Published in final edited form as:
J Alzheimers Dis. 2013 ; 33(Suppl 1): S371–S376. doi:10.3233/JAD-2012-129022.

Biomarkers for Alzheimer's disease: Showing the way or leading us astray?

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Ask any Alzheimer's disease (AD) patient, any caregiver, or anyone at risk of Alzheimer's for advice on how to approach research, and you are likely to get a single answer: "Work faster!" While science moves at breakneck speed in other arenas, we seem to crawl along in the AD field, constrained by the bottleneck of clinical trials. And with good reason: Since it takes 400 subjects followed for 18 months to detect a disease-modifying effect in established dementia, 700 subjects and 3 years to detect an effect in mild cognitive impairment, and 5000 subjects and (at least) 5 years to detect preventive effects in healthy elderly, it is little wonder that the field moves so slowly. The manuscripts identified for discussion here have the common theme of trying to advance the pace of research by identifying surrogate markers of disease with the goal of developing clinical trial models which are smaller, faster, and more efficient than our current approach, which relies on clinical measures as the primary outcomes. These manuscripts also illustrate the importance of multi-center collaborations, as all of them represent cooperative efforts, with our modest contribution from Oregon reflected in our "middle author" status on most of them.

Each paper attempts to advance our understanding of cerebrospinal fluid (CSF) and plasma biomarkers of AD, and the following discussion organizes the papers into three themes: 1) New purposes for "old" biomarkers, 2) New biomarkers for old purposes, and 3) The Biomarker Holy Grail: Biomarkers as primary outcome measures for drug development.

New purposes for "old" biomarkers

Li's 2007 paper on "CSF tau/Abeta42 ratio for increased risk of mild cognitive impairment" (1) took the approach of examining whether CSF biomarkers which are known to be useful for diagnosis of AD might also be used to predict subsequent cognitive decline in healthy elderly subjects. Although the findings of increased CSF tau, decreased CSF Abeta42, and increased tau/Abeta42 ratio in AD compared to control subjects has been widely reproduced, there was little evidence prior to this report for the prognostic value of this CSF "signature". At the same time, virtually every prior study with age matched controls found a proportion of control subjects with an "AD profile" in CSF. Whether these subjects represent a "preclinical" form of AD or simply illustrate a false positive rate in CSF was hotly debated. The challenge of finding subject populations free of "silent" AD pathology prevented resolution of the question. Variability in the ELISAs used also confounded the issue. Li and colleagues addressed these issues by measuring CSF tau and CSF Abeta42 using a consistent ELISA method in a single laboratory in 129 cognitively healthy individuals ranging in age from 21 to 100 years of age, along with reference groups of subjects with MCI and AD. As in most other studies, a proportion of cognitively normal older subjects had an "AD profile" in CSF, but none of the subjects younger than age 53 had that CSF picture. Assuming that healthy younger subjects are unlikely to harbor silent pathology, Li et

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al defined a normal CSF tau:Abeta 42 ratio based on the findings in all subjects aged less than 53 years. Using this newly established norm, they then noted that 16% of older, cognitively healthy subjects had a CSF profile outside that norm, suggestive of AD, and postulated that those individuals had clinically silent AD brain pathology. Those subjects with a high CSF tau:Abeta42 ratio were more likely to carry an Apolipoprotein E4 allele, and more likely to progress to mild cognitive impairment over an average of 42 months of follow-up, further supporting the hypothesis that those subjects harbored AD brain pathology which was silent at the time of CSF collection. The authors used the term “latent AD” to describe those subjects with normal cognition but CSF evidence of AD brain pathology. The Washington University group and others published similar findings at about the same time, and subsequent studies, including the Alzheimer’s Disease Neuro-imaging Initiative, have confirmed the contention that CSF evidence of AD pathology predicts subsequent decline. The revised consensus definitions of AD, recognizing the existence of “latent AD” make it difficult to believe that this was a fairly novel observation less than 5 years ago.

