Fluid biomarkers for diagnosing dementia: rationale and recommendations for Canadian Physicians.

Pedro Rosa-Neto MD. PhD (1),
Ging-Yuek Robin Hsiung, MD (2) MHSc.
Mario Masellis MD (3).

(1) McGill Centre for Studies in Aging (MCSA), McGill University, Montreal, QC, Canada. Douglas Research Institute, McGill University, Montreal, QC, Canada.

(2) Division of Neurology, Department of Pathology, Department of Medicine, University of British Columbia, Canada

(3) Research Institute, Sunnybrook Health Sciences Centre. University of Toronto

Abstract

Literature data suggest that plasma amyloid beta ($A\beta_{1-40/2}$) peptides are elevated in populations at risk for Alzheimer's disease (AD) and progressively decline during the conversion from Mild Cognitive impairment to AD. At present, there is no consensus regarding the optimal methodology for conducting quantification of plasma $A\beta_{1-40}$ or $A\beta_{1-42}$. Further, there is insufficient evidence supporting clinical applications for plasma $A\beta_{1-40}$ or $A\beta_{1-42}$ measures. Therefore, they are not recommended either for primary care or specialists, though CSF $A\beta_{1-42,}$ total tau and p-tau can be considered at the tertiary care level for selected cases to improve diagnosis certainty, particularly in those cases presenting atypical clinical features. CSF biomarkers are not recommended for screening in normal healthy subjects for the purpose of assessing future risk of developing AD. Fluid biomarkers must be sampled using standardized methodology, in a certified, centralized facility, and transported under appropriate conditions.

Definitions and abbreviations

AD-P: Alzheimer's pathology: Spectrum of brain macroscopic and microscopic pathological features associated with the AD clinical phenotype. Classic features of AD-P are senile neuritic (amyloid) plaques and neurofibrillary tangles, synaptic loss, and vascular amyloid deposits.

AP: Amyloid plaques: Extracellular deposits of fibrillary beta-amyloid abundant in the cortex of AD patients. Amyloid plaques are commonly classified as diffuse or dense-core based on their morphology and positive or negative staining with Thioflavin-S or Congo Red. [1]

APOE4: An allele of the apolipoprotein E gene (APOE; chromosome 19) associated with a high risk for AD.

Biomarker: Characteristic measured as an indicator of biological or pathogenic processes, or of pharmacologic response.

ELISA: enzyme-linked immunosorbent assay

Molecular probe (imaging agent): Molecular imaging agents are probes used to visualize, characterize, and measure pathological and/or pathophysiological processes in living systems. Both endogenous molecules and exogenous probes can be used as molecular imaging agents.

Molecular target: A biological process of interest for quantification with molecular probes.

MRI: Magnetic Resonance Imaging

Neurodegeneration: Progressive loss of structure or function of neurons, including neuronal death.

Neurofibrillary tangles (NFTs): Hyperphosphorylated and misfolded intraneuronal aggregates of tau protein.

PET: Positron Emission Tomography: Imaging technique that utilizes a tracer concentration of short-lived radiopharmaceuticals targeting a biological process of interest. High-resolution PET produces 3D images quantifying a biological process reaching up to pico molar (10^{-9}-10^{-12} M) concentrations.

p-tau-181; phosphorlyated tau (p-tau) in the 181-threonine position

Radiopharmaceutical: A radioisotope labelled molecule intended for use in the diagnosis and/or monitoring of a disease in humans.

t-tau: concentration of total tau on the CSF

tau protein: microtubule-associated protein tau present in neurons and astrocytes. Hyperphosphorylation of tau protein occur in Alzheimer's disease.

xMAP; Novel high-throughput technology allowing simultaneously measures the concentration of a large number of different analytes ($A\beta_{1-42}$, t-tau and p-tau181) in real-time

Introduction

Technological advances bring the promise of preclinical diagnosis of dementias using fluid biomarkers. Fluid biomarkers ultimately provide quantitative information regarding biosynthesis, the concentration and kinetics of biomolecules as well as their respective metabolites in biological fluids. Today high-throughput analytical platforms are available for detailed analysis of fluid biomarkers and at some point in the near future advanced proteomics techniques will possibly reveal complex signatures for all neurodegenerative diseases.

Significant developments have been obtained with quantification of cerebrospinal (CSF) and plasma concentrations of amyloid beta ($A\beta_{1-42}$), total tau (t-tau) and phosphorlyated tau (p-tau) in the 181-threonine position ($p\text{-}tau_{181}$). However, despite significant developments and favorable results obtained using large cohorts of dementia patients, there are important factors such as variability across laboratories that hamper the use of these techniques in clinical settings. This manuscript aims to summarize this literature and provide recommendations to physicians in Canada.

A. Framework for using biomarkers in the diagnosis of dementia

Biomarker is defined as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [2]. Diagnostic or "core" biomarkers should express the underlying molecular pathology of a disease. In AD, core biomarkers reflect amyloid load, neurofibrillary tangle (NFT) pathology, as well as axonal degeneration. As such, biomarkers serve to identify in living individuals a variety of neuropathological features previously detected only by the analysis of specimens from biopsy or necropsy [3-5]. The availability of biomarkers for quantifying in vivo AD pathology (AD-P) has propelled advances on the understanding of AD as a dynamic clinicopathological entity. In contrast with the cross sectional nature of neuropathology assessments, biomarker measures allows for longitudinal observations necessary to describe the temporal

progression of neuropathology in neurodegenerative diseases. Indeed, the value of imaging or fluid biomarkers for supporting the diagnosis of AD in living individuals has been acknowledged in the 2010 NIA-AA criteria [6].

B. Neurobiology of CSF biomarkers for AD

During the last years, research in AD has elaborated a construct called the amyloid cascade hypothesis, which posits that a defect in Aβ-peptide metabolism, a major chemical constituent of amyloid plaques, triggers a downstream cascade of neurodegenerative events leading to dementia [7-9]. This "amyloidocentric" disease model supports the basis for the classification of biomarkers as biomarkers of amyloid deposition or biomarkers of neurodegeneration (see table 1).

Table 1. Biomarkers for diagnosis of AD.

Neuropathological Processes	Process of interest	Method	Outcome	Interpretation
Amyloid deposition	Fibrillar amyloid availability	[^{11}C]PIB PET	Increased retention	Amyloid deposition
	Fibrillar amyloid availability	[^{18}F]Florbetapir PET	Increased retention	Amyloid deposition
	Fibrillar amyloid availability	[^{18}F]Florbetapen PET	Increased retention	Amyloid deposition
	Fibrillar amyloid availability	[^{18}F]Flutametamol PET	Increased retention	Amyloid deposition
	$A\beta_{1-42}$ CSF concentrations	$A\beta_{1-42}$	Decline in CSF concentrations	Amyloid deposition
	$A\beta_{1-42}$ serum levels	$A\beta_{1-42}$	Decline in serum concentrations	Amyloid deposition
Neurodegeneration (Downstream)	Brain metabolism	[^{18}F]FDG PET	Brain hypometabolism	Synaptic depletion
	Total tau CSF concentrations	Lumbar puncture	Increased CSF concentration	Cell depletion
	Tau-181 CSF concentrations	Lumbar puncture	Increased CSF concentration	Tau phosphorylation
	Brain atrophy	MRI	Decreased volume loss	Atrophy
Non AD	Brain lesions	MRI	Brain	Tumor, vascular disease
	Metabolism	[^{18}F]FDG PET	Brain	LBD, FTD
	R/O LBD	[^{123}I]Ioflupane (DAT)	Brain	Reduction of dopamine transporters
	R/O CJD	Lumbar puncture 14-3-3	CSF	Neuronal depletion

1 CSF Biomarkers of Amyloid Accumulation

Biomarkers of amyloid deposition refer to indices of amyloid deposition obtained using either Positron Emission Tomography (PET) or analysis of CSF or plasma, with brain fibrillary Aβ-peptide deposits characteristic of AD detected by increased retention of amyloid imaging PET agents or declines in Aβ-peptide$_{1-42}$ CSF concentrations. Such methods constitute powerful means for in vivo detection of pathological brain amyloid concentrations. [10 11]

Mega-aggregates of Aβ-peptide form the core of amyloid plaques in AD. Amyloid beta ($A\beta_{1-40/2}$) peptides result from the proteolysis of an integral membrane protein called amyloid precursor protein (APP). APP is degraded by

two enzymatic processes called nonamyloidogenic and amyloidogenic (Figure 1). In the nonamyloidogenic pathway, APP is cleaved by an alpha-secretase (A disintegrin and metalloprotease domain, ADAM10) precluding formation of the amyloidogenic peptides and leading to the release of soluble APPs- alpha into the extracellular space. The $A\beta_{1\text{-}40/2}$ peptides are secreted to the extracellular space via the amyloidogenic pathway, which refers to the sequential APP proteolysis by the enzymes beta and gamma secretases. Both peptides are hydrophobic and tend to form aggregates in aqueous environments. Once in the extracellular space, $A\beta_{1\text{-}42}$ molecules undergo a massive aggregation process, which generates the inert core of an amyloid plaque. Importantly, neurotoxic soluble $A\beta_{1\text{-}42}$ aggregates (oligomers) are transiently formed during the process of plaque formation [12]. In fact, oligomeric forms of Aβ are increased in the CSF of AD patients [13, 14]. The toxicity of $A\beta_{1\text{-}42}$ oligomers has been extensively demonstrated by numerous studies (see review) [15]. From the biomarker perspective, while amyloid PET agents detect abnormal fibrillar amyloid load in AD brain, CSF measures detect declines in $A\beta_{1\text{-}42}$ concentrations. In fact, $A\beta_{1\text{-}42}$ CSF concentrations and amyloid load detected by PET [^{11}C]PIB are correlated in a exponential fashion [16 17]. Low $A\beta_{1\text{-}42}$ CSF concentrations possibly occur due to the retention of $A\beta_{1\text{-}42}$ moieties on amyloid plaques, the so-called sink effect.

