## Discussion

# Alzheimer Research Forum Live Discussion: Making a BioMark on Alzheimer Disease[1]

*http://www.alzforum.org/res/for/journal/trojanowski3/biomarker_transcript.asp*

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**Tom Fagan:** I am Tom Fagan from the Alzheimer Research Forum and I will be moderating today. John Trojanowski, would you like to start the ball rolling?

**John Trojanowski:** Okay, Tom. Briefly, we will focus here on chemical analyte biomarkers in various bodily fluids such as cerebrospinal fluid (CSF), urine, blood, etc., and while genetics may come up, too, we will save imaging biomarkers for another time. Concerning apolipoprotein E (ApoE): It does seem to be critical to consider this for cohort assignment as demonstrated in the recent NEJM paper from R. Peterson [1] on mild cognitive impairment (MCI), and we will do this as well in the Alzheimer Disease Neuroimaging Initiative (ADNI) study.

**Anne Fagan:** John, what is your gut feeling about the potential utility of urine showing decent biomarkers?

**John Trojanowski:** So far, urine has seemed fruitful for studies of isoprostanes, although it depends on which one is measured and the method, but I am not certain if other analytes in urine are informative [2–4].

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**Anne Fagan:** We are already seeing some differences in levels of A$\beta$42 in ApoE4-positive versus ApoE4-negative folks in our antecedent study, but not other CSF markers, although the data are very preliminary. It suggests that perhaps A$\beta$42 is the first thing to change. We must verify with follow-up, of course.

**Tom Fagan:** Anne, do you have indications that urine analysis might prove useful?

**Anne Fagan:** Tom, we are not collecting urine. It may be too far down the line (i.e., processed) to be meaningful, but the idea is very clinically appealing.

**John Trojanowski:** Les, do you have information on other urine analytes?

**Les Shaw:** John, for AD, no.

**John Trojanowski:** Anne, what do the receiver operating curves (ROCs) look like in the studies you mention, and perhaps the group would benefit from a definition of "antecedent," as this is not on everyone's radar screen? [ROCs can provide useful information on the sensitivity and specificity of clinical measurements.]

**Anne Fagan:** True, John. We have not done formal ROC analysis yet. I use "antecedent" to mean biomark-

ers that indicate presence of neuropathologic AD (i.e., brain changes), but prior to clinical symptoms. Morris and Price and others at our Alzheimer Disease Research Center (ADRC) have published findings showing that a good percentage of clinically normal elderly folks who die from other causes actually have AD pathology but no clinical symptoms [5,6]. Our goal is to be able to identify these folks since clinical variables, by definition, cannot define the presence of preclinical disease. The percentage of "preclinical" subjects is about 30 percent.

**John Trojanowski:** Anne, so antecedent is restricted to prodromal AD as in MCI, or might it be earlier in populations such as those with familial AD (FAD) or Down syndrome?

**Anne Fagan:** Good question, John. I guess Down syndrome would also be considered preclinical, as would FAD cases prior to impairment. They would be a good population to study. For what it is worth, I consider "prodromal" to mean prior to any cognitive symptoms, including prior to cognitive symptoms characteristic of MCI.

**John Trojanowski:** I agree with Anne that these analytes are the rising stars for early AD diagnosis with a promise to be useful for sorting out MCI, too, but more work is needed on this, and we need to add a new possibility: lipoxygenase metabolites, as per work from Domenico Pratico at the University of Pennsylvania [7].

**Les Shaw:** Anne, what will it take to convert to a true diagnostic biomarker in terms of studies and data needed?

**John Fryer:** John, what is the patient population you are collecting from and is it longitudinal?

**John Trojanowski:** The ADNI population is well described in the ADNI website (www.loni.ucla.edu/ADNI) where the redacted grant is publicly available, but briefly, the study is a 3-year longitudinal of 400 MCI, 200 AD, and 200 normal controls balanced for ApoE status. I can add that the Penn Biomarker Core led by Les Shaw and me will perform CSF tau, A$\beta$, isoprostane, and homocysteine measures, as well as plasma homocysteine isoprostanes and urine isoprostanes, and we will cover as many species of A$\beta$, tau, and isoprostanes as we are capable of measuring.

