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Prion Disease: A Challenging Diagnosis

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Abstract

Introduction: Human prion diseases are a group of rare encephalopathies resulting in rapidly progressive dementia and ultimately death. While there are no effective treatments for any form of prion disease, prompt and efficient diagnosis is essential to prevent the spread of the self-propagating protein, which may occur through aerosols, and avoid unnecessary or invasive testing. Diagnosis relies largely on physical examination, with many nonspecific findings, and laboratory testing, which has wide ranges of reported accuracy and high false positive rates with diseases such as Alzheimer's dementia.

Methods: Patients who underwent testing for prion disease were retrospectively identified from the electronic health records at a single-center university hospital. Presenting symptoms, as well as laboratory, radiographic, and electroencephalogram findings, were recorded and analyzed by group of final diagnosis, including prion disease, not prion disease, and undiagnosed.

Results: There were 27 patients identified, two who had a final diagnosis of prion disease, 20 who had a formal diagnosis other than prion disease, and five who remained undiagnosed until death. There was a high degree of overlap in presenting symptoms. A high rate of false positive laboratory values, higher than previously reported, occurred for both the protein 14-3-3 and total Tau in cerebrospinal fluid. Magnetic resonance imaging ruled out prion disease most often. Testing with Rt-QuIC was the most diagnostic laboratory test. Both patients with a diagnosis of prion disease developed pneumonia and died of respiratory failure, and a total of nine patients required intubation for respiratory infections.

Conclusion: Diagnosing prion disease remains a challenge due to nonspecific physical exam findings and symptoms and the high false positive rates of the laboratory algorithm. Testing with Rt-QuIC should be performed in patients that are critically ill or may have diseases known to cause high false positive rates of 14-3-3 or total Tau. Proper personal protective equipment should be used for any aerosol-generating procedure in patients who may have prion disease.

Introduction

Human prion disease is a group of rare, spongiform encephalopathies caused by alteration of a naturally occurring protein resulting in rapidly progressive dementia, neurologic symptoms, and ultimately death.[1-4] Prion disease in humans is a transmissible spongiform encephalopathy known as Creutzfeldt-Jakob Disease (CJD) and can be broken down into sporadic (sCJD), familial, acquired (including iatrogenic), and variant (vCJD).[2, 5] Of all subgroups, sporadic CJD (sCJD) comprises roughly 85% of cases and is the most widely studied.[2]

While there are no effective treatments for any form of CJD, prompt diagnosis is essential to prepare both the patient and family for the inevitable outcomes, to prevent unnecessary testing, and to prevent iatrogenic spread of the self-propagating, aberrant prion protein.[6-9] While most commonly spread through direct contact with instrumentation contaminated with central nervous system (CNS) contents, it has been demonstrated that aerosolized prion protein can infect mice with high efficiency.[10] The spread of CJD is rare but deadly, and the safest and most unambiguous cleaning of surgical equipment requires incineration.[8] To ensure proper decontamination and safety precautions, a difficult diagnosis must first be made.

To definitively diagnose sCJD, a brain biopsy must show aberrantly folded prion protein (PRP^{sc}).[11] Since a brain biopsy is strictly diagnostic and has no bearing on treatment, a number of clinical criteria have been developed to classify *probable* and *possible* prion disease that largely rely on physical examination.[11, 12]

Physicians must have knowledge of the presentation and diagnosis of prion disease to properly treat patients, decontaminate instruments properly, and prevent the spread of CJD. We retrospectively identified patients at a single center who underwent testing for prion disease. We sought to identify and characterize the incidence of specific presenting symptoms and physical exam findings and investigate the efficacy of the laboratory testing algorithm used at our institution.

Methods

This study was approved by the University of Louisville Institutional Review Board (IRB number 17.1323), and informed consent was waived as this was a retrospective study.

Patient population

Patients were identified using electronic health records at the University of Louisville Hospital between 2014 and 2019. Patients were included if they had a CSF sample analyzed for markers of prion disease. Electronic health records of readmissions were also reviewed, and local obituaries were searched for deaths after admission.

Study definitions

Diagnostic group was determined per the Centers for Disease Control and Prevention (CDC) guidelines for diagnosis of sCJD and vCJD, presented in **Figure 1**.[13, 14] Patients without a diagnosis, CJD or otherwise, at the time of death were separated into the *undiagnosed group.*

MRI criteria

Positive criteria by magnetic resonance imaging (MRI) were defined by Fragoso *et al*. and the CDC.[2, 13, 14] These results were further separated into *typical* findings, corresponding to the most commonly identified MRI signs in sCJD, and *atypical* findings, corresponding with findings indicative of vCJD or the sCJD variants.

EEG criteria

Positive electroencephalogram (EEG) criteria were defined as the presence of periodic sharp wave complexes.

