EDITORIAL

Alzheimer's disease biomarkers: walk with deliberate haste, don't run blithely on?

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The term biomarker originated from petroleum engineering, when Wolfgang Seifert at Chevron used it to describe the origin of hydrocarbons in petroleum. Since then, the term biomarker has evolved and even become popular in medicine and other disciplines. A quick survey of original publications indexed in PubMed containing the term "biomarker" in the title or abstract has increased 45-fold from 1994 to 2012, and the number of publications which contain "Alzheimer's disease (AD)" and "biomarker" also increased from under 10 in 2000 to over 300 in 2013. While this is most probably an underestimate of all biomarker-related work (after all, the search term "biomarker" poorly predicts biomarker studies), the increasing use of this term in the era of title character count and abstract word count can be viewed as scientists' interest in this topic. Compared to our colleagues focused on more accessible organ systems, clinical neuroscientists rarely have the opportunity to analyze sufficient sampling from the brain in the ante-mortem phase to elucidate the sequence of events leading to cognitive decline. We are thus often at a fork in the road where we must decide to wait until autopsy for tissue studies, or to use imperfect methods to infer time-dependent changes in living patients. These imperfect methods constitute many of the current biomarkers in use or under investigation, and this special cluster of *Acta Neuropathologica* has assembled a series of original papers and reviews which highlight their potential values and as well as challenges and controversies in basic and clinical research in AD to highlight the relatively rapid pace of their translation.

Within Alzheimer's disease (AD) biomarker research, much discussion has focused on the prediction of neuritic plaques and neurofibrillary tangles in keeping with the neuropathologic diagnosis for AD. We often compare ante-mortem biomarker changes to post-mortem neuropathologic changes. Using this approach, AD biomarkers will always be imperfect as only the cumulative biomarker changes across the lifetime of a subject will amount to the neuropathologic changes seen at death. Here lies an intrinsic paradox in the value of any AD biomarker: biomarker changes may correspond perfectly with neuropathologic changes early in the disease course, but our analysis is limited to comparison of ante-mortem biomarker with post-mortem neuropathologic changes. The recognition of this paradox is crucial especially when there are few longitudinal biomarker studies to associate the trajectory of biomarker changes (instead of absolute levels) towards end-of-life neuropathologic analysis. Many of the controversies in determining the sequence and topology of AD pathology also center around this paradox. That said, the goal of much biomarker research is to advance this field to the point where a panel of biomarkers of AD and related neurodegenerative diseases can replace post-mortem neuropathology studies as the ante-mortem diagnostic "gold standard". To that end, in this special cluster, Drs. Braak, Zetterberg, Del Tredici, and Blennow directly addressed the contradictory hypotheses on the emergence of AD pathophysiology [2]. Whereas cross-sectional pathologic series have shown neurofibrillary tangles in the absence of or well before the emergence of neuritic plaques in cognitively normal young and middle-aged people who have died from non-AD related causes, cerebrospinal fluid (CSF) characterization of "AD pathology" (using levels of beta-amyloid 1–42

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[Aβ42] and total Tau [t-Tau]) identifies many more cognitively normal subjects with "pathologic" Aβ42 but normal t-Tau levels than the opposite combination (pathologic t-Tau, normal Aβ42) that would support the neuropathologic observations. These observations would seem to challenge the very foundation of pre-clinical AD staging using biomarkers [9], and the authors set out to resolve the contradiction by re-thinking the stage-wise progression of AD in its pre-symptomatic and symptomatic phase.

