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Cerebrospinal Fluid Biomarkers in Mild Cognitive Impairment and Dementia

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Abstract

Given the magnitude of the public health problem of dementia in the elderly, there is a pressing need for research, development, and timely application of biomarkers that will identify latent and prodromal illness as well as dementia. Although identification of risk factors and neuroimaging measures will remain key to these efforts, this review focuses on recent progress in the discovery, validation, and standardization of cerebrospinal fluid (CSF) biomarkers, small molecules and macromolecules whose CSF concentration can aid in diagnosis at different stages of disease as well as in assessment of disease progression and response to therapeutics. A multimodal approach that brings independent information from risk factor assessment, neuroimaging, and biomarkers may soon guide physicians in the early diagnosis and management of cognitive impairment in the elderly.

Keywords

Alzheimer's disease; biomarkers; cerebrospinal fluid; Lewy body disease; vascular cognitive impairment

PREVALENT CAUSES OF COGNITIVE IMPAIRMENT AND DEMENTIA

Cognitive impairment and dementia in the elderly are public health problems that already cause untold suffering and are poised to overwhelm health care delivery systems in the coming decades. The most prevalent causes of cognitive decline are Alzheimer's disease (AD), vascular cognitive impairment (VCI), and Lewy body disease (LBD) [1,2]. While there are other primary causes of cognitive impairment and dementia in older adults, such as fronto-temporal lobar degeneration (FTLD), their prevalence is not comparable to the three mentioned.

AD, VCI, and LBD commonly are co-morbid and overlap sufficiently in their presentation, neurocognitive profiles, standard laboratory test results, and neuroimaging findings. Thus, accurate clinical distinction [3,4] is a challenge and reveals dementia in the elderly to be a

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complex, often convergent, phenotype. Moreover, AD, VCI, and LBD are syndromes, or clinic-pathologic entities, with potentially multiple causes. At least three genetic mutations can cause rare autosomal dominant forms of early-onset AD. While, risk of the more common late-onset AD is associated with inheritance of the ε4 allele of *APOE*, the polymorphic genetic locus that encodes apolipoprotein E (ApoE) [5].

The cellular and molecular processes that underlie these common dementing illnesses are known only in part. For AD and LBD in particular, complex molecular cascades appear to derive from accumulation of abnormal forms of proteins, a proposed class of diseases so called "protein-misfolding diseases" or "proteinopathies" [6]. Key among these abnormal proteins are Aβ peptides and tau in AD, and α-synuclein in LBD [7]. Important pathogenetic contributors to each disease may include innate immune activation, excitotoxicity, mitochondrial dysfunction, and increased oxidative damage, all of which may contribute to regional loss of synapses and ultimately death of neurons. The intersection of these processes with VCI is unclear. Population-based studies have highlighted a more important role for VCI from small vessel disease (SVD), rather than macroscopic lesions from large vessel disease (LVD), as a risk factor for dementia [1,2]. It is possible that ischemic injury simply adds to damage from protein misfolding diseases like AD or LBD. Alternatively, microangiopathy from VCI-SVD may intersect mechanistically with protein misfolding diseases, especially the congophilic angiopathy of AD [8].

Most of the neuropathologic literature on these common dementing illnesses is limited to data from specialized centers with patients and volunteers who represent a more highly selected, and perhaps biased, cohort than the general elderly population. Only a few population-based studies of cognitive impairment and dementia have reported comprehensive pathologic evaluations [1,2,9–12]. In each case, VCI and LBD have been observed to have an important role along with the more generally recognized contribution of AD. Indeed, we recently estimated the population-attributable risk for clinical dementia from AD, VCI, and LBD in older women and men from the metropolitan Seattle area as 45%, 33%, and 10%, respectively [1].