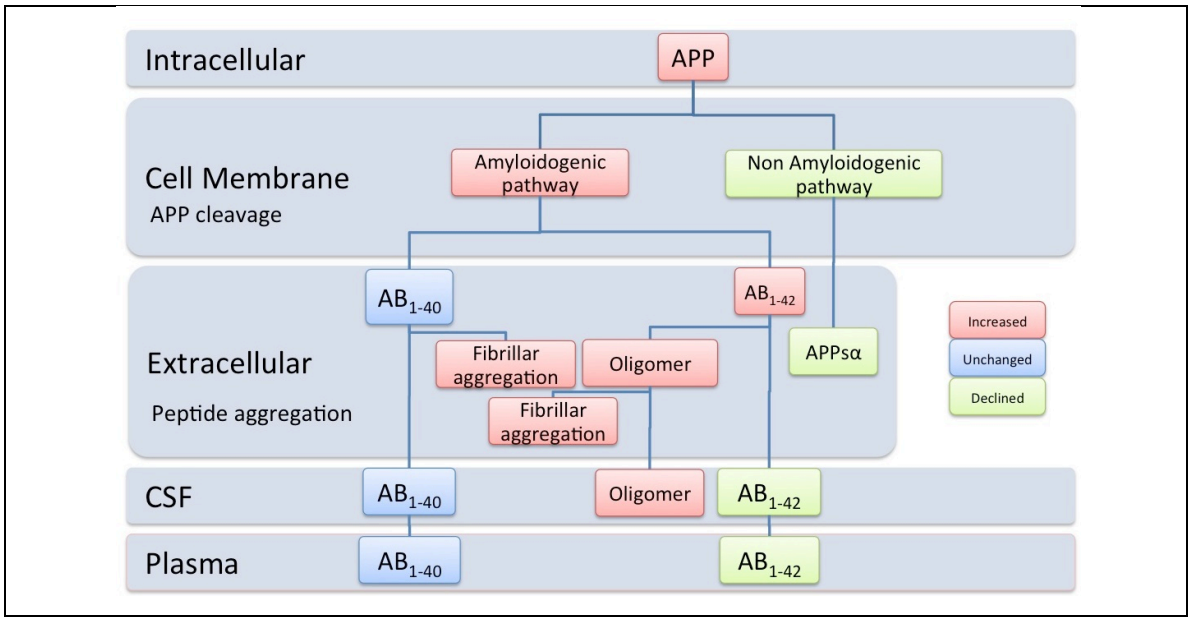

Figure 1. Schematic representation of APP metabolism and biomarker of amyloid pathology in various biological compartments. Color pallet indicates processes that have increased, declined or remained unchanged in AD. Note that in AD higher $A\beta_{1-42}$ retention in the extracellular compartment (brain tissue) due to peptide aggregation leads to declines of $A\beta_{1-42}$ in the CSF. In the plasma, it is debatable whether declines are present in individuals with AD.

2 Plasma Biomarkers of Amyloid Accumulation

Although several plasma biomarkers—incuding plasma concentration of clusterin, c-reactive protein and acethylcholinesterase—have been tested in the context of dementia, core AD biomarkers constitute a less invasive alternative for lumbar punctures [18, 19] [18, 19]. Plasma $A\beta_{1-42}$ or $A\beta_{1-42}$/ $A\beta_{1-40}$ levels have been studies in AD, MCI as well as in populations at risk using enzyme-linked immunosorbent assays (ELISA) and multiplex platforms [20-35]. In general these results show elevated $A\beta_{1-42}$ or $A\beta_{1-42}$/ $A\beta_{1-40}$ plasma concentrations in asymptomatic carriers of AD mutations as well as patients with Down syndrome [36] [33]. In cases of sporadic AD , there is evidence suggesting that low plasma levels of $A\beta_{1-42}$ or $A\beta_{1-42}$/ $A\beta_{1-40}$ ratios characterize individuals with AD [27, 37] (Figure 1). However, there is no correlation between $A\beta_{1-42}$ or $A\beta_{1-40}$ plasma and CSF [38] [39] [40]. Overall, these results need to be replicated due to disagreement between studies, although a recent meta-analysis of plasma core biomarkers in AD indicates that $A\beta_{1-42}$/ $A\beta_{1-40}$ predict progression to dementia [25]. Methodological limitations in terms of sampling and analysis require further validation.

3 CSF Biomarkers of Neurodegeneration

Today, PET [^{18}F]-fluorodeoxyglucose ([^{18}F]FDG), MRI volumetry, t-tau or p-tau are the most relevant biomarkers of neurodegeneration, providing information regarding neurodegenerative processes present in carriers of AD-P [10]. Assuming the “amyloidocentric” framework proposed by the amyloid cascade hypothesis, declines in brain function revealed by biomarkers of

neurodegeneration occur as a result of amyloid toxicity, although the mechanisms linking amyloid pathology and neurodegeneration remain elusive [15].

T-tau and p-tau constitute the classic CSF biomarkers for neurodegeneration (Figure 2). The tau protein is a constituent of neuronal microtubules, which are cell structures responsible for the motility of proteins and organelles within the neuron [41]. The functional expression of microtubules is modulated via phosphorylation of numerous serine and threonine residues (phosphorylation sites) present in the tau protein [42]. Abnormal tau hyperphosphorylation deposits in AD are observed within neurons, NFTs, or dystrophic neurites present in neuritic plaques [43]. In MCI, tau and t-tau concentrations increase nearly 30-40%. [44] [45], with such concentrations increasing by nearly 40-50% in AD. Synaptic injury or cellular death contributes to the leakage of tau and p-tau to the extracellular space. In fact, CSF t-tau concentration is also increased in patients with encephalitis, trauma and stroke [46-48]. CSF tau concentration is useful for distinguishing AD patients from control subjects as well as from non-AD forms of dementia, although overlap at the level of pathology often exists [49, 50].

Despite numerous threonine and serine phosphorylation sites present in tau protein, AD is better characterized by hyperphosphorylation at the position 181 (p-tau$_{181}$) or 232 (p-tau$_{232}$). Particularly, discrimination between AD and dementia with Lewy bodies or frontotemporal dementia is maximized by p-tau$_{181}$ or p-tau$_{231}$ [51]. Indeed, Aβ_{1-42} / p-tau ratio provides the highest diagnostic performance in terms of identifying the predementia stage of AD with a sensitivity of 83% and specificity of 72% [44] [52] [53] [54].

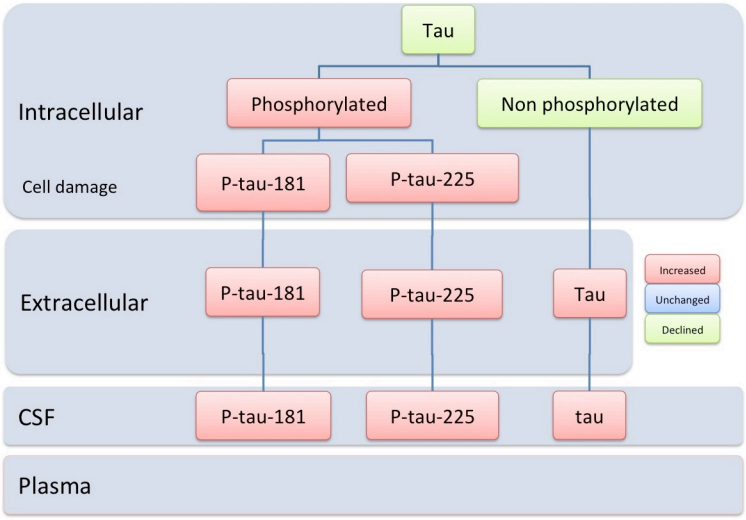

Figure 2. Schematic representation of biomarkers of tau pathology in various biological compartments. Color pallet indicates processes that have increased, declined or remained unchanged in AD. It has been proposed that the leakage of tau into the extracellular and CSF space is secondary to brain damage. Note in AD increased concentrations of p-tau in all compartments.

4 Progression of Biomarkers During the Course of the Disease: Dynamic Biomarker of AD Pathological Cascade (DBAPC).

Cross-sectional biomarker and longitudinal biomarker data provided the empirical basis for an AD model called the "dynamic biomarker of AD pathological cascade" (DBAPC). Proposed by Jack and collaborators, this model is widely accepted by the research community as a model of biomarker progression from asymptomatic to dementia phases of AD [10]. The DBAPC hypothesis incorporates the entire clinical spectrum of AD comprising preclinical, MCI due to AD and dementia stages as well as their specific biomarker signatures. Moreover, biomarker abnormalities are assumed as a surrogate for progressive neuropathological changes and follow a non-linear progression as shown in Figure 3. Similarly to the amyloid cascade hypothesis, the DBAPC hypothesis assumes amyloidosis as an early event leading to a cascade of successive neurodegenerative processes (i.e. tau-pathology, synaptic depletion and cell loss) resulting in dementia [10, 55-57]. This assumption stems from observations in clinical populations as well as in carriers of PS1, PS2 and APP

mutations. Particularly regarding CSF biomarkers, low concentration of $A\beta_{1-42}$ and high CSF tau and has been observed in carriers of genetic forms of AD approaching the age of progression to symptomatic AD. The DBACP model predicts decline of $A\beta_{1-42}$ followed by sequential tissue functional abnormalities (hypometabolism) and release of tau and p-tau in the CSF, and brain atrophy detectable by MRI [6-17]. The model also proposes that [10, 58] memory and functional declines occur as a function of neurodegeneration [10].