In a second review to resolve another controversy, Drs. Jack, Barrio, and Kepe keyed in on the research and clinical significance of cerebral amyloid imaging [4]. The accurate visualization of neuritic plaques non-invasively during life would significantly enhance the understanding on the natural history of AD. This potential is highlighted by the landmark publication by Clark and colleagues, which compared cerebral amyloid burden (measured by florbetapir) and neuritic plaque count/area (measured by immunohistochemistry) obtained within 1 year of each other [3]. The scientific controversy has been further fueled by regulatory topics in the US and the financial implications of the clinical use of this technology and other AD biomarkers. Per the Cluster Editors' request, Dr. Jack focused on cerebral amyloid imaging's association with other AD biomarkers (cognitive decline, CSF studies, neuropathologic diagnosis), while Drs. Barrio and Kepe focused on topographical distribution of cerebral amyloid burden from ante-mortem imaging and post-mortem neuropathologic characterization. Using the study by Clark et al. [3] as a springboard, a two step clustering analysis including age, amyloid PET SUVR, Aβ immunoreactive area, CERAD peak plaque count, and average cortical plaque count in those who underwent amyloid PET and autopsy in the same year identified two clusters which largely replicated the pathologic AD diagnosis (accurate in 45 out of 46 cases). This suggests that presence of two distinct groups with each forming a centroid rather than a normally distributed population (Fig. 1). Whereas analyzing both clusters together showed strong correlation between florbetapir uptake with Aβ immunoreactive area ($R = 0.684$, $p < 0.001$), CERAD peak plaque count ($R = 0.472$, $p < 0.001$) and average cortical plaque count ($R = 0.517$, $p < 0.001$), limiting the analysis to the AD cluster significantly diminished all these associations ($R = 0.199$, $p = 0.330$ for Aβ immunoreactive area; $R = 0.158$, $p = 0.439$ for CERAD peak plaque count; $R = 0.033$, $p = 0.874$ for average cortical plaque count). Based on this re-interpretation of published data, Drs. Jack, Barrio, and Kepe were all correct, while the application of cerebral amyloid imaging in diagnosis may be much greater than its use in staging or therapeutic monitoring (until at least the quantitative nature of cerebral amyloid imaging is determined).
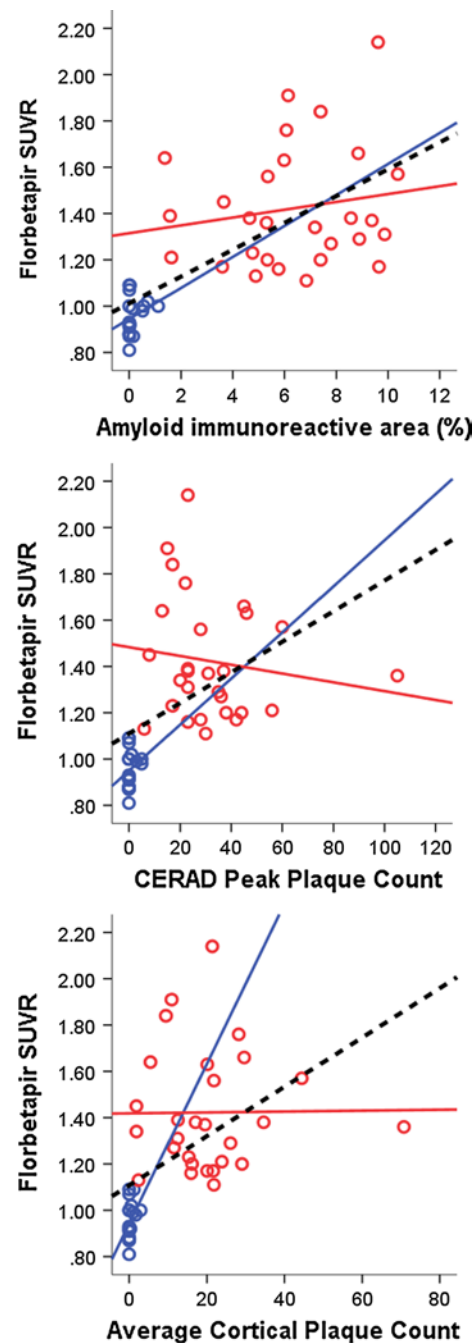

Fig. 1 Correlation between ante-mortem cerebral amyloid burden by imaging and post-mortem amyloid burden by neuropathologic analysis. Ante-mortem Aβ amyloid burden was determined by florbetapir retention expressed in standard uptake value ratio (SUVR). Post-mortem Aβ amyloid burden was determined by quantitative measures of plaque count or amyloid immunoreactivity (the data shown are from Clark et al. [3]). *Dotted black lines* represent line fits when subjects with (*red circles*) and without (*blue circles*) AD were analyzed together. *Solid blue lines* represent line fits when subjects without AD were analyzed alone, and *solid red lines* represent line fits when subjects with AD were analyzed alone