STAGES OF ILLNESSES THAT CAN CULMINATE IN DEMENTIA

AD, VCI-SVD, and LBD appear to converge in their effect on older individuals to produce a clinical syndrome of progressive decline in cognitive function. Most clinical research has dealt with this continuum by defining discrete stages: normal for age, mild cognitive impairments or behavioral changes that exceed those expected for age, but fall short of consensus criteria for dementia, and dementia. Multiple clinico-pathologic studies focusing on AD have shown that AD-type changes are common in older individuals rigorously demonstrated to be cognitively and behaviorally normal [13]. These findings demonstrate that a substantial subset of clinically normal older individuals actually has latent AD, a conclusion buttressed by neuroimaging and biomarker measures [14–17]. Indeed, the proposal that AD progresses from (i) latent stage, with some structural or molecular damage, but no functional or behavioral changes, through (ii) prodrome, with greater damage and mild functional or behavioral changes, to (iii) dementia, with substantial and irreversible damage that provokes cognitive and behavioral abnormalities (Table 1) was first introduced over thirty years ago [18]. Emerging data also suggest that a similar chronic disease model may apply also to VCI and LBD [1,2,8,19].

RISK FACTORS, BIOMARKERS, AND DISEASE SURROGATES

With this background in mind, we turn to measures to assess disease state and stage. Risk factors are identifiable events or conditions associated with increased probability of disease, such as heritability. For instance, recent twin studies of AD [20] show heritability estimates

of 0.5 or higher. As mentioned above, biomarkers are molecules whose concentration in tissue or biological fluids aid in diagnosis or assessment of disease progression and response to therapeutics. Disease surrogate is a concept from clinical trials that describes a trait used as a substitute for the clinical endpoint of interest.

Before covering the existing cerebrospinal fluid (CSF) biomarkers for geriatric dementia, it is important to evaluate both the nature and quality of the data as well as the extent of methodologic development. To do so, we suggest a hierarchy of five levels of biomarker development: Level 1 - initial association, Level 2 - confirmation in a larger and more complex independent sample, Level 3 - validation by other laboratories using a reproducible quantitative assay, Level 4 – standardization across sites and use as disease surrogates in clinical trials, and Level 5 - widespread clinical use in the primary care setting (Table 2).

While initial studies appropriately may use idiosyncratic assays (Level 1), progressing to reproducible quantitative analysis requires uniform assays with authentic standards, quality assurance, and known performance characteristics. Moving forward also must take into account the potential effects of other common illnesses and commonly used drugs or supplements [21,22], age [23,24], gender [25], diet, level of physical activity, and circadian variation [26] (Levels 2 and 3). Once these challenges are met, markers may be used at multiple sites as disease surrogates in clinical research (Level 4) and finally may be adopted for widespread application in clinical laboratories (Level 5).

PROPOSED CSF BIOMARKERS FOR PREVALENT DISEASES THAT CAN CULMINATE IN DEMENTIA

Alzheimer's disease

By far, the majority of data are for possible or probable AD [27] as diagnosed by expert physicians in tertiary medical centers. Since many of these centers have active research programs that include autopsy and neuropathologic classification of dementing illnesses, the performance characteristics for expert clinical diagnosis of AD are known. Speaking broadly, sensitivity (vs. neuropathological confirmation) for an expert clinical diagnosis of AD is about 90 to 95%, while specificity is about 50 to 60% because it is difficult to discern clinically the commonly comorbid VCI and LDB. The limited specificity presents a particularly important problem for interpreting biomarker studies that rely on expert clinical diagnosis: the possible or probable AD group is a mixture of patients with dementia deriving from AD processes alone as well as a large subset of AD plus VCI or AD plus LBD. This problem can be overcome by restricting biomarker studies to only include individuals who subsequently undergo autopsy for "gold standard" classification [4,28,29]. However, besides the obvious drawback of greatly limiting the number of cases, this approach assumes that the combination of neuropathologic processes present at the time of death also were present at the time that the biomarker was quantified, which may not always be the case.