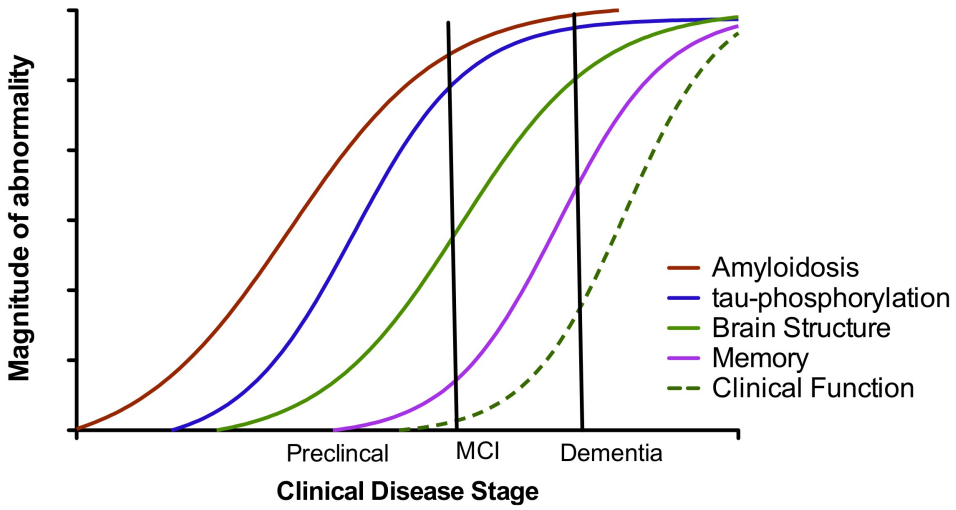

Figure 3. The dynamic biomarkers of AD pathological cascade (DBAPC), as proposed by Jack and collaborators. This model predicts a preclinical and MCI stage of AD characterized by predominance of amyloid pathology and a dementia phase characterized by amyloid pathology, neurodegenerative changes and subclinical cognitive impairment (adapted from Jack et al 2009 [10]).

C. Methodological recommendations for CSF sampling

The best practices for CSF sampling and analysis remain a work in progress. Considerable variability in absolute concentrations and cutoff values of AD biomarkers has been found between different centers using the same assay [59]. Recently standard operating procedures (SOPs) for xMAP-Luminex multiplex

platform have shown in intralaboratory variation in the order to 5% and intra-laboratory variation between 10—20-% [60].

In principle it lumbar puncture (LP) procedure should follow a standard operating procedure in order to minimize the chances of false positive results. There is no evidence supporting that meal consumption would have an impact CSF concentrations of $A\beta_{1-42}$ or tau protein. The Alzheimer's biomarkers standardization initiative (ABSI) recommendations for CSF biomarker are summarized on Table 2.[61].

It is not a recommendation to perform lumbar punctures during the morning, since circadian variation on $A\beta_{1-42}$ CSF concentrations initially reported was not replicated [62 63]. Aliquoting is not a major issue since the gradient of $A\beta_{1-42}$ concentrations is negligible if the CSF sample remains between 10–40 mL [60]. Importantly, it is crucial to comply exclusively with containers and vials recommended by the CSF analytical infrastructure since $A\beta_{1-42}$ readily adsorbs to test tube polystyrene, glass and even to polypropylene walls [64, 65]. Therefore If CSF is sampled with the atraumatic needle, make sure that the syringe utilized for CSF sampling complies with the recommendations of the CSF analytical infrastructure. Hemorrhagic CSF sampling might induce false positive results due to the high concentration of proteins present in the contaminating blood. As a rule, erythrocyte count above >500/lL in the CSF requires centrifuge the CSF before freezing. In order to assures best stability, it is recommended freeze and store at -80°C immediately after CSF collection. Immediate freezing after collection avoids protein degradation in CSF by proteases [66].

D. CENTRALIZED ANALYTICAL INFRASTRUCTURE

It is crucial to send the samples to a state of the art CSF infrastructure with appropriate control population.

Table 2. Summary of ABSI recommendations for preanalytical and analytical aspects for AD biomarker testing in CSF [61]		
1	CT or MRI performed before LP`	LP should not be performed in cases where there is high intracranial pressure or where there is a mass lesion in the brain.
2	Concomitant medication	LP should not be performed in patients treated with anticoagulants (e.g., warfarin). Treatment with platelet aggregation inhibitors is not a contraindication.
3	Diurnal variation	No diurnal variation.
4	CSF gradient/volume	No gradient observed. No requirement for a certain fraction. Minimum volume of 1.5 mL.
5	Meal consumption	No need for fasting.
6	Position	LP may be performed with the patient either sitting or lying down. The position of the patient does not affect the results.
7	Location	Vertebral body L3–L5. The incision point of the needle (L3–L4 or L4–L5) does not affect the results.
8	Disinfection/anesthesia	Disinfection will reduce the risk of local infection. Local anesthetics introduce a risk of adverse effects, but can be given to patients who worry about local pain during LP.
9	Needle	Use a small diameter (0.7 mm and 22 G), preferably nontraumatic needle. A small-gauge needle will make a smaller hole in the dura, aiding healing, and an atraumatic needle will reduce the chance of blood contamination in the CSF.
10	Rest	Leave the patient to rest for half an hour after LP. Prolonged bed rest or other procedures will not influence the risk of post-LP headache.
11	Tubes and aliquotation (type, volume, homogeneity)	Each laboratory should use the same PP tube. Glass or polystyrene tubes should in no circumstances be used. Tubes of the smallest volume should be used, and these should be filled to at least 50% of their volume.
12	Documentation of sampling/aliquotation	It is important to have carefully recorded and validated details concerning each stored sample so that any investigator when using these samples has a precise history of the sample.
	Centrifugation (speed and temperature)	Centrifugation only required for visually hemorrhagic samples. Centrifuge as soon as possible—within 2 hours of LP (on site or at nearest laboratory). Speed has no effect; however, recommend 2000g for 10 minutes at room temperature (controlled).
13	Time and temperature before storage	Samples may be sent by regular post (transport 5 days).
14	Method of freezing (liquid nitrogen, dry ice, slow freezing @ -20oC or 80oC.	Freezing at -80oC for storage. No difference between methods of freezing.
15	Length of storage (when frozen)	Storage: -20oC for less than 2 months. Note: No evidence of any effect for up to 2 years at -80oC.
16	Number of freeze / thaw cycles	Limit the number of freeze/thaw cycles to 1–2.
17	Interfering substances (hemolysis)	Traumatic LP: Discard first 1–2 mL. Samples with an erythrocyte count of 500/mL should not be used without centrifugation.

E. Limitations for the use of Biomarkers in Clinical Practice

Biomarkers of amyloid deposition and neurodegeneration tremendously advance the identification of AD-P in populations at risk for developing dementia. However, the process of applying these novel technologies in clinical settings is challenging due to the complex interface between research and clinical practice.

Moreover, normative standards for fluid and imaging biomarker technologies are still a work in progress.

Hitherto, it has been unclear whether a single or a combination of biomarkers is required to assess populations at risk for developing dementia. In fact, further population-based studies are necessary to define cut-off values, stability and the likelihood of progression to dementia for all biomarkers. Further research is also necessary to identify those biomarkers capable of identifying individuals "on the verge of conversion" from those carriers of amyloid pathology who will remain stable for several years. Unfortunately, the current body of knowledge regarding biomarkers fails to provide a robust empirical framework for evaluating clinical scenarios characterized by ambiguous, indeterminate or conflicting results involving multiple biomarkers. Ditto, it is applicable regarding those clinical cases suggestive of mixed pathology. Thus, further studies are necessary to prioritize biomarkers and outline their validity in numerous clinical and research scenarios.

F. CSF Biomarkers in AD and Other Dementias

CSF biomarkers in AD

Much has occurred over the past decade in terms of the development and validation of CSF biomarkers for the diagnosis of AD and related dementias. CSF is considered to be a good source of biomarkers for neurodegenerative diseases since pathological brain changes are expected to be reflected in the CSF owing to its constant contact with cerebral tissue [67].

The most comprehensively studied CSF biomarker in AD is the measurement of the 42 amino acids beta-amyloid peptide ($A\beta_{42}$), since it is the main component of the amyloid plaques seen in AD. The dynamics of Aβ metabolism in CSF has recently been examined in a study using a radioactive [13C] isotope labeling coupled with mass spectrometry analysis [68]. It was calculated that the average fractional synthesis rate of Aβ in CSF was 7.6

percent, while the fractional clearance rate was 8.3 percent, implying that production and clearance rates were not significantly different in healthy adults [68]. The rate is comparable, though slightly faster, to the turnover of Aβ measured in mouse models using in vivo microdialysis [69]. Significant diurnal variation in $A\beta_{42}$ levels was also found, with lowest level occurring in the morning [62]. Moreover, Aβ levels fall during sleep, and chronic sleep deprivation may increase $A\beta_{42}$ production [70]. Therefore, it is important to control for the timing of CSF $A\beta_{42}$ collection in order to minimize variability.