Since the data will be made public rapidly, all the approximately 50 sites participating in this will have access to these data.

**Mony de Leon:** What is the most specific diagnostic CSF marker in AD?

**Anne Fagan:** Mony, currently only autopsy findings are definitive. A number of CSF analytes (A$\beta$42, tau, perhaps isoprostanes or sulfatide) are promising, but so far have not fulfilled criteria for a true diagnostic biomarker.

**John Fryer:** Has anyone proposed to use an additional patient population, such as Parkinson disease (PD), to determine which changes are specific to Alzheimer's versus neurodegeneration?

**Anne Fagan:** John F., we just did some analyses on PD CSF (with and without cognitive impairment). So far, no difference between the groups on our standard CSF analytes (A$\beta$s, tau, etc.).

**June Kinoshita:** Anne, to clarify, are you finding no difference between PD with and without dementia, or between AD and PD?

**Anne Fagan:** June, between PD with and without dementia, hot off the press as of yesterday afternoon – not published yet, and still preliminary, of course. There are differences between AD and PD, as perhaps one would expect.

**June Kinoshita:** Anne, at the pathology level, do PD patients with dementia have AD pathology while PD patients without dementia have no AD pathology? I imagine it is not quite so clear-cut, but it is interesting that you do not see differences at the biomarker level.

**Anne Fagan:** June, these samples are from a big bank in Rochester. I do not think any autopsy information is available. I will have to check on that.

**John Trojanowski:** I agree with Anne, and add that Lewy body variant of AD (LBVAD) may be possible to distinguish from PD or PD with dementia (PDD) by the AD biomarker profile of CSF tau, A$\beta$, and isoprostanes, but I do not know about sulfatides, which I neglected to mention also is on the roster of analytes the ADNI Biomarker Core will examine in CSF.

**Anne Fagan:** John T., we are still working out some methodological bugs with sulfatide, but our published data were very promising and warrant exploration. Glad to hear you are doing that.

**John Trojanowski:** Okay, Anne, and we had input from folks at Washington University on how to do the sulfatide assays, and these data also will be made public on the ADNI website within a few months of study completion.

**Anne Fagan:** Another issue is ultimate ease of measurement. It seems like some of the metabolites and various other things are measured by labor-intensive methods such as high-performance liquid chromatography (HPLC). I guess we need to find the marker first and then hone down the measurement issues.

**John Trojanowski:** While some of these tests are labor-intensive, Les does mass spectrometry measurements on many analytes other than for AD, so perhaps he can give a sense of labor, costs, and turnaround for this type of measurement, as they are routine in some pathology and laboratory medicine diagnostic units.

**Tom Fagan:** All, given the potential labor required, is cost a major impediment to validating biomarkers?

**Anne Fagan:** Tom, I do not think cost should be an issue yet, but it will be ultimately as things are taken to the clinic.

**John Trojanowski:** I do not think this is a problem, Tom, but perhaps Les can weigh in here as this is an area with which he has expertise in laboratory diagnostics.

**Les Shaw:** Most major medical centers and clinical laboratories have mass spectrometry equipment and expertise for certain types of tests – toxicology, drug monitoring, metabolic diseases. Once the equipment and the expertise to run these are in place, the costs are very competitive compared with costs for immunoassays, for example, and that type of cost is reasonable. I foresee more mass spectrometry applications – for example, the low-molecular-weight biomarkers, once clinically validated – coming into routine practice in the future.

**Tom Fagan:** Anne, what about enrollment numbers? Is ADNI or your study sufficiently powerful statistically, or will a broader net need to be cast, eventually?

**Anne Fagan:** Tom, the answer for our study is likely no. A wider net would be good. Even though we defined our enrollment numbers according to proper power calculations, I predict greater numbers will eventually be needed, especially if the effect is relatively small, as one would perhaps predict in a preclinical disease state.