With these limitations in mind, Table 3 summarizes current knowledge for biomarkers of AD identified in cross-sectional studies. Investigations of biomarkers for AD are dominated by cross-sectional studies for CSF $A\beta_{42}$ and tau species at every stage of disease, achieving Level 2 in latency, Level 3 in prodrome, and Level 4 in dementia. CSF $A\beta_{42}$ levels are decreased about 50% in patients with MCI and AD compared to controls [30], associated with enhanced $A\beta_{42}$ deposition in brain [17]. In contrast, CSF total tau (T-tau) is increased 2- to 3-fold while some phosphorylated tau (tau-P) species, e.g., tau-P231 or tau-P181, can be increased by up to one or two orders of magnitude in MCI and AD patients compared to controls [31–36]. Unfortunately, none of these changes in CSF protein concentration is specific to AD [37], with virtually all studies showing substantial overlap in CSF values among patients with AD and VCI or LBD [28,30,32–34,38]. Since reduced CSF $A\beta_{42}$ and

increased CSF tau both are characteristic, although not specific, changes for prodromal and dementia stages of AD, most groups combine results of both assays [39–41]. Indeed, the CSF tau/$A\beta_{42}$ ratio can distinguish individuals with very mild cognitive impairments from controls [17]. Recently, some have proposed including other Aβ species in this ratio to improve accuracy for classifying dementia from AD [42].

Cross-sectional investigations of AD latency deserve special consideration. Most have used inherited risk factors or mutations to identify apparently healthy older adults who are likely to subsequently develop cognitive impairment. Some [43], but not all [44], investigators have observed that CSF $A\beta_{42}$ levels are decreased to dementia-like levels in cognitively normal elderly who inherited an ε4 allele of *APOE*, while a third group observed decline in CSF $A\beta_{42}$ levels beginning in the sixth decade of life of individuals with normal cognition, and an enhancement of this decline in individuals with ε4 allele of *APOE* [23], consistent with decreased CSF $A\beta_{42}$ as a biomarker of latent AD. Interestingly, a cognitively normal volunteer with a family history of AD who had exceptionally high CSF $A\beta_{42}$ concentration and an inherited disease-causing mutation in *PSEN1* was recently identified [24]. Together these results suggest that AD latency itself may have different stages: early increased CSF $A\beta_{42}$ with little parenchymal deposition followed by decreased CSF $A\beta_{42}$ secondary to parenchymal deposition. Alternatively, the pattern of changes in CSF biomarkers may differ among those with *APOE* ε4 vs. a disease-causing mutation. Some [32,44], but not all [43], investigators have observed increased CSF tau or tau-P231 concentrations in cognitively normal older adults who inherited an *APOE* ε4 allele, suggesting that increased CSF tau and tau-P231 may be biomarkers of at least one stage or form of AD latency.

Longitudinal analyses of CSF by multiple groups have shown that decreased CSF $A\beta_{42}$ and increased CSF T-tau or phosphorylated tau species predict subsequent conversion from MCI to AD over follow-up extending to 6 years (Level 3) [35,45–50]. In addition, increased baseline CSF activity of BACE1, an enzyme central to the generation of Aβ peptides, predicts progression from MCI to dementia stage of AD [51]. Others have observed that cross-sectional differences in CSF $A\beta_{42}$, T-tau, and tau-P181 concentrations in MCI or AD patients far exceed interval changes over an average of 21 months, suggesting that these measures may not be especially sensitive markers of disease progression in prodromal or dementia stages [52]. Similar measures may also prove helpful in identifying latent AD. An empirically defined CSF tau/$A\beta_{42}$ ratio cutoff correctly identified cognitively normal individuals who subsequently developed MCI or AD over follow-up extending to 42 months; however, the total number of clinical events in this group was small and further follow-up as well as validation in other centers is needed (Level 1) [16].

