
Global Biomarker Standardization Consortium

Global Biomarkers Standardization Consortium

AAIC 2020 Meeting

Wednesday, July 22, 2020

8:00am-11:00am CDT/3:00-6:00pm CEST/1:00-4:00PM GMT

Meeting Summary

Attendees (Names taken from webinar attendee information if a name was listed. This is not a complete list of attendees. There were ~110 attendees): Maria Carrillo, Chris Weber, Rebecca Edelmayer, Emily Meyers Heather Snyder, Keith Fargo, Suzanne Schindler, Robert Rissman, Kaj Blennow, Henrik Zetterberg, Charlotte Teunissen, Inge Verberk, Britta Brix, Oskar Hansson, Elisabeth Thijssen, Jeff Dage, Michelle Mielke, Stephen Zicha, Steven Greenberg, Gill Farrar, Jonathan Schott, Leslie Shaw, José Luis Molinuevo, Adrian Jones, Agneta Nordberg, Akinori Nakamura, Alexander Jethwa, Andrea Benedet, Anne Fagan, Ashleigh Herd, Benjmain Levno, Beth Snitz, Blaine Roberts, Carolina Schipke, Cecelia Gabriel Garcia, Chiaki Yoda, Danielle Graham, Danni Li, Douglas Galasko, Eli Shobin, Erik Stoops, Eugeen Vanmechelen, Ezequiel Surace, Frances-Catherine Quevenco, Gabrielle Strobel, Garth Ringheim, Gina Jerome, Helen Hu, Hiroshi Sekiya, Ian Sherriff, Ishu Venkat, Jackie Jones-Laughner, Jamie Eberling, Joel Braunstein, John Lawson, Julia Gray, Julie Collens, June Kaploq, Keith Fargo, Kelley Faber, Kevin Yarasheski, Kira Sheinerman, Kristina Malzbender, Ksenia Musaelyan, Kwasi Mawuenyega, Sylvain Lehmann, Leyla Anderson, Lynn Bekris, Manu Vandijck, Maria Julieta Russo, Maria Pascual, Marta del Campo, Melissa Budelier, Naoki Kaneko, Nathalie Le Bastard, Nathan Yates, Nicholas Ashton, Nicolas Barthelemy, Nina Silverberg, Noelia Fandos, Omar Ehsan, Pallavi Sachdev, Patrick van Zalm, Pendro Pesini, Pedro Rosa-Neto, Philip Verghese, Rachel Henson, Randy Slemmon, Rianne Esquivel, Ricardo Allegri, Richard Dennis, Robert Bowser, Robert Dean, SangYun Kim, Sasha Bozeat, Sebastien Boulo, Sergio Castillo, Shinichi Iwamoto, Silvia Fossati, Steven Chance, Sudhir Sivakumaran, Sunil Pandit, Takashi Kato, Tatiana Foroud, Tharick Pascoal, Thomas McAvoy, Thomas Register, Tiernan O'Malley, Tobias Bittner, Tony Bannon, Vesna Kostanjevecki, Wesley Horton, William Chen, Yuan Moucon, Zahinoor Ismail

Welcome

Christopher Weber

- Dr. Chris Weber opened the meeting by welcoming the GBSC membership. GBSC was established over 10 years ago to gather researchers, clinicians, industry, regulatory and government leaders in Alzheimer's disease and all dementia to drive research to standardize and validate biomarker assays for use.

Impact of COVID-19

Laboratory and Institutional updates

Suzanne Schindler
Robert Rissman

- Dr. Schindler highlighted issues to consider in order to restart in-person visits amid the COVID-19 pandemic. Local infection rates and safety precautions must be considered when resuming in-person clinic visits. Procedures should continue to be re-evaluated as the environment changes.
- From a biofluid perspective, Dr. Rissman shared that his laboratory uses universal precautions, assuming all specimens are infectious, and COVID-19 samples are not treated any differently. Adjustments made since the pandemic include an increase in decontamination processes. Lab staff are essential to maintain equipment and staffing is mostly normal though on an irregular schedule. Sites are slowly beginning to reopen with strict collection procedures.