**Patrick Lynn:** Has anyone seen robust biomarkers for AD in serum or plasma? This is an area of our research.

**Anne Fagan:** Patrick, we have not looked carefully at plasma yet, although we collect it (fasted) so we can go back at any time as interesting possibilities emerge. Tough to work with, given all the proteases, etc.

**Hasan Siddiqi:** How about using SELDI-TOF (surface-enhanced laser desorption and ionization-time of flight) mass spectroscopy to look for novel biomarkers in plasma?

**Anne Fagan:** SELDI-TOF is certainly a possibility. I predict biomarkers that mark brain processes will be in low abundance, especially in plasma/serum. Tough to do, though.

**Les Shaw:** Mass spectroscopy methods for certain drugs and toxins, for example, and metabolic diseases are now fairly standard for routine or semi-routine testing. Most drug study method validations using mass spectroscopy and using it for the clinical trials is now what is typical, and I foresee this more and more for many of the biomarkers we are interested in for AD. What is needed more than anything, for the methodology part, is lab-to-lab comparisons and standardization.

**Tom Fagan:** Les, what is being done to standardize among different labs? I would imagine this must be one of the major aims of the ADNI, right?

**Anne Fagan:** Standardization will be a major issue (dare I say, problem?) and one that must be attacked head-on at the inception of studies, if possible.

**John Trojanowski:** To add to what Les mentions, this standardization is a major effort and focus of the Biomarker Core which will interact with pharmaceutical companies and diagnostic companies to make the best academic/industry standards for the analytes that we measure. So we are hopeful that CLIA-approved

AD diagnostics will come out of this effort 3–5 years hence. (CLIA, or Clinical Laboratory Improvement Amendments, is a branch of Centers for Medicare and Medicaid Services that regulates all laboratory testing carried out on humans in the US).

**John Fryer:** John T. and Anne, how often are your patients imaged and does the imaging include amyloid imaging agents?

**John Trojanowski:** John F., the imaging is magnetic resonance imaging (MRI) and positron emission tomography (PET) at different schedules for the different cohorts. No imaging ligands are part of this study.

**Anne Fagan:** John F., our studies include MRI as well as Pittsburgh compound B (PIB) PET. Imaging will be done over time, but I cannot recall offhand the intervals. I know CSF will be taken every 3 years. Perhaps it will be the same for imaging. I will have to go back to the grant to be sure.

**John Trojanowski:** In ADNI, there is no PIB, and the CSF is to be from about 30 percent to 50 percent or more of all subjects obtained at baseline and after 1 year. Other fluid draws are more frequent and over longer periods of the study, and this, too, is all available on the ADNI website by accessing the grants.

**Hasan Siddiqi:** Does anyone think the biggest issue with plasma biomarkers is specificity/sensitivity in relation to CSF biomarkers?

**Anne Fagan:** I do not have a feeling for that, Hasan.

**Clary Clish:** Hi, I am Clary Clish from Gene Logic, Inc. I am developing a liquid chromatography-mass spectrometry (LC-MS)-based biomarker analysis platform for a wide range of disease states including AD.

**Tom Fagan:** Clary, can you give us some idea of the scope of your project, how many patient samples, etc.?

**Clary Clish:** My efforts are focused on small, endogenous molecules, and I am just getting started on AD. Method development is going to begin with CSF, plasma, and urine samples from preclinical subjects. We are going to target compounds that are in some way tied to the pathophysiology and potentially dysregulated.

**Anne Fagan:** For what it is worth, we have also begun a CSF proteomics project using two-dimensional difference gel electrophoresis (2D-DIGE)-based methods combined with LC-MS–a fishing expedition, but one that we hope will pay off down the line.

**June Kinoshita:** Most (dare I say, all?) of the biomarker candidates that are being looked at are based on studies in AD patients. How do we know that the same biomarkers will indicate the presence of preclinical stages? What do you all think about casting a wider net and carrying out some proteomic and genomic fishing expeditions? Good idea or waste of dollars?

**Anne Fagan:** June, our efforts on antecedent biomarkers dovetail nicely with our work on AD subjects. We also are collecting CSF/plasma from very mild and mild AD cases. Maybe I am naïve, but I think that what one finds in clinical AD will probably be found earlier; it just depends how far back we can detect the change. Also, proteomics projects are tough to get funded, especially with the present NIH funding situation.

