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Cyril Helbling and Mari L DeMarco.
Eye Catching Advancement for Creutzfeldt–Jakob Disease Diagnostics
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Guests: Dr. Mari DeMarco from St. Paul's Hospital and the Department of Pathology and Laboratory Medicine at the University of British Columbia in Vancouver, Canada, and Cyril Helbling, a Ph.D. student in the DeMarco Lab at the University of British Columbia.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett.

Prion diseases including Creutzfeldt-Jakob disease, or CJD, are characterized by the aggregation of misfolded proteins in the brain causing rapid neurodegeneration and ultimately death. CJD was identified in the 1920s, but a full century later, most cases still require evaluation of brain tissue in a post-mortem specimen to make a formal diagnosis.

Recent years have seen advances in anti-mortem testing such as the measurement of 14-3-3 and misfolded prion protein in cerebrospinal fluid, which are now the mainstays of CJD diagnosis in many countries. However, as CSF requires an invasive collection, researchers have explored the possibility of measuring prion protein in other sample types that are more convenient to collect but introduce additional technical and interpretive challenges.

What specimen types are under consideration? What new insights does it provide and does it make sense to implement in the patient care setting?

A perspective article appearing in the April 2024 issue of *Clinical Chemistry* describes the latest advances in the diagnosis of protein misfolding disorders, explores the use of tear fluid and other specimen types, and discusses the expected impact on the clinical laboratory.

In this podcast, we are pleased to speak with both authors of this perspective article. Dr. Mari DeMarco is a Clinical Chemist at St. Paul's Hospital and a Clinical Professor in the Department of Pathology and Laboratory Medicine at the University of British Columbia in Vancouver, Canada. Cyril Helbling is a Ph.D. student in the DeMarco Lab at the University of British Columbia.

And Dr. DeMarco, we're going to start off with you. Can you explain how seed amplification assays detect protein misfolding diseases like Creutzfeldt-Jakob disease?

Mari DeMarco: Seed amplification assays have quite a unique design from the perspective of the landscape of assays used in clinical laboratories to detect proteins. They are kinetic assays that rely on the formation of protein aggregates that's specifically formed in the presence of pathologies like prion diseases, which include Creutzfeldt-Jakob disease.

In Creutzfeldt-Jakob disease, a protein called the prion protein misfolds and aggregates. And while these aggregates form in the brain, there are also smaller version of these aggregates that are also found in other tissues and fluids like cerebrospinal fluid. Seed amplification assays aim to detect this misfolded structure in biofluids by first amplifying amount of the aggregate present, and this is really related to analytical sensitivity. So they do this by adding more of the building blocks of these aggregates, which are the monomeric version of that protein.

These monomeric building blocks are added to the patient's sample and then the sample is put under conditions that really promote aggregate formation. And then how we differentiate samples that have these endogenous aggregates present versus those that do not relies on the kinetics of aggregate formation. These assays are designed so that seeded amplification, that is when there's that aggregate present, has faster aggregation kinetics as compared to spontaneous aggregation that can occur in the absence of a pre-formed aggregate starting off the process.

It sounds a little easier than it actually is in practice as these assays require careful design and validation to ensure this separation between seeded versus spontaneous aggregation.

Bob Barrett: Well, I want to talk about these assays a little more. Are these seed amplification assays widely used and are they limited to prion diseases like Creutzfeldt-Jakob disease?

Mari DeMarco: Well, the assay design as I described might sound a little unusual. However, these assays are used around the globe for the detection of prion diseases. They are relatively complex to perform, particularly with high consistency. They also take a lot of time to run because of this aggregation phase that we're waiting for and this might take multiple days, 2, 3, 4 days before you get a readout with the assay.

As such, these assays are typically run in centralized labs such as by a country's CJD Surveillance Center and not routinely run in clinical labs. We are however seeing the continued expansion of the use of seed amplification assays to other protein misfolding diseases beyond prion diseases, most notably the synucleinopathies, which include

Parkinson's disease, dementia, dementia with Lewy bodies, and multiple system atrophy.

Seed amplification assays have performed well in this particular proteinopathy and are advancing into clinical care, really following in the footsteps of the prion seed amplification assays.

Bob Barrett: Well thank you, doctor. Let's get to our Ph.D. student, Cyril Helbling. Cyril, in the study you describe in your perspective article, the authors used tear fluid, which is different from most other studies. Can you help us understand just why they did it this way?

Cyril Helbling: Yes, so there are several reports on ophthalmological symptoms in Creutzfeldt-Jakob disease, disease transmission, and detection of misfolded prion protein in eye tissues that guided the authors to focus on tear fluid. First, there are reports of prion pathology affecting eye structures, resulting in visual disturbances and blindness in some of the sporadic Creutzfeldt-Jakob disease cases.

