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Q&A

Biomarkers, Assays, and Therapies for Alzheimer Disease

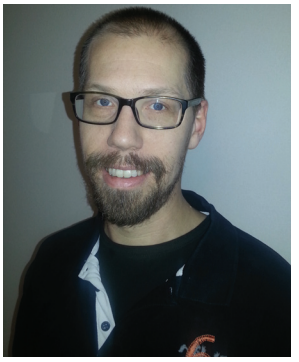
Moderators: Daniel R. Beriault[1*] and Eleftherios P. Diamandis[1]
Experts: Erik Portelius,[2] Armand Perret-Liaudet,[3] and Stephen Salloway[4]

Alzheimer disease (AD) is a devastating neurodegenerative disorder characterized by a progressive decline in cognitive function. In 2010, an estimated 36 million people worldwide had AD or a related dementia, with this number projected to double by 2030. The social and economic burden of AD is well documented and will be amplified by this increase in prevalence.

The initial pathophysiologic changes of AD are found in the hippocampus region of the brain, disrupting memory and the ability to learn. AD progression is linked to nerve cell dysfunction and cell death due to the accumulation of 2 protein aggregates: β-amyloid (Aβ)[5] and tau. In the cerebrospinal fluid (CSF), these proteins are biomarkers for AD. Cleavage of the amyloid precursor protein (APP) generates varying lengths of Aβ peptides (38–43 amino acids) that accumulate in the extracellular space. Of these monomers, Aβ-42 is the major form associated with AD. In addition, tau entanglement is also associated with AD and consists of insoluble hyperphosphorylated tau protein in the intracellular space. Both total tau (t-tau) and phosphorylated tau (p-tau) proteins are measured and associated with AD. Currently, clinical trials are testing therapies that target these proteins in hopes to delay or halt the cognitive decline in AD patients.

In this Q&A, we discuss the current state and future direction of biomarkers, assays, and therapies for AD with 3 experts.

Established Alzheimer biomarkers include β-amyloid and tau protein in CSF, as well as imaging techniques that involve MRI and positron emission tomography (PET). What are the limitations of these biomarkers that restrict them mostly to research purposes?



Erik Portelius: In the recently updated diagnostic criteria for AD (the International Working Group-2 criteria), the CSF AD biomarkers (Aβ-42, t-tau, and p-tau) and neuroimaging with Pittsburgh compound B (PiB)-PET were included. Although MRI may mirror the disease progression and help to characterize the clinical phenotype, it does not qualify as a pathophysiological biomarker for the underlying disease process.

There is a high concordance between decreased CSF Aβ-42 concentrations and positive PiB-PET. Limitations of PiB-PET include its relatively high cost, as compared with lumbar puncture, and limited availability of scanning facilities for this procedure in proximity to memory clinics. In addition, the nonspecific binding of the radiotracers used with PiB-PET to white matter makes standardization of quantitative measurements complicated. The need for standardization of quantitative measurements holds true also for the CSF biomarkers. The major limitation with CSF biomarkers is that they require CSF sampling by lumbar puncture. Although this is a safe procedure, some patients get post–lumbar puncture headache.

[1] Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada; [2] Associate Professor in Neurochemistry, Department of Neuroscience and Physiology, University of Gothenburg, Sweden; [3] Clinical Biochemist, Head of Neurochemistry Unit, Department of Biochemistry and Molecular Biology, Hospices Civils de Lyon, France [4] Director of Neurology and the Memory and Aging Program, Butler Hospital, and Professor of Neurology and Psychiatry, the Warren Alpert Medical School of Brown University, Providence, RI.
* Address correspondence to this author at: University of Toronto, 90 Meadow Wood Cres. Stoney Creek, ON L8J3Z8, Canada. Fax 416-619-5521; e-mail dan.beriault@mail.utoronto.ca.

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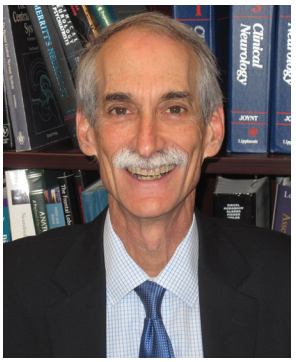
[5] Nonstandard abbreviations: Aβ, β-amyloid; CSF, cerebrospinal fluid; APP, amyloid precursor protein; t-tau, total tau; p-tau, phosphorylated tau; PET, positron emission tomography; PiB, Pittsburgh compound B; ADNI, AD Neuroimaging Initiative; IVD, in vitro diagnostic; RUO, research use only; CRM, certified reference material; RMP, reference measurement procedure; BACE, β-secretase.