Other established CSF biomarkers may also have some value in defining aspects of AD which are relevant to prognosis. Bowman et al illustrated the prognostic value of the CSF albumen index, a well established measure of blood brain barrier (BBB) impairment, in patients with AD(2). This paper described a population of 40 subjects who participated in a multi-faceted AD biomarker study, with clinical, imaging, and CSF measurements at baseline, 3 months and 12 months in the study. This highly motivated and highly informative cohort, recruited and characterized a decade before the Alzheimer’s Disease Neuroimaging Initiative, has affectionately been described as “the fabulous forty,” and has yielded many novel observations about biomarkers and disease course (3) (4) (5) (6) (7). In the Bowman BBB paper, the serial CSF measurements permitted demonstration of the stability of the CSF albumen index over time, with persistent elevation in 22% of subjects indicating BBB dysfunction in a substantial proportion of individuals diagnosed with probable AD according to established research criteria. Previous literature suggested that BBB dysfunction was common in vascular but not Alzheimer’s dementia, so the possibility that this population of subjects was contaminated by vascular dementia was explored but not supported by the data. There was no significant relationship between CSF albumen index and vascular risk factors, Hachinski Ischemia Score, or white matter hyperintensity burden on MRI. Autopsy results in these subjects also supported the clinical impression of AD rather than vascular dementia. At the time of the publication, thirteen subjects had come to brain autopsy, four with BBB dysfunction and 9 without, and all met Reagan criteria for a pathologic diagnosis of AD. (Since the time of the publication, a total of 25 brain autopsies have been completed on this cohort, with the presence of AD confirmed in all cases and no cases of vascular dementia). These data consequently showed, with fairly good certainty, that a sizeable proportion of reliable diagnosed AD patients have compromise of the blood brain barrier. The cause of this compromise is uncertain, with possible reasons for BBB dysfunction ranging from amyloid angiopathy to silent atherosclerosis / arteriosclerosis, but no conclusion is possible at this point.

What was perhaps more interesting was not the cause, but the apparent consequence of BBB impairment in AD patients: those patients with BBB impairment tended to progress more rapidly than subjects with preserved BBB function. While this may be interpreted to mean that BBB dysfunction is simply a marker of more malignant disease, it also raises the possibility that the BBB may represent a target for therapy. Others have pointed out that homocysteine-lowering strategies may result in improvement in the integrity of the BBB (8), but definitive trials have not yet been conducted and further research in this area is warranted.

New biomarkers for old purposes

The identification and utilization of CSF biomarkers for diagnosis, prognosis, and, as we will discuss shortly, development of therapeutics in AD has increased interest in the development of additional biomarkers of AD mechanisms, as well as identification of CSF biomarkers for other neurodegenerative diseases. These interests coincided with the development of new proteomics technologies, which were applied in the next two papers for review. In “Detection of biomarkers with a multiplex quantitative proteomics platform in cerebrospinal fluid of patients with neurodegenerative disorders” (4), Abdi et al used an unbiased proteomics approach to identify differences between CSF from patients with AD, Parkinson’s disease (PD), Lewy body dementia (LBD), and healthy aging. A proprietary method for labeling proteins, iTRAQ (isobaric raggin for relative and Absolute protein Quantification) was followed by identification of peptides on tandem mass spectrometry, using one of many newly available technologies. One of the unique and important features of this particular study was that the AD and LBD diagnoses were pathologically confirmed, reducing the confounding effects of mis-classification on clinical grounds, a problem in 10% of AD patients and a significant proportion of LBD patients defined on the basis of clinical criteria alone.

Using these methods, more than 1500 CSF proteins were identified, with quantitative changes compared to healthy control subjects in 388 proteins in AD, 282 proteins in PD, and 380 proteins in LBD. Disease-specific changes were seen for 163 proteins in AD, 72 proteins in PD, and 101 proteins in LBD. Sixteen proteins were selected for experimental confirmation from this list based on the identification of two or more peptides by two independent protein databases, unique disease association of the protein, known biological function, and commercial availability of antibodies to the protein. Western blot studies on individual subject CSF samples confirmed group differences in several proteins consisting of ApoC1, ApoH, ceruloplasmin, chromogranin B, B-fibrinogen, haptoglobin, T-cadherin, and Vitamin D binding protein (VDBP). When the diagnostic utility of combinations of these markers was evaluated in individual samples, AD, PD, and DLB could be separated from controls with 95% specificity, demonstrating how proteomics technology might be used to define novel biomarkers of disease. However, both the discovery and validation portions of this study used very small numbers of subjects, and the manuscript thoroughly reviews how different published proteomics approaches have identified different groups of proteins, citing small group sizes as the most likely reason for the variability. The paper concludes with a call for a validation study of the best candidates in a larger sample.

