Emerging Modalities that Support Differential Diagnoses of Creutzfeldt-Jakob Disease and other Neurodegenerative Disorders

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Abstract

Creutzfeldt-Jakob disease (CJD) is a transmissible spongiform encephalopathy (TSE) that afflicts humans. Like all TSEs, CJD belongs to a family of incurable neurodegenerative disorders characterized by the accumulation of misfolded prion proteins in the central nervous system. Due to the similarities between CJD and other neurodegenerative diseases, such as an extremely low rate of incidence and older age of onset, CJD is frequently misdiagnosed as a rapidly progressive dementia disorder. This review examines several different case reports that highlight the challenges in differentiating CJD from another neurodegenerative diseases. Secondly, as no test exists that can reliably diagnose a living individual suspected of CJD, this review investigates techniques that are currently being developed for CJD diagnosis that are ante-mortem. Recent studies indicate that new accurate and sensitive approaches, such as neurofilament biomarkers and prion seeding assays, introduce a promising outlook into the diagnostic capacity for CJD. Indeed, properly discriminating CJD and its variants from other diseases is important for a patient's subsequent treatment plan and the family's peace of mind. Recent studies indicate that neurofilament light chains are a promising surrogate biomarker candidate in the field of TSE diagnostics.

Introduction

As with all transmissible spongiform encephalopathies (TSEs), CJD is caused through the accumulation of misfolded prion proteins in the central nervous system. Scrapie prion isoforms (PrP^{Sc}) are the pathogenic form of normal prion proteins (PrP^{C}) and induce a conformational change in PrP^{C} alpha-helical structure to that of beta-pleated sheets, resulting in the formation of indigestible amyloid plaques. Seeding between cells produces a highly augmented conversion of PrP^{C} to PrP^{Sc}, although the mechanism remains nebulous. PrP^{C} may sporadically misfold, leading to a prion disease (1). According to the Centers for Disease Control and Prevention, sporadic CJD (sCJD) has a disease rate of approximately 1 case per 1 million people of the total population per year (2). Even more terrifying, 85% of all cases are sporadic, meaning that PrP^{C} misfolds without ingestion of infected meat, familial history, or contaminated medical instruments.

Many symptoms of CJD are similar to those of other neurodegenerative diseases. For example, some patients with CJD experience a shuffling gait, where the patient never fully lifts his/her feet off the ground to ambulate (3). A shuffled gait is one of the most common presentations of Parkinson's disease (PD). Other shared manifestations of neurodegenerative disease include ataxia, severe cognitive decline, a personality change, amnesia, myoclonic jerking, difficulty swallowing/speaking, coma, and eventual death (4). Discerning between other neurodegenerative disorders and CJD on a molecular level without a reliable test is difficult enough, but the heterogeneity of symptoms for all aforementioned pathologies only compounds the diagnosis. The challenge for health care providers lies in the discriminating CJD from other neurodegenerative diseases with the same symptoms. A screening technique that can detect CJD antemortem is essential.

Researchers and clinicians attempt to identify CJD in two phases. First, they extract blood serum or cerebrospinal fluid (CSF) from potential CJD patients to look for a possible surrogate biomarker, such as neurofilament light chains (NFL). Secondly, standard assays, like RT-QuIC, amplify any detected amount of PrP^{Sc} found in either the CSF or blood serum samples to obtain a final diagnosis. If there is PrP^{Sc} is present, even at miniscule quantities, then amplification should confirm its presence (5). This article examines several studies that assess the application of NFL as a surrogate biomarker, as well as the application of RT-QuIC assays to facilitate the conversion of PrP^{C} to PrP^{Sc}.

Biomarkers are measurable biomolecules found in bodily fluids or tissues that can be signs of normal or abnormal biological conditions (6). They are an integral part of medicine, with applications in clinical and research settings and must be reliably reproduced for objective measurement. Unfortunately, prion diseases are one group of disorders that remains without an officially accepted biomarker. Ascertaining a clinical end point for patients suffering from CJD is difficult without a measurable outcome, leaving patients and their families in a state of uncertainty. Abu-Rumeilah et al. claims that the insufficient availability of reproducible biomarkers for CJD significantly limits the ability of clinicians to apply a broad screening program (7). Currently, 14-3-3 protein, t-tau, and NFL are some of the most researched biomarker candidates for CJD diagnosis. Moreover, 14-3-3 protein is the only candidate blood biomarker authorized by the World Health Organization (WHO) (5). As auspicious as these candidate biomarkers appear, most are still combined with a secondary assay to confirm prion activity. Research continues to explore the clinical and research utility of NFL as a biomarker for CJD.