Also, in cases of iatrogenic transmission of prion disease due to cornea graft from person with Creutzfeldt-Jakob disease have been reported. All this led the World Health Organization to rank the eye in the high infectivity category in the Infection Control Guidelines for transmissible spongiform encephalopathies along with brain and spinal cord tissue.

Second, in cases of variant Creutzfeldt-Jakob disease, also known as mad cow disease, misfolded prion proteins have been detecting using immunohistochemistry in different eye structures, including the retina and the optic nerve.

Finally, another study used seed amplification assays to detect misfolded prion protein within eye structures of sporadic Creutzfeldt-Jakob disease. The retina and cornea had a relatively strong seeding activity compared to cerebrospinal fluid.

Bob Barrett: So what assay modifications were required in order to perform testing on tear fluid?

Cyril Helbling: In order to adapt to cerebrospinal fluid seed amplification assay to tear fluid, two major modifications were necessary. First, most common cerebrospinal fluid seeding assay use a truncated recombinant hamster prion protein sequence as monomeric source. For the tear fluid seeding assay, they used recombinant human full-length prion protein sequence with the E200K variant. This variant is interesting to use because in silico and in vitro studies suggest that this protein increases the misfolding and aggregation propensity of

monomeric prion protein into pathologically misfolded prion oligomers.

This modification may have been used to improve the analytical sensitivity of the tear fluid seed amplification assay. The need for greater analytical sensitivity can also be seen from the changing assay time from 50 hours for cerebrospinal fluid to 150 hours for tear fluid.

The second modification concerns the specimen collection procedure. Tear fluid was collected using a Schirmer test. A small strip of filter paper is inserted in the lower lid of the eye for about 10 minutes and then removed. For sample processing, the filter paper was cut into pieces and incubated with a reaction buffer. The tear fluid solution was then extracted by centrifugation and subjected to the typical seed amplification assay workflow.

Bob Barrett: Well, I guess the next obvious question is, so what happened? How well did this test method perform using this new matrix?

Cyril Helbling: So in the discovery and validation cohorts, the assay had a sensitivity in the range of approximately 78 to 85%. A specificity of 100% was obtained in both the discovery and validation cohorts. These cohorts were composed of individuals with sporadic, familial, and asymptomatic familial Creutzfeldt-Jakob disease as well as controls. More precisely, a positive response was obtained in 8 out of 9 and 4 out of 5 sporadic Creutzfeldt-Jakob disease patients in the discovery and validation cohorts respectively.

For the familial Creutzfeldt-Jakob disease patients, 3 out of 4 in the discovery cohort and 1 out of 1 in the validation cohort yielded a positive seeding assay signal. In the asymptomatic familial Creutzfeldt-Jakob disease group, 4 out of 5 and 2 out of 3 had a positive seeding assay interpretation. And none of the 26 controls in the discovery cohorts and none of the 68 controls in the validation cohort were positive to the tear fluid seed amplification assay.

Bob Barrett: Well finally Dr. DeMarco, let's look ahead. Will doctors be seeing an option to collect tear fluid instead of cerebrospinal fluid in the near future?

Mari DeMarco: Well, I do have to first emphasize that these are preliminary findings that we're discussing and so far un-replicated. And as Mr. Helbling noted there are still some shortcomings to overcome with this testing but the benefits of this ease of collection of tear fluid versus cerebrospinal fluid is striking and will drive continued investigations, I'm sure.

As far as clinical implementation, to put in context, patients with suspected prion diseases, they're likely getting a lumbar

puncture anyway to rule out other diseases of the central nervous system that could be causing their symptoms. Routine CSF testing is helpful in identifying other causes of rapidly progressive neurological decline that may present similarly to CJD.

As such, we generally have [CSF] available, or it's going to be collected for these patients anyway, and such a lumbar puncture is not really a large barrier to testing. So in the near future, perhaps where we would see such a development of seed amplification assays gaining greater traction and solving a current challenge would be in a clinical trial setting.

This could include improving screening protocols for those entering into a clinical trial, identifying those with or without the pathology. And again because of that ease of collection with tear fluid, being able to perform repeat collections become so much easier and this could become potentially a tool for monitoring response to therapy in that way. And as such really open up new workflows for clinical research, which is very exciting.

Also a great potential interest is the application of this type of fluid to other proteinopathies where seeding amplification assays have also shown to be successful in detecting protein aggregates in CSF. So can we translate this maybe to other proteinopathies?

I'm very much looking forward to future developments in this area whether it be for prion disease or related proteinopathies like Parkinson's disease and Alzheimer's disease.

Bob Barrett: That was Dr. Mari DeMarco and Cyril Helbling from the University of British Columbia in Vancouver, Canada. They wrote a perspective article in the April 2024 issue of *Clinical Chemistry* highlighting advances in testing for Creutzfeldt-Jakob disease and other protein misfolding disorders. I'm Bob Barrett. Thanks for listening.