Global Biomarker Standardization Consortium

GBSC Working Group Updates

QC Program (CSF/Plasma)

Kaj Blennow

- The QC program started in 2009 aimed at assessing analytical and assay variability for CSF and blood in different clinical labs and to monitor assay performance. Results from the program have shown that samples can be compared between labs and from batch to batch with little to no difference. Assays tested for the core biomarkers include: INNOTEST, Euroimmune, Meso-Sclae, Elecsys and Lumipulse. For NfL, the assays tested include: Uman/IBL, UGOT, Meso-Scale and Quanterix. In total, 118 different assays have been tested in the CSF QC program. Data was presented for A β 42, A β 40, total tau and p-tau. New NfL assays have acceptable data, but with additional data and time will become even better. In the future, we invite more labs to participate and expand to blood biomarker assays (A β 42, A β 40, NfL, total tau and p-tau181). Next steps may be to move forward with next publication for the QC program.

Round Robin Studies (CSF/Plasma)

Henrik Zetterberg

- A manuscript draft of plasma A β results discussed at AAIC 2019 is in progress and will be circulated to the work group soon. Plan to have a short report for DADM.
- The plasma NfL round robin that began in 2019 by contacting labs/kit vendors and the final list of labs and assays completed in 2020. Assays being tested include: Basel Homebrew, Basel HDX, MAGQU, Quanterix, Roche, Olink, MesoScale and ProteinSimple. Each lab will be provided with 40 EDTA plasma samples and 40 serum samples with an additional 5 of each plasma and serum to be done as duplicates, which will be blinded. In addition a mix of certified reference materials samples that are spiked with NfL or CSF. Results from this study will be obtained in September 2020. Outcome will improve knowledge on how assays compare to each other and if there are candidate reference materials to standardize measurement.

Certified Reference Materials/Methods (CSF/Plasma)

Britta Brix

- Previously, Euroimmun, Fujirebio and Roche formed a group to test and calibrate A β 42 CRM on A β 1-42 assays. This small study found that CRM reproducibility was strongly increased while bias of assays decreased. All companies decided to continue using CRMs to implement in commercial tests. In 2019 Fujirebio and EUROIMMUN shared data on re-standardized commercial tests and found that CRM values are directly comparable however still observed a difference when looking at CSF samples. A second commutability study (now delayed due to pandemic) to be performed by Drs. Blennow and Zetterberg will run the different methods side by side to ensure no difference in handling/sample shipment. This next study may help answer whether we need to improve further or is this a difference we can live with.
- There is an increased need for sets of well characterized (e.g. with clinical data and PET, MRI imaging data) samples (CSF and blood) to be used by groups building diagnostics for assay validation purposes. Discussions regarding a biorepository and sample distribution for this purpose are ongoing. Robert Rissman is discussing the opportunity with the Alzheimer's Association and other partners.

SABB Workgroup Updates (Plasma)

Charlotte Teunissen / Inge Verberk

- Charlotte Teunissen shared an update on the SABB initiative, which addresses the pre-analytical phase to establish standardized protocol as early as possible for blood biomarkers. How do certain variations affect biomarkers? The SABB initiative aims to gain a consensus on SOP that is not analyte or platform specific. A SOP proposal was presented.
- Inge Verberk shared recommendations so far. 10 samples per protocol were used. Results obtained with Simoa 4-plex, PeopleBio MSD and EUROIMMUN ELISAs. Work is ongoing, and focusses on EDTA

Global Biomarker Standardization Consortium

sample tubes (note: other tube types lead to different biomarker results thus might result in different pre-analytical recommendations). Data on the delay in processing between sampling and centrifugation indicate that <3hrs RT or >3hrs 2-8°C is recommended. For storage, <24hrs at 2-8°C and >24hrs at -20°C is recommended with long term storage at -80°C. Repeated freeze thaw experiments indicate that up to 1 cycle is feasible. Next steps to incorporate data from Araclon, C2N and Shimadzu assay and p-tau isoforms.

CSF Pre-Analytical Consortium Manuscript

Oskar Hansson

- The CSF Pre-Analytical Consortium was created to validate a consensus protocol for CSF handling before analyses with input from academia and industry. Protocol uses “fresh” CSF directly collected from lumbar puncture in LoBind tubes without any further handling before analyses. Main findings from literature search were shared. Protocol recommendations include that the first 2mL of CSF sample is not used with no further handling at the collection site. Transport at 2-8°C for <14 days, however if at RT <2days is recommended. At testing site, no further handling is recommended and measurements should be done immediately. Freezing CSF samples (-20°C or -80°C) after collection allows for extended time between transport to analysis. Blood contamination can be mitigated if samples are stored at 2-8°C or -20°C with or without centrifugation (shown in AB42 MSD and Aβ40/Aβ42 MSD). This paper is under final review with the workgroup members and will be submitted to *Alzheimer's & Dementia* later this year.

Provocative Research Discussion

Plasma p-tau Presentation

Elisabeth Thijssen

- pTau is an essential key in AD and FTLD-tau pathology. Lots of binding in early onset AD and almost no binding in healthy elderly. Different assays have been developed using the AT270 antibody to detect tau (Eli Lilly MSD, Simoa). Eli Lilly assay has recently been optimized and has shown that fold change is bigger, more clustered data with no extreme outliers.
- 2020 is quite a year for p-tau181 in plasma with robust results that correlate strongly with tau-PET binding. Plasma and CSF p-tau trajectories are similar with longitudinal plasma p-tau showing increase 16 years before symptom onset in familial AD.
- Also p-tau217 that shows a similar pattern to p-tau181 and very strongly correlated.

Panel Discussion: Plasma p-tau application and future impact

Panel: Jeffrey Dage, Kaj Blennow, Oskar Hansson, Charlotte Teunissen

Moderator: Henrik Zetterberg

- Overall discussion on this topic indicated that though the difference between p-tau181 and p-tau217 may not be huge, p-tau217 may be better numerically. P-tau217 has greater increases during the symptomatic phases and is at much lower levels in those without AD. Reason why performance between each biomarker is different, is not known (antibody used, phosphorylation site, cleavage site). In brain tissue with tau pathology categorized by Braak stages, there is higher levels of P-tau181 in 0-II compared with P-tau217. Also, P-tau217 levels increase more between III-IV and V-VI than P-tau181. P-tau217 levels increase more across stages while pT181 seems to

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reach a plateau when compared relative to Braak staging of postmortem tissue. Will p-tau217 be a good marker for early diagnosis?

- A ratio of p-tau217/p-tau181 has seen value in CSF, but not in plasma.
- A total tau assay has not yet been developed that replicates what is seen in CSF. Data may be available soon that addresses total tau epitopes by taking advantage of work with mass spectrometry.
- Some interesting papers to address what p-tau is a marker of: track TBI group showed p-tau increases dramatically after TBI and goes down after 2-3 days. This could be opening of BBB to release these markers and then recovery. Could a low-grade BBB impairment influence p-tau levels in AD?
- Combining p-tau antibodies in assays has been attempted, but sensitivity was not high enough. Perhaps with new technologies there will be more opportunities.
- What do we need to do for clinical practice/clinical trials? Plasma work has moved faster and with additional studies on the pre-analytical phase and some basic standardization work, this can be used for clinical trials. Clinical practice will need to wait.

Global updates from outside the GBSC

Biofluid Based Biomarker PIA Update

Michelle Mielke

- Membership in the BBB PIA has doubled over the last 5 years, partly due to extending from blood based biomarkers to include all biofluid based biomarkers. Vast majority are professional faculty and industry members, with a push to include young investigators and student members.
- Primary goals of the BBB PIA :
 - To develop awards for best abstracts on PIA day
 - To enhance cross-collaborations within PIAs: FTD PIA, Down Syndrome PIA, Neuropsychiatric Syndromes PIA, Immunity and Neurodegeneration PIA
 - To continue with existing work groups and developing new work groups: context of use and statistical methods; saliva working group; exosome working group just getting under way.
 - Create a template for reporting CSF fluid in clinics - need to harmonize reporting protocol
- BBB PIA has active communication through a LinkedIN site, quarterly newsletters and a twitter account.

FNIH Plasma A β Work Group

Stephen Zicha

- The FNIH Plasma A β project started over 2 years ago as part of biomarkers consortium with the desire to validate new plasma A β assays published in 2018 in a rigorous manner. This collaborative project aims to determine whether plasma A β 40/42 assay increases the probability of identifying patients with amyloid positivity to improve clinical trial efficiency, reduce costs in early stages of Alzheimer's disease.
- The project consists of two different studies. Study 1 aims to determine whether any of the platforms should be removed from consideration moving forward by evaluating the different assays on standard valuation and quantification criteria. Study 2 will compare the different assays on clinical utility at early stages of disease.
- Samples for this project are sourced from ADNI for Study 1 and potentially TRC-PAD for Study 2. There are 6 project partners to evaluate 6 assays (ADx, Quanterix, Roche Diagnostics, Shimadzu,

Global Biomarker Standardization Consortium

University of Gothenberg and Washington University in St. Louis).

- The Plasma A β project as launched, however Study 1 was delayed to September 2020 due to COVID-19.
- Feasibility assessment is ongoing for the potential tau buy-up option to review plasma p-tau assays that are currently available (p-tau181, p-tau217, and 231).

MarkVCID – Research Updates

Steven Greenberg

- The goal of MarkVCID is to identify and validate biomarkers for small vessel disease-related VCID to the point of being ready for application to clinical trials. Instrumental validity and biological validity are the desired properties of a biomarker. Sites that participated in MarkVCID proposed 11 candidate biomarkers including MRI and fluid markers and the validation process is ongoing. Fluid biomarkers include plasma endothelial growth factors, exosome endothelial Inflammatory factors, Plasma NFL, CSF placental growth factor
- Instrumental validation for fluid-based kits and imaging-based kits to measure validity of the assay and site specific validity. Participant enrollment, clinic visits and some analysis have been paused due to COVID-19 and are resuming slowly.

Discussion: The Future Use of CSF and Amyloid PET in Routine Clinical Practice

Panel: Gill Farrar, Jonathan Schott, Leslie Shaw, and José Luis Molinuevo

Moderator: Maria Carrillo

Discussion Overview

Dr. Les Shaw provided an overview of CSF biomarkers and their relationship to amyloid PET and how to potentially use these biomarkers in clinical practice. In the setting of clinical practice, the use of advanced diagnostic procedures (CSF biomarkers and amyloid PET) is considered if a question on cognitive complaint remains. In comparison of A β 42 and A β 40/A β 42 ratio in CSF and amyloid PET, both measures show high concordance, with the A β 40/A β 42 ratio a few percentage points higher. The ATN framework provides a useful approach that can utilize CSF biomarkers to define presence/absence of amyloid, tau and neurodegeneration pathology. Using participant samples from ADNI with a diagnosis of MCI, the rate of progression to dementia over 4 years can be elucidated using the ATN criteria. This is further defined when the genetic history is included. The ATN criteria permits a combination of biofluid and imaging (eg, MRI-based hippocampal volume) and genetic biomarkers and likely plasma biomarkers will become very important.

Panel Discussion

- How can we use CSF and amyloid PET biomarkers in context of clinical practice, especially with the potential approval of a therapeutic agent?
- Gill Farrar: From a patient perspective, access to biomarkers is essential for diagnosis. There are logistical and potentially procedural issues relating to both the use of PET and CSF, so I think it is necessary to be clear how to apply the information derived from both tests for consistent clinical decision making.
- Jonathan Schott: Practice across the UK varies considerably. In routine practice, we use CSF on clinical basis as the first line investigation to look for molecular biology underlying disease and then utilize PET. Why are biomarkers not being used in clinic? Accuracy of tests: there are issues regarding standardization and cut points, with a lot of work to be done. Ability of tests: start to move from group studies to individual studies to put together individual patient predictors. Potential use of a negative biomarker test? Availability: PET availability is limited in UK and CSF is still limited though more

Global Biomarker Standardization Consortium

available. Affordability: cost can be a large factor for clinical use. Apathy: in absence of disease modifying treatment, should we bother?

- Jose Luis Molinuevo: Both CSF and PET correlate with pathology and are great biomarkers for diagnosis. Concordance between CSF and amyloid PET increases in clinical groups, when there is symptomatology the concordance is much better. Hence, for selecting patient candidates of a DMT treatment in a clinical population, both CSF and amyloid PET will be interchangeable and other factors may come into play (affordability, accessibility, etc). In Spain the use of CSF is broader than in the US/UK. Many centers use CSF routinely for diagnosis, PET is much more expensive and that is why it comes second.
- Maria Carrillo: Early Alzheimer's disease is the intended population for the biologic submitted to the FDA. In those stages of patients, is CSF equivalent (or more sensitive) to amyloid PET? If/when a therapy is available and use of CSF and amyloid PET increases, we will need to discern if there are gaps between these methods of measuring amyloid positivity to be sure we are capturing an equivalent population for treatment.
- Les Shaw: Among the things that we greatly need in terms of more studies are special populations, minorities, for example, which has become a huge issue in the US.
 - MC: the New IDEAS study was approved by the Centers of Medicare and Medicaid and will include 4000 samples from minority populations and will grow a biorepository for validation purposes.

New Business and Concluding Remarks

Revising Appropriate Use Criteria (AUC) for
Amyloid and Tau & Concluding Remarks

Maria Carrillo

- The Alzheimer's Association is undertaking the revision of the AUC criteria along with the Society of Nuclear Medicine Molecular Imaging. This will be launching by the end of July and we anticipate that this will include amyloid PET and tau PET.