Stephen Salloway: Lot-to-lot and interplatform variability of CSF assays and uncertainty in determining clinically relevant cutpoints limit the use of CSF measures of Aβ-42 and tau in both clinical trials and clinical diagnosis. For example, problems using CSF markers to determine eligibility led to adjustments in biomarker cutpoints during 2 recent treatment trials for prodromal AD. In the avagacestat trial, the initial Aβ-42 cutpoint of <200 pg/mL was broadened to include a ratio of total tau/Aβ-42 of 0.39 on the basis of the AD Neuroimaging Initiative (ADNI) data. Fifty-three percent of subjects met the Aβ-42 cutpoint criterion, 90% of subjects met the tau/Aβ-42 ratio, and 43% of subjects met both. In the Scarlet Road trial of gantenerumab, the Aβ-42 cutpoint used to determine eligibility was recalibrated to mimic that used in ADNI.

Multisite acquisition and longitudinal measurements of change on amyloid PET pose numerous technical and analytical challenges for this imaging biomarker. These include interscanner variability (between sites and manufacturers), scanner upgrades, intersubject variability over multiple time points, comparing data from multiple tracers, and selection of the best reference region. Detection of change in cortical amyloid accumulation is limited by the relatively small rate of change during the study period in relation to the variance in the reference region. Use of subcortical white matter regions as the reference may have lower variability and may help improve detection of change in cortical uptake. The Centeloid Project is developing a method to standardize measurement across amyloid tracers.

Armand Perret-Liaudet: Limitations of these procedures include: a lack of consensus for cutpoint decisions coupled with a lack of standardization in the clinical indications; no consensus on preanalytical and analytical steps; and lack of standardization in the interpretation of results.

The avagacestat trial cited by Professor Salloway is a very nice example of the heterogeneity of worldwide biomarker cutpoints linked to different analytical assay principles. In the ADNI studies, the measures were done using a multiplex approach with the INNO-BIA AlzBio3 test adapted on the Luminex platform. In contrast, in Europe, the INNOTEST® is primarily used. Although both tests are produced by the same manufacturer (Fujirebio), 2–3-fold differences in concentration were reported for Aβ-42 and the resulting cutpoints (<200 vs <500 pg/mL for INNO-BIA AlzBio3 vs INNOTEST, respectively). In this specific case, the problem will not be solved because INNOTEST assays have been customized and validated for in vitro diagnostic (IVD) use, meeting the quality criteria for diagnostic purposes (therefore for research trials), whereas INNO-BIA AlzBio3 was designed and validated for research use only (RUO). The probability that Fujirebio will shift the multiplex platform from RUO to IVD is very low.

Currently, ELISA kits are used to quantify Aβ and tau protein CSF concentrations. What are the limitations of these assays and are there gold standard methods with which they may be compared? If possible, can you highlight any new methods being developed that may overcome these limitations?

Erik Portelius: There is variability in the measurements of Aβ-42, t-tau, and p-tau between clinical centers and laboratories. The discrepancy has been attributed to both preanalytical and analytical factors which in the end influence the concentration of the measured analytes. To implement the broad-scale use of these methods on different technology platforms, standardization of all steps included in the analysis is needed.

Currently, there are no certified reference materials (CRM) and reference measurement procedures (RMP) available for Aβ-42, t-tau, and p-tau. If we had CRM and RMP at hand, they could be used to harmonize the different assays used so that values could be compared worldwide. However, this work is ongoing and recently candidate RMPs, based on solid-phase extraction and isotope-dilution LC-MS/MS for the quantification of Aβ1–42, were published in a joint effort together with the IFCC Scientific Division Working Group on CSF proteins.

Similar RMP projects also have been initiated for t-tau and p-tau but they are lagging behind the Aβ1–42 work by some years. Through this ongoing standardization process, AD biomarkers will become integrated in clinical routine. However, to integrate the AD biomarkers, fully automated assays have to be developed.

Armand Perret-Liaudet: The main limitation of these assays is the lack of standardization among methods. The nature and environment of the standards may be different (i.e., proteins, peptides in variable matrix), and there can be differences in epitope recognition by different antibodies (patent issues). Moreover, the assays have poor

reproducibility, and inadequate instructions for use, not in accordance with the ISO 15189 scheme.

In addition, there is no gold standard outside the postmortem analysis, considering that the comparative CSF analysis must be done very shortly before death, which is a big problem in many reports. Development and validation of a reference standard by mass analysis and a strict validation of the assays that fulfill the European Medicines Agency and the US Food and Drug Administration criteria are possible solutions.

Stephen Salloway: An international consortium is working to harmonize CSF methods for use in clinical research.

There has been significant interest in developing new biomarkers for AD, with a focus on plasma biomarkers. How close are we to developing an accurate plasma biomarker panel? Do you expect these plasma biomarkers to be more useful than current imaging techniques?

Armand Perret-Liaudet: The problem with plasma biomarkers from central nervous system diseases is 2-fold: low specificity with potential peripheral production/catabolism of the biomarkers, and low sensitivity with lower concentrations in circulation than in CSF. Furthermore, matrix problems cannot be ignored. We are far from having a validated panel of plasma biomarkers for etiological diagnosis of cognitive disorders in elderly patients for whom comorbidities (autoimmune, vascular, etc.) are not infrequent. It is because of these issues that previously promising studies were not able to be fully reproduced. A good example of this was seen in December 2013 when a company stopped its development strategy for a transcriptomic blood signature of AD due to an insufficient level of diagnostic accuracy.

I do not expect that plasma biomarkers will be more or less useful than current imaging techniques because the information given or expected is absolutely different. For MRI, we have information indicating the presence of atrophy, diffuse or focal changes; moreover, imaging supplies other types of information regarding vascular comorbidities (leukopathy, ischemic lesions, angiopathy, etc.) or ventricular dilation that can explain partially the clinical presentation. We need more experience with determination of amyloid load by imaging techniques to establish the utility of this measure (it seems good enough to exclude an Alzheimer pathology).

In my opinion, given our expectation to increase the accuracy of diagnosis at an early stage, we are going in the wrong direction with plasma biomarkers. The neurologists will never use them as screening tests because they already have clinical and neuropsychological tests that enable them to accurately identify about 70% of AD patients (typical memory impairment). To identify the others, we have the CSF biomarkers to increase the accuracy of clinical diagnosis. Therefore, what place do plasma biomarkers have in diagnosis of AD? I am convinced that the future of plasma biomarkers is in the development of disease stage biomarkers that are sensitive to efficacy of therapy.

Erik Portelius: There are numerous studies showing that multivariate protein signatures and, more recently, that a panel of lipids can be used as blood-based biomarkers to both differentiate AD from controls and to predict AD. However, these studies need replication and the findings must be validated in larger independent cohorts. The results are intriguing but we are a long way from having a new blood-based biomarker with specificity and sensitivity values similar to the core AD biomarkers, including PET.

In addition, a possible confounder for plasma biomarkers that has to be carefully investigated is that their likely degradation by plasma proteases or in the liver will influence the results.

The identification of a new AD biomarker in blood or plasma would provide a very valuable clinical tool as CSF sampling and imaging with tracers are more costly and invasive. In addition, simple and repeated sampling might be possible, thus allowing dynamic changes to be measured over time at regular clinic visits.

Stephen Salloway: The development of plasma biomarkers would be a major step forward in the detection of AD risk. At this point we have not yet developed an assay that is ready for research or clinical use. There is some evidence that plasma Aβ-42 increases early in autosomal dominant AD owing to overproduction, but this finding has not been replicated in sporadic AD where decreased clearance of Aβ-42 is more salient. Mapstone et al. (*Nat Med* 2014;20:415–8) reported detection of AD risk in a panel of plasma phospholipids. This work requires replication and testing in a variety of risk populations. The development of plasma biomarkers is being pursued worldwide by groups focusing on proteomic, metabolomic, genetic, and epigenetic markers. Plasma-based biomarkers to determine risk and outcome will be critical to the eventual development of primary prevention strategies.

If possible, can you highlight any intriguing non-plasma biomarkers that are currently being developed for AD?