**John Trojanowski:** Further on ADNI, while the analytes we measured were chosen with their potential promise to be informative (based on published reports) and budget in mind, there are opportunities of add-on studies to the ADNI biomarker work, and folks interested in this should check the ADNI website. But realize that requests for add-on studies are vetted by a resource allocation review committee (RARC) of advisors to ADNI, and it will not be possible to apply to duplicate studies of analytes already covered by the ADNI proposal.

**Anne Fagan:** John T., I am sure you have thought about data management for the ADNI. Do you have someone that is spearheading this specific part?

**John Trojanowski:** Anne, the data collection is in the petabyte domain of intensity, and it is coordinated by the Clinical Core at the University of California, San Diego, under the direction of Leon Thal in collaboration with Art Toga at the laboratory for neuroimaging (LONI) at UCLA. This is all Web-based, and data mining will be possible by frequent postings of data on a public website at the ADNI website. And, June, focused research will be possible through add-on studies to ADNI as mentioned above, but funding for this will be through competitive grant applications, following approval by the RARC, to access banked ADNI biosamples.

**Anne Fagan:** John T., will people be able to download data and use them at their discretion or will there be some guidance/policing of who does what with what data?

**John Trojanowski:** Anne, yes, this will be possible with approvals, access codes, etc., and the data will be de-identified, but the idea is to encourage data mining and publication with consultation with an ADNI publication committee.

**Anne Fagan:** John T., a publication committee is a great idea.

**John Trojanowski:** Further on Anne's query about data management, an attractive aspect of the ADNI is the fact that all data – clinical, biomarker, imaging, etc. – will be made public so bioinformatics and biostatics gurus can mine the data for developing better data analysis tools and new correlations, etc. My questions to Anne are: Will the data collected in the study she mentions be made public and can investigators apply to add-on studies to examine aliquots of the biosamples collected for this new study?

**Anne Fagan:** Also, to answer your questions, John T., we are in the process of developing a relational database for our antecedent study (termed ACS for Adult Child Study) for in-house analyses; it is tricky even to coordinate four to six investigators within the project, let alone between institutions. Hopefully this proof-of-principle database can then be adopted by other institutions for their own in-house analyses. Ultimately, we will make the data public, but there are some important issues that need to be addressed first, such as what I just brought up: how to make sure that the data are "used" appropriately, for lack of a better word. And yes, our samples will certainly be available to other investigators through our usual (competitive) tissue request and review process. Information can be found on our ADRC website (alzheimer. wustl.edu/adrc2/Research/ResourcesRequest.htm).

**John Ciallella:** Is anyone tracking blood glucose as a biomarker, given a possible link between diabetes and AD?

**Anne Fagan:** Great question, John C. We have not proposed to test blood glucose, but certainly could do so with appropriate collaborations. The AD/insulin/diabetes connection is very interesting to me, although I am just learning about it.

**Nancy E. Lombardo:** Earlier this week during the Alzheimer Research Forum chat on insulin resistance, AD, and depression [8], Suzanne de la Monte, Natalie Rasgon, June, myself, and others were wondering whether the current leading studies of biomarkers for AD, including the National Institute on Aging (NIA) neuroimaging study, are including biomarkers for insulin resistance, especially in the brain.

**John Trojanowski:** Nancy, this is not covered in the ADNI biomarker core list of analytes to be studied at the University of Pennsylvania, and we selected analytes, the ones mentioned above, with budget limitations in mind, but others can pursue studies of other analytes through the ADNI add-on study mechanisms, and funding for this must be obtained by the investigators wishing to study the other analytes, so there are mechanisms in place for hypothesis-driven as well as exploratory metabolomic, proteomic, and other "omic" approaches. Since the samples are precious and limited, the RARC will adjudicate who will gain access to samples. Also, a certain percentage of the aliquots will be held in reserve until the end of the ADNI grant to enable us to take advantage of new technologies that emerge in the next few years.