This review aims to provide a critical assessment of current literature that discusses emerging diagnostic modalities for CJD. An ideal examination would be sensitive and specific for CJD and able to distinguish the proteopathy from other neurodegenerative diseases with comparative presentations.

Methods

The databases utilized for this research included PubMed, sourced exclusively through the Geisinger Commonwealth School of Medicine library collection, and Google Scholar. The following keywords and terms were used to facilitate the literature search: *Creutzfeldt-Jakob disease, proteopathy, neurofilament light chain, biomarker,* and *transmissible spongiform encephalopathy*. Care was taken to ensure that the articles chosen were recent and relevant to the topic, with the oldest source utilized having been published in 2009.

Discussion

Discriminating CJD from other proteopathies

CJD shares many symptoms with other more common degenerative diseases, like Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and dementia disorders. Patients experience a personality change, cognitive decline, ataxia, loss of gross motor faculties, and eventual death. The changes associated with CJD, however, are more pronounced and sudden when compared to AD, ALS, and other disorders (8). This review investigated multiple case reports that display the challenges of neurodegenerative diagnosis. Case reports often cite the complications in identifying CJD from a pool of non-specific symptoms that may apply to multiple neurodegenerative conditions. One report discussed a case of CJD in an otherwise healthy 76-year-old male who, following a 6-month period of rapidly progressive dementia, presented a wide range of neurodegenerative symptoms including: ataxia, aphasia, myoclonus, akinetic mutism, hallucinations, and progressive dementia. The patient expired within 8 weeks of hospitalization, and an autopsy was performed. Postmortem tissue analysis confirmed that he had CJD. Pathologists reported micro-spongiosis, the classic hallmark of TSEs, as well as neuronal death, and gliosis of the basal ganglia, hippocampus, cerebellum, and cortex. This patient had no family history of dementia or CJD, as was the situation with two other case reports (9). These involved patients who possessed no known family history of dementia, CJD, or malignancy. Both investigative teams relied on supplementary tests to support a probable CJD diagnosis for each patient, although none of these assessments officially confirm the presence of a prion disease (10 ,11).

Diffusion-weighted magnetic resonance imaging (DW-MRI) can reveal much about the pathological changes of a patient's brain and is regarded as one of the most valuable techniques for investigating a possible CJD case. Different variants of CJD often exhibit subtle but distinct characteristics that may show up on a DW-MRI. DW-MRI combines routine MRI imaging with the diffusion of water molecules over the brain; movement/localization of the water molecules contrasts with the original MRI, exposing the brain's topography. Regions of high neuronal loss, as is seen in CJD, will reflect intense regions of water perfusion (12). Kwon et al. utilized DW-MRI using gadolinium for assessing a 76-year-old male patient who experienced an abrupt decline in cognitive and physical functioning over a 2-week period. The patient passed away only 5 weeks after symptom onset. A DW-MRI found bilateral hyperintensities of the cortical gyri, which is a common characteristic of sCJD (10). Tomizawa et al. encountered an extremely unique case in which a patient with genetic CJD (gCJD) mimicked dementia with Lewy bodies (DLB). A 75-year-old female patient deteriorated over the course of 3 years, experiencing dementia, parkinsonism, depression, rigidity, bradykinesia, and a resting hand tremor. Although she initially scored a 15 on the Mini Mental State Examination (MMSE), her disorientation and cognitive dysfunction continued to decline, and her score plummeted to 4 when tested again at age 77. Genetic counseling concluded that a valine-to-isoleucine point mutation at codon 180 of *PRNP* had resulted in gCJD. Her case is unusual due to the unique nature of her DW-MRI results. In cases where sCJD has been reported to mimic DLB, hyperintensities had consistently shown up early during surveillance of a patient. This patient, however, exhibited radiological data accordant with patients who have suffered from DLB for almost 3 years. Cortical hyperintensities were not found until 39 months after she had presented at the hospital. These findings make sense, as gCJD has been known to have a slower pathogenic course than other CJD variants. Information obtained from DW-MRI findings could help discriminate between the various forms of CJD (11).