The three original articles in this cluster address critical gaps in many biomarker studies: longitudinal changes in biomarker levels and the multi-factorial nature of biomarker alterations. Longitudinal changes in biomarkers for neurodegenerative diseases deserve special attention because they are hypothesized to undergo significant deviations from normal long before clinical symptoms appear, and their rates of change—if detectable—in the symptomatic phase or the immediately pre-symptomatic phase may help extrapolate the original rate of disease onset. Using data from the multi-center North American Alzheimer's Disease Neuro-Imaging Initiative (ADNI), Toledo, Xie, and colleagues show that subgroups of subjects (with normal or abnormal cognition) undergo significant changes in Aβ42 or p-Tau$_{181}$ levels [11]. The magnitude of Aβ42 change was most significant for those with baseline CSF Aβ42 levels near the cut-off for AD diagnosis and those without the APOE ε4 allele, and future studies will need to address whether these changes can be replicated within each clinical subgroup (e.g., MCI with APOE ε4, MCI without APOE ε4). Zhang and colleagues examined the same CSF AD biomarkers using historical CSF samples from DATATOP (deprenyl and tocopherol antioxidative therapy for Parkinsonism) study, [12] and observed hints of association between baseline AD biomarker levels and subsequent development of parkinsonian symptoms. More importantly, they found the longitudinal CSF t-Tau levels to correlate with longitudinal clinical decline in parkinsonism, even though most patients had normal t-Tau levels throughout the study period. Finally, combining data from ADNI and Parkinson's progression marker initiative (PPMI), investigators from the first two original papers in this cluster teamed up to determine how biomarkers of two diseases may influence each other [10]. Together, these papers encourage current and future studies to re-examine how we view and analyze biomarkers in neurodegenerative diseases including AD, Parkinson's disease (PD), and frontotemporal lobar degeneration (FTLD) among others. First, in examining subjects with the disease of interest against a healthy cohort, studies must determine whether longitudinal changes in biomarkers specific to the disease (e.g., CSF Aβ42 in AD) reflect disease progression, aging in association with other factors (e.g., absence of APOE ε4 allele), or a combination of the two. As we see in AD, biomarker changes in one subject may be associated with the same clinical decline as that associated with little or no changes in the same biomarkers in another subject suffering from the very same disease. Second, studies must empirically account for other factors which may influence biomarkers under investigation, including co-existing neurological or other neurodegenerative diseases (e.g., cerebrovascular disease or the development of Lewy bodies and/or full blown PD in AD patients) and changes in other proteins which may interfere with biomarker measurement. For example, in addition to the effects of AD and PD progression on CSF t-Tau levels, t-Tau levels are significantly increased in prionopathies (without associated changes in p-Tau$_{181}$ levels) but remain largely in the normal range in diverse tauopathies other than AD. So is CSF t-Tau level a marker of neuronal injury, [9] the cumulative sum of longitudinal t-Tau change (over years or even decades), a downstream effect of altered cellular tau metabolism, or a surrogate marker of another insidious neurodegenerative process? Finally, can the change or absence of change in biomarker levels be reliably used to monitor outcomes in clinical settings? Answers to these questions are likely to be complex and likely cannot be easily answered in monopathic studies such as ADNI or PPMI. However, examining these questions in a multi-pathic approach will be essential in providing a true model for complex human diseases, and may require future studies to include subjects with multiple forms of neurological disorders. On the other hand, AD is heterogeneous and not just a plaque and tangle disease since common comorbid pathologies include cerebrovascular disease as well as the inclusions that define PD and FTLD due to TDP-43 pathology.

On the heels of updated definitions for clinical [1, 6, 9] and neuropathologic [7] characterization of AD, this may be an opportune moment to re-visit the goals of AD biomarkers [8]. Previously defined criteria for AD biomarkers have focused on diagnostic performance, validation, and clinical feasibility. These remain critical factors in designing and interpreting biomarker studies, but key properties of AD biomarkers must now reflect the advances in the fundamental AD pathophysiology and the characterization of non-AD pathologic changes that can exist with or without AD pathology (Table 1). The ideal AD biomarkers should still reflect the pathologic landmarks of AD (plaques, tangles, neuronal loss), but these biomarkers, which correlate with *disease diagnosis*, may or may not quantitatively predict the *disease burden*. Because of concurrent AD and non-AD pathology, the ideal AD biomarkers should also characterize *AD-associated* changes (e.g., synaptic changes, inflammation) and *AD-independent* changes (e.g., α-synuclein or TDP-43 inclusions). AD-associated changes may be neuro-protective or toxic, and how AD pathology drives disease progression and responds to therapeutic interventions should be determined in appropriate models. The specificity of biomarkers can be enhanced by detecting the core non-AD drives of neurodegeneration as well as other changes (e.g., different cell signaling pathways) specific to the non-AD disorders. Validation remains essential at the technical level as well as the cohort level, and causes for validation failure (referral bias, biological variability according to geographic locales, protocol variation) need to be determined. This underscores the importance of the

