

**Global Biomarker Standardization Consortium
AAIC In Person Meeting
July 21, 2018
Chicago, IL, USA**

Update on QC Program-Kaj Blennow

- Ongoing project since 2009, 3 QC samples of pooled CSF are analyzed 3 times per year, 2 unique samples and 1 identical sample, last testing point is round 27. The 2 unique samples are to measure comparisons between labs and the 1 identical sample is to measure longitudinal changes. The goal of the program is to have a tool to measure laboratory performance and accuracy of testing.
- A β 42 between labs comparison – Utilized Innostest ELISA, 1 lab result was compared to 61 labs in the round, there was a range from 600-1200 pg/ml with a CV of 18.7% (data round 17 (2015)).
- Batch to batch variability on the Innostest ELISA, 5 different batches for A β 42, p-tau and t-tau- in the round 24 there were no batch to batch differences.
- Results from 2014-2018 for the various platforms (assays and automated, different implementation dates and number of labs participating) were CSF A β 42: 7-20%, A β 40 – CVs from 10-24%, CSF total tau 4%-14%; p-tau Innostest is 11.1% and Elecsys is 2%: the automated platforms had lower CVs.
- Currently there is no available funding and it was raised if the program is still necessary? Does the QC program have a role? Should it be free of charge to academic proficiency programs?
- Is it time to expand the program with additional CSF biomarkers? Expand to blood biomarker?
- Comments/Questions from Audience
 - There were extreme high and low data reports, this could provide an opportunity to see how they differ from the normal outcomes, was this looked into? The QC program does not have the resources to contact each of the labs. Labs might have used the same kit but modified the protocol. This should subside when the standardization to the certified reference materials and calibration are adopted.
 - Long-term stability for analytes have been measured internally in Kaj's lab with an internal control sample, 2-year storage was fine.

- There is a role for the QC program: to improve the CVs; batch to batch with fully automated system; blood based biomarkers and new upcoming markers in CSF; also after the implementation of the CRMs.
- Approach the College of American Pathology Chemistry Resource Committee for collaboration in the USA, also include the manufacturers
- Manufacturers are appreciative of this program because it allows them to build credibility around CSF and the precision for blood will be higher.
- The Alzheimer's Association still has the potential to fund the program but needs a new mandate especially when there is potential for FDA approval, need to restructure the mandate of what the QC program is doing.
- In Europe they use the analytes on a routine basis and use the Alzheimer's Association QC Program for quality control, if the program stops they will have to implement their own program.

Blood-Based Biomarkers in the QC Program – Henrik Zetterberg & Kaj Blennow

- The Alzheimer's Association QC program has shown historically plasma A β data was variable.
- Pannee *et al* published in Neuroscience Letters that A β profiles in plasma are similar to CSF and there was a trend for a decrease in plasma A β 42/40 ratio in AD.
- Randy Bateman's group (Ovod et al. Alzheimer's and Dementia) published amyloid positive individuals had reduced plasma A β 42/40 ratios.
- Nakamura's paper in Nature 2018 showed a biomarker signal for the A β 40/42 ratio.
- Zetterberg *et al.* 2012, plasma tau showed an increase in AD but overlap was larger than in CSF, there is a tau signal that is reproducible.
- Simoa can be used to measure low concentrations of neurofilament light (NfL) in CSF and blood. Plasma NfL and CSF NfL had a correlation.
- In familial Alzheimer's disease symptomatic carriers had increased plasma NfL, with the levels increasing 10 years before onset.
- In multiple sclerosis patients' serum NfLs were controlled after natalizumab treatment but they had side effects and treatment was stopped.
- It is time to include plasma A β 42, A β 40 and NfL.

Biofluid Based Biomarkers PIA – Michelle Mielke & Henrik Zetterberg

- The PIA has expanded from just blood based to Biofluid Based Biomarker (BBB) PIA. Several of the executive committee members are also involved in the GBSC leadership.
- 2017-2018 goals were to include more students and trainees in the PIA and this was incorporated in to their PIA Day with student presentations. The PIA was successful in hosting a NfL Featured Research Session during AAIC.

- Establishment of working groups:
 - Development of saliva biomarkers - led by Sid O'Bryant
 - Goal is to perform a literature review and then identify markers and methods more beneficial to move forward.
 - Context of use- led by Jeff Dage
 - Goal is to define specific contexts of use and clarifying study design and sample sizes that need to occur to validate those context of use. The working group is drafting the first white paper.
 - Reference Ranges for both blood and CSF
 - Looking for input from GBSC
- The BBB PIA extended an invitation to collaborate with GBSC to move biomarkers forward.