The utility of bioomarkers in the diagnosis of AD and differentiation from other dementias have been reviewed extensively [71-74]. AD patients typically exhibit a low level of $A\beta_{42}$ but high levels of CSF t-tau and p-tau_{181} . This characteristic pattern is regarded as the "signature profile of AD" in CSF [71, 75, 76]. Amalgamation of data from the Alzheimer Disease Neuroimaging Initiative (ADNI) studies generated a model for the temporal ordering of AD biomarkers, which suggests that Aβ amyloid biomarkers (such as CSF $A\beta_{42}$ or PET amyloid imaging) are the first to become abnormal, followed by changes in neurodegenerative biomarkers (CSF t-tau, p-tau, [^{18}F]FDG PET, and structural MRI) with the onset of clinical symptoms [77, 78]. These findings have been confirmed in a number of cross-sectional studies and prospective cohorts [50, 51, 79], including several with pathological confirmation of the diagnosis [80-84]. When the diagnosis of dementia was ambiguous on the basis of clinical presentation alone, CSF biomarkers improved diagnostic accuracy, and correlated with autopsy confirmation in up to 82% of cases [85, 86]. In another study applying CSF biomarkers in a specialized dementia clinic, knowledge of CSF biomarker profiles changed the diagnosis in 10% of the cases, and confidence in the diagnosis increased for one third of the patients [87]. Using a ratio of either p-tau/$A\beta_{42}$, or t-tau/$A\beta_{42}$ in differentiating AD from other dementias, the sensitivity was reported to be up to 92%, and specificity 86%, with an overall accuracy of 90% for the presence of pathologic neuritic plaque in the brain [82, 88] ENREF 65. Accuracy was particularly high using this combination of CSF biomarkers in differentiating

AD from frontotemporal dementia (FTD), progressive supranuclear palsy (PSP), Parkinson's Disease with Dementia (PDD), corticobasal degeneration (CBD), but not as clear for dementia with Lewy Bodies (DLB) and vascular dementia (VaD), possibly because of the propensity for mixed pathology DLB and VaD [82, 85, 89, 90]. Patients with Creutzfeldt-Jakob disease (CJD) demonstrated an extremely high CSF t-tau with relatively normal levels of p-tau and $A\beta_{42}$ [83].

CSF biomarkers in MCI – predicting conversion to AD

An important utility of CSF biomarkers is to predict the likelihood of conversion from MCI to AD dementia. A number of studies support the notion that the CSF "signature profile of AD"—comprising low $A\beta_{42}$ and high t-tau or p-tau—has good diagnostic accuracy in terms of distinguishing between normal ageing and AD (> 85%) and a positive predictive value (>90%) in terms of predicting conversion to dementia in patients with MCI [54]. Large-scale longitudinal studies of MCI cohorts consistently demonstrate that the presence of this "AD signature" in CSF has a good diagnostic accuracy (>80%) in discriminating patients with MCI who progress to AD ("MCI converters") from those who remain cognitively stable ("MCI-stable" patients) and healthy controls [54], as well as those MCI patients who progress to non-AD dementias [91]. These findings have been extensively replicated by different research groups worldwide [51, 74, 75, 92, 93] _ENREF_64, and further reinforced by meta-analyses of different datasets [94, 95] _ENREF_68. The converging evidence thus suggests that the presence of this "AD signature" in the CSF is a strong predictor of dementia outcome due to AD-P, with an increased odds ratio of up to 20 [96]. MCI patients who convert to AD generally have a CSF biomarker pattern indistinguishable from that found in patients with dementia of the AD type. Conversely, MCI patients who display non-progressive deficits over time have a CSF biomarker pattern most similar to that found in healthy older adults [97]. CSF t-tau and p-tau are robust predictors of AD outcome, and are also associated with a more rapid

progression from MCI to AD [98]. These findings can be applied to enrich clinical trials via recruitment of MCI subjects who are most likely to progress to clinical AD [99].

CSF biomarkers in normal elderly

While the intention of CSF biomarkers is to diagnose patients with dementia in the prodromal phase and to predict progression in patients with mild symptoms, it has been shown in different cohorts that the "characteristic CSF profile of AD" can be seen in up to one third of healthy elderly individuals [100, 101]. Several longitudinal studies have shown that lower CSF $A\beta_{42}$ and higher t-tau or p-tau levels, even in healthy subjects at baseline, are correlated with future decline in cognitive functions [102, 103] and faster progression of brain atrophy [104], suggesting that the CSF changes are consistent with the presence of significant AD-P at baseline [105]. In another study, high CSF levels of t-tau or p-tau was correlated with more severe impairment in memory, mental speed, and executive functioning, which not explained by disease severity, implying that high p-tau or t-tau may reflect a more aggressive disease course [106].

CSF vs. imaging biomarkers

In comparison to structural MRI, CSF appears to perform less well in detecting changes over time. While both whole-brain atrophy rate as measured by MRI and CSF levels of $A\beta_{42}$, t-tau, and p-tau all provide complementary information in patients with MCI and AD [107], baseline MR imaging and FDG-PET measures were more responsive to clinical changes than CSF measures in MCI subjects [108-111]. Additionally, structural MRI change was a better predictor of subsequent cognitive/functional change than CSF biomarkers. While MRI and CSF provide complimentary predictive information about time to conversion from amnestic MCI to AD—with the combination of the 2 resulting in increased

predicted power relative to either source alone—it was found that MRI was a better predictor of future clinical/functional decline than the CSF biomarkers tested [111]. Furthermore, when $A\beta_{42}$ and t-tau are used together, p-tau does not appear to have additional value for the purpose of predicting progression from MCI to AD, although p-tau appears to be more specific to AD pathology, while t-tau can be elevated in other neurodegenerative conditions [77, 112, 113]. However, a more recent study found that p-tau decreases at a rate of 2.2 pg/mL/yr and correlates better with cognitive functioning than either $A\beta_{42}$ or t-tau, possily reflecting neuronal loss in AD [114].

Other potential CSF biomarkers

In addition to $A\beta_{42}$, t-tau and p-tau, additional CSF biomarkers have been proposed, though they have yet to be widely replicated. For example, CSF epithelium-derived factor and haptoglobin are measures of oxidative damage, and may help with differentiating AD from other forms of dementia [115]. Sphingomyelin, a class of phospholipids involved in neurodegenerative processes, is significantly elevated in AD compared to controls, with potential utility as an AD biomarker in terms of studying lipid metabolism in the brain [116]. Similarly, lipoprotein receptor (LR11) has been implicated in the pathogenesis of AD, and with levels significantly increased in AD compared to controls [117]. In studies using targeted proteomic screening approach, novel biomarkers including C3, CgA, IL-1alpha, I-309, NrCAM and VEGF were found to further improve differentiation between AD and non-AD dementia, with altered levels of IL-1alpha and TECK being associated with subsequent cognitive decline [118]. In addition, oligomeric $A\beta$ species have been implicated in the pathophysiology of AD, and therefore may correlate with the onset of disease. Novel assays of misfolded protein for the detection of soluble $A\beta_{40}$ and $A\beta_{42}$ oligomers in CSF have shown promise in terms of greater accuracy in differentiating patients with MCI and AD

from normal controls, as compared to the usual methods based on fibrillar forms of the peptide [14, 119, 120].

CSF biomarkers in other dementias

While there have been extensive efforts in defining biomarkers of AD-P, the need for biomarkers of other forms of dementia may be even more acute. For instance, we now know that frontotemporal lobar degeneration (FTLD) is associated with three distinct pathological subtypes due to abnormal protein accumulation from tau (FTLD-tau), TDP-43 (FTLD-TDP), and FUS (FTLD-FUS). However, their clinical presentations are highly variable and heterogeneous, which includes behavioural variant FTD, progressive non-fluent aphasia, and semantic dementia, and can often be confused with logopenic and behavioural variant of AD. While a number of studies have demonstrated utility of CSF biomarkers in differentiating AD from FTLD variants, few have demonstrated good utility in differentiating between FTLD subtypes [79, 121]. Targeted multiplex proteomics screening found that Fas, agouti-related peptide, adrenocorticotropic hormone, and several chemokines (IL-23, IL-17) may have utility in differentiating FTLD-TDP from FTLD-tau [122]. These novel findings require further replication and validation.

Vascular cognitive impairment (VCI) and vascular dementia (VaD) are conditions that often coexist with AD [123]. While some studies have found utility of $A\beta_{42}$, t-tau and p-tau in differentiating AD from VaD [124], this has not been consistently replicated . This may, in part, be due to the co-existence of AD and VCI/VaD in a given study sample. Several studies have found that the elevation of CSF/serum albumin index may be a useful measure of disruption to the blood-brain barrier due to VaD [125-129]. Another potential biomarker for VaD is CSF sulfatide, an acidic glycophospholipid presented in myelin sheaths of oligodendrocytes that was found to be 200% higher in VaD patients compared to controls and AD patients [130, 131]. This marker was found to be decreased in a

study of MCI and early AD compared to controls, but its exact mechanism remains unclear [132]. Neurofilament (NFL) is a cytoskeletal component concentrated in larger myelinated axons. A few studies found that CSF NFL elevation is associated with the presence of white matter changes, whereas CSF NFL is normal in AD [133-135]. Other markers of inflammation including interleukin-6 (IL6) and metalloproteinase-9 (MMP9) are elevated in VaD or its precursor state, but not in AD [135-140]. A caveat of these inflammatory markers is that they may be influenced by other disease states, for instance viral meningitis, and must be interpreted with caution.

