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 (Aβ_{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 Aβ_{1-40} or Aβ_{1-42}. Further, there is insufficient evidence supporting clinical applications for plasma Aβ_{1-40} or Aβ_{1-42} measures. Therefore, they are not recommended either for primary care or specialists, though CSF Aβ_{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 fibrillar 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.  

| 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: | phosphorylated 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 |
| xMAP: | Novel high-throughput technology allowing simultaneously measures the concentration of a large number of different analytes (Aβ1-42, 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 (Aβ$1$-$42$), total tau (t-tau) and phosphorlyated tau (p-tau) in the 181-threonine position (p-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.

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. 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>
<thead>
<tr>
<th>Table 1. Biomarkers for diagnosis of AD.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuronal Pathological Processes</td>
</tr>
<tr>
<td>----------------------------------</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
<tr>
<td>Amyloid deposition</td>
</tr>
</tbody>
</table>

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 fibrillar Aβ-peptide deposits characteristic of AD detected by increased retention of amyloid imaging PET agents or declines in Aβ-peptide CSF concentrations. Such methods constitute powerful means for in vivo detection of pathological brain amyloid concentrations.

Mega-aggregates of Aβ-peptide form the core of amyloid plaques in AD. Amyloid beta (Aβ1-40/42) 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 Aβ_{1-40/2} 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, Aβ_{1-42} molecules undergo a massive aggregation process, which generates the inert core of an amyloid plaque. Importantly, neurotoxic soluble Aβ_{1-42} 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 Aβ_{1-42} 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 Aβ_{1-42} concentrations. In fact, Aβ_{1-42} CSF concentrations and amyloid load detected by PET [^{11}C]PIB are correlated in an exponential fashion 16, 17. Low Aβ_{1-42} CSF concentrations possibly occur due to the retention of Aβ_{1-42} 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 Aβ1-42 retention in the extracellular compartment (brain tissue) due to peptide aggregation leads to declines of Aβ1-42 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—including plasma concentration of clusterin, c-reactive protein and acetylcholinesterase—have been tested in the context of dementia, core AD biomarkers constitute a less invasive alternative for lumbar punctures 18, 19, 18, 19. Plasma Aβ1-42 or Aβ1-42/ Aβ1-40 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 Aβ1-42 or Aβ1-42/ Aβ1-40 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 Aβ1-42 or Aβ1-42/ Aβ1-40 ratios characterize individuals with AD 27, 37 (Figure 1). However, there is no correlation between Aβ1-42 or Aβ1-40 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 Aβ1-42/ Aβ1-40 predict progression to dementia 25. Methodological limitations in terms of sampling and analysis require further validation.

3 CSF Biomarkers of Neurodegeneration

Today, PET [18F]-fluorodeoxyglucose ([18F]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.  

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. The functional expression of microtubules is modulated via phosphorylation of numerous serine and threonine residues (phosphorylation sites) present in the tau protein. Abnormal tau hyperphosphorylation deposits in AD are observed within neurons, NFTs, or dystrophic neurites present in neuritic plaques. In MCI, tau and t-tau concentrations increase nearly 30-40%, 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. 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.

Despite numerous threonine and serine phosphorylation sites present in tau protein, AD is better characterized by hyperphosphorylation at the position 181 (p-tau\textsubscript{181}) or 232 (p-tau\textsubscript{232}). Particularly, discrimination between AD and dementia with Lewy bodies or frontotemporal dementia is maximized by p-tau\textsubscript{181} or p-tau\textsubscript{231}. Indeed, Aβ\textsubscript{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%.
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. 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. 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](image)

**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-%. 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 on CSF concentrations of Aβ_{1-42} or tau protein. The Alzheimer’s biomarkers standardization initiative (ABSI) recommendations for CSF biomarker are summarized on Table 2.

It is not a recommendation to perform lumbar punctures during the morning, since circadian variation on Aβ_{1-42} CSF concentrations initially reported was not replicated. Aliquoting is not a major issue since the gradient of Aβ_{1-42} concentrations is negligible if the CSF sample remains between 10–40 mL. Importantly, it is crucial to comply exclusively with containers and vials recommended by the CSF analytical infrastructure since Aβ_{1-42} readily adsorbs to test tube polystyrene, glass and even to polypropylene walls. 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/μL in the CSF requires centrifuge the CSF before freezing. In order to assure 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.

**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

<table>
<thead>
<tr>
<th>No.</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CT or MRI performed before LP</td>
</tr>
<tr>
<td></td>
<td>LP should not be performed in cases where there is high intracranial pressure or where there is a mass lesion in the brain.</td>
</tr>
<tr>
<td>2</td>
<td>Concomitant medication</td>
</tr>
<tr>
<td></td>
<td>LP should not be performed in patients treated with anticoagulants (e.g., warfarin). Treatment with platelet aggregation inhibitors is not a contraindication.</td>
</tr>
<tr>
<td>3</td>
<td>Diurnal variation</td>
</tr>
<tr>
<td></td>
<td>No diurnal variation.</td>
</tr>
<tr>
<td>4</td>
<td>CSF gradient/volume</td>
</tr>
<tr>
<td></td>
<td>No gradient observed. No requirement for a certain fraction. Minimum volume of 1.5 mL.</td>
</tr>
<tr>
<td>5</td>
<td>Meal consumption</td>
</tr>
<tr>
<td></td>
<td>No need for fasting.</td>
</tr>
<tr>
<td>6</td>
<td>Position</td>
</tr>
<tr>
<td></td>
<td>LP may be performed with the patient either sitting or lying down. The position of the patient does not affect the results.</td>
</tr>
<tr>
<td>7</td>
<td>Location</td>
</tr>
<tr>
<td></td>
<td>Vertebral body L3–L5. The incision point of the needle (L3–L4 or L4–L5) does not affect the results.</td>
</tr>
<tr>
<td>8</td>
<td>Disinfection/anesthesia</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>9</td>
<td>Needle</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>10</td>
<td>Rest</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>11</td>
<td>Tubes and aliquotation (type, volume, homogeneity)</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>12</td>
<td>Documentation of sampling/aliquotation</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>13</td>
<td>Centrifugation (speed and temperature)</td>
</tr>
<tr>
<td></td>
<td>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).</td>
</tr>
<tr>
<td>14</td>
<td>Time and temperature before storage</td>
</tr>
<tr>
<td></td>
<td>Samples may be sent by regular post (transport 5 days).</td>
</tr>
<tr>
<td>15</td>
<td>Method of freezing (liquid nitrogen, dry ice, slow freezing @ -20°C or 80°C.</td>
</tr>
<tr>
<td></td>
<td>Freezing at -80°C for storage. No difference between methods of freezing.</td>
</tr>
<tr>
<td>16</td>
<td>Length of storage (when frozen)</td>
</tr>
<tr>
<td></td>
<td>Storage: -20°C for less than 2 months. Note: No evidence of any effect for up to 2 years at -80°C.</td>
</tr>
<tr>
<td>17</td>
<td>Number of freeze/thaw cycles</td>
</tr>
<tr>
<td></td>
<td>Limit the number of freeze/thaw cycles to 1–2.</td>
</tr>
<tr>
<td>18</td>
<td>Interfering substances (hemolysis)</td>
</tr>
<tr>
<td></td>
<td>Traumatic LP: Discard first 1–2 mL. Samples with an erythrocyte count of 500/mL should not be used without centrifugation.</td>
</tr>
</tbody>
</table>

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 (Aβ42), 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 Aβ\(_{42}\) levels was also found, with lowest level occurring in the morning \(^{62}\). Moreover, Aβ levels fall during sleep, and chronic sleep deprivation may increase Aβ\(_{42}\) production \(^{70}\). Therefore, it is important to control for the timing of CSF Aβ\(_{42}\) collection in order to minimize variability.

The utility of biomarkers in the diagnosis of AD and differentiation from other dementias have been reviewed extensively \(^{71-74}\). AD patients typically exhibit a low level of Aβ\(_{42}\) 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 Aβ\(_{42}\) 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/Aβ\(_{42}\), or t-tau/Aβ\(_{42}\) 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 \(^{82, 88}\). 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 \cite{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 Aβ$_{42}$ \cite{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 Aβ$_{42}$ 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 \cite{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 \cite{54}, as well as those MCI patients who progress to non-AD dementias \cite{91}. These findings have been extensively replicated by different research groups worldwide \cite{51,74,75,92,93,ENREF_64}, and further reinforced by meta-analyses of different datasets \cite{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 \cite{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 \cite{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. These findings can be applied to enrich clinical trials via recruitment of MCI subjects who are most likely to progress to clinical AD.

**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. Several longitudinal studies have shown that lower CSF Aβ42 and higher t-tau or p-tau levels, even in healthy subjects at baseline, are correlated with future decline in cognitive functions and faster progression of brain atrophy, suggesting that the CSF changes are consistent with the presence of significant AD-P at baseline. 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.

**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 Aβ42, t-tau, and p-tau all provide complementary information in patients with MCI and AD, baseline MR imaging and FDG-PET measures were more responsive to clinical changes than CSF measures in MCI subjects. 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 \textsuperscript{111}. Furthermore, when A\textsubscript{β42} 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 \textsuperscript{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\textsubscript{β42} or t-tau, possibly reflecting neuronal loss in AD \textsuperscript{114}.

**Other potential CSF biomarkers**

In addition to A\textsubscript{β42}, 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 \textsuperscript{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 \textsuperscript{116}. Similarly, lipoprotein receptor (LR11) has been implicated in the pathogenesis of AD, and with levels significantly increased in AD compared to controls \textsuperscript{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 \textsuperscript{118}. In addition, oligomeric A\textsubscript{β} 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\textsubscript{β40} and A\textsubscript{β42} 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

**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. 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. These novel findings require further replication and validation.

Vascular cognitive impairment (VCI) and vascular dementia (VaD) are conditions that often coexist with AD. While some studies have found utility of Aβ42, t-tau and p-tau in differentiating AD from VaD, 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. 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. This marker was found to be decreased in a
Fluid biomarkers 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 Aβ42 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\textsuperscript{159}. Potential sources of variation include: 1) pre-analytical conditions such as different lumbar puncture protocol, diurnal variation of Aβ\textsubscript{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\textsuperscript{160, 161}.

**Recommendations for CCCDTD4 regarding biomarkers in AD and related dementias:**

Preamble: Currently, the most established CSF biomarkers for AD pathology are Ab\textsubscript{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 3-5-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


