in clinical, pathologic, and even biomarker studies [33]; however, any interaction beyond functional remains enigmatic.

Other Neurodegenerative Diseases

Although the CSF 14-3-3 protein has been a valuable diagnostic aid for Creutzfeldt-Jakob disease (CJD) for a decade [34], its quantification can be problematic [35]. More recently, attention has focused on "extremely high" (at least 10-fold higher) tau levels in CSF as a biomarker of CJD. A recent meta-analysis concluded that the sensitivity and specificity for extreme elevations in CSF tau both exceeded 90% for CJD when compared with controls, AD, VBI, DLB, or VBI [36]. However, direct comparison of CSF 14-3-3 and CSF tau as biomarkers for sporadic CJD showed that each yielded similar results [34]. Although concentration of CSF tau and some of its phosphorylated isoforms have been reported to decrease in FTLD [37], this same meta-analysis concluded that the sensitivity and specificity for CSF concentration of total tau and tau phosphorylated at amino acid 181 were both approximately 80% [36]. Recently, others have reported the discovery of a panel of CSF biomarkers that discriminated between two different forms of FTLD [38].

Free Radical Injury and Inflammation

Unlike the investigations reviewed above that focused on specific diseases, other biomarkers are being developed that reflect specific mechanisms of stress, injury, or response to injury in the central nervous system. Because these mechanisms may be shared by multiple diseases, the emphasis is not on the diagnostic utility of these markers. Rather, they may prove very useful in estimating disease progression or pharmacologic action of therapeutics, or as additions to a panel of disease-specific diagnostic markers. Biomarkers have been tested most extensively for two mechanisms of injury or response to injury: free radical injury and inflammation.

Free radical stress refers to pathologic states in which free radical production is increased, whereas free radical injury occurs when this stress exceeds the system's capacity to detoxify free radicals. It is important to realize that the free radical injury is an indiscriminate process in which a complex array of biochemical reactions occurs simultaneously. It is most typically quantified using chemical modification of nucleic acids, proteins, or lipids as end points. Because the range of biochemical reactions that occur under conditions of free radical stress and injury is large, so is the number of potential biomarker candidates. The National Institutes of Health-sponsored BOSS (Biomarker of Oxidative Stress Study) concluded that of the products of free radical injury, F_2-isoprostanes (F_2-IsoPs) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) showed the best performance characteristics as quantitative in vivo biomarkers of free radical injury under experimental conditions [39]. 8-OHdG has been reported to be increased in CSF from AD patients compared with controls [40], and numerous studies have investigated CSF F_2-IsoPs as potential biomarkers of neurodegenerative diseases [41].

Esterified F_2-IsoPs have been measured in brain tissue of both mice and humans. In the cerebrum or hippocampus of some transgenic mouse models of AD that deposit $A\beta$ in plaques, esterified F_2-IsoPs are elevated early in the course of pathology and increase further as the mice age [42]. Esterified F_2-IsoPs are also elevated in human brain tissue from individuals with MCI or AD [43]. Although esterified F_2-IsoPs are not detectable in human CSF, free F_2-IsoPs can be measured at levels of picogram per milliliter. CSF F_2-IsoPs measured with stable isotope dilution assays (using either deuterated 8-iso-$PGF_{2\alpha}$ or 8,12-iso-$iPF_{2\alpha}$-VI) are consistently elevated in patients with AD compared with controls [44–46]. Although initially reported also as elevated [47], it has since been shown that plasma F_2-IsoPs are not reproducibly different between patients with AD and controls [48–50]. CSF F_2-IsoPs levels do not correlate strongly with the severity of dementia, and the increase is observed early in the symptomatic course of AD [47, 51]. Elevated CSF F_2-IsoP levels accompany reduced $A\beta_{42}$ levels in presymptomatic carriers of familial AD mutations [52]. The concentration of CSF F_2-IsoPs has also been shown to increase during AD progression in a longitudinal study using sequential lumbar punctures [51]. Two small studies have indicated that when combined with other CSF biomarkers such as $A\beta_{42}$ and tau, measuring F_2-IsoPs may improve diagnostic classification of AD relative to controls [46, 47].

The potential utility of CSF F_2-IsoPs to assess antioxidant treatment effects has been the focus of several studies. In a naturalistic study analyzing antioxidant supplement use, CSF F_2-IsoPs levels in patients with AD was measured at baseline and 12 months later [53]. Patients who did not take any supplements had increased F_2-IsoPs after 12 months, whereas those who took vitamins E plus C showed no changes in CSF F_2-IsoP levels after 12 months. In a recent clinical trial, patients with AD were randomized to receive vitamin C and α-lipoic acid, a combination of "cytosolic" antioxidants, α-tocopherol, coenzyme Q, or placebo, for 16 weeks. CSF was obtained at baseline and at the end of the 16-week treatment period. A significant decrease in CSF F_2-IsoPs was observed in the group who received cytosolic antioxidants relative to the group who received placebo [12]. This finding suggests that F_2-IsoPs may be useful in evaluating suppression of free radical injury to the central nervous system by drugs. Although encouraging, establishing the clinical significance of these findings would require long-term assessment with clinical end points.