**Tom Fagan:** All, is anyone following FAD patients in preclinical stages?

**Anne Fagan:** Tom, we are not following FAD families. Alison Goate here at Washington University is doing some genetic studies with them. It would be great to get CSF/plasma, but these families are all over the world. Again, it brings up the issue of variability in sample collection and analysis protocols between labs.

**June Kinoshita:** Tom, regarding FAD subjects, there is one study that I know of with individuals who are positive for a FAD mutation and have not developed symptoms yet. I think there are more people out there who would volunteer if we could only find a way to connect with them. That is where the early-onset AD (EOAD) Web page idea comes in.

**Tom Fagan:** June, yes, that was one of the things on my mind. It also occurs to me that many FAD patients might be slightly scared to get involved, for fear of finding out the worst, but as treatments start to become available, they might be more inclined.

**Anne Fagan:** June, what is the EOAD Web page idea?

**June Kinoshita:** Anne, the idea of the EOAD Web page is to set up an informational page on the Alzforum directed to people with early-onset AD in their families and for researchers who are interested in working with them. They have some different medical needs and issues, for example, genetic counseling, family issues, etc., and might benefit from support and advice from other EOAD families. They also may have a very high level of motivation to participate in research because their children may have the mutations. So an EOAD online community would support patients and families and also nurture alliances between affected individuals and researchers.

**Anne Fagan:** June, great idea with the FAD families.

**John Fryer:** Anne, how long is this longitudinal study currently funded for?

**Anne Fagan:** John F., the study just got funded for 5 years – not enough time to do any validation, of course, but we hope it will continue for many years.

**Anne Fagan:** What do people think about the concept of incentives for researchers to adopt certain collection and analytical procedures in order to better compare data between labs? Too controlling? I think one of the big problems with biomarkers, especially antecedent studies, is lack of enough statistical power. How can we as a scientific community pool our resources (samples, data, etc.) to move forward at a more rapid pace?

**Les Shaw:** Anne, That is a good way to bring up the importance of the collection and procedures standardization needs. I think building the spirit of collaboration and promoting this are key.

**Anne Fagan:** Les, I agree wholeheartedly.

**Clary Clish:** Anne, I think your idea about standardization, particularly for sample collection and storage, is a good one. The analytical protocols that follow afterward are more challenging, as they may be hardware-dependent.

**Anne Fagan:** I absolutely agree with you, Clary. Even in my own project, we use two different protocols to measure $A\beta42$. While the values are highly correlated, they are not the same, and that is using the same experimenter and the exact same samples.

**Les Shaw:** Further to collaboration, too, I believe we in academia benefit a lot from engaging with our pharma colleagues who bring certain strengths to the table like issues of standardization, since they have FDA hoops to go through for drug studies.

**John Fryer:** John, how comprehensive is the neurologic examination of your patients over time and will this be on the ADNI Web posting also, or will the Health Insurance Portability and Accountability Act (HIPAA) regulations prevent that?

**John Trojanowski:** John F., there is not enough room here to mention the inclusion/exclusion criteria to be used at the approximately 50 participating ADNI sites, but these are available for identifying normal, MCI, and AD patients in the ADNI grants that are available to the public at the ADNI website, and moreover, the statements of purpose (SOPs) for all of the studies to be done, including biomarkers, which was an amazing accomplishment by Les Shaw, will go up on the ADNI website too, so we hope that others will take advantage of this public access to ADNI data and study design as we all go forward with biomarker studies for AD as well as related dementias such as frontotemporal dementias (FTDs), Parkinson disease with dementia (PDD), etc.

**Hasan Siddiqi:** Is tau a current neuroimaging target?

**Tom Fagan:** Hasan, I do not believe there are any ligands yet that are useful for tau imaging, but there was a paper recently on natural tau fluorescence, but no indication that this might be useful *in vivo* [9].

**June Kinoshita:** Hasan, I believe Gary Small at UCLA has been working on tau imaging ligands.

**Tom Fagan:** Okay, folks, we are nearing the end of our scheduled hour. I just want to thank John, Les, and Anne for agreeing to devote some of their valuable time to this chat.

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