Data collection

Data were collected on 9 cognitive symptoms, 4 cerebellar symptoms, 10 behavioral symptoms, 7 general symptoms, 8 motor symptoms, 3 sensory symptoms, 3 visual symptoms, and seizure tendencies. Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Louisville.[15, 16] According to the company, "REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources."

Data analysis

Data were analyzed using Stata/IC 16.1 (StataCorp LLC, College Station, TX).[17] Categorical variables were reported as frequency and percentage whereas continuous variables were described as mean, range, and standard deviation (SD). Sensitivity and specificity of lab testing were calculated with standard equations. The patients were classified based on the final diagnosis and separated into 5 groups: *definite prion disease, probable prion disease, possible prion disease, non-CJD,* and *undiagnosed.* Summary statistics of clinical data were presented for each group.

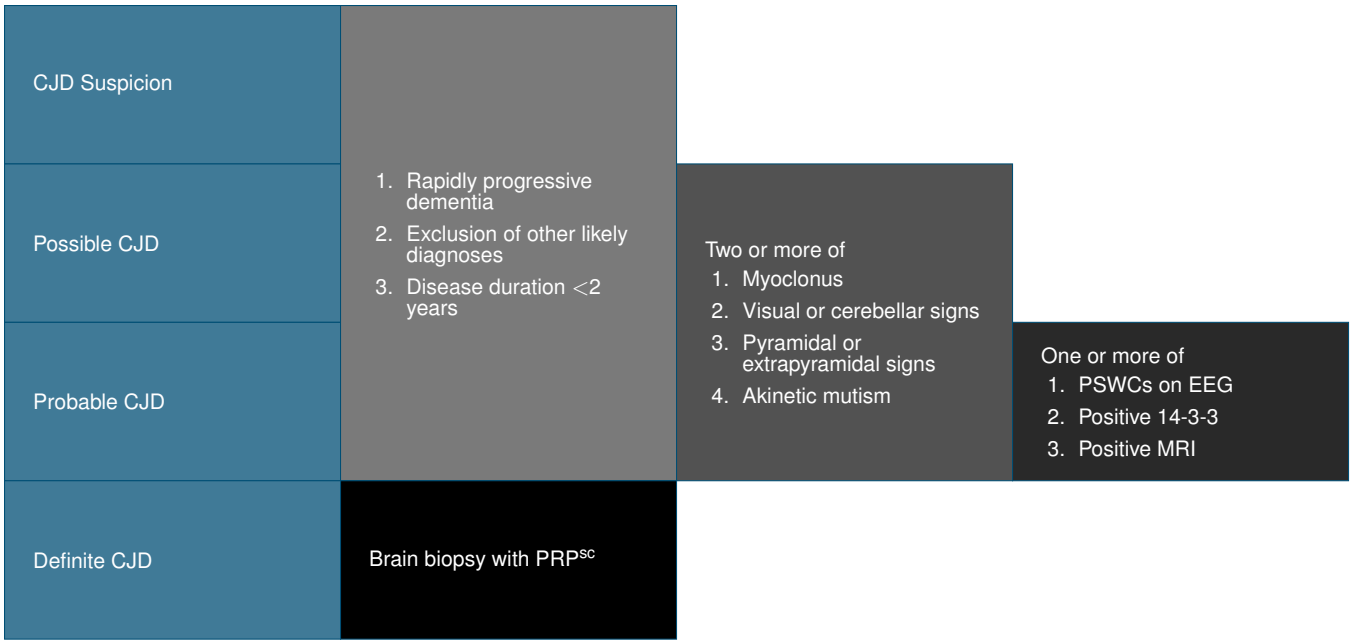

Figure 1. Diagnostic criteria for CJD, adapted from the CDC.
Abbreviations: PSCW, periodic sharp wave complexes; EEG, electroencephalogram, PRP^{sc}, prion protein scrapie isoform.

Results

The study included 27 patients, 13 males and 14 females. There was one patient with biopsy-confirmed prion disease and one patient who met criteria for *probable CJD* after presenting with rapidly progressing dementia and with Rt-QuIC positivity. There were 20 patients who had a final diagnosis other than CJD. No patients met CDC criteria for *possible* prion disease. Five patients did not have a final diagnosis. Of these, all died within two years of admission (mean 182 days until death; range 8–461; SD 203) and had paraclinical tests suggestive of CJD.

The most common presenting symptoms for the patients with *definite* or *probable* CJD were memory loss, decreased alertness, disorientation, executive dysfunction, myoclonus, and confusion. In the *non-CJD* group, confusion, aphasia/dysphasia, memory loss, decreased alertness, and disorientation were most frequently documented. Data are presented in **Table 1**.