Dementia with Lewy Bodies (DLB) and Parkinson's Disease with dementia (PDD) are also common causes of cognitive decline in the elderly. Like VCI and VaD, DLB can often co-exist with AD in patient populations [141]. Several studies have shown that levels of CSF Aβ are decreased in DLB and PDD, which is also predictive of future cognitive decline [118, 142-144]. However, in these studies, subjects were diagnosed clinically without pathological confirmation. It is possible that these patients had mixed AD/DLB, or that DLB pathology *per se* can cause a drop in CSF Aβ levels.[89, 145-147] In contrast, while t-tau and p-tau levels in DLB may be similar or slightly lower than those of controls, they are significantly higher in AD compared to DLB, and therefore can be used to differentiate AD from DLB [148, 149]. There is, moreover, emerging evidence that measurement of specific forms of α-synuclein in CSF may contribute to the diagnosis of PDD and DLB. However, studies have been mixed, and further validation is required before this can be put forward as a diagnostic test for PDD or DLB [90, 143, 150-158].

Limitations of CSF Biomarkers in Clinical Practice

Despite the high sensitivity and specificity reached in various research cohorts and populations using a combination of $A\beta_{42}$ and t-tau or p-tau in diagnosing AD and predicting progression in amnestic MCI, several methodological limitations remain before this finding can be translated into

broader clinical practice. While the measurements of CSF concentrations of these biomarkers using enzyme-linked immunosorbent assay (ELISA) (e.g. Innogenetics, Ghent, Belgium) or multiplex techniques (e.g. xMAP; Luminex, Austin TX, USA) have acceptably low coefficients of intra-laboratory variability (5-10%), the high inter-laboratory variation (20-30%) hinders comparison of data generated in different settings [159]. Potential sources of variation include: 1) pre-analytical conditions such as different lumbar puncture protocol, diurnal variation of $A\beta_{42}$ levels, sample handling and aliquot storing (e.g. polypropylene vials) prior to experimentation; 2) analytical conditions such as the different methods for the determination of the concentrations of biomarkers; and 3) post-analytical variations, which includes the definition of norms for patients and controls used in defining the cutoff points. These are major obstacles in instituting multi-centre studies. The establishment of gold-standard protocols to be shared by different laboratories and the recent launch of a multi-centre quality control program with participation of commercial and academic laboratories around the world will hopefully overcome these limitations in the near future [160, 161].

Recommendations for CCCDTD4 regarding biomarkers in AD and related dementias:

Preamble: Currently, the most established CSF biomarkers for AD pathology are Ab_{42} , t-tau, and p181-tau.

To primary care physicians:

1. Plasma amyloid are not recommendable for clinical practice.
2. CSF biomarkers for the diagnosis of AD are not recommended for use by primary care physicians to evaluate subjects with memory loss with typical presentation of AD (Grade B, Level 2). When such specialized testing in an individual with atypical presentation is considered, a referral of the patient to a specialist with expertise in assessing dementia and cognitive changes is recommended.

To specialists:

1. CSF biomarkers are not recommended in the usual diagnosis of AD when all other clinical criteria are met, unless in specific situations when confirmation for the presence of AD pathology is beneficial, for instance, when considering participation in a clinical trial of disease modifying therapy (Grade B, Level 2).
2. CSF biomarkers can be considered in cases in which there are atypical features and diagnostic confusion, such as differentiating frontal variants of AD from FTD, or cases of progressive aphasia which may be due to AD-P or FTLD pathology (Grade B, Level 2).
3. CSF biomarkers may improve diagnostic certainty and prognostication in cases of MCI, for example, if the patient and family would like to know the likelihood of progression over the next 3- to 5-year period (Grade A, Level 1).
4. CSF biomarkers are not recommended for screening in normal healthy subjects for the purpose of assessing future risk of developing AD. (Grade B, Level 3).
5. There is insufficient evidence for the use of CSF biomarkers in differentiating AD from DLB and VaD. (Grade C, Level 3)
6. When a decision to obtain CSF biomarkers is made, a combination of Ab42, total tau, and/or p-tau measurements can be used (Grade A, Level 1). Biomarkers measurements should be performed at a centralized facility (commercial or academic) with a track record in producing high quality, consistent data. (Grade B, Level 2).

References

1. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological Alterations in Alzheimer Disease. Cold Spring Harbor Perspectives in Biology 2011;3:-.
2. Group. BDW. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001;69:89-95.
3. Rabinovici GD, Rosen HJ, Alkalay A, et al. Amyloid vs FDG-PET in the differential diagnosis of AD and FTLD. Neurology 2011;77:2034-2042.
4. Rabinovici GD, Jagust WJ, Furst AJ, et al. Aβ amyloid and glucose metabolism in three variants of primary progressive aphasia. Ann Neurol 2008;64:388-401.

5. Silverman DH, Small GW, Chang CY, et al. Positron emission tomography in evaluation of dementia: Regional brain metabolism and long-term outcome. JAMA 2001;286:2120-2127.
6. Jack Jr CR, Albert MS, Knopman DS, et al. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers & Dementia 2011;7:257-262.
7. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science 1992;256:184-185.
8. Wirths O, Multhaup G, Bayer T. A modified beta-amyloid hypothesis: intraneuronal accumulation of the beta-amyloid peptide - the first step of a fatal cascade. Journal of Neurochemistry 2004;91:513-520.
9. Marcello E, Epis R, Di Luca M. Amyloid flirting with synaptic failure: Towards a comprehensive view of Alzheimer's disease pathogenesis. European journal of pharmacology 2008;585:109-118.
10. Jack CR, Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 2009;9:119-128.
11. Ikonomovic MD, Klunk WE, Abrahamson EE, et al. Post-mortem correlates of in vivo PiB-PET amyloid imaging in a typical case of Alzheimer's disease. Brain 2008;131:1630-1645.
12. Seeman P, Seeman N. Alzheimer's Disease: beta-Amyloid Plaque Formation in Human Brain. Synapse 2011;65:1289-1297.
13. Gao CM, Yam AY, Wang X, et al. A beta 40 Oligomers Identified as a Potential Biomarker for the Diagnosis of Alzheimer's Disease. PLoS One 2010;5:-.
14. Fukumoto H, Tokuda T, Kasai T, et al. High-molecular-weight beta-amyloid oligomers are elevated in cerebrospinal fluid of Alzheimer patients. Faseb J 2010;24:2716-2726.
15. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide. Nature Reviews Molecular Cell Biology 2007;8:101-112.
16. Tolboom N, van der Flier WM, Yaqub M, et al. Relationship of Cerebrospinal Fluid Markers to 11C-PiB and 18F-FDDNP Binding. Journal of Nuclear Medicine 2009;50:1464-1470.
17. Weigand SD, Vemuri P, Wiste HJ, et al. Transforming cerebrospinal fluid Aβ42 measures into calculated Pittsburgh compound B units of brain Aβ amyloid. Alzheimers & Dementia 2011;7:133-141.
18. Finch N, Baker M, Crook R, et al. Plasma progranulin levels predict progranulin mutation status in frontotemporal dementia patients and asymptomatic family members. Brain 2009;132:583-591.
19. Schrijvers EMC, Koudstaal PJ, Hofman A, Breteler MMB. Plasma clusterin and the risk of Alzheimer disease. JAMA: The Journal of the American Medical Association 2011;305:1322-1326.
20. Blasko I, Jellinger K, Kemmler G, et al. Conversion from cognitive health to mild cognitive impairment and Alzheimer's disease: prediction by plasma amyloid beta 42, medial temporal lobe atrophy and homocysteine. Neurobiol Aging 2008;29:1-11.

21. Cammarata S, Borghi R, Giliberto L, et al. Amyloid-beta(42) Plasma Levels are Elevated in Amnestic Mild Cognitive Impairment. Journal of Alzheimer's disease : JAD 2009;18:267-271.
22. Fei M, Jianghua W, Rujuan M, Wei Z, Qian W. The relationship of plasma A beta levels to dementia in aging individuals with mild cognitive impairment. Journal of the neurological sciences 2011;305:92-96.
23. Graff-Radford NR, Crook JE, Lucas J, et al. Association of low plasma Abeta42/Abeta40 ratios with increased imminent risk for mild cognitive impairment and Alzheimer disease. Arch Neurol 2007;64:354-362.
24. Hansson O, Zetterberg H, Vanmechelen E, et al. Evaluation of plasma Abeta(40) and Abeta(42) as predictors of conversion to Alzheimer's disease in patients with mild cognitive impairment. Neurobiol Aging 2010;31:357-367.
25. Koyama A, Okereke OI, Yang T, Blacker D, Selkoe DJ, Grodstein F. Plasma Amyloid-β as a Predictor of Dementia and Cognitive Decline: A Systematic Review and Meta-analysis. Arch Neurol 2012.
26. Lambert J-C, Schraen-Maschke S, Richard F, et al. Association of plasma amyloid beta with risk of dementia The prospective Three-City Study. Neurology 2009;73:847-853.
27. Lewczuk P, Kornhuber J, Vanmechelen E, et al. Amyloid beta peptides in plasma in early diagnosis of Alzheimer's disease: A multicenter study with multiplexing. Exp Neurol 2010;223:366-370.
28. Lopez OL, Kuller LH, Mehta PD, et al. Plasma amyloid levels and the risk of AD in normal subjects in the Cardiovascular Health Study. Neurology 2008;70:1664-1671.
29. Mayeux R, Schupf N. Blood-based biomarkers for Alzheimer's disease: plasma Aβ40 and Aβ42, and genetic variants 10.1016/j.neurobiolaging.2011.09.004 : Neurobiology of Aging | ScienceDirect.com. Neurobiol Aging 2011.
30. van Oijen M, Hofman A, Soares HD, Koudstaal PJ, Breteler MMB. Plasma Abeta(1-40) and Abeta(1-42) and the risk of dementia: a prospective case-cohort study. The Lancet Neurology 2006;5:655-660.
31. Okereke OI, Xia W, Selkoe DJ, Grodstein F. Ten-year change in plasma amyloid beta levels and late-life cognitive decline. Arch Neurol 2009;66:1247-1253.
32. Schupf N, Tang MX, Fukuyama H, et al. Peripheral Abeta subspecies as risk biomarkers of Alzheimer's disease. Proceedings of the National Academy of Sciences 2008;105:14052-14057.
33. Schupf N, Patel B, Pang D, et al. Elevated plasma beta-amyloid peptide A beta(42) levels, incident dementia, and mortality in Down syndrome. Arch Neurol 2007;64:1007-1013.
34. Seppälä TT, Herukka S-K, Hänninen T, et al. Plasma Abeta42 and Abeta40 as markers of cognitive change in follow-up: a prospective, longitudinal, population-based cohort study. Journal Of Neurology Neurosurgery And Psychiatry 2010;81:1123-1127.
35. Sundelof J, Giedraitis V, Irizarry MC, et al. Plasma beta amyloid and the risk of Alzheimer disease and dementia in elderly men. Arch Neurol 2008;65:256-263.
36. Ringman JM, Younkin SG, Pratico D, et al. Biochemical markers in persons with preclinical familial Alzheimer disease. Neurology 2008;71:85-92.