Another extensively studied CSF biomarker is measurement of F_2-isoprostanes (F_2-IsoPs). Unlike the disease-oriented $A\beta_{42}$ and tau biomarkers, F_2-IsoPs are mechanism-specific; they quantitate *in vivo* free radical damage to lipid. In cross-sectional studies, increased CSF concentrations of this biomarker have achieved Level 1 in individuals with an *APOE* ε4 allele [44], Level 2 in MCI [53,54], and Level 3 in AD dementia [22,53,55–59]. These associations are strengthened by findings from brain samples from patients who died with prodromal or dementia stage AD showing increased F_2-IsoPs or related molecules [60–62]. Longitudinal studies have shown a 1 year interval increase in CSF F_2-IsoP concentrations in patients with mild dementia from AD [22,23,63] that was suppressed by antioxidant supplementation [22].

EXPERIMENTAL BIOMARKERS

Several laboratories are employing relatively unbiased discovery approaches to identifying biomarkers for neurodegenerative diseases. Several groups are using proteomics of CSF in

cross-sectional studies to identify partially overlapping ensembles of CSF proteins that are associated with the dementia stage of AD (Level 1). These are often from relatively small numbers of individuals and only twice have had autopsy confirmation of three dementing illnesses [29,64]. One group identified a panel of 17 potential biomarkers by surface-enhanced laser desorption/ionization that could distinguish between MCI individuals who progressed to dementia over 4 to 6 years versus those who remained stable during this period [65]. We are aware of only one CSF proteomics-discovered ensemble of proteins that has been adapted to a multianalyte profile (MAP) and confirmed in a relatively large number of individuals from multiple centers with different neurodegenerative diseases [66]. MAP analysis of eight optimal proteins (tau, brain-derived neurotrophic factor, IL-8, $A\beta_{42}$, α_2-microglobulin, vitamin D binding protein, ApoAII, and ApoE) gave results that agreed with expert clinical diagnosis for 95% of controls, 75% of patients with AD, and 95% of patients with Parkinson's disease.

Vascular cognitive impairment

The issues surrounding the limitations in expert clinical diagnosis of AD are present and perhaps even more pronounced for VCI [8]. As we note above, limitations in clinical diagnosis are both the motivation for biomarker development, but also one of its greatest challenges as long as the "gold standard" remains neuropathologic assessment despite its limitations.

VCI ranges from local territorial infarcts from LVD, for which structural neuroimaging provides unsurpassed insight into lesion size and progression, to widespread SVD that is not as easily assessed or distinguished from processes of AD by clinical examination or current standard neuroimaging techniques [3]. Thus, while neuroimaging plays a central role in evaluating ischemic injury from LVD, biomarkers may contribute to the evaluation of damage from SVD. It is beyond the scope of this review to summarize peripheral biomarkers of vascular disease that may be useful in establishing risk for VCI. Several CSF biomarkers of VCI have been proposed including increased concentrations of T-tau or phosphorylated tau species, sometimes combined with normal $A\beta_{42}$ levels in patients with dementia (Level 2) [34,67,68]; however, all are limited by broad overlap with values from patients thought clinically to have AD without VCI. Another CSF candidate is low molecular weight neurofilament protein, which is increased in individuals with extensive white matter changes (as seen by MRI) and in patients with dementia from VCI, but also in patients with AD (Level 1) [69]. One longitudinal study reported that normal CSF tau levels in combination with MRI evidence of presumed white matter ischemic injury are characteristic of MCI patients less likely to progress to dementia [70].

Lewy body disease

LBD is characterized by regional intraneuronal accumulation of Lewy bodies, structures composed of several proteins that prominently include α-synuclein. While Lewy body accumulation in neocortical regions of brain is a strong correlate of dementia [1], clinical criteria that reliably distinguish dementia from LBD (called Dementia with Lewy Bodies or DLB) versus AD are still under development. Thus biomarker studies without autopsy disease classification may have groups enriched for AD or DLB, but these will be cross-contaminated to an unknown extent [68].