In total, 14 patients had both CSF 14-3-3 and total Tau (normal 0–1149 pg/mL) positive for prion disease. Otherwise, five patients had a positive 14-3-3 but negative Tau, eight were negative for both, and four patients had inconclusive tests due to blood in the CSF sample. Based on final diagnoses and not including those in the undiagnosed group, our sensitivity of total Tau in CSF was 100%, and specificity was 50%. Our sensitivity of CSF 14-3-3 was 100%, and specificity was 37.5%.

Also, total CSF Tau was quantitatively higher in the *CJD* group (6011 pg/mL, 1807–10215, SD 5945) than the *non-CJD* group (2053 pg/mL, 0–7353, SD 2059), though the differences were not statistically significant.

Total CSF protein was lower in the *CJD group* at an average of 12.5 mg/dL (range 8–17, SD 6.4) than in the *non-CJD* group with an average of 105.0 mg/dL (14.8–930, SD 204.1), though the difference was not statistically significant due to limited sample size.

Fourteen patients with a positive or inconclusive 14-3-3 had reflex Rt-QuIC testing, of which one was positive. All but one patient had an MRI brain performed, and of those, four patients had findings consistent with sCJD, three had atypical findings consistent with vCJD or sCJD variants, and 14 patients had findings leading to a non-CJD diagnosis.

No patients had periodic sharp wave complexes identified on EEG, though seven were noted to have seizures or seizure tendencies, and 15 were noted to have moderate to severe background slowing consistent with encephalopathy. Data are presented in **Table 2**.

In total, 19 patients were admitted or transferred to the ICU, and nine of those patients were intubated. Both patients with prion disease were intubated and developed pneumonia contributing to respiratory failure and eventual death, as did a third patient with a final diagnosis other than prion disease.

Table 1. Frequency data of symptoms, including only symptoms with at least one data point reported.

Symptom	Definite/Probable CJD (*n*=2)	Non-CJD (*n*=20)
Memory Loss	100%	40%
Decreased Alertness	100%	30%
Disorientation	100%	25%
Executive Dysfunction	100%	10%
Myoclonus	100%	0%
Confusion	100%	55%
Aphasia/Dysphasia	50%	45%
Personality Change	50%	10%
Apathy	50%	5%
Social Isolation	50%	0%
Gait Change	0%	20%
Agitation	0%	20%
Seizures	0%	20%
Anger/Aggression	0%	20%
Agitation	0%	20%
Bizarre Behavior	0%	15%
Tremor	0%	15%
Irritability	0%	15%
Urinary Incontinence	0%	15%
Gastrointestinal	0%	15%
Tremor	0%	15%
Pyramidal Symptoms	0%	10%
Apraxia	0%	5%
Weight Loss	0%	5%
Limb Ataxia	0%	5%
Fatigue	0%	5%
Headache	0%	5%
Handwriting Changes	0%	5%
Fasciculations	0%	5%

Table 2. Diagnostic data per group.

	Definite CJD (*n*=1)	Probable CJD (*n*=1)	Non-CJD (*n*=20)	Undiagnosed (*n*=5)
Age (years)*	64	70	57.4±18.3 (18–79)	64.4±6.3 (54–69)
CSF Total Tau (*n*)	1 pos.	1 pos.	10 pos., 10 neg.	1 pos., 4 neg.
CSF Total Tau (pg/mL)*	1,807	10,215	2,053.6±2,059.1 (0–7,353)	740.2 [12–1,630 ± 607.5]
CSF 14-3-3	1 pos.	1 pos.	10 pos., 6 neg., 4 inconcl.	3 pos., 2 neg.
CSF Protein (mg/dL)*	17	8	105±204.1 (14.8–930)	48.5±19.9 (21.6–64)
Rt-QuIC		1 pos.	13 neg.	2 neg.
EEG Findings				
n	1	1	16	3
Background Slowing	1	1	10	3
Seizures	0	0	2	1
Seizure Tendencies	0	1 GPED	3 PLED	0
MRI Findings				
n	1	1	19	5
Typical Findings	1	1	2	0
Atypical Findings			1	2
Normal			5	0
Other Diagnosis			11†	3
Brain Biopsy	1 pos.		1 neg.	

* Mean±standard deviation (range).

† PRES, toxic leukoencephalopathy, psychiatric (2), CNS lymphoma, acute myeloid leukemia, NORSE/FIRE seizures, autoimmune epilepsy, acute demyelinating encephalitis, CNS vasculitis (2), neurosyphilis (2), herpes encephalitis, dementia (3), stroke (2), metabolic encephalopathy.