Stephen Salloway: Tau PET tracers are undergoing rapid development and offer considerable promise as a marker of AD pathogenesis and potentially as an outcome measure in AD clinical trials. Tau PET appears to have a more dynamic rate of change than amyloid PET in

both the mild cognitive impairment and mild dementia, and possibly the preclinical stages of AD. Tau PET could be validated as a surrogate biomarker if interventions that lower or reverse tau accumulation correlate with clinical benefit.

Erik Portelius: Previous studies have shown that neuronal and synaptic loss occurs early in the AD brain and the synaptic pathology has also been shown to correlate better with the cognitive deficits in AD patients than, e.g., CSF Aβ-42. Thus, biomarkers reflecting the loss of synapses may add another aspect for the diagnosis of AD as well as increase our understanding of disease progression and pathology.

There are some intriguing CSF biomarkers, including neurogranin, which seem to reflect the synaptic pathology in AD. We recently showed that the postsynaptic protein neurogranin is increased in CSF from AD patients as compared to healthy controls and that the concentrations are increased even in the mild cognitive impairment stage. The CSF neurogranin concentrations also correlated with a more rapid change in cognition during clinical follow-up.

Armand Perret-Liaudet: Validation of the nonradioactive amyloid PET and development of tau imaging are promising approaches.

There have been several recently published clinical trials testing various therapies for AD. What have we learned from these trials and what seems to be the most challenging aspect with regard to treating this disease?

Erik Portelius: Two major clinical trials with γ-secretase inhibitors have recently been published (semagacestat and avagacestat). Both trials were negative; higher doses worsened the cognition in people with mild-to-moderate AD, with side effects including skin cancer. There seems to be an enormous complexity around the biological function of γ-secretase and one should not forget that it is extremely hard to generate drugs with the exact characteristics that you look for. For example, we know that γ-secretase has more than 100 substrates, including notch.

We have also recently seen 2 immunization trials (bapineuzumab and solanezumab) which both missed their primary goal. It should be noted that it was found that 36% of non–APOE (apolipoprotein E) ε4 carriers enrolled in the bapineuzumab trial did not fulfill the criteria for AD. Thus, they did not have the pathology against which the drugs were directed. However, the solanezumab trial showed some intriguing data in the mild AD group with improved cognition.

Based on the reported clinical trials, there are divergent opinions on whether Aβ-42 therapies should be abandoned. Clearly more work needs to be done to learn more about these drugs and how best to design these clinical trials before we abandon such therapies.

Armand Perret-Liaudet: For amyloid-based trials, the experimental models based upon transgenic mice overexpressing amyloid are not representative of AD in its sporadic phenotype. Focusing on amyloid is not enough and therapies directed at amyloid potentially could be detrimental by solubilizing aggregates into soluble oligomers, which were shown to be the most neurotoxic entities. However, it will be interesting to see if amyloid-based therapies are useful in genetic cases (amyloid precursor protein and presenilin 1 or 2) of AD. It is my opinion that, for many of the completed trials, therapy was introduced at stages too late to show a beneficial effect.

Stephen Salloway: I will highlight a few key points from lessons learned from recent phase 3 AD clinical trials:

Use of amyloid PET in recent phase 3 trials for mild–moderate AD dementia has demonstrated that approximately 20% of subjects did not meet the amyloid cutpoint value and the amyloid-negative subjects had a slower rate of decline. Future AD clinical trials should include markers of amyloid to determine eligibility.

Amyloid deposition may have its most prominent effect in AD pathogenesis early in the disease course and modifying amyloid may have only limited benefit in mild–moderate dementia. Future amyloid-based trials are likely to have the greatest impact in earlier stages of AD.

The primary goal of AD treatment research is to treat the right target, using the right drug(s), and at the right stage to produce a major positive impact on the clinical course of AD. Though we have demonstrated a mild degree of amyloid lowering in a few recent trials, it is not clear that we have achieved sufficient target engagement to produce a clinical benefit. It may also be true that amyloid-based monotherapy approaches have only limited impact and combination treatments that target multiple mechanisms may be required. Two or more compounds may need to be tested together to demonstrate efficacy when neither component has demonstrated definitive efficacy alone. The combination could include antiamyloid drugs with different targets or an antiamyloid and an anti-tau drug.

In your opinion, what are some of the promising new therapies currently in the pipeline?

Stephen Salloway: The field looks forward to expanding the current approaches beyond amyloid-based treatments and to develop tau-based treatments, especially anti-tau monoclonal antibodies. These could potentially be combined with one or more antiamyloid approaches.

Erik Portelius: Two promising therapies are γ-secretase modulators and inhibitors of β-secretase (BACE). Several pharma companies have shown that BACE inhibition lowers CSF Aβ by up to 80%–90%. This reduction has been shown in animal models as well as in clinical studies on healthy volunteers and AD patients.

A γ-secretase modulator that could shift the cleavage preference of the γ-secretase complex from Ala42 to Gly37, Gly38, and Val39, thus generating shorter, less toxic, hydrophobic and aggregation-prone peptides, should be a good candidate to reduce the risk of building up plaques. However, so far no clinical study on humans has been published. Thus, the efficacy in humans remains to be shown.

Armand Perret-Liaudet: Note the recent promising results in mice and a small cohort of AD patients using the synergistic effect of 2 GABA (γ-aminobutyric acid aminotransferase)-ergic drugs currently used in alcoholic patients [Orgogozo JM. 7th Clinical Trials on Alzheimer's Disease conference in Philadelphia, November 2014, for the patient study; and the mouse results in a publication in *Scientific Reports*, January 2015 (5:7608)]. The balance between excitation/inhibition activities is one of the main targets of therapy for cognition preservation and recovery (potentially in synergy with amyloid and/or tau therapies).

The literature highlights the consequence of Aβ and tau protein aggregation in AD pathophysiology. In terms of treatment, do you believe we need to target both of these proteins to show appreciable effects in cognition?

Erik Portelius: My short answer is yes. In my opinion, it is not 100% clear what neurotoxic species are causing the neurodegeneration in the brain. Experimental studies have shown that oligomers may be the toxic species which effects long-term potentiation but this is extremely difficult to prove in humans. However, we do know that both tau and Aβ accumulate in the AD brain for decades and most likely they are, at some level, connected.

Armand Perret-Liaudet: Yes. As cognition impairment is more related to paired helical filaments, at least a trial focusing on tau is needed.

Stephen Salloway: Yes, the eventual treatment of AD will include 2, 3, 4, or more drugs in combination. These will include antiamyloid, anti-tau, neuroprotective, and antiinflammatory approaches. The medication interventions will eventually be combined with risk reduction and lifestyle modification strategies for maximal benefit.

Do you have any additional insights you would like to share (such as analytical concerns, biomarkers, or intriguing ideas pertaining to AD)?

Stephen Salloway: I propose the following to move the AD field forward:

1. Fund AD research on par with the magnitude of the problem and commensurate with other major diseases such as heart disease and cancer.
2. Promote open access, real-time data sharing, similar to the ADNI model.
3. Stimulate broad engagement of the scientific community and develop new models of public–private partnership.
4. Develop paradigms and incentives that increase collaboration in drug development.
5. Validate surrogate biomarkers, establish clear evidence of target engagement, and develop biomarkers of disease progression.
6. Train a new cadre of young investigators from a wide range of disciplines.

Armand Perret-Liaudet: For the development of biological biomarkers, we need:

1. Etiological biomarkers for AD on par with other neurodegenerative diseases. For AD, we have the target biomarkers in CSF, tau and amyloid; now we need standardization.
2. Biomarkers of disease progression: cleaved amyloids, neurofilaments, orexin, prion protein, cleaved tau, and potential synapse markers. The field is competitive and very promising.
3. Lastly, we need biomarkers that are sensitive to efficacy of therapy. Logistically, blood biomarkers would be better than CSF.

The accumulation of proteins into big aggregates is not the main problem. The real problem seems to be more linked to the oligomerization of monomers that gives rise to neurotoxic oligomers, which occur well before the presence of plaques. There were crucial reports in *Nature* highlighting the role of each type of oligomer. For example, the amyloid pentamer was shown as the more neurotoxic entity, thus representing a new approach for therapy.

Erik Portelius: I strongly believe that we have much more to learn within the biomarker discovery and development area. The instruments are getting more sensitive and we are at the same time getting more and more insight into the pathological processes occurring in the brain.

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significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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