We are aware of a single large (> 30 individuals per group) multicenter cross-sectional study of CSF biomarkers for DLB versus AD using commercially available kits to measure CSF $A\beta_{42}$, T-tau, and tau-P181 (Level 3). The study concluded that CSF tau-P181 is the most significant variable for distinguishing DLB from AD with an overall accuracy of 80% [71], confirming a smaller previous study [68]. Another group reported that the relative

abundance of CSF $A\beta_{37}$ and $A\beta_{42}$ discriminates between relatively small sample sizes of DLB and AD (Level 1) [72]. While there is an emerging literature on quantification of CSF α-synuclein in patients with Parkinson's disease, we are unaware of any similar published investigation of patients with DLB.

Multimodal approach

A likely outcome of knowledge gained from investigation of risk factors, structural and functional neuroimaging, and biomarkers will be the definition of more homogenous groups of patients to assist group selection in clinical trials. For instance, this is now common practice for the *APOE* ε4 allele risk factor [17,23,32,43–45]. Recent studies combining biomarkers with neuroimaging [22,57] are a critical area of research as the field moves from Level 3 to Level 4 biomarkers. For example, will determining CSF tau (or a tau-P) and $A\beta_{42}$ levels carry the same, significantly more, or significantly less information as a PET imaging or structural MRI [73–75]? We expect that evaluation of genetic risk, structural or functional changes as assessed by neuroimaging, and some combination of biomarkers measured in body fluids or by imaging probes will contribute to the standard workup for geriatric dementia in the near future.

SUMMARY

Dementia in the elderly is a complex convergent phenotype that derives substantially from AD, VCI, and LBD which combine variably in individuals. Despite "pure" forms of each of these pathologic entities, the evidence no longer supports viewing them as distinct illnesses in elderly populations outside of highly referred groups in tertiary medical centers. The core challenge to the investigation of risk factors, structural and functional imaging, and biomarkers will be the efficient and economical assessment of the relative contribution of these three processes to the different stages of cognitive impairment in older individuals from the general population [34]. The challenges will almost certainly be greatest for latent disease where therapies are likely to be most effective, but where the threshold for toxicity will be lowest. New knowledge from these investigations will fuel testing and development of mechanism-specific interventions by identification of appropriate subgroups and provision of objective measures of disease suppression. Success with new therapies will, in turn, be likely to spur widespread application of multimodal ensembles of risk factor assessment, neuroimaging, and biomarker measurement in the clinical management of cognitive impairment and dementia in the elderly.

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Table 1

Stages of Dementia

	Normal	Latency	Prodrome	Dementia
Clinical	Age appropriate		CIND or MCI	Dementia
Pathological	None	+	+ or ++	++ or +++

Cognitive Impairment, No Dementia (CIND), and Mild Cognitive Impairment (MCI) are examples of defined clinical states meant to reflect cognitive and behavioral impairments that exceed those appropriate for age, but yet do not meet consensus criteria for dementia.

Table 2

Levels of Biomarker Development

Level 1	Initial Associations	Association with expert diagnosis, progression through disease stages, or response to therapeutics
Level 2	Confirmation	Estimation of biomarker performance in a larger, independent sample that includes multiple related diseases
Level 3	Validation	Similar estimates of performance as in Level 2, but in independent samples from multiple sites
Level 4	Clinical Research	Standardization of quantitative assay and use as disease surrogate in clinical research
Level 5	Primary Care	Adopted as part of standard work-up in primary care setting

Table 3

CSF Biomarkers for AD*

	Latency	Prodrome	Dementia
Level 1	F_2-IsoPs tau-P231	BACE1	Many
Level 2	T-tau or $A\beta_{42}$	F_2-IsoPs	8-member MAP
Level 3	None	$A\beta_{42}$ and T-tau tau-P181 tau-P231	tau-P181 tau-P231 F_2-IsoPs
Level 4	None	None	$A\beta_{42}$ and T-tau
Level 5	None	None	None

* see references in text