Abbreviations: GPED, generalized periodic epileptiform discharges; PLED, periodic lateralized epileptiform discharges; PRES, posterior reversible encephalopathy syndrome; NORSE, new onset refractory status epilepticus; FIRES, febrile infection related epilepsy syndrome; CNS, central nervous system; pos., positive; neg., negative; inconcl., inconclusive.

Discussion

Patients with definite and probable CJD presented with similar symptoms to those with a final diagnosis that was not CJD, with overlap in memory loss, decreased alertness, confusion, and aphasia or dysphasia. Only executive dysfunction and myoclonus did not have a high degree of overlap and were more often associated with CJD. This made it difficult to differentiate between conditions and identify patients for further testing. We also identified a substantial proportion of positive tests for CSF total Tau and 14-3-3 in patients with final diagnoses other than CJD.

At our institution, initial testing for prion disease includes CSF t-Tau and 14-3-3. Tau is a microtubule stabilization protein found within neurons that is elevated in disease processes affecting the central nervous system, specifically strokes, traumatic brain injuries, and many forms of dementia.[5] CSF t-Tau is reported to have a sensitivity of 87–90% and specificity of 67–75%.[18, 19]

Levels of the protein 14-3-3 in CSF provide an indication of neuronal injury.[5] Similarly, disease processes such as CNS inflammation, strokes, and particularly Alzheimer's dementia (AD) also present with elevated levels of 14-3-3.[5] CSF 14-3-3 is reported to have sensitivity of 61–95% and specificity of 40–92%.[18, 19] Since the incidence of Alzheimer's dementia (AD) is much more frequent than prion disease, the pre-test probability of 14-3-3 in CSF is higher for AD than it is for prion disease.[5, 20] In the present study, there was a lower specificity of both tau and 14-3-3. Likewise, the final diagnoses for our patients included multiple cases of strokes and dementia.

Reasons for the false positive tests could also include the comorbidity or severity of illnesses in our patients as these tests were not studied extensively in critically ill patients.[5, 19] Of the 27 patients identified, 19 were admitted or transferred to an ICU. These patients were critically ill, with nine of them requiring mechanical ventilation. Standard workup for CJD, such as a thorough psychiatric and neurologic examination, were difficult. In these critically ill patients, the higher frequency of false positive results further complicated the diagnostic workup.

After 2015, once available in the United States, real-time quaking-induced conversion (Rt-QuIC) was reflexively tested if CSF 14-3-3 was positive. Rt-QuIC is an assay that uses a patient's CSF specimen to seed reaction wells containing recombinant prion protein. A positive test occurs when conformational change of proteins results in amyloid formation. Rt-QuIC is described as an optimal method of prion protein detection without requiring brain biopsy.[5] The reported sensitivity for the majority of sCJD subtypes is 91–100%, and it has a specificity of 98.5–100%.[5, 21, 22] Therefore, testing with Rt-QuIC would be of benefit even in the absence of positive 14-3-3, especially in patient populations that could have a higher pre-test probability of non-CJD diagnoses.

With every sample, CSF protein was also analyzed. In the present study, CSF protein was higher in patients with a non-CJD final diagnosis. Direct neuronal injury or disruption of the blood-brain barrier causing protein release is not associated with prion disease.[5] While the differences in CSF protein were not statistically significant for our limited sample size, there may be a negative correlation. Further study could be directed towards this negative correlation.

Of the five patients in our sample who did not have a final diagnosis, only two had Rt-QuIC testing, which was negative, and two patients had a positive 14-3-3 and MRI findings consistent with vCJD. All five died within two years of presentation. While rare, variant CJD in these patients is possible, especially since the utility of Rt-QuIC in the diagnosis of vCJD is limited. In situations like these, where the workup is ambiguous and no other causative illness is found, or in the case of critically ill patients, non-standard laboratory tests may be of benefit. The nonspecific CSF biomarkers currently being investigated for the diagnosis of prion disease include Tau subtype ratios, neuron-specific enolase, S100 beta, and alpha-synuclein. While their sensitivities and specificities vary, and they are not included in current diagnostic algorithms, their utility lies in otherwise ambiguous workups.[5, 18]

Of all patients in our sample tested for prion disease, nine (33%) required intubation for respiratory infections. A recent review confirmed that endotracheal intubation and extubation are aerosol-generating procedures.[23] Since it has been demonstrated in a mouse model that prion protein can be spread through aerosols, it is important that proper personal protective equipment be employed for any aerosol-generating procedure performed on patients with prion disease.[10] As testing is often ambiguous, aerosol precautions should be taken with any patient in whom there is suspicion of prion disease.

Conclusions

The presenting symptoms in patients with prion disease largely overlap with patients whose final diagnosis is not prion disease. There is a high false positive frequency of both CSF total Tau and CSF 14-3-3, especially in critically ill patients. The systematic incorporation of Rt-QuIC may increase diagnostