

Supplement: Clinical features, disease course, supporting investigations and diagnoses of variant Creutzfeldt-Jakob disease patients

The diagnostic criteria used for variant Creutzfeldt-Jakob disease are those published by the World Health Organization and discussed further by Heath *et al.* (World Health Organization, 2003; Heath *et al.*, 2010). The terminology used to classify Western blot patterns for disease-associated prion protein follows published criteria (Hill *et al.*, 1999; Parchi and Saverioni, 2012).

Case 1

A thirty-three-year-old Saudi engineer with a past medical history of remote tonsillectomy and aseptic meningitis in 1998 presented in December 2003 with a 10-month history of psychiatric symptoms, and cognitive and neurological dysfunction. In February 2003, his family first noticed increasing irritability, withdrawal and agitation. His symptoms progressed to paranoia, bizarre behavior, workaholism and sleep/wake cycle disturbances. By October 2003, he had auditory hallucinations, increased depressive symptoms, slurred speech, non-fluent aphasia, a hyperkinetic movement disorder (mixture of chorea and dystonia), an unsteady and scissoring gait, and sensory symptoms causing self-mutilation and cellulitis. His grandfather had died of dementing illness in his 80s.

At admission, he had global cognitive impairment with verbal output limited to incomprehensible speech that was preceded by slurring and characteristically high-pitched sounds. He had normal extraocular movements, facial symmetry, and deep tendon reflexes. Plantar responses were flexor, but he had mild spasticity and paratonia in all limbs.

Extensive studies of blood, urine, and cerebrospinal fluid were non-diagnostic. The CSF was weakly reactive for 14-3-3 protein by Western immunoblot, and was scored as negative. Electroencephalography revealed only diffuse, non-specific slowing. *PRNP* gene analysis demonstrated homozygosity for methionine at codon 129 and no pathogenic mutations. Brain magnetic resonance imaging (MRI) showed increased T2 and FLAIR hyperintensities in the posterior thalami, consistent with the pulvinar sign and supporting a diagnosis of probable vCJD. Tonsil biopsy was not possible due to distant tonsillectomy. A right frontal cortical biopsy revealed vacuolation and strong deposition of PrP^{Sc} in florid plaques, with Western immunoblotting demonstrating Type 2B proteinase K-resistant PrP^{Sc}, diagnostic for definite vCJD.

Over the next 8 months, his condition deteriorated to an akinetic mute state complicated by frequent aspiration pneumonias. He was treated supportively and experimentally with chlorpromazine and quinacrine. His condition continued to deteriorate and he remained in an akinetic-mute state for seven years until he died in 2010. No autopsy was performed.

Case 2

A 23-year-old health-professional student with no significant medical history was referred to the University of California, San Francisco Medical Center in November 2006, following a six-month history of progressive neurological decline in multiple domains. His clinical syndrome had begun in April 2006 with insomnia, decreased concentration, and episodes of uncontrollable and inappropriate laughter. Additional symptoms included delusions (ideas of reference),

impairment of short-term memory, and palinopsia. One month later, he developed extrapyramidal symptoms – difficulty initiating gait, and dystonic posturing of the right hand while walking. By July, he required assistance walking due to ataxia, and was wheelchair-bound one month later. His cognition deteriorated, affecting his language. He began speaking in short phrases, and later in monosyllabic utterances. Seven months after onset, when seen at UCSF, he was globally aphasic, had no blink to threat, but had intact brainstem reflexes. His motor examination revealed spasticity of all extremities, withdrawal of the arms and triple flexion in the legs with painful stimuli, and no purposeful spontaneous movements. Occasional myoclonic jerks occurred in all limbs. Deep tendon reflexes were brisk with some spread, including a jaw jerk and extensor plantar responses.

An extensive panel of CSF and serum tests for viral, bacterial, fungal, mycobacterial, and parasitic agents proved non-diagnostic. Evaluations for a wide range of toxic exposures, autoimmune, endocrine, and metabolic disorders were also negative. CSF 14-3-3 protein showed weak immunoreactivity, and was scored as negative. *PRNP* gene analysis demonstrated homozygosity for methionine at codon 129 and the absence of pathogenic mutations. Electroencephalography revealed marked background slowing, intermittent triphasic complexes with anterior predominance, and no epileptiform activity. Magnetic resonance imaging of the brain revealed abnormal T2/FLAIR hyperintensity in the bilateral thalami, putamina, and bodies of the caudate, consistent with the pulvinar sign. Restricted diffusion was also seen in these regions, as well as within the left superior and middle temporal gyri, and the superior margin of the right cingulate gyrus.

Due to high suspicion for vCJD, brain and adenoid (due to tonsillectomy) biopsies were performed. Lymphoid follicles in the adenoid tissue demonstrated positive staining for PrP^{Sc}, consistent with probable vCJD. Immunohistochemistry of brain (X frontal) cortical tissue for the PrP^{Sc} protein (3F4 antibody following hydrolytic autoclaving) (Kascsak *et al.*, 1987; Hill *et al.*, 1999) demonstrated widespread PrP^{Sc} single plaque-like masses and clusters as well as pericellular encrustations of stellate-shaped cells with PrP^{Sc} deposits around astrocytes, capillaries, and clusters of plaque-like structures, consistent with vCJD. As with Case 1, H&E stained neocortex showed spongiosis and florid plaques. The patient was discharged to home hospice and passed away eight months after onset of obvious symptoms, without autopsy.

Case 3

A 23 year old right-handed gentleman presented with a one-year history of social withdrawal and a marked decline in spontaneous verbal fluency. Six months after the onset of behavioral changes he developed difficulty walking and gait instability, leading to his first falls around 8 months after onset. He gradually lost the ability to walk independently without falling. Speech deteriorated to the point that he could barely stutter short words. He developed intermittent urinary incontinence and occasional bowel incontinence. He became dependent for all activities of daily living. Around a year after his first symptoms he had made attempts to bite his mother and had several episodes of inappropriate laughter. There were no clear hallucinations, but at times he rubbed his hands as if cleaning something off of them. At the same point in time he lost his appetite and significant weight. There was no history of obvious pain apart from a recent suggestion of headaches based on his gestures and expressions.

His family history was unremarkable apart from his having a healthy identical twin brother, with the same epidemiologic history although it was unclear if his brother also had visited England in 1996. There was no history of drug or toxin exposure and he was sexually active until the beginning of his disease. He was not a vegetarian. His vaccination schedule was complete and up to date. There was no history of skin rash or other systemic complaints.

His neurological examination 12 months after onset was remarkable for absent spontaneous verbal fluency and severe compromise in naming, repetition, and comprehension. He could only reproduce simple sounds. He had significant cognitive and motor impairment and perseveration, as well as parietal and waxy flexibility. He scored 6/30 on the MoCA, identifying the three animals, correct clock drawing, and orientation to year and city. There was possible complete astereognosia in the left hand and mild astereognosia with his right hand; he was not able to name any objects with his left hand, and could name three simple objects from five that were presented to the right hand. His cognitive impairment might have influenced these findings. There was moderate ideomotor and visuospatial apraxia. He had difficulty controlling the position of the left hand in the space with a significant left drift, and paresis compatible with occipitoparietal and frontal deficits. There was mild dysmetria with the right arm and leg. There was full range of extraocular movements, but difficulty generating saccades particularly to the left, and pursuit was compromised by motor impairment and inattention. Deep tendon reflexes were normal. Frontal release signs, enhanced startle reflex, and myoclonus were absent. Vibration and tactile sensation seemed preserved.

CBC, ESR, B12, glucose, liver function test, CK, creatinine, sodium, potassium, chloride, protein electrophoresis, TSH, anti double-stranded DNA antibody, C3, C4, complement, serum protein electrophoresis, serology for Lyme's disease, RPR, HIV, and urine 24 hour copper were normal or negative. CSF findings were negative or normal including protein 47mg/dl, IgG index, OCBs, and weak immunoreactivity for 14-3-3 protein by immunoblot that was scored negative as it fell below cutoff. EEG at 10 months after symptom onset showed general slowing with no rhythmic or epileptiform activity. At 13 months, EEG showed additional intermittent focal non-epileptiform disturbances affecting much of the right frontal, central and temporal regions, consistent with generalized encephalopathy, but no PSWCs.

Two brain MRI scans done at 10 and 13 months after onset showed T2 and DWI hyperintensity and ADC map hypointensity involving right frontal and parietal and bilateral occipital cortices with bilateral, symmetrical involvement of the putamen, caudate, and posterior-mesial thalamus (more in the pulvinar, configuring the hockey stick sign) (Supplementary Figure, panel F). Prion gene (*PRNP*) sequencing revealed homozygosity (ATG/ATG) encoding methionine (Met/Met) at codon 129 and heterozygosity (GAG/AAG) encoding glutamate and lysine at codon 219, with no pathogenic mutation. Tonsillar tissue histopathology showed accumulation of prion protein in follicular dendritic cells and macrophages within germinal centers, supportive of a probable diagnosis of variant Creutzfeldt-Jakob disease. Western blot of a tonsillar biopsy (3F4 antibody) was positive for protease resistant prion protein (PrP^{Sc}) with a glycosylation pattern consistent with Type 2B PrP^{Sc}. The relative intensities of the di-, mono- and unglycosylated bands of immunoreactivity on this blot were also consistent with the 4t glycosylation type as *per* the terminology of Hill *et al.* (Hill *et al.*, 1999).

The patient's history combined with the results of neuroimaging, lumbar puncture, EEG, blood tests, genetic testing, Western blot and tonsillar histopathology thus support a diagnosis of probable vCJD. At 13 months after onset, he was started on oral doxycycline 400mg daily. By 14 months after onset of symptoms, he had rapidly become mute with severe behavioral disturbances such as agitation. He remained in this state until he died in 2011, approximately 18 months after onset.

Supplementary references

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