37. Pesaresi M, Lovati C, Bertora P, et al. Plasma levels of beta-amyloid (1-42) in Alzheimer's disease and mild cognitive impairment. Neurobiol Aging 2006;27:904-905.
38. Mehta PD, Pirttila T, Patrick BA, Barshatzky M, Mehta SP. Amyloid beta protein 1-40 and 1-42 levels in matched cerebrospinal fluid and plasma from patients with Alzheimer disease. Neuroscience Letters 2001;304:102-106.
39. Hansson O, Zetterberg H, Vanmechelen E, et al. Evaluation of plasma Abeta(40) and Abeta(42) as predictors of conversion to Alzheimer's disease in patients with mild cognitive impairment. Neurobiol Aging 2010;31:357-367.
40. Vanderstichele H, Van Kerschaver E, Hesse C, et al. Standardization of measurement of beta-amyloid((1-42)) in cerebrospinal fluid and plasma. Amyloid-Journal of Protein Folding Disorders 2000;7:245-258.
41. Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. Proc Natl Acad Sci USA 1975;72:1858-1862.
42. Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nature reviews Neurology 2010;6:131-144.
43. Hall GF, Lee VM, Kosik KS. Microtubule destabilization and neurofilament phosphorylation precede dendritic sprouting after close axotomy of lamprey central neurons. Proc Natl Acad Sci USA 1991;88:5016-5020.
44. Mattsson N, Zetterberg H, Hansson O, et al. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. JAMA: The Journal of the American Medical Association 2009;302:385-393.
45. Petersen RC, Aisen PS, Beckett LA, et al. Alzheimer's Disease Neuroimaging Initiative (ADNI): clinical characterization. Neurology 2010;74:201-209.
46. Zetterberg H, Hietala MA, Jonsson M, et al. Neurochemical aftermath of amateur boxing. Arch Neurol 2006;63:1277-1280.
47. Ost M, Nylen K, Csajbok L, et al. Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury. Neurology 2006;67:1600-1604.
48. Hesse C, Rosengren L, Andreasen N, et al. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. Neuroscience Letters 2001;297:187-190.
49. Tapiola T, Lehtovirta M, Ramberg J, et al. CSF tau is related to apolipoprotein E genotype in early Alzheimer's disease. Neurology 1998;50:169-174.
50. Andreasen N, Minthon L, Davidsson P, et al. Evaluation of CSF-tau and CSF-A beta 42 as diagnostic markers for Alzheimer disease in clinical practice. Arch Neurol 2001;58:373-379.
51. Hampel H, Buerger K, Zinkowski R, et al. Measurement of phosphorylated tau epitopes in the differential diagnosis of Alzheimer disease: a comparative cerebrospinal fluid study. Archives of general psychiatry 2004;61:95-102.
52. Perneczky R, Tsolakidou A, Arnold A, et al. CSF soluble amyloid precursor proteins in the diagnosis of incipient Alzheimer disease. Neurology 2011;77:35-38.
53. Hansson O, Stomrud E, Vanmechelen E, et al. Evaluation of Plasma A beta as Predictor of Alzheimer's Disease in Older Individuals Without Dementia: A Population-Based Study. Journal of Alzheimer's disease : JAD 2012;28:231-238.

54. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol 2006;5:228-234.
55. Mortimer J, Gosche K, Riley K, Markesbery W, Snowdon D. Delayed recall, hippocampal volume and Alzheimer neuropathology - Findings from the Nun Study. Neurology 2004;62:428-432.
56. Jack Jr CR, Bernstein MA, Borowski BJ, et al. Update on the Magnetic Resonance Imaging core of the Alzheimer□s Disease Neuroimaging Initiative. Alzheimers & Dementia 2010;6:212-220.
57. Jack CR, Lowe VJ, Weigand SD, et al. Serial PIB and MRI in normal, mild cognitive impairment and Alzheimer's disease: implications for sequence of pathological events in Alzheimer's disease. Brain 2009;132:1355-1365.
58. Sperling R. Preclinical Alzheimer’s Disease Workgroup. National Institute on Aging and Alzheimer's Association Lead Effort to Update Diagnostic Criteria for Alzheimer's Disease 2010:1-14.
59. Hort J, Bartos A, Pirttila T, Scheltens P. Use of cerebrospinal fluid biomarkers in diagnosis of dementia across Europe. European Journal of Neurology 2010;17:90-96.
60. Kang J-H, Vanderstichele H, Trojanowski JQ, Shaw LM. Simultaneous analysis of cerebrospinal fluid biomarkers using microsphere-based xMAP multiplex technology for early detection of Alzheimer’s disease. Methods (San Diego, Calif) 2012.
61. Vanderstichele H, Bibl M, Engelborghs S, et al. Standardization of preanalytical aspects of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: A consensus paper from the Alzheimer's Biomarkers Standardization Initiative. Alzheimers & Dementia 2012;8:65-73.
62. Bateman RJ, Wen G, Morris JC, Holtzman DM. Fluctuations of CSF amyloid-beta levels: implications for a diagnostic and therapeutic biomarker. Neurology 2007;68:666-669.
63. Bjerke M, Portelius E, Minthon L, et al. Confounding Factors Influencing Amyloid Beta Concentration in Cerebrospinal Fluid. International Journal of Alzheimer's Disease 2010;2010:1-11.
64. Lewczuk P, Beck G, Esselmann H, et al. Effect of sample collection tubes on cerebrospinal fluid concentrations of tau proteins and amyloid beta peptides. Clinical Chemistry 2006;52:332-334.
65. Perret-Liaudet A, Pelpel M, Tholance Y, et al. Cerebrospinal fluid collection tubes: a critical issue for Alzheimer disease diagnosis. Clinical Chemistry 2012;58:787-789.
66. You J-S, Gelfanova V, Knierman MD, Witzmann FA, Wang M, Hale JE. The impact of blood contamination on the proteome of cerebrospinal fluid. PROTEOMICS 2005;5:290-296.
67. Reiber H. Dynamics of brain-derived proteins in cerebrospinal fluid. Clin Chim Acta 2001;310:173-186.
68. Bateman RJ, Munsell LY, Morris JC, Swarm R, Yarasheski KE, Holtzman DM. Human amyloid-beta synthesis and clearance rates as measured in cerebrospinal fluid in vivo. Nat Med 2006;12:856-861.