Plasma A β Biomarker –Katsuhiko Yanagisawa

- Blood is a good source of information to predict brain pathology.
- Katsuhiko's lab is planning to conduct a larger scale validation study using similar, different cohorts, and also including longitudinal samples. Also, plans to form an international collaboration team.
- They will accept external request for sample analysis starting at the end of September and they are willing to work with the GBSC on AD biomarkers for AD research and clinical trials.

Panel Discussion: Blood-Based Biomarker Consortium – Kaj Blennow, Susan DeSanti, Hartmuth Kolb, Colin Masters, Michelle Mielke, Sid O'Bryant, Holly Soares

Moderator: Rebecca Edelmayer

- Panel was developed due to the interest in use and validation of blood based biomarkers for AD and wanted to convene a panel to discuss their initiatives they are working with.
- Susan DeSanti -PPSB
 - The PPSB is the scientific board, comprised of a group of companies, associated with ADNI.
 - For biofluid based biomarkers they are convening a meeting with companies that are developing assays and the companies will present at the fall PPSB F2F meeting. 12 companies presented at the 2016 meeting and were asked to present if made progress since 2016 methods that are available for use now and that are in development that will be used in the future.
- Hartmuth Kolb –FNIH

- Foundation of NIH, pool resources and address pre-competitively issues in AD research. Key issue is validation for biomarkers, pre-analytical standardization, assay platforms, clinical validation, FNIH has several projects either started or in the design phase. One project is validation of inflammatory markers in blood and CSF in AD and major depression subjects, also working on plasma A β project.
- Colin Masters
 - Why do blood tests work? A β in the brain goes up ~3.8 fold over natural history of disease and CSF drops 50% and blood goes down 25%. Colin used A β 40/42 because this allows for a more normal distribution.
 - Could a blood test work better than a PET scan, CSF? The blood test allows for more specificity than a PET scan. A β in CSF drop dramatically as it crosses the threshold. There is a dramatic drop in CSF A β in the dynamic range as one crosses the normality to abnormality threshold and this is what is picked up in the blood. CSF A β is diurnally and circadian variable but hypothetically in the blood it is in equilibrium with the CSF and is more stable.
- Michelle Mielke – BBB PIA
 - Biofluid biomarkers are needed for screening and disease progression.
- Holly Soares
 - Blood based tests are becoming more sophisticated in approach to pre-analytical factors.
 - Screen failure rates are 70%, \$15K/patient, if this percentage can be decreased based on a blood test that would save costs.
- Kaj Blennow
 - International Federation of Clinical Chemistry (IFCC) wants to develop reference measurement procedures and reference materials with plasma A β using mass spectrometry.
 - There should be several assays available for comparison and the IFCC plans to do a Round Robin study for this year to compare immune assays and mass spectrometry assays for A β , they still need a large number of plasma samples and a gold standard of CSF positivity or amyloid PET positivity.
 - Kaj's lab has generated a set of new NfL antibodies.
 - Working with Roche on neurotool kit project to develop high precision methods for blood biomarkers
- Sid O'Bryant
 - Multi-tiered diagnostic methods (ex. blood test screening into a confirmatory PET scan or LP) should be incorporated for Alzheimer's disease.

- Expansion of blood based biomarkers in other neurodegenerative diseases has occurred and Sid will report on using proteomics data to predict incident MCI and AD in Down's syndrome.
- Those interested in race and ethnicity's impact AD, there are several large scale cohorts, one is Sid's HABLE study consists of 1000 Mexican Americans, 1000 non-Hispanic whites; 3T MRI, plasma, cognitive testing, multiple time points collected, data samples will be available to the community.
- The potential New IDEAS study will add a biorepository.
- The 1st ever prospective study of blood based biomarkers in AD will start recruiting in November/December, all subjects will be from primary care and will get amyloid PET scans, 3T MRI, stored blood, will not have PET tau. These samples will be a resource for the community.
- Questions to the panel
 - How to support a coordinated effort to advance and accelerate these initiatives forward? How can the Alzheimer's Association help?
 - A sample repository that is well characterized in their disease stages and a standardized pre-analytic protocol. Also, there is a need for multiple samples so there can be a comparison between the clinical standard and the ideally collected sample.
 - PPSB had an initiative looking at CSF that was collected by ADNI, incorporated a program where companies presented proposals on how to use that and they were vetted. Every study that was proposed could come to an outcome useful to the community. Including this same type of initiative for blood.
 - Need for FDA advice, to know the studies will be able to use for registration purposes. In the FNIH meetings members from FDA attend.
 - Need to know how to collect and process blood from a clinical standpoint.
 - Should we collect a plasma sample without EDTA or with an addition to EDTA? Include a protease cocktail?
 - AIBL has collected with platelet stabilizers and tubes go into liquid nitrogen after fractionation.
 - A β , NfL, and tau are the priorities for optimal collection.
 - There should be an open sharing information forum available for plasma A β 40 and A β 42, then make a standard of operating procedures.

Integration of Certified Reference Material – Henrik Zetterberg and Britta Brix

- CRMs released on Dec 1, 2017, the next step is for company implementation.

- Euroimmun, FujiRebio, and Roche collaborated on investigating consistency between the CRM adjusted concentrations measured in CSF samples using different Beta amyloid 1-42 assays.
- Study design
 - 3 CRM samples, Frozen pooled CSF (N=15),
 - Pre-analytical handling
 - CRM & CSF pools: thawed 30 min at RT while roller mixing, short spin down
 - Problem: the tubes with frozen CSF were very small. Roche and Fujirebio needed to use a kind of adapter to be able to measure them - > limitation of study.
 - Measurement:
 - CRM samples: 2 aliquots were measured at the beginning and end of the run; 2 runs were performed.
 - Other samples: 1 aliquot was measured in each run in randomized order, 2 runs were performed
 - 2 determinations from each aliquot
- Measurement results
 - Original concentrations and re-calibrated using CRM samples (CRM-adjusted) concentrations.
 - Original concentrations: Good overall precision across all sample types and all different systems; and high correlation between the systems but there is a large bias possibly due to pre-analytical problems.
 - Original vs. re-calibrated (CRM-adjusted) concentrations: there was a correlation between the original and recalibrated in all systems, original concentrations had a bias of -25-50% at median concentrations but after calibration the re-standardized concentration was from -0.2-2.2% at median concentration. There is no significant bias between the re-standardized concentrations.
- After re-calibration they were able to measure the samples at their target values.
- The companies that participated are committed to commercialize the re-calibrated assays and will continue to work together and run broader Round Robin studies with more samples. Other companies are invited to join.

Panel discussion with Britta Brix, Henrik Zetterberg, Sandra Rutz and Manu Vandijck

- It was huge improvement and challenge but it was successful for the companies.
- How to take into account that some companies did not re-calibrate to the standards? The FDA will take this into account and will state this.

- Can you recalculate the old data with the new assay? Roche is currently working on this and the optimal recalculation would be if there is a concentration factor between the methods.
- The companies have laid the groundwork for a universal cut point.
- The companies want a broader set of samples and quantify it with LCMS methods.
- Depending on the clinical applications and population, universal cut offs are not used and needs flexibility.
- Les Shaw can incorporate mass spectrometry
- A question from the audience was will the Alzheimer's Association be able to make a pool of 15 samples that the companies can use? The Association is always open to suggestions and will discuss this further.
- Will do the same for tau and p-tau.

LP Video – Charlotte Teunissen

- The LP video was shown, it is available in English, Dutch and additional languages. If interested in the video contact Charlotte.
- There was a suggestion for the video to include a dramatization of the drip method and to detail the CSF quantity that will be removed but mention that this is replaced very quickly.
- It was also suggested for the voice over to slow down.
- Object to the word spinal tap, suggested CSF collection
- The needle in the beginning is big.

CSF Appropriate Use Criteria (AUC) Update – Les Shaw

- A workgroup (WG) consisting of experts in the field, was convened February 2017 by the Alzheimer's Association to develop appropriate use criteria with the purpose to assist healthcare practitioners with guidance based on evidence and the experience of WG members, and ethical standards for patient care-on the appropriate and inappropriate use of LP and CSF AD biomarker testing.
- The WG builds on the published 2013 Johnson et al. Amyloid PET AUC and intended to support clinicians in identifying appropriate patients for LP and CSF testing, while also taking in to account the cost-effective use of limited healthcare resources. The goal is to have the AUC an important resource for policy makers & 3rd party payers.
- The AUC did not provide recommendations for the research use of CSF biomarker testing or rule out conditions other than AD or MCI-AD as possible causes of cognitive decline.
- The CSF Appropriate Use Criteria timeline for publication is fall 2018.

CSF Pre-Analytics Protocol Update – Jim Hendrix

- The Alzheimer's Association convened a WG comprised of various companies and academic participants to develop a consensus around a CSF pre-analytical protocol.
- The objective of the pre-analytical protocol is for it to be utilized in clinical practice and simple to use.
- The Sarstedt tubes are available for ordering.