Global Biomarkers Standardization Consortium (GBSC) Meeting
Summary Notes AAIC 2015

Update on QC Program – Kaj Blennow

The principle of the QC-CSF program is to monitor the performance of biomarkers between labs (>90 labs participating) and across time (ongoing since 2009)

- 17 rounds have been completed; for each round 3 QC samples (pooled CSF) are sent out to each lab (2 unique for inter-lab comparisons and one that remains the same for longitudinal)
- Results have been getting better and the coefficient of variation (CV) has been going down in later rounds
- Several new assay developments and formats have been tried:
  - <u>Innotest</u>: Between lab CV has gone down when looking across rounds 7-17; CV is in the range of 14-16% for AB42, T-Tau and P-Tau
    - Data has also shown that pooled CSF aliquots are stable for >3.5 years – this is important for clinical trials
  - <u>AlzBio3</u>: no clear trend for reduced variability (AB42, P-Tau, T-Tau) across rounds (between lab CV ranges from ~8 to ~35%); fewer labs make the data less certain
  - <u>MesoScale</u>: between lab CV shows trend for reduced variability (AB42, AB40); again few labs make data less certain
  - <u>EuroImmune/ADx</u> – this has just been added, have few labs and few rounds but CV appears to be getting better (below 10% for AB42 and AB40)

<u>Sources of variability for CSF biomarkers measurements</u>
(see slide for details; also see pub: http://www.ncbi.nlm.nih.gov/pubmed/23622690)
- Variability can come from preanalytic, analytical and assay manufacturing factors
- Analytical factors (lab and technician procedures) and assay quality and stability have a large impact on variability outcomes

<u>Automation of ELISAs may help decrease variability</u>
- Manual versus pipetting robot: CV went from 14.2% to 11% (130-230 QC samples; Innotest)
- In the future, we may need to look at fully automated clinical analyzers to reduce variability as much as possible (no lab or tech variability); this will also allow for all samples to be run at once and it's faster (<30 min)
  - <u>Roche Diagnostics – Cobas Elecsys</u> – data from first rounds show minimal variability (1.9 – 4.6% for AB42)
  - <u>Lumipulse G1200 and G600II</u> – these are not yet in the QC program but hope to add; initial in-house data shows CVs of 2-5% for AB42 and 1-3% for T-Tau

Update on Abeta SRM RMP, including correlations with amyloid PET – Kaj Blennow

Update on SRM RMP

- The IFCC Work Group and GBSC are working together to develop "Golden Standards" for a Reference Measurement Procedure (RMP) and Certified Reference Material (CRM)

- Reference Measurement Procedure (RMP) - must be a high precision method for absolute quantification
    - Mass spec based technique – no antibodies involved; allows quantification without interference (matrix effects)
    - Use of certified calibrator (amino acid analysis)
    - Tested in Round Robin Studies
    - Intended Use: To set the level in the Certified Reference Material
    - Candidate Reference Methods for CSF AB42 have been published
        - http://www.ncbi.nlm.nih.gov/pubmed/24842955
        - http://www.ncbi.nlm.nih.gov/pubmed/24625802

- Certified Reference Material (CRM) – golden standard CSF – exact levels set using RMPs
    - Large aliquoted CSF pool
    - Commutability tested between assays
    - Testing for long term stability, etc.
    - Intended Use: distribute to kit vendors and large labs; harmonize CSF levels between assay formats; assure stability between production lots

Update on correlations of CSF AB with Amyloid PET

Recent studies looking at correlation between CSF AB and Amyloid PET

- Study: 118 patients with cognitive complaint; examined the relationship between CSF AB42 (Innotest ELISA) and amyloid PET (flutematomol)
    - http://www.ncbi.nlm.nih.gov/pubmed/25155658
    - Concurrence is very high (92%); when add cutoffs for CSF and PET it increases to 97%
- Study: 103 patients with cognitive complaint; examined the relationship between CSF AB42 (SRM mass spec) and amyloid PET (flutematomol) (Shahim, et al, unpublished)
    - Amyloid PET and AB42 - correlate well, but not completely; higher concordance between PET and AB42/40 ratio

Applying absolute biomarker cut-off levels in AD

- Challenging because even if we have exact biomarkers, how to do we apply cutoffs when there is overlap between AD and normal aging as well as AD and other pathologies?
- There is a continuum of pathology between AD and aging – this gives a continuum in CSF AB levels and PET measures
- What are the pros and cons of reporting actual values instead of "positive" or "negative"?

