Real-time Quaking-induced Conversion Assay for the Diagnosis of Sporadic Creutzfeldt-Jakob Disease in a Living Patient

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ABSTRACT: Creutzfeldt-Jakob disease (CJD) is the most common prion disease in humans with an incidence of one case per million inhabitants worldwide. The sporadic form of CJD (sCJD) is spontaneous and accounts for 85% of cases. Its symptoms include rapidly progressive dementia, ataxic gait, personality changes, myoclonus, coma, and eventually death. The challenging diagnosis is currently made by a combination of clinical criteria and supporting tests such as electroencephalography (EEG), magnetic resonance imaging (MRI) findings, and cerebrospinal fluid (CSF) studies. These modalities can be falsely positive or negative in some cases. Therefore, true confirmation usually requires a postmortem brain biopsy. We present a case of a 58-year-old woman who was diagnosed with sporadic form CJD by the novel Real-time Quaking-induced Conversion (RT-QuIC) assay. It is based on an ultrasensitive detection of the pathogenic prion protein in the CSF that directly detects a prion protein rather than a surrogate marker of neurodegeneration such as 14-3-3 or tau protein. The RT-QuIC assay has emerged as the most sensitive and specific CSF study to accurately diagnose sCJD in a living patient, without the need for invasive brain biopsy. The emergence of the nasal brushing RT-QuIC assay may further revolutionize the future of combating prion diseases.

KEYWORDS: Prion diseases, sporadic Creutzfeldt-Jakob disease, RT-QuIC assay, rapidly progressive dementia, proteopathies, diagnostic test, real-time quaking-induced conformation

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Case

A 58-year-old female with no previously known medical history was brought in by her daughter due to unsteady gait for the past 6 weeks and unable to ambulate without assistance. For the past week, the patient's mental status was declining with increased forgetfulness and disorganized speech. The patient had no history of falls or head trauma, headache, dizziness, fever, cough, night sweats, animal bite, or recent exposure to ill contacts. She has no history of tobacco, alcohol consumption, or use of illicit drugs. She was born and raised in Ghana and moved to the United States many years ago. Her last visit to Ghana was 2 years prior to hospitalization. Her vaccination history was unknown.

On presentation her temperature was 99.0° Fahrenheit (F), heart rate of 70 beats per minute (bpm), respiratory rate of 20 breaths per minute, blood pressure (BP) of 123/66 mmHg, and 97% oxygen saturation breathing ambient air. Her physical exam showed a well-groomed woman who was in no acute distress. There were no cranial nerve abnormalities. Her oropharynx was clear, moist, without oral thrush, and her neck was supple, without thyromegaly or lymphadenopathy. There was no nuchal rigidity. Her cardiovascular and pulmonary exams were normal. She had normal sensation, muscle tone, strength, and symmetric reflexes. She had a negative Romberg test. Brudzinski and Kernig signs were absent. She was noted to have positive dysdiadochokinesia and an ataxic shuffling gait. She was oriented to her name and that she was in a hospital but did not know the hospital name. She knew the year and month but not the day of the month. She could not understand why she was there and her affect was flat with dull mood. Mini-Mental State Examination (MMSE) score was 23/30.

Computed tomography (CT) scan of the head without contrast ruled out acute hemorrhage and showed an age-indeterminate left cerebellar lacunar infarct. She was treated for a possible cerebrovascular accident and placed on telemetry. However, the findings did not account for the acuity of change in mental status. Carotid Doppler ultrasound and echocardiogram were normal. Thyroid-stimulating hormone (TSH), vitamin B12, and folate levels were within normal limits and rapid plasma reagin (RPR) test was nonreactive. Urinalysis and urine drug screen were negative and blood work did not show any electrolyte abnormalities, renal dysfunction, or anemia. She had a reactive HIV-1 antibody test. This was determined to be a false positive after further testing confirmed by undetectable HIV RNA viral load and a CD4T-Cell count of 1449 cells/μL. Cryptococcal antigen was negative.