Table 1 Previous and proposed criteria for AD biomarkers

	Reagan/NIA Working Group (1998)	Proposed (2013)
Detecting AD	Sensitivity >80 % in detecting key AD changes compared to control	– Sensitivity >80 % in detecting clinical-neuropathologic AD (disease diagnosis)
		– May correlate with quantitative post-mortem changes in AD brains (disease burden)
		– Provides information on neurodegenerative and neuro-protective changes
Detecting non-AD	Specificity >80 % in differentiating AD from other disorders	– Detects non-AD degenerative etiologies (a-synuclein, TDP, Tau)
		– Identifies non-etiologic changes common or specific to different neurodegenerative disorders
Replication	Findings confirmed in two independent laboratories	Findings confirmed in two independent cohorts, and causes for non-replication analyzed
Feasibility	Easy to perform	Ease of performance and standardization analyzed as part of biomarker development
	Cheap and non-invasive	Cost and invasiveness evaluated on the basis of application (e.g., community screening, diagnosis, clinical trials)

systematic collection of the appropriate imaging and CSF biomarker data in longitudinal studies using highly standardized methodologies instead of selection of one biomarker over another for convenience or cost purposes. These efforts are underway in a number of studies and will require continued long-term commitment of the resources needed for these multi-modal studies. The feasibility of any biomarker will also need to be determined according to the application. From a cost and ease of performance perspective, cerebral amyloid imaging or CSF multi-analyte profiling are much more feasible in the targeted clinical trial arena than in the community screening setting. Finally, the conceptualization of what an AD biomarker is should transition from a single biomarker model to a multi-modal biomarker panel. This brings to mind the story of the six blind men each describing a different part of the elephant as its whole. Even though every isolated description could sufficiently distinguish the elephant from a mouse, none gave an accurate picture of the majestic beast unless all descriptors were combined. As the reviews and original articles point out in this cluster, there exist complex interactions between biomarkers within and between modalities (CSF, MRI, PET imaging). The biomarker panel's composition should also demonstrate flexibility according to application. For example, a clinical trial targeting Tau hyperphosphorylation may need *simultaneous* CSF biomarkers reflecting AD and non-AD etiologies, Aβ and Tau imaging (which has recently been reported to be feasible and capable of distinguishing AD from corticobasal degeneration) [5], as well as structural and functional MRI, but a diagnostic pathway may instead involve the sequential use of a blood screening test (sensitive and cheap), structural MRI, and Aβ and Tau biomarkers from CSF or PET imaging. Because the one-size-fits-all approach is no longer

sufficient to characterize the clinical and neuropathological spectrum of AD, AD biomarkers must evolve into quantitative and qualitative measures of AD and related diseases individualized to the patient and the application. The good news is that steady and deliberate progress in biomarker research is now bringing into sharper focus how these goals can be achieved in the very near future.

References

1. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC et al (2011) The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging and Alzheimer's Association Workgroup. Alzheimers Dement 7(3):270–279
2. Braak H, Zetterberg H, del Tredici K, Blennow K (2013) Intraneuronal tau aggregation precedes diffuse plaque deposition, but amyloid-β changes occur before increases of tau in cerebrospinal fluid. Acta Neuropathol (this issue). doi:10.1007/s00401-013-1139-0
3. Clark CM, Schneider JA, Bedell BJ, Beach TG, Bilker WB, Mintun MA et al (2011) Use of florbetapir-PET for imaging B-amyloid pathology. JAMA: J Am Med Assoc 305(3):275–283
4. Jack CR, Barrio JR, Kepe V (2013) Cerebral amyloid PET imaging in Alzheimer's disease. Acta Neuropathol (this issue). doi:10.1007/s00401-013-1185-7
5. Maruyama M, Shimada H, Suhara T, Shinotoh H, Ji B, Maeda J et al (2013) Imaging of tau pathology in a tauopathy mouse model and in Alzheimer patients compared to normal controls. Neuron 79(6):1094–1108
6. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH et al (2011) The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging and the Alzheimer's Association Workgroup. Alzheimers Dement 7(3):263–269
7. Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW et al (2012) National Institute on Aging-Alzheimer's

Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol 123(1):1–11

8. Reagan Research Institute and NIA Working Group (1998) Consensus report of the Working Group on: "Molecular and Biochemical Markers of Alzheimer's Disease". The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group. Neurobiol Aging 19(2):109–116

9. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM et al (2011) Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7(3):280–292

10. Toledo JB, Korff A, Shaw LM, Trojanowski JQ, Zhang J (2013) CSF alpha-synuclein improves diagnostic and prognostic performance of CSF tau and amyloid beta in Alzheimer's disease. Acta Neuropathol (this issue). doi:10.1007/s00401-013-1148-z

11. Toledo JB, Xie SX, Trojanowski JQ, Shaw LM (2013) Longitudinal changes in CSF Tau and beta-amyloid biomarkers for up to 48 months in ADNI. Acta Neuropathol (this issue). doi:10.1007/s00401-013-1151-4

12. Zhang J, Mattison HA, Liu C, Ginghina C, Auinger P, McDermott MP et al (2013) Longitudinal assessment of tau and amyloid beta in cerebrospinal fluid of Parkinson disease. Acta Neuropathol (this issue). doi:10.1007/s00401-013-1121-x