- o In the US actual values are not reported, but in other countries they are – clinicians use this for making Dx and are informed they need to be careful in borderline cases

CSF Round Robin – Les Shaw

Overview of Round Robin Study – 4 Center collaborative study of mass spec technology for measurement of AB42 in 12 samples (CSF pools)
- Use of common sample preparation methodology; different high purity AB42 standards used
- 4 labs; 3 different mass spec systems; 3 different HPLC systems; 4 different calibration matrices
  - o Results for 12 pools: performance within centers CV=4.8%; performance between centers CV = 12.2%
- Efforts to reduce variability
  - o Developed a response factor for each lab using one sample; reconstruct each calibration curve; this reduced CV from 12.2% to 8.3%
  - o This tells us that use of a common calibrator will improve interlab results

IRMM preparation of AB1-42 common calibrator – being used for:
- Ring Trial – follow up interlab study – all 4 centers are participating and using common standard AB42 calibrator prepared by IRMM; design and oversight provided by IRMM (see IRMM/IFCC Project Overview slides)
  - o Ring Trial has been completed by 5 labs
- Pilot study to compare 2 candidate reference methods - 2 labs using 10 CSF pools and common calibrator - initial results show good concordance (see slide for details)

Clinical Relevance of Methods
- Evidence that data is actually clinically relevant - study in mild AD patients show good concordance between mass spec and AlzBio3 immunoassay for AB42 (N=41 AD and 41 controls), showing that the mass spec method had comparable AUC (both >0.9, and p=0.22 for comparison of the AUCs). The mass spec accuracy-based values were greater in conc value by more than 4 and significantly correlated: N=158, R^2=0.72).

Applications of mass spec based analysis
- Can use mass spec method to make comparisons to new and existing immunoassays; this will be important for Commutability Study
  - o Provides an accuracy-based anchor for comparison of other methods
- Can use mass spec method to study various metabolites in various patient populations; look at differences related to age, pathologies, etc. e.g., can assess AB42/40 ratio for AD amyloid pathology detection
  - o There is interest in moving forward with reference methods for AB40, but it is a matter of time, drive and other factors as to how quickly this will happen

Summary - the goal is to gain certification of these methods and then create reference materials, the ultimate goal is give AB reference materials actual values; this could ultimately be shared with diagnostic companies and used clinically.

IRMM/IFCC Project Overview – Henrik Zetterberg & Ingrid Zegers

AB42 Reference Material - Standardization

- Aim: Setting up a traceability chain linking results on routine samples to a common reference, the international system of units (SI)
- Goal: make it possible to compare lab to lab and over time
- Manufacturers cannot get long-term stability unless assays are anchored to a reference material

All steps in the creation of certified reference material (CRM) must be carefully considered, this is not a fast or easy process, but we are making progress on the many steps (see slide for details)

- The Ring Trial is focused on amino acid validation of the AB42 calibrator batch
  - AB42 Calibrator Batch
    - Use: calibration of LC-MS measurements for the value assignment of the CRM
    - Format: recombinant AB42 in 20% acetonitrile; 1% ammonium hydroxide
    - Concentration: determined by amino acid analysis and purity assessment
    - Initial results from the Ring Trial (5 labs, 6 data sets) shows acceptable value assignment of the CRM (uncertainty for value assignment =3.7%) (see slide for details)

Problems with amino acid assessment:

- Having problem with in-house repeatability and between amino acids – this means there is a problem with applying their methods to AB peptide, this is common for this type of work
  - Possible explanations/solutions are being worked on and include checking purity, optimizing digestion conditions, freeze-drying of samples before measurements to prevent interaction between solvent an HCl
  - Ideal conditions are being identified and optimized and will be repeated on actual calibrant (ongoing)

Commutability Studies

The reference material must behave in the same way as a patient sample

- They have done a study on this – recently submitted for publication (Bjerke, CCLM, submitted; see slide for details)
  - Looking at 2 studies and 10 methods:
    - Both the aCSF and the spiked CSF do not look good
    - The ideal form will be pooled CSF without spiking

Standardization of CSF Processing

The pooled CSF requires exact conditions and all vials must be filled to same volume, work is being done to identify and optimize these conditions:

  - Manual filling versus machine filling => machine is better

- o Stirring/not stirring => stirring is better
- o Flash freezing versus slow freezing => slow freezing is better
- o Using these conditions, variability was reduced (overall, method and between vial)

Decision on format of the matrix reference material:
- CSF pool, no spiking, 0.5 ml/vial, store at -70
- We will need 3 levels of reference material (low, med, high) because the user cannot dilute
- A year long stability study will be conducted

Next steps: The first level has been filled, next step is to study homogeneity, then process the other 2 levels and make value assignment for CRM

Discussion/Questions:
- What is the longest stability the group has seen for CSF AB? - about 15 years.
- Can these pools be used to measure the stability of tau and other analytes? - yes.

CSF Biomarker Qualification – Diane Stephenson
Objective of Critical Path Institute's CAMD CSF Biomarker Team: FDA biomarker qualification for CSF analytes (AB42, T-Tau, P-Tau) for the purpose of enrichment in clinical trials at the pre-dementia stage

CAMD Biomarkers team is aiming for FDA Qualification Prognostic Application - AD and PD
- Alzheimer's Disease Biomarkers
    - o Low hippocampal volume
    - o CSF biomarkers for Aβ, T-Tau and P-Tau
- Parkinson's Disease Biomarkers
    - o Dopamine transporter neuroimaging

Progress since last AAIC GBSC Meeting
- FDA issues Letters of Support (LoS) to CAMD (March 2015)
- http://www.alzforum.org/news/research-news/fda-gives-nod-alzheimers-and-parkinsons-biomarkers
- FDA Website to LoS:
  http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/UCM439713.pdf
- CSF Biomarker LoS
    - o Highlights potential value of CSF biomarkers and encourages further evaluation
    - o Can help increase visibility of biomarkers and identify gaps to address on the path to qualification
        - LoS pointed out the need for data sharing and employing consensus standards (AD CDISC standards originating from ADNI) for data harmonization
        - LoS pointed out that the FDA/EMA will not endorse/validate any specific test system or validation process

- o They will need data showing that methods/assays produce measures that are specific, reliable and reproducible

- FDA has provided feedback on what is needed to move forward towards biomarker qualification
  - o Enhanced data sharing and collaborative efforts among consortia
  - o Increased communication about the value and progress of consortia efforts
  - o Global data/specimen repositories which can support expanded contexts of use for biomarkers
  - o Upfront conversations about context of biomarker use; this drives the level of evidence needed

FDA Request for Comments – released Feb 2015
https://www.federalregister.gov/articles/2015/02/13/2015-02976/identifying-potential-biomarkers-for-qualification-and-describing-contexts-of-use-to-address-areas

- Seeking feedback and information on promising, novel biomarker candidates in areas important for drug development
- Looking for proposed context of use and the type of evidence needed to support qualification
  - CAMD AD Biomarkers teams submitted two candidate biomarkers
    - o CSF Neurogranin
    - o Tau PET Imaging

FDA Qualification of 1st Clinical Biomarker Qualified for Prognostic Use (July 2015)
What can we learn from this?
- Consortia driven
- 5 clinical studies (N>1000 patients), all observational, no RCTs
- Only one dataset was used to get this passed/qualified
- Had several different assays (all immunoassays) with different platforms and different cutpoints
- Bridging between assays was considered but does not appear to be required in advance
- Overall, this is considered encouraging news for the team

Next Steps – What is needed for success?
Context of Use > Level of Evidence > Qualification
- Assay analytical performance – Reliability and Reproducibility
- Clinical Validation – possible studies:
  - o ADNI
    - ADNI goals align with CAMD goals; not overlapping, but synergistic
    - All CAMD regulatory achievements were enabled by ADNI
  - o BMS Avagacestat
  - o DESCRIPA
  - o WashU ADRC
  - o Others?

Next Steps – How can GBSC help?