In “CSF multianalyte profile distinguishes Alzheimer’s and Parkinson’s disease” (5), the same group of investigators do just that in 95 control subjects, 48 AD subjects, and 40 PD subjects. The panel of 8 novel CSF biomarkers includes haptoglobin and VDBP from the previous study, as well as several other biomarkers implicated in other studies. They also measured tau and Abeta 1–42 in the CSF samples in order to provide a frame of reference for comparison with other CSF biomarker studies. This study confirmed that eight of the ten CSF biomarker were useful in distinguishing control subjects from AD or PD, with a couple also helpful in distinguishing AD from PD. In order of ability to discriminate groups, those proteins included tau, Brain derived neurotrophic factor (BDNF), IL-8, Abeta 1–42, β2-microglobulin, VDBP, ApoAII, and ApoE. The discriminatory power of the biomarkers was most powerful when all eight were combined, and this multianalyte profile was also helpful in distinguishing a small group of frontotemporal dementia patients, and in dividing Mild Cognitive Impairment patients into an “AD-like” group and a group that did not have the CSF signature of AD.

These two studies, as well as a great many other studies exploring the potential of CSF biomarkers of neurodegenerative disease, identified the potential for developing CSF panels which might simultaneously probe multiple disease pathways for diagnostic and other purposes. However, CSF collection is an invasive procedure, so many investigators attempted to develop plasma biomarkers of AD, since blood collection is more readily accepted by both patients and clinicians. Probably due to the limitations imposed by the blood-brain barrier, most of these attempts yielded limited or negative results until a strategy was developed for incorporating a large panel of plasma biomarkers in a single multi-analyte profile.

In “Classification and prediction of clinical Alzheimer diagnosis based on plasma signaling proteins” (9), Ray et al approached the problem with a large panel of cytokines, chemokines, and other signaling proteins analyzed with a proprietary software which identified combinations of proteins which distinguished AD from control plasma. In this study, 120 signaling proteins were measured in each of 259 plasma samples from AD and control subjects. Half of the samples were then designated as a “training set” and a statistical algorithm was applied to determine which combinations of plasma proteins distinguished AD from control subjects, ultimately identifying 18 of the 120 proteins as having discriminatory power. The proteins in this panel included ANG-2, CCL5, CCL7, CCL15, CCL18, CXL8, EGF, G-CSF, GDNF, ICAM-1, IGFB-6, IL-1α, IL-3, IL-11, M-CSF, PDGF-BB, TNF-α, and TRAIL-R4. This newly defined multianalyte plasma profile was then used to examine the remaining specimens, defined as the “test” samples, and the panel was able to identify 90% of AD samples and 88% of control samples correctly. While this was a neat trick, it has limited practical value because the distinction between patients and controls is already feasible on clinical grounds. A tougher distinction, and the one in need of a biological test, is the distinction between subjects destined to develop AD in the future from subjects destined to “age successfully.” This more challenging issue was approached in the same paper by comparing Mild Cognitive Impairment (MCI) patients who were destined to remain stable and MCI patients who were destined to progress to AD or to other dementias. The next experiment in this report consequently compared plasma biomarker levels in a population of 47 MCI patients, 17 of whom remained stable, 22 of whom declined to AD, and 8 of whom progressed to other types of dementia. The 18-protein panel classified 20 of the 22 MCI subjects who progressed to AD as “AD”, while none of the eight MCI subjects who progressed to other types of dementia were classified as “AD” based on the plasma profile. Among the 17 MCI subjects who remained stable, 7 were classified by the biomarker panel as AD and 10 as controls. The enthusiastic response to this report was based on the potential that a straightforward blood test could assist not only in the diagnosis of AD, but in the prediction of future AD in subjects at risk. Efforts to reproduce and validate these results in independent samples are ongoing.