A large observational and experimental literature supports a role for free radical injury in AD, PD, and VBI. Although several sources of increased free radical stress have been proposed in these diseases, one that is shared by all three is activation of inflammatory responses in brain. There are differences in the cellular components and ways in which inflammation is mediated in the brain ("neuroinflammation") compared with the periphery. Very briefly, although all cells in brain can participate in neuroinflammatory responses, the major cellular player is microglia. As in peripheral organs, activation of neuroinflammation can have both beneficial and deleterious effects that depend upon the type, degree, and length of activation. Substantial experimental evidence shows that activation of neuroinflammatory mechanisms can damage neurons in a variety of models, including mouse models of AD, PD, and VBI [54]. The balance between the beneficial and deleterious effects of neuroinflammation in patients with these diseases is not yet clear; however, individual molecular components of neuroinflammation are being investigated.

Cytokines and chemokines have been measured in many observational studies of neurodegenerative diseases with mixed results. They occur at low concentrations and extremely sensitive assays are required for their detection in CSF or plasma. Findings have been inconsistent for many of the inflammatory biomarkers [12]. A few cytokines or chemokines have been found to be increased in CSF from individuals with MCI, suggesting that activation of those signaling pathways may occur relatively early in the clinical expression of AD. Examples include monocyte chemoattractant protein-1, interleukin

(IL)-8, IL-1 receptor type II, and IL-18 [55, 56]; these observations require larger-scale replication and follow-up of patients to determine their predictive value.

Multiplex assays that allow the simultaneous measurement of panels of cytokines, chemokines, and other secreted molecules are becoming increasingly available. There are a few published studies to date of inflammatory biomarkers using multiplex analyses of CSF from AD versus controls [57–59] or AD versus PD versus MCI versus controls [60], providing Level I or in some instances Level II information for clinically symptomatic stage of disease. Studies are underway that will help to establish whether there is consistent alteration in a set of inflammatory proteins in latent or prodromal stage of AD or PD.

Therapeutic interventions that target inflammatory pathways also have been examined with biomarkers. In one recent study, patients with AD were randomized to either a high dietary intake of omega-3 fatty acids or to placebo. Subjects in both groups underwent lumbar punctures after completing 6 months of treatment. CSF levels of $A\beta_{42}$ and tau were no different between groups, and there was no difference in IL-6, tumor necrosis factor-α (TNF-α), and soluble IL-1 receptor type II levels [61].

Plasma or serum inflammatory molecules as biomarkers of AD have failed to discriminate consistently patients with AD from controls. However, others have investigated whether levels of plasma inflammatory biomarkers may predict the future development of AD. For example, in the Framingham study, cytokine release by peripheral blood mononuclear cells (PBMCs) was analyzed in elderly community-dwelling subjects. Subjects with the highest extent of PBMC production of IL-1β and TNF-α had an increased risk of developing incident AD [62]. In the Rotterdam study, elderly subjects with higher α1-antichymotrypsin and IL-6 plasma levels had an increased risk of incident dementia, which remained significant for incident AD [63]. In a population-based study, levels of plasma CRP were increased in individuals with MCI relative to controls [64].

Conclusions

Biomarkers are one type of laboratory testing being developed in response to the therapeutic imperative for diseases that cause cognitive impairment and dementia. The role of biomarkers is already transforming the organization and conduct of clinical trials, and if successful will likely contribute in the future to the medical management of patients with these diseases. Despite the obvious utility of practicality of blood- or urine-based biomarkers, so far results from these fluid compartments have not been reproducible. In contrast, substantial progress has been made in CSF biomarkers.

In this paper, we reviewed the stages of CSF development for several common and unusual diseases that cause cognitive impairment and dementia. We outlined five stages of biomarker development: initial association, confirmation, validation, standardization, and finally clinical application. The most progress has been made in diagnostic CSF biomarkers for AD followed by PD. Furthermore, we highlighted biomarkers of mechanisms of injury or response to injury that included free radical injury and immune response. Future applications of diagnostic or mechanistic biomarkers will likely focus on diagnosis of latent or early-stage disease, assessment of disease progression, mechanism of injury, and response to experimental therapeutics.

Acknowledgments

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Table 1

Clinical and laboratory testing for predicting versus measuring disease

Predict	Risk	Determine likelihood of developing disease based on genetics, past events, etc.		
		Normal	Mild Impair	Dementia
Actual	**Clinical Data** Degree & character of functional impairment			
	Laboratory Data Disease type & burden	None	+	++	+++
	Chronic Disease Model	*No Disease*	*Latency*	*Prodrome*	*Full Expression*

Table 2

Five-level ranking for the development of biomarkers

	Level of biomarker development
Level I	Initial association in disease versus control
Level II	Confirmation in separate cohorts with same assay
Level III	Validation in separate cohorts with a different assay
Level IV	Standardized application in multicenter clinical investigations
Level V	Incorporation into best medical practice

Table 3

Cerebrospinal fluid biomarkers for Alzheimer's disease

	Latent	Prodrome	AD dementia
Level I	Several	Many	Many
Level II	Several	Many	Many
Level III	$A\beta_{42}$ and tau species	F_2-isoprostanes	F_2-isoprostanes
Level IV	None yet	$A\beta_{42}$ and tau species	$A\beta_{42}$ and tau species
Level V	None yet	None yet	None yet

AD: Alzheimer's disease

Table 4

CSF biomarkers for Parkinson's disease

	Latent	Prodrome (cognitive)	PD, DLB, or MSA
Level I	None yet	Several	Several
Level II	None yet	Several	Decreased CSF DJ-1
Level III	None yet	Decreased CSF Aβ_{42} with no change in CSF tau	Decreased CSF SNCA
Level IV	None yet	None yet	None yet
Level V	None yet	None yet	None yet

CSF: cerebrospinal fluid; DLB: dementia with Lewy bodies; MSA: multiple system atrophy; PD: Parkinson's disease; SNCA: α-synuclein.