69. Cirrito JR, May PC, O'Dell MA, et al. In vivo assessment of brain interstitial fluid with microdialysis reveals plaque-associated changes in amyloid-beta metabolism and half-life. J Neurosci 2003;23:8844-8853.
70. Kang JE, Lim MM, Bateman RJ, et al. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. Science 2009;326:1005-1007.
71. Hampel H, Frank R, Broich K, et al. Biomarkers for Alzheimer's disease: academic, industry and regulatory perspectives. Nat Rev Drug Discov 2010;9:560-574.
72. Forlenza OV, Diniz BS, Gattaz WF. Diagnosis and biomarkers of predementia in Alzheimer's disease. BMC Med 2010;8:89.
73. Hampel H, Burger K, Teipel SJ, Bokde AL, Zetterberg H, Blennow K. Core candidate neurochemical and imaging biomarkers of Alzheimer's disease. Alzheimers Dement 2008;4:38-48.
74. Mulder C, Verwey NA, van der Flier WM, et al. Amyloid-beta(1-42), total tau, and phosphorylated tau as cerebrospinal fluid biomarkers for the diagnosis of Alzheimer disease. Clin Chem 2010;56:248-253.
75. Hampel H, Blennow K, Shaw LM, Hoessler YC, Zetterberg H, Trojanowski JQ. Total and phosphorylated tau protein as biological markers of Alzheimer's disease. Experimental gerontology 2010;45:30-40.
76. Englund H, Degerman Gunnarsson M, Brundin RM, et al. Oligomerization partially explains the lowering of Abeta42 in Alzheimer's disease cerebrospinal fluid. Neurodegener Dis 2009;6:139-147.
77. Trojanowski JQ, Vandeerstichele H, Korecka M, et al. Update on the biomarker core of the Alzheimer's Disease Neuroimaging Initiative subjects. Alzheimers Dement 2010;6:230-238.
78. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 2010;9:119-128.
79. de Souza LC, Corlier F, Habert MO, et al. Similar amyloid- burden in posterior cortical atrophy and Alzheimer's disease. Brain 2011;134:2036-2043.
80. Buerger K, Ewers M, Pirttila T, et al. CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. Brain : a journal of neurology 2006;129:3035-3041.
81. Clark CM, Xie S, Chittams J, et al. Cerebrospinal fluid tau and beta-amyloid: how well do these biomarkers reflect autopsy-confirmed dementia diagnoses? Arch Neurol 2003;60:1696-1702.
82. Tapiola T, Alafuzoff I, Herukka S-K, et al. Cerebrospinal Fluid beta-Amyloid 42 and Tau Proteins as Biomarkers of Alzheimer-Type Pathologic Changes in the Brain. Arch Neurol 2009;66:382-389.
83. Schoonenboom NS, Reesink FE, Verwey NA, et al. Cerebrospinal fluid markers for differential dementia diagnosis in a large memory clinic cohort. Neurology 2012;78:47-54.
84. Shaw LM, Vanderstichele H, Knapik-Czajka M, et al. Qualification of the analytical and clinical performance of CSF biomarker analyses in ADNI. Acta neuropathologica 2011;121:597-609.
85. Bian H, Van Swieten JC, Leight S, et al. CSF biomarkers in frontotemporal lobar degeneration with known pathology. Neurology 2008;70:1827-1835.

86. Le Bastard N, Martin JJ, Vanmechelen E, Vanderstichele H, De Deyn PP, Engelborghs S. Added diagnostic value of CSF biomarkers in differential dementia diagnosis. Neurobiol Aging 2010;31:1867-1876.
87. Kester MI, Boelaarts L, Bouwman FH, et al. Diagnostic impact of CSF biomarkers in a local hospital memory clinic. Dement Geriatr Cogn Disord 2010;29:491-497.
88. Bloudek LM, Spackman DE, Blankenburg M, Sullivan SD. Review and meta-analysis of biomarkers and diagnostic imaging in Alzheimer's disease. Journal of Alzheimer's disease : JAD 2011;26:627-645.
89. Bibl M, Mollenhauer B, Esselmann H, et al. CSF amyloid-beta-peptides in Alzheimer's disease, dementia with Lewy bodies and Parkinson's disease dementia. Brain : a journal of neurology 2006;129:1177-1187.
90. Mollenhauer B, Bibl M, Trenkwalder C, et al. Follow-up investigations in cerebrospinal fluid of patients with dementia with Lewy bodies and Alzheimer's disease. Journal of neural transmission 2005;112:933-948.
91. Riemenschneider M, Wagenpfeil S, Diehl J, et al. Tau and Abeta42 protein in CSF of patients with frontotemporal degeneration. Neurology 2002;58:1622-1628.
92. Arai H, Ishiguro K, Ohno H, et al. CSF phosphorylated tau protein and mild cognitive impairment: a prospective study. Exp Neurol 2000;166:201-203.
93. Heister D, Brewer JB, Magda S, Blennow K, McEvoy LK. Predicting MCI outcome with clinically available MRI and CSF biomarkers. Neurology 2011;77:1619-1628.
94. Diniz BS, Pinto Junior JA, Forlenza OV. Do CSF total tau, phosphorylated tau, and beta-amyloid 42 help to predict progression of mild cognitive impairment to Alzheimer's disease? A systematic review and meta-analysis of the literature. World J Biol Psychiatry 2008;9:172-182.
95. van Rossum IA, Vos S, Handels R, Visser PJ. Biomarkers as predictors for conversion from mild cognitive impairment to Alzheimer-type dementia: implications for trial design. Journal of Alzheimer's disease : JAD 2010;20:881-891.
96. Hertze J, Minthon L, Zetterberg H, Vanmechelen E, Blennow K, Hansson O. Evaluation of CSF biomarkers as predictors of Alzheimer's disease: a clinical follow-up study of 4.7 years. Journal of Alzheimer's disease : JAD 2010;21:1119-1128.
97. Haldenwanger A, Eling P, Kastrup A, Hildebrandt H. Correlation between cognitive impairment and CSF biomarkers in amnesic MCI, non-amnesic MCI, and Alzheimer's disease. Journal of Alzheimer's disease : JAD 2010;22:971-980.
98. Blom ES, Giedraitis V, Zetterberg H, et al. Rapid progression from mild cognitive impairment to Alzheimer's disease in subjects with elevated levels of tau in cerebrospinal fluid and the APOE epsilon4/epsilon4 genotype. Dement Geriatr Cogn Disord 2009;27:458-464.
99. Lorenzi M, Donohue M, Paternico D, et al. Enrichment through biomarkers in clinical trials of Alzheimer's drugs in patients with mild cognitive impairment. Neurobiol Aging 2010;31:1443-1451, 1451 e1441.
100. Andreasen N, Vanmechelen E, Vanderstichele H, Davidsson P, Blennow K. Cerebrospinal fluid levels of total-tau, phospho-tau and A beta 42 predicts development of Alzheimer's disease in patients with mild cognitive impairment. Acta Neurol Scand 2003;107:47-51.

101. De Meyer G, Shapiro F, Vanderstichele H, et al. Diagnosis-independent Alzheimer disease biomarker signature in cognitively normal elderly people. Arch Neurol 2010;67:949-956.
102. Andersson C, Blennow K, Almkvist O, et al. Increasing CSF phospho-tau levels during cognitive decline and progression to dementia. Neurobiol Aging 2008;29:1466-1473.
103. Stomrud E, Hansson O, Zetterberg H, Blennow K, Minthon L, Londos E. Correlation of longitudinal cerebrospinal fluid biomarkers with cognitive decline in healthy older adults. Arch Neurol 2010;67:217-223.
104. Tosun D, Schuff N, Truran-Sacrey D, et al. Relations between brain tissue loss, CSF biomarkers, and the ApoE genetic profile: a longitudinal MRI study. Neurobiol Aging 2010;31:1340-1354.
105. Bouwman FH, Schoonenboom NS, Verwey NA, et al. CSF biomarker levels in early and late onset Alzheimer's disease. Neurobiol Aging 2009;30:1895-1901.
106. van der Vlies AE, Verwey NA, Bouwman FH, et al. CSF biomarkers in relationship to cognitive profiles in Alzheimer disease. Neurology 2009;72:1056-1061.
107. Sluimer JD, Bouwman FH, Vrenken H, et al. Whole-brain atrophy rate and CSF biomarker levels in MCI and AD: A longitudinal study. Neurobiol Aging 2010;31:758-764.
108. Walhovd KB, Fjell AM, Brewer J, et al. Combining MR imaging, positron-emission tomography, and CSF biomarkers in the diagnosis and prognosis of Alzheimer disease. AJNR Am J Neuroradiol 2010;31:347-354.
109. Fjell AM, Walhovd KB, Fennema-Notestine C, et al. CSF biomarkers in prediction of cerebral and clinical change in mild cognitive impairment and Alzheimer's disease. J Neurosci 2010;30:2088-2101.
110. Arlt S, Brassen S, Jahn H, et al. Association between FDG uptake, CSF biomarkers and cognitive performance in patients with probable Alzheimer's disease. Eur J Nucl Med Mol Imaging 2009;36:1090-1100.
111. Vemuri P, Wiste HJ, Weigand SD, et al. MRI and CSF biomarkers in normal, MCI, and AD subjects: predicting future clinical change. Neurology 2009;73:294-301.
112. Jack CR, Jr., Vemuri P, Wiste HJ, et al. Evidence for ordering of Alzheimer disease biomarkers. Arch Neurol 2011;68:1526-1535.
113. Scheurich A, Urban PP, Koch-Khoury N, Fellgiebel A. CSF phospho-tau is independent of age, cognitive status and gender of neurological patients. J Neurol 2010;257:609-614.
114. Seppala TT, Koivisto AM, Hartikainen P, Helisalmi S, Soininen H, Herukka SK. Longitudinal changes of CSF biomarkers in Alzheimer's disease. Journal of Alzheimer's disease : JAD 2011;25:583-594.
115. Abraham JD, Calvayrac-Pawlowski S, Cobo S, et al. Combined measurement of PEDF, haptoglobin and tau in cerebrospinal fluid improves the diagnostic discrimination between alzheimer's disease and other dementias. Biomarkers 2011;16:161-171.
116. Kosicek M, Kirsch S, Bene R, et al. Nano-HPLC-MS analysis of phospholipids in cerebrospinal fluid of Alzheimer's disease patients--a pilot study. Anal Bioanal Chem 2010;398:2929-2937.