The Biomarker Holy Grail: Biomarkers for development of therapeutics

As noted at the outset, this interest in biomarkers is driven by the urgent need for more efficient clinical trial designs, which theoretically could be achieved with appropriate biomarkers as surrogate markers of disease. The last two papers for discussion describe the most productive use of biomarkers for this purpose to date. These two papers describe Phase 2 studies of Eli Lilly’s gamma secretase inhibitor semagacestat, which relied on biomarkers as the primary outcome measures, permitting critical dose-finding decisions and advancing the clinical development of this agent with remarkable efficiency by relying on biomarkers for “go-no go” decisions.

In “Effects of a gamma secretase inhibitor in a randomized study of patients wth Alzheimer disease” (10), Siemers et al describe a remarkably efficient randomized double blind placebo

controlled trial in 70 patients with mild to moderate AD at six clinical sites. Participants had CSF collected prior to randomization and again after 6 weeks of study drug, at a time point when traditional clinical outcome measures would not be informative, and in a sample far smaller than that necessary to detect clinical effects. They saw a 38% reduction in plasma Abeta 1–40 and non-significant trends to lower CSF levels of Abeta at the dose of 40 mg per day, and concluded that higher doses might be required to achieve more robust effects on Abeta production. Although this design does not permit conclusions about the clinical consequences of Abeta modulation (other than tolerability), the efficiency achieved with this design, compared to a typical Phase 2 trial in AD, is tremendous, and permitted a rational decision to increase the dose in the next phase of study.

Doses of 100 and 140 mg per day were evaluated in the next phase of clinical study and reported by Fleisher et al in “Phase 2 safety trial targeting Amyloid beta production with a gamma secretase inhibitor in Alzheimer’s disease.” (11) This study randomized 51 subjects with mild to moderate AD to either placebo (n=15), study drug at 100 mg per day (n=), or study drug at 140 mg per day (n=). The treatment period was 14 weeks, with study drug recipients all receiving 60 mg per day for the first two weeks, 100 mg per day for the next 6 weeks, and then randomized to 100 or 140 mg per day for the last 6 weeks. As predicted, the increased dose produced a greater effect on plasma Abeta, with 58.2% reduction by the 100 mg dose and 64.6% reduction by the 140 mg dose. Although the change in CSF Abeta levels were not significant, trends toward an association between CSF drug level and change in CSF Abeta were appreciated (p=0.06–0.07). Safety signals were also appreciated in this study, with three drug rashes and three adverse events leading to study drug discontinuation, including one small bowel obstruction. Unblinding issues were also raised by three instances of hair color change. No effects of study drug were identified on clinical outcome measures, including the ADAS-cog and an Activities of Daily Living scale, which was not surprising due to the the small sample size and short duration of follow-up, accentuating the value of biomarkers in small, brief trials. The ultimate outcome was that these Phase 2 studies permitted a rational decision about dose for a Phase 3 trial in a very efficient manner, potentially defining a paradigm for biomarker-driven AD drug development.

Hopeful message turns into cautionary tale

While the papers discussed here provide reason for optimism with respect to identification, validation, and utilization of surrogate markers of AD for the development of novel AD therapeutics, the semagacestat, story has an unhappy ending which should serve as a cautionary tale as the field moves in this direction. Based on the promising results in the Phase 2 studies just described, two long-term Phase 3 placebo controlled trials of semagacestat were initiated. In August 2010, Eli Lilly halted the trials and terminated the development of semagacestat when the Data and Safety Monitoring Board found that semagacestat was associated with worsening of clinical measures of cognition and ability to perform activities of daily living (12). This unfortunate outcome serves as a sobering reminder that while surrogate markers of disease can play a role in preliminary drug development, we are not yet at a point where surrogate markers can be used to confidently predict clinical outcomes.

On the other hand, the demonstration that semagacestat engaged the therapeutic target in these Phase 2 studies makes the Phase 3 results potentially more powerful and more significant in the long run. We have had many disappointing outcomes in Phase 3 trials in AD, usually without compelling evidence that the target has been engaged (13) (14, 15). Although it remains to be determined whether the apparent acceleration of disease progression by semagacestat was due to the intended effect on Abeta or due to an “off-target” effect, the biomarker-based drug development program has armed analysts with data

which may permit insights which can advance the field as a whole. Both established and yet to be defined biomarkers will continue to play an important role in the development of AD therapeutics, even as we recognize the imperative to proceed with caution.

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