Although DW-MRI is heralded as an excellent diagnostic tool for detecting some characteristics associated with CJD, it is just one of many ancillary assessments that are performed to extract supportive data for a probable CJD case in question. None of these preliminary tests provide data that is clinically accepted as criteria for a positive CJD diagnosis. A sensitive and specific test for CJD has yet to be created, but there are several emerging procedures reported in the literature that have had promising experimental results.

Neurofilament light chains

NFL is a subunit of a heteropolymer known as a neuronal intermediate filament (NIF), which is a major constituent of the axon architecture and the most plentiful feature of mature neuronal cytoskeletons. NFL are highly localized to axons, where they support axonal strength and growth. Elevated NFL concentrations may indicate the presence of axonal and white matter degeneration. Several studies have examined the potential for NFL to act as a biomarker for CJD when extracted from blood and CSF samples. In a study comparing NFL concentrations among two independent cohorts composed of patients suspected of sCJD, NFL levels were significantly increased in individuals confirmed to be positive for sCJD. Each cohort contained non-sCJD patients who, although initially suspected of sCJD, were excluded because their supplementary test results did not meet the diagnostic criteria for a probable prion disease. Investigators noted that when compared to all other neurodegenerative disorders, NFL concentrations were highest in cases of sCJD. NFL might be a candidate biomarker for the differential diagnosis of sCJD (13).

Wang et al. conducted a meta-analysis of 36 studies that examined NFL efficacy as a biomarker in blood and CSF. Axonal degeneration in the CNS resulting from neurodegenerative disorders disperses NFL into the interstitial fluid surrounding the neurons, which eventually migrates into the blood and CSF. Because the populations differed across a variety of laboratory locations, investigators utilized a ratio of means method to approximate the difference in NFL concentration between controls and cases. NFL levels

were found to be significantly elevated among cases of dementia, ALS, Huntington's disease, and CJD. However, NFL concentrations were not significantly increased in cases of PD, but they were increased in PD-related disorders. The investigation concluded that NFL may be a promising biomarker for a differential diagnosis between certain neurodegenerative disorders, but not without the supplementary assessments or presentation of clinical symptoms (14). These findings are congruent with results from another investigation that compared the diagnostic potency of 3 different biomarkers: 14-3-3, t-tau, and NFL. Abu-Rumeileh et al. found that NFL performance was highly specific to distinguishing between the different forms of CJD. It was evident that the degree of sub-cortical pathology strongly affected the concentrations of NFL, possibly explaining why NFL was so able to differentiate between different types of CJD. However, NFL did not perform to the same degree of sensitivity as t-tau, which had an overall sensitivity of 91.3% for all CJD types. Summarized, studies show that NFL biomarkers have the potential to contribute to a differential diagnosis of CJD but may not be appropriate to alone definitively confirm a prion disorder (7).

As mentioned, axonal degradation disseminates NFL particles in the CSF and blood (13). Countless diseases and pathological phenomena can be screened using a blood sample containing some measurable biomarker. Utilizing a blood biomarker for CJD diagnosis might prove more pragmatic and less invasive than having to extract CSF for analysis, which involves a lumbar puncture (18). Currently, the WHO has yet to endorse a blood biomarker capable of detecting CJD. If NFL fragments from axonal degeneration leaked into the interstitial fluid and migrated to the CSF and blood, then the concentrations of both compartments should be comparable. Thus, a blood sample could be representative of prion activity in the central nervous system (15). Steinacker et al. looked at the difference in NFL levels between blood serum and CSF samples. The retrospective study called for CSF and serum samples to be taken from patients 103 patients in total: 60 controls, both with and without dementia, and 43 cases of CJD. It should be noted that of the 43 cases, 4 had been labeled as probable, but not validated neuropathologically. Of those individuals neuropathologically authenticated to be CJD positive, increases in serum NFL corresponded to measurements of increased NFL found in the CSF. Perhaps blood serum could provide an alternative to CSF extraction (5).

Real-time quaking induced conversion assay

One case report describes a living patient who was diagnosed with sCJD using an experimental RT-QuIC assay. This technique is interesting because RT-QuIC does not detect a surrogate biomarker, such as NFL or 14-3-3, but the PrP^{Sc} itself. The assay is quickly becoming one of the most specific and sensitive modalities to detect CJD antemortem using a CSF sample. Unfortunately, the patient passed away before a follow-up could ensue and any postmortem biopsy of her neural tissue could not be found, so sCJD could not be precisely diagnosed. However, an RT-QuIC assay of her CSF sample proved positive for sCJD, as confirmed by the National Prion Disease Pathology Surveillance Center. Investigators utilized the standard procedure for CJD diagnosis via a RT-QuIC assay.

The 58-year-old female patient presented to the hospital after a 6-week history of serious decline. Her daughter disclosed that her mother had experienced an unsteady gait and general confusion for the past 2 months. Within the last week, the patient's walk worsened to an ataxic "shuffled" gait, and she displayed dysdiadochokinesia, the inability to execute quick and alternating gestures. The investigative team performed a lumbar puncture to extract CSF for the study. The patient displayed myoclonic jerks, bruxism, and a total loss of orientation with regards to time, space, and self over the course of the study.

RT-QuIC assays exploit the seeding mechanism of PrP^{Sc} by exposing patient-derived samples of CSF, assumed to contain PrP^{Sc}, to recombinant PrP (rPrP), a surrogate substrate that imitates PrP^{C} found naturally in the brain. If PrP^{Sc} is present, then it should amplify the rapid conversion of rPrP to PrP^{Sc}, eventually resulting in aggregations of amyloid fibrils. The protocol involves distributing rPrP substrate across a 96-well plate already seeded with $rPrP^{Sc}$ samples; experimental RT-QuIC usually utilizes $rPrP^{Sc}$ to seed as well (3). It should be noted that rPrP can only be sourced from truncated recombinant protein, from the vole rodent, if investigators plan to seed the recombinant protein with PrP^{Sc} (16). The aforementioned investigative team most likely seeded each well with the patient's CSF sample. Applied heat and vigorous shaking of the wells causes the amyloid plaque formation and degradation to occur at a faster rate. PrP^{Sc} seeds continue to convert rPrP, generating even more seeds at an exponential rate. Amyloid plaques are identified using thioflavin T dye (ThT), which contains fluorophores that bind to amyloid substrates and generate a measurable fluorescence spectrum that can be interpreted in real time. However, the study did report that limitations were imposed by several factors. First, not possessing autopsy results of the patient's neural tissue made it impossible to verify the RT-QuIC results. Second, there are many possible factors that could contribute to variable results in future RT-QuIC analyses, such as speed and temperature of thermal mixer appliances, human error in distribution of seeds across a well-plate, pH of the solutions, and intervals of shaking (3).

Timed protein seeding assay

The process behind a RT-QuIC amplification assay is similar to a timed protein seeding assay, which also aims to quantify the conversion of PrP^{C} to PrP^{Sc} in real time. Quantification is measured and interpreted via a fluorescence spectrum graph. Gray et al. sought to investigate the in vitro infectivity of prions in elk neural tissue; chronic wasting disease is a prion disorder that afflicts cervids. This study is novel because amyloid fibril formation has not yet been timed in non-human animals. As the paper mentions, RT-QuIC assays and amyloid seeding assays both utilize rPrP as a substrate for conversion by PrP^{Sc}. Researchers combined a sample of rPrP with varying brain homogenate solutions that were either positive or negative for PrP^{Sc}. Should the sample homogenate be positive for PrP^{Sc}, then the conversion rates would be identified on the ThT signal times on the fluorescence spectrum. The study concluded that the ThT signal times are the specific diagnostic component of the assay (17).

Conclusion

In summary, there is still a significant amount of research to be conducted before a sensitive and specific CJD diagnostic test can be employed for routine use. Scientists have made impressive strides in the field of prion research, exploring different biomolecules and their capacity to serve as potential biomarkers. Although NFL have demonstrated a role as a potential biomarker for the differential diagnosis of CJD subtypes, such as sCJD and gCJD, they do not perform to the same caliber as other neural substrates, such as t-tau or 14-3-3. NFL might be a good tool to combine with the latter substrates to maximize the precision of a CJD diagnosis.

Although other substrates have proven more effective at diagnosing CJD outright, such as protein 14-3-3 and t-tau, NFL has proven the most specific to the subtype of CJD. Additionally, researchers have continued to refine commonplace laboratory techniques for the application of prion detection. RT-QuIC assays are used to amplify the concentration of PrP^{Sc} from a patient sample by exposure to a concentration of PrP^{C}, indicating the absence or presence of prions. A diagnostic test is urgently needed to provide patients and their families an answer antemortem. Furthermore, a proper examination procedure would allow health care providers to provide their patients with the best care possible, as well as develop new treatments.

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