117. Ikeuchi T, Hirayama S, Miida T, et al. Increased levels of soluble LR11 in cerebrospinal fluid of patients with Alzheimer disease. Dement Geriatr Cogn Disord 2010;30:28-32.
118. Hu WT, Chen-Plotkin A, Arnold SE, et al. Novel CSF biomarkers for Alzheimer's disease and mild cognitive impairment. Acta neuropathologica 2010;119:669-678.
119. Santos AN, Torkler S, Nowak D, et al. Detection of amyloid-beta oligomers in human cerebrospinal fluid by flow cytometry and fluorescence resonance energy transfer. Journal of Alzheimer's disease : JAD 2007;11:117-125.
120. Gao CM, Yam AY, Wang X, et al. Abeta40 oligomers identified as a potential biomarker for the diagnosis of Alzheimer's disease. PLoS One 2010;5:e15725.
121. Bibl M, Mollenhauer B, Lewczuk P, et al. Cerebrospinal fluid tau, p-tau 181 and amyloid-beta38/40/42 in frontotemporal dementias and primary progressive aphasias. Dement Geriatr Cogn Disord 2011;31:37-44.
122. Hu WT, Chen-Plotkin A, Grossman M, et al. Novel CSF biomarkers for frontotemporal lobar degenerations. Neurology 2010.
123. Woodward M, Mackenzie IRA, Hsiung GY, Jacova C, Feldman H. Multiple brain pathologies in dementia are common. European Geriatric Medicine 2010;1:259-265.
124. de Jong D, Jansen RW, Kremer BP, Verbeek MM. Cerebrospinal fluid amyloid beta42/phosphorylated tau ratio discriminates between Alzheimer's disease and vascular dementia. J Gerontol A Biol Sci Med Sci 2006;61:755-758.
125. Bowman GL, Kaye JA, Moore M, Waichunas D, Carlson NE, Quinn JF. Blood-brain barrier impairment in Alzheimer disease: stability and functional significance. Neurology 2007;68:1809-1814.
126. Skoog I, Wallin A, Fredman P, et al. A population study on blood-brain barrier function in 85-year-olds: relation to Alzheimer's disease and vascular dementia. Neurology 1998;50:966-971.
127. Wallin A, Blennow K, Rosengren L. Cerebrospinal fluid markers of pathogenetic processes in vascular dementia, with special reference to the subcortical subtype. Alzheimer Dis Assoc Disord 1999;13 Suppl 3:S102-105.
128. Wallin A, Pirttila T. Biomarkers in vascular dementia. In: Wahlund LO, Erkinjuntti T, Gauthier S, eds. Vascular Cognitive Impairment in Clinical Practice. Cambridge: Cambridge University Press, 2009: 77-91.
129. Chalbot S, Zetterberg H, Blennow K, et al. Blood-cerebrospinal fluid barrier permeability in Alzheimer's disease. Journal of Alzheimer's disease : JAD 2011;25:505-515.
130. Fredman P, Wallin A, Blennow K, Davidsson P, Gottfries CG, Svennerholm L. Sulfatide as a biochemical marker in cerebrospinal fluid of patients with vascular dementia. Acta Neurol Scand 1992;85:103-106.
131. Tullberg M, Mansson JE, Fredman P, et al. CSF sulfatide distinguishes between normal pressure hydrocephalus and subcortical arteriosclerotic encephalopathy. Journal of neurology, neurosurgery, and psychiatry 2000;69:74-81.
132. Han X, Fagan AM, Cheng H, Morris JC, Xiong C, Holtzman DM. Cerebrospinal fluid sulfatide is decreased in subjects with incipient dementia. Ann Neurol 2003;54:115-119.

133. Bjerke M, Andreasson U, Rolstad S, et al. Subcortical vascular dementia biomarker pattern in mild cognitive impairment. Dement Geriatr Cogn Disord 2009;28:348-356.
134. Wallin A, Sjogren M. Cerebrospinal fluid cytoskeleton proteins in patients with subcortical white-matter dementia. Mech Ageing Dev 2001;122:1937-1949.
135. Bjerke M, Zetterberg H, Edman A, Blennow K, Wallin A, Andreasson U. Cerebrospinal Fluid Matrix Metalloproteinases and Tissue Inhibitor of Metalloproteinases in Combination with Subcortical and Cortical Biomarkers in Vascular Dementia and Alzheimer's Disease. Journal of Alzheimer's disease : JAD 2011.
136. Rosenberg GA, Sullivan N, Esiri MM. White matter damage is associated with matrix metalloproteinases in vascular dementia. Stroke 2001;32:1162-1168.
137. Adair JC, Charlie J, Dencoff JE, et al. Measurement of gelatinase B (MMP-9) in the cerebrospinal fluid of patients with vascular dementia and Alzheimer disease. Stroke 2004;35:e159-162.
138. Galimberti D, Venturelli E, Fenoglio C, et al. Intrathecal levels of IL-6, IL-11 and LIF in Alzheimer's disease and frontotemporal lobar degeneration. J Neurol 2008;255:539-544.
139. Blum-Degen D, Muller T, Kuhn W, Gerlach M, Przuntek H, Riederer P. Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. Neurosci Lett 1995;202:17-20.
140. Wada-Isoe K, Wakutani Y, Urakami K, Nakashima K. Elevated interleukin-6 levels in cerebrospinal fluid of vascular dementia patients. Acta Neurol Scand 2004;110:124-127.
141. Woodward M, Brodaty H, Boundy K, Ames D, Blanch G, Balshaw R. Does executive impairment define a frontal variant of Alzheimer's disease? International Psychogeriatrics 2010;22:1280-1290.
142. Johansen KK, White LR, Sando SB, Aasly JO. Biomarkers: Parkinson disease with dementia and dementia with Lewy bodies. Parkinsonism Relat Disord 2010;16:307-315.
143. Kasuga K, Tokutake T, Ishikawa A, et al. Differential levels of alpha-synuclein, beta-amyloid42 and tau in CSF between patients with dementia with Lewy bodies and Alzheimer's disease. Journal of neurology, neurosurgery, and psychiatry 2010;81:608-610.
144. Kester MI, van der Vlies AE, Blankenstein MA, et al. CSF biomarkers predict rate of cognitive decline in Alzheimer disease. Neurology 2009;73:1353-1358.
145. Alves G, Bronnick K, Aarsland D, et al. CSF amyloid-{beta} and tau proteins, and cognitive performance, in early and untreated Parkinson's Disease: the Norwegian ParkWest study. Journal of neurology, neurosurgery, and psychiatry 2010.
146. Compta Y, Marti MJ, Ibarretxe-Bilbao N, et al. Cerebrospinal tau, phospho-tau, and beta-amyloid and neuropsychological functions in Parkinson's disease. Mov Disord 2009;24:2203-2210.
147. Mollenhauer B, Bibl M, Wiltfang J, et al. Total tau protein, phosphorylated tau (181p) protein, beta-amyloid(1-42), and beta-amyloid(1-40) in cerebrospinal fluid of patients with dementia with Lewy bodies. Clin Chem Lab Med 2006;44:192-195.

148. Parnetti L, Tiraboschi P, Lanari A, et al. Cerebrospinal fluid biomarkers in Parkinson's disease with dementia and dementia with Lewy bodies. Biol Psychiatry 2008;64:850-855.
149. Otto M, Lewczuk P, Wiltfang J. Neurochemical approaches of cerebrospinal fluid diagnostics in neurodegenerative diseases. Methods 2008;44:289-298.
150. Reesink FE, Lemstra AW, van Dijk KD, et al. CSF alpha-Synuclein Does Not Discriminate Dementia with Lewy Bodies from Alzheimer's Disease. Journal of Alzheimer's disease : JAD 2010.
151. Aerts MB, Esselink RA, Abdo WF, Bloem BR, Verbeek MM. CSF alpha-synuclein does not differentiate between parkinsonian disorders. Neurobiol Aging 2011.
152. Ballard C, Jones EL, Londos E, Minthon L, Francis P, Aarsland D. alpha-Synuclein antibodies recognize a protein present at lower levels in the CSF of patients with dementia with Lewy bodies. Int Psychogeriatr 2010;22:321-327.
153. Tokuda T, Qureshi MM, Ardah MT, et al. Detection of elevated levels of alpha-synuclein oligomers in CSF from patients with Parkinson disease. Neurology 2010;75:1766-1772.
154. Aarsland D, Kurz M, Beyer M, Bronnick K, Piepenstock Nore S, Ballard C. Early discriminatory diagnosis of dementia with Lewy bodies. The emerging role of CSF and imaging biomarkers. Dement Geriatr Cogn Disord 2008;25:195-205.
155. Spies PE, Melis RJ, Sjogren MJ, Rikkert MG, Verbeek MM. Cerebrospinal fluid alpha-synuclein does not discriminate between dementia disorders. Journal of Alzheimer's disease : JAD 2009;16:363-369.
156. Ballard CG, Jones EL. CSF alpha-synuclein as a diagnostic biomarker for Parkinson disease and related dementias. Neurology 2010;75:1760-1761.
157. Mollenhauer B, Schlossmacher MG. CSF synuclein: adding to the biomarker footprint of dementia with Lewy bodies. Journal of neurology, neurosurgery, and psychiatry 2010;81:590-591.
158. Waragai M, Sekiyama K, Sekigawa A, Takamatsu Y, Fujita M, Hashimoto M. alpha-Synuclein and DJ-1 as Potential Biological Fluid Biomarkers for Parkinson's Disease. Int J Mol Sci 2010;11:4257-4266.
159. Mattsson N, Blennow K, Zetterberg H. Inter-laboratory variation in cerebrospinal fluid biomarkers for Alzheimer's disease: united we stand, divided we fall. Clin Chem Lab Med 2010;48:603-607.
160. Mattsson N, Zetterberg H, Bianconi S, et al. Gamma-secretase-dependent amyloid-beta is increased in Niemann-Pick type C: a cross-sectional study. Neurology 2011;76:366-372.
161. Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat Rev Neurol 2010;6:131-144.