Due to the rapid decline in her mental function in the last 2 months, a lumbar puncture was performed to rule out infectious causes. Empiric intravenous acyclovir was begun to treat possible herpes simplex meningoencephalitis pending results.

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Cerebrospinal fluid (CSF) testing was performed for gram stain, culture, cell count, protein and differential, India ink stain, glucose, protein, herpes simplex virus (HSV) polymerase chain reaction (PCR), Venereal Disease Research Laboratory (VDRL), fluorescent treponemal antibody absorption (FTA-Abs), anti-N-methyl-D-aspartate (NMDA) receptor antibodies, and 14-3-3 proteins. The CSF was clear and colorless, and a total of 3 cells/μL were present, with 1 WBC cell/μL, 1 RBC cell/μL, and a differential of 100% lymphocytes. Cerebrospinal fluid protein was 40.7 mg/dL and glucose 71 mg/dL. Gram stain, culture, VDRL, and HSV PCR were negative. All serology, paraneoplastic, and vasculitis biomarkers returned negative and acyclovir was discontinued.

Fluid-attenuated inversion recovery (FLAIR) magnetic resonance imaging (MRI) sequence showed high intensity involvement of the caudate nucleus and putamen. Diffusion-weighted imaging (DWI) of MRI showed apparent increased signals of the bilateral supratentorial cortical ribbon and caudate nucleus (Figure 1). An electroencephalogram demonstrated diffuse slow wave activity. She was given supportive treatment for dementia. She was later discharged home on her daughter's request with home services and physical therapy. The 14-3-3 proteins CSF test result was pending.

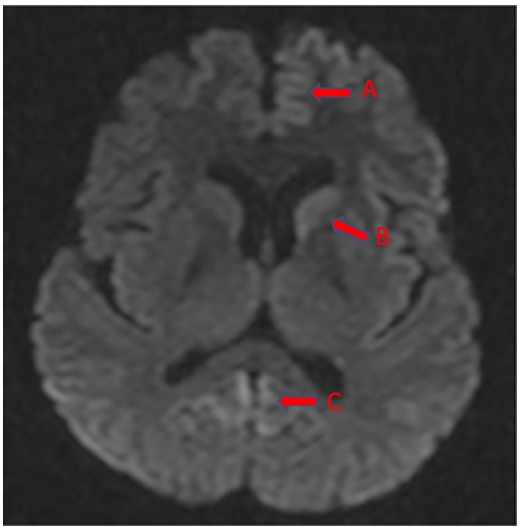

Figure 1. Faint areas of apparent scattered increased signals of the (A and C) bilateral supratentorial cortical ribbon and (B) caudate nucleus on DWI of MRI.

Two weeks later, she was brought to the hospital and readmitted due to further worsening of her mental status. She had fallen 3 times in the last 3 days without any head trauma. Although her vitals and chemistry panel were unchanged, she was more somnolent and had periodic myoclonic jerks of her extremities at irregular intervals. She developed severe bruxism and spasticity. She was not oriented to self, place, or time. Repeat CT and MRI imaging of the brain did not show any new changes. A repeat electroencephalography (EEG) was consistent with severe diffuse cerebral disease with 3 to 4 Hz triphasic waves seen throughout (Figure 2).

The 14-3-3 proteins study of the CSF sent out in her first admission returned positive. The National Prion Disease Pathology Surveillance Center reported a positive result of the Real-time Quaking-induced Conversion (RT-QuIC) assay and elevated T-tau protein levels. The diagnosis of sporadic form Creutzfeldt-Jakob disease (sCJD) was made and the Centers for Disease Control and Prevention (CDC) was notified. No brain biopsy was required for clinical management. The results and prognosis were discussed with her daughter and the patient was given supportive management and discharged to a long-term care facility.

The patient was readmitted 36 weeks later after diagnosis due to a percutaneous endoscopic gastrostomy (PEG) tube malfunction that required replacement. The infection had rendered her nonverbal and unable to consume adequate oral intake due to progressive spasticity and myoclonus. Although she was awake, there was deterioration in her mental status. Vital signs showed temperature of 98.7°F, heart rate of 73 bpm, respiratory rate of 18 breaths per minute, BP of 131/71 mmHg, and 99% oxygen saturation on room air. Her serum chemistry panel, complete blood count (CBC), and other routine labs were unremarkable. Due to her severe bruxism, a PEG tube placement could not safely be performed, even with deep sedation and general anesthesia. Discharge to hospice care was

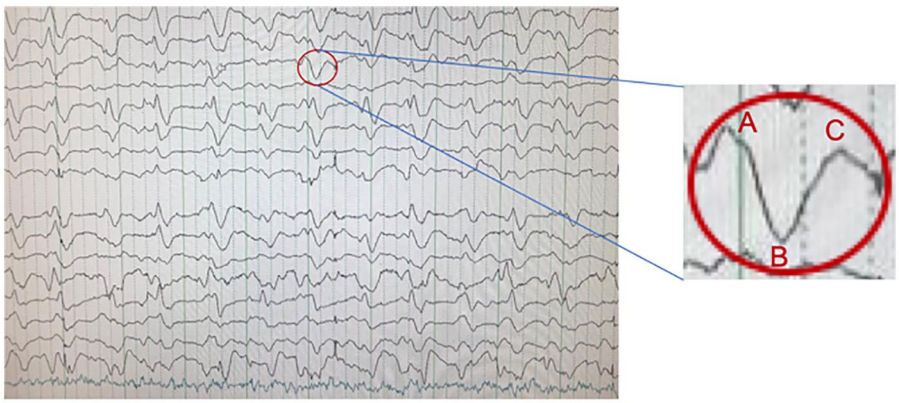

Figure 2. Electroencephalography (EEG) revealed 3 to 4 Hz triphasic waves with a (A) negative upward deflection, (B) positive downward deflection, and (C) negative upward deflection demonstrating diffuse cerebral disease.

deemed appropriate and comfort measures were recommended to her family. Unfortunately, the patient was lost to follow-up. Results of an autopsy and postmortem testing, if performed, could not be obtained.

Discussion

Proteopathies are a group of disorders in which highly infectious proteins are abnormal in both structure and function. These misfolded proteins are called prions and can cause conformational and functional changes in proteins around them and lead to severe damage of cells, tissues, and organs.[1] One such proteopathy is Creutzfeldt-Jakob disease (CJD), a subtype of a group of diseases known as transmissible spongiform encephalopathies (TSE). The process of detecting proteopathies in the past, specifically CJD, had been a combination of clinical criteria and a series of extensive testing, sometimes unreliable due to false-positive results and overlapping medical conditions. Clinically, CJD includes a wide array of neurological symptoms like rapidly progressive dementia, personality changes, cerebellar ataxia, and myoclonus.[2] Routine lab work including CBC, comprehensive metabolic panel (CMP), C-reactive protein, and blood cultures often do not aid in its diagnosis. Further testing like MRI, EEG, and CSF protein analyses are used for the diagnosis. The gold standard is the brain biopsy.

Magnetic resonance imaging (FLAIR and DWI) can show increased cortical signals and hyper-intensities in the basal ganglia and thalamic regions of the brain. However, a study showed that these signaling patterns varied among the different CJD subtypes and were not found in all patients.[3] Electroencephalographies show abnormal periodic sharp and slow wave complexes but are usually only observed 8 to 12 weeks after the onset of clinical symptoms.[4] Furthermore, both EEG and CSF 14-3-3 proteins were studied for the diagnosis of CJD on 1003 patients, of which only 805 were confirmed to have the disease. The results indicated that EEG had a sensitivity of 66% and a specificity of 74%.[5] CSF 14-3-3 protein detection showed a 94% sensitivity and 84% specificity for an accurate diagnosis.[5]

Other tests include detecting tau, neuron-specific enolase, and S100B to support the diagnosis of CJD. Diagnostic tests such as PCR and enzyme-linked immunosorbent assay (ELISA) are unable to detect prion particles as they do not contain genetic material. The most reliable form of diagnosing CJD depends on detecting neuronal changes such as spongiosis, astrocytic gliosis, and most importantly PrPSC deposition using immunohistochemical methods.[2] The modified western blot demonstrating proteinase K (PK)-resistant PrPSC is an additional definitive test that can be performed, including in cases when there are minimal spongiform changes present on histopathology.[6,7] The disadvantage of these tests is that their use is generally during the terminal stage of disease or in the postmortem state.

To detect the sporadic form of CJD (sCJD) at a preclinical stage, an ultrasensitive prion deduction technique called the real-time quaking-induced conformation (RT-QuIC) assay is becoming more prevalent.[8] The RT-QuIC technique was developed based on the prion proteins' ability of misfolding recombinant PrP (rPrP) to PrPSC and forming amyloid fibrils. The rPrP which mimics normal PrPSC is expressed in *Escherichia coli* as a substrate to amplify the seeded PrPSC.[9,10] The rPrP substrate is placed in 96-well plates with the seeded PrPSC and then heated and shaken vigorously to induce the formation of amyloid fibrils. These fibril aggregates can be detected using a dye called ThT. The constant shaking continues to break apart the fibrils and form more reactive PrPSC seeds at an exponentially increasing rate. The cycles of shaking and the interaction of the fibrils with ThT result in a change in its fluorescence emission spectrum that can be read in real time, expediting the process.

Investigations were conducted to assess the specificity and sensitivity of RT-QuIC by examining CSF samples with and without CJD and analyzing the same CSF sample in different laboratories to assess the reliability and accuracy of the results.[11] These investigations showed that the test had 96% sensitivity and 100% specificity for diagnosing sporadic CJD and was regarded as an effective antemortem diagnostic test in clinical practice when run on CSF specimens.[12,13] Another study showed that nasal brushing samples may be used to diagnose sCJD because olfactory neuronal cells are also infected by prions. The RT-QuIC assay revealed higher seeding activity and a stronger and faster response to olfactory mucosa than CSF with 97% sensitivity and 100% specificity.[14] However, sensitivity and specificity are dependent on the quality of the RT-QuIC assay done as many factors such as temperature, truncation of rPrP substrates, shaking speed, shaking interval, pH, and concentrations of denaturant and detergent can alter the outcome.[8,12]

This case report highlights the importance of using RT-QuIC in making an accurate diagnosis in patients at high risk or with neurological symptoms due to suspected CJD. Antemortem diagnosis is rare and conducting quarantine measures can be crucial, particularly when acquired (vCJD) and familial (fCJD) forms of the disease must be excluded. There is no therapy for this ultimately fatal infectious disease and prion particles remain nearly indestructible. Using RT-QuIC with correct technique when setting the assay parameters is necessary for a timely diagnosis and may reduce prion transmission risk by providing valuable data to the CDC. The advent of the nasal brushing RT-QuIC assay may further revolutionize the future of combating prion diseases.

Author Contributions

All authors contributed equally to this work. Conception and design of case report: VDR, AH, NS, SJ, SR, MES, JB; acquisition of data: VDR, AH, NS, SJ, SR, MES, JB; analysis and/or interpretation of data: VDR, AH, NS, SJ, SR, MES, JB. Drafting the manuscript: VDR, AH, NS, SJ, SR, MES, JB; revising the manuscript critically for important intellectual content: VDR, AH, NS, SJ, SR, MES, JB. Approval of the

version of the manuscript to be published: VDR, AH, NS, SJ, SR, MES, JB.

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