- Sharing of analytical performance data; the new biofluid recommendations from ADNI3 is what the FDA is looking for
- Employ CDISC AD standards for CSF biomarkers in ongoing and future studies
- Patient informed consent to enable data sharing of clinical samples

LPs and CSF in Clinical Research – John Morris

There is high variability in success rates for lumbar puncture
- Rates are influenced by country, culture and center
- DIAN – Procedure completion rates vary across participating centers:
  - Baseline rates range from 45-99%; Follow up rates range from 0-96%

Survey – Factors Influencing LP Feasibility
- Building upon European multi-center study of LP headache (Kaj Blennow)
- Administered over 2-year period to the 27 NIA-funded US Alzheimer's Disease Centers (ADCs)
- Surveyed medical staff that request the LP and the LP participants (pre and post LP)
- Goal: Examine potential factors influencing (1) LP consent and (2) LP complications
- Findings – ADC LP Experience:
  - All centers were collecting CSF for research (100%); many were also using it for diagnosis (62%) and industry-supported studies (68%)
  - Most CSF assays are for AD protein concentrations; AB (88%), Tau (88%)
  - Most medical staff use brochures to educate the participants and spend about 6-10 min discussing risks/benefits
  - Most medical staff requesting LPs had never had LP themselves (92%)

Factors that impact participant agreement to undergo LP
- Sex - Males (81%) more likely than females (73%) to consent
- Race – consent rates vary: Caucasians (80%), African Americans (64%), Asian (45%)
- Dementia status – 93% of persons with AD (or their caregiver) agreed vs. 72% of persons with MCI or cognitively normal
- Previous LP – Participants who had undergone LP in the past (90%) were more likely to consent than people who had not (70%)
- Previous complication of headache – no effect
- Precious complication non-headache – less likely to consent

Incidence of Complications
- No persons in the study experienced severe complications (eg, subdural hematoma, infection)
- 29% reported some complication; of those experiencing complications, most were mild LP headache lasting for less than 1 day

- Of <u>all</u> participants who had LPs:
  - 11% of participants had LP headache
  - 3% non-specific headache
  - 2% (each) – nausea, dizziness, nerve root pain
  - 1% (each) – vasovagal response, back pain, neck stiffness

<u>Factors that contribute to LP complications</u>
- Factors associated with <u>lower incidence</u> of complications:
  - Use of atraumatic needle (Sprotte)
  - Gravity drip method of LP
  - Male sex
  - Older age
- Factors associated with <u>higher incidence</u> of complications
  - Hemorrhage
- Factors that <u>did not affect incidence</u> of complications
  - Needle gauge
  - Presence or absence of dementia
  - Position (supine vs. upright)
  - Previous LP complication
  - History of migraine

<u>LP Standardization</u>
- There is lack of standardization for LP procedure – there is variability in terms of fasted state, time of day (circadian rhythm), needle type/gauge, methods and volume of CSF collected
  - This is something we could address and work to improve, possibly through education and training

<u>Next Steps</u>
- Pool the US data with the European data; look for similarities and differences
- Consider addition analyses with NACC data on participants

<u>Discussion</u>
What can be done to improve consent and standardization?
- Attendees asked John to share how WashU was able to succeed in both standardization of LP and high consent rates – could this be a model that is disseminated more widely?
  - WashU has put great effort into training and standardization; the main focus is getting the message across on the high value of CSF assays and how much this advances research

- ADNI has also led a campaign to help improve consent and has seen significant improvement
  - They used educational materials and focused on health care providers, not patients

- There was group interest in possibly holding a workshop at AAIC focused on LP consent and standardization (see later discussion in section on Educational Workshops)

Alzheimer's Association Educational Workshops – Keith Fargo
Keith provided an overview of the Educational Workshops offered at AAIC 2015
- Neuropathology was the first workshop to be offered in 2014; in 2015 Animal Models and Neuroimaging were added
- All workshops are offered the Friday before the conference starts; Neuropathology is a full day; the others are a half day
- The workshops are didactic in nature and aimed at: new researchers, researchers that may be new to that field/topic and researchers that would like a refresher
  - Currently the workshop attendees are about half students, half those coming for a refresher

Group Discussion – are workshops something this group is interested in? How can the Alzheimer's Association support that?

Ideas for Workshop Topics:
- Educational Workshop on assay/analytical performance for biomarkers – this could help researchers outside of the GBSC get up to speed on the status of standardization efforts and keep these in mind in when they are looking for novel biomarkers

- Educational Workshop on how to analyze quantitative data – biomarker data cutpoints, grey zones – more education and sharing of knowledge is needed here

- Educational Workshop on LPs in research settings – this could help increase consent and allow for building of a cohort and repository of CSF samples; focus on education materials for research cohorts to address things like cultural barriers, etc.

