Project Title: Improving the sensitivity of CSF RT-QuIC by examining cases with indeterminate results

Principal Investigator
PI: Jaime Noguez, PhD: CSF Section Head, National Prion Disease Pathology Surveillance Center, Case Western Reserve University, Cleveland, OH, USA
Director Collaborators:
Shashi Shetty, PhD, CLIA Lab Director, National Prion Disease Pathology Surveillance Center, Case Western Reserve University, Cleveland, OH, USA
Brian Appleby, MD, Director, National Prion Disease Pathology Surveillance Center

Objectives for Non-Treatment Related Study
Aim 1: Determine the final diagnoses of patients with indeterminate CSF RT-QuIC testing results through review of autopsy, clinical information, and death certificate data

Aim 2: Determine characteristics associated with indeterminate CSF testing results by examining demographic, specimen quality, and clinical characteristics of cases

Aim 3: Apply changes to RT-QuIC protocols to improve results in indeterminate cases

Aim 4: Apply changes in Aim 3 to false negative CSF RT-QuIC cases to improve assay sensitivity

Summary of Accomplishments:
Since 2015, the National Prion Disease Pathology Surveillance Center (NPDPSC) has offered the CSF RT-QuIC test, with the number of samples submitted increasing annually, reaching approximately 7,000 specimens per year. Analysis of test performance revealed that around 10% of autopsy-confirmed prion disease cases yield false-negative results, while approximately 1% produce indeterminate results. To enhance diagnostic accuracy—particularly for prion disease subtypes prone to false negatives—this project focused on characterizing indeterminate cases and investigating potential improvements.

Between 2016 and 2021, 211 cases were reported with indeterminate CSF RT-QuIC results. A comprehensive review of patient demographics and clinical data showed a near-equal male-to-female distribution and an average patient age of 65-66 years. Of these cases, 108 patients were confirmed deceased through database searches and obituaries. Among the deceased, 52 had a known diagnosis, with 36 cases confirmed as prion disease via autopsy. Surprisingly, several of these indeterminate results were linked to prion subtypes MM1-2, MM1, and MV1-2—subtypes typically well-detected by RT-QuIC. Subsequent RT-QuIC testing on brain homogenates from these cases demonstrated seeding activity in all, suggesting that indeterminate results may stem from either low concentration of abnormal prion protein in the CSF or the presence of inhibitory substances.

Further analysis of CSF RT-QuIC curves for the 211 indeterminate cases identified two primary curve types: Type A, characterized by a typical signal shape but below the positivity threshold, and Type B, featuring an uncharacteristic curve shape and delayed signal detection. These types were equally distributed among cases. CSF RT-QuIC requires each patient sample to be tested in quadruplicate, with results measured from four separate wells of a 96-well plate. Analysis revealed that Type A curves generally had more wells showing seeding activity and shorter lag times before signal detection compared to Type B curves. A simulation was conducted to assess the impact of reducing the RT-QuIC run time from the standard 60 hours to

30-40 hours. Preliminary data suggested that this modification could successfully eliminate Type B indeterminate results without compromising the test's ability to detect prion disease. Initial findings indicate that Type B indeterminate results may not be linked to prion disease, but further investigation is required to validate these observations.

To further enhance test accuracy, a preanalytical preparation step was developed using iron oxide magnetic bead extraction to concentrate abnormal prion protein in patient samples and remove potential inhibitory substances that could suppress RT-QuIC signals. This technique was applied to 17 of the 36 autopsy-confirmed prion disease cases that had originally produced indeterminate results. Re-testing with this enhanced specimen preparation enabled the reclassification of indeterminate results into definitive positive or negative outcomes. Notably, 12 of the 17 cases (70%) were reclassified as positive for prion disease, demonstrating the potential of this method to improve diagnostic clarity.

Key Findings and Implications

- At the NPDPSC, approximately 10% of autopsy-confirmed prion disease cases yield false negatives, while ~1% produce indeterminate results.

- This study found that indeterminate results were frequently associated with MM1-2, MM1, and MV1-2 subtypes upon autopsy, despite their typically strong detection rates in the CSF RT-QuIC test.

- Reducing the RT-QuIC run time may help eliminate late-lag-time indeterminate cases caused by non-prion disease conditions without compromising the detection of prion disease.

- Implementing a preanalytical sample preparation step using iron oxide magnetic beads shows promise for concentrating low levels of abnormal prion protein and removing potential interferences. This approach could decrease the number of indeterminate CSF RT-QuIC results, allowing for more definitive positive or negative classifications.

These findings reinforce CSF RT-QuIC as a highly effective diagnostic tool for prion disease while identifying key areas for refinement. The insights gained from this study will help guide future protocol enhancements to improve diagnostic accuracy, particularly for challenging prion subtypes. Additionally, these refinements have the potential to reduce the turnaround time from sample collection to result reporting, ultimately aiding clinicians and families in timely decision-making.

Next Steps

The findings of this study are promising but remain preliminary. Further experiments are necessary using more indeterminate samples to validate the results. If confirmed, these findings will be leveraged to enhance the clinical CSF RT-QuIC testing method at the NPDPSC, ultimately improving diagnostic accuracy and patient care. The results of this research will be submitted for publication.