Questions & Answers

Soluble Biomarkers for Alzheimer's Disease Webinar - Q&A

Soluble Biomarkers for Alzheimer's Disease Webinar was held on August 22, 2024. Here is a comprehensive compilation of all the questions raised during the webinar, along with their detailed answers.

1. Measuring p-Tau217 in blood appears promising for Alzheimer's Disease diagnostics. Do you believe other markers remain important?

Prof Zetterberg: There are now several good assays for p-Tau217, and p-Tau217 standardization has just started with IFCC. If you are interested in joining that project, please contact me as I chair that work group. In addition to p-Tau217, I think it is beneficial to have several p-Tau forms measured, since they could be used for staging Alzheimer's disease pathology. In addition to p-Tau, even some other types of Tau, for example, brain-derived Tau, which is a CNS-specific form of Tau that you can measure in blood, is a promising marker for Alzheimer's and other neurodegenerative conditions.

All in all, combining multiple biomarkers can provide a more accurate staging of Alzheimer's disease pathology, and reduces the risk of misdiagnosis by identifying consistent patterns associated with the disease. A multi-marker approach including NfL, p-Tau217, GFAP, and brain-derived Tau, in minimum, can enhance diagnostic accuracy. Future research may expand this panel to include additional biomarkers, say up to 5 to 10, for more precise disease classification.

2. How do you in Medix Biochemica decide on new mAb development projects, which analytes target?

Emilia Galli: We carefully follow customer requests and do internal evaluation of the cases in terms of market potential and technical feasibility. We also stay updated with scientific literature to identify emerging and interesting analytes.

3. How do blood samples compare to CSF samples in the analysis of brain-derived analytes?

Prof Zetterberg: Measuring CNS-derived proteins in blood presents analytical challenges due to low concentrations and complex matrix, so it requires more for the test in terms of sensitivity and specificity. CSF biomarkers often correlate more closely with brain pathology, but blood biomarkers are improving. However, the correlation between CSF and plasma remain most commonly at 0,6 to 0,9, so not close to 1, since the blood levels are affected by e.g. kidney function and fluid dynamics.

Some markers, or conditions, show unexpected breakage in the correlation between CSF and plasma levels. For example, GFAP is more accurate in blood than CSF, for reasons not fully understood currently, but could possibly be linked to pH-induced structural changes. Also, peripheral neuropathies and ALS can disrupt the correlation between blood and CSF biomarkers, suggesting unique disease-specific mechanisms. As we expand our use of blood-based biomarkers in larger cohorts, we must be prepared for unexpected findings and continue to refine our understanding of their relationship to neurological diseases.

4. Which assay platforms do you use in Medix Biochemica to evaluate new mAb candidates?

Emilia Galli: Typically, we would evaluate the antibody candidates in fluoroimmunoassay having one of the assay components labelled with Europium lanthanoid. As was evident from my presentation, we most often determine the kinetics before launch. This data is added to the product sheet and has been very appreciated by our customers. In some cases, we may also test candidates using lateral flow or CLIA if found relevant.

5. Any emerging markers of interest?

Prof Zetterberg: I think the synaptic markers are really interesting and could be valuable early indicators of neurodegenerative pathology. In most neurodegenerative diseases, the synapse is the first one to be impaired. And once the synapse is lost, the axon is lost, and then the eventually the neuronal soma. From blood-based biomarkers, beta-synuclein and SNAP-25 show promise in reflecting synaptic dysfunction. There is still research needed to better understand the relationship between synaptic markers in blood and brain changes, and this could be approached by collaborating with neuroimaging experts.

6. What kind of sustainability actions do you take in Medix Biochemica?

Emilia Galli: Thank you for bringing up this important topic. At Medix Biochemica, we are actively keeping our eyes open for various sustainability initiatives, let it be replacing harmful chemicals, or reducing plastics in our packaging.

Last year, Medix Biochemica joined the EcoVadis sustainability rating system and earned a Bronze medal on our first assessment, scoring better than over 70% of all companies rated by the system. This year, we improved our sustainability actions and performed even better, scoring better than 84% of all companies and attaining a Bronze medal again. Our top-performing areas were environment, labor & human rights, and ethics.

Related to mAb production, as I mentioned in my presentation, we have a long-standing practice of producing our mAbs in vitro with serum-free medium, which applies to hybridomas as well as recombinants.

7. To what extent does the test platform influence assay sensitivity compared to the monoclonal antibodies used?

Emilia Galli: Antibodies are the backbone of many diagnostic tests. They provide high specificity by binding only to the intended target, and high sensitivity by detecting even smallest amounts of the target molecule in sample matrix. However, the sensitivity of monoclonal antibodies can only be enhanced to a certain extent. Further gains in sensitivity rely on advanced technologies. Achieving optimal results requires a combination of high-performance antibodies and cutting-edge, ultrasensitive platforms.

For instance, novel methods like digital ELISA (or single molecule array, Simoa) can be 100 to 1000 times more sensitive than standard ELISA, enabling the detection of very low concentrations of biomarkers. Also, methods combining DNA-tagged antibodies with PCR amplification, such as Proximity Extension Assay (PEA), enable highly sensitive detection of proteins. In PEA, pairs of DNA-tagged antibodies bind to a target protein, bringing their DNA tags close together and then can be extended by polymerase. The DNA template can then be quantified by qPCR, allowing precise detection of low-abundance proteins.

Another advanced approach is immunoprecipitation mass spectrometry (IP-MS), which pairs precise protein capture by antibodies, with mass spectrometry analysis for highly specific and sensitive detection of target molecules.

8. **Early in the presentation there were two graphs showing how A42/A40 combined is a very good marker but there was also an A38 marker listed. Is A38 not viewed as a viable marker even when combined with either A42/A40? If so, why not?**

Prof Zetterberg: Yes, Abeta38 is very interesting and it could even be protective against the amyloid cascade. I think we should work more on this amyloid beta form, both as a biomarker but potentially also a target for intervention (increasing its concentration might inhibit Abeta42 fibrillization).

9. **How should one think about different p217 clones. I guess none of the data you showed was done with the Medix Biochemica clone. Would each new clone have to be validated in a large clinical cohort or is correlation to golden standard with a few samples enough?**

Prof Zetterberg: Yes, to prove equivalence this could be enough. To prove superiority, really large cohorts may be needed (given how well the current assays work).

Should you have any additional inquiries or need further clarification, please feel free to contact us at medix@medixbiochemica.com

View the webinar on demand at: medixbiochemica.com/alzheimers-disease-biomarkers-webinar

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