- Educational Workshop - Scientific reproducibility, basic data responsibility, helping new investigators become aware of standardization efforts from the start

Educational Workshop Logistics/Questions:
- The first step to have Chairs identified and in place to lead the workshop, the Chairs then put together the faculty for the educational component
  - Association can help with the logistics and speakers

- Can the Educational Workshops be held during the conference instead of beforehand?
  - This is difficult with the overlap of the many preconferences and PIA Meetings; we need to ensure that workshops are not up against competing sessions, but we can consider all options

- Can we consider a wandering fee structure – allowing attendees to pay one price but attend several of the workshops, going in and out since they occur somewhat simultaneously?
  - This is a good idea and as we add more workshops we'll need to consider fee structure options

Blood Based Biomarker PIA – Sid O'Bryant
The blood based biomarker (BBB) PIA has grown exponentially; currently at about 400 members
Founding Goals of Blood Based Biomarker PIA:
- Standardization and Best Practices
- Increase data sharing and access, virtual repository
- Establish validated blood biomarkers
- Study blood biomarkers across neurodegenerative diseases

Standardization Issues
- Lack of standardization is a barrier; less than 50% of academic findings replicate in industry
- What are the main concerns?
  - Inconsistent findings in the literature; failure to replicate findings
  - Different protocols across labs and large-scale studies
  - Failure to learn from CSF literature
    - The blood biomarkers PIA really came together because of the standardization initiatives around CSF – this is where the ideas are coming from; it is critical to keep sharing this work to the broader community

Standardization Initiative
- There have been efforts towards standardization of blood biomarkers and several recent publications from the working group:
  - O'Bryant et al (2015) http://www.ncbi.nlm.nih.gov/pubmed/25282381
  - Henriksen et al (2014) http://www.ncbi.nlm.nih.gov/pubmed/23850333
  - Snyder et al (2014) http://www.ncbi.nlm.nih.gov/pubmed/24365657

- PIA working group study: examined serum and plasma samples across platforms (data presented at 2015 AAIC)

Next Steps/Future Goals
- *Alzheimer's and Dementia: Diagnosis, Assessment and Disease Monitoring* (OA Journal) is partnering with PIA for special collection of articles "Latest Advances in the Science of Blood-Based Biomarkers"

- How can the blood biomarkers and the CSF biomarkers groups learn from each other?

- o Data and working group initiatives from BBB PIA can be leveraged to assist in global standardization efforts
- o The BBB PIA members are interested in looking for blood based tools that filter into what CSF and Imaging are doing
- o The CSF group has the most experience in standardization and harmonization; other groups could learn from this
  - It would be useful to include blood biomarkers researchers in these discussions
  - It will also be important to consider including other neuroscience/neurodegenerative researchers beyond AD

Questions/Discussion
- There was a question about why there seem to publications and press about blood biomarkers but nothing really "sticks" – why?
  - o Sid shared that it could be due lack of reproducibility because the research is discovery-based and not designed for laboratory developed tests (LTDs)
  - o There is difficulty in moving from discovery-based platforms to LTDs but it's starting to gain traction

- Several attendees discussed the interest to collaborate and share samples (serum, plasma)
  - o Challenges include getting the agreements in place, having a strong proposal, and needing the funding and will to move this forward
  - o NINDS is doing something like this for PD with 10 U01s – could we use this as a model?

Open Discussion on Future GBSC Activities
Wrapping up the discussion, Maria and Jim posed to the group the need to move this conversation and the vast amount of knowledge gained by this collaboration beyond this room and out into the AD field and scientific community at large. What are some ideas and options to make this happen?

- From the group discussion it sounds like educational workshops and becoming a PIA could be good options for outreach and broadening to a wider audience

  - o Nest steps – consider ideas for Educational Workshops discussed during this meeting (biomarker standardization, data analysis/cutpoints, LPs, data reproducibility) and continue to take ideas; the Association can help organize this and move it forward

  - o Next steps – look to leadership in the group for interest and options possibly move toward becoming a PIA – either our own PIA or perhaps in conjunction with the Blood Based Biomarkers PIA – possible consolidating to form a Fluid Biomarkers PIA

- Due to time these questions were posed to the group to consider, please email Jim with thoughts and suggestions; Jim will also be following up by email after AAIC

The next GBSC meeting is scheduled for October 13 at 9 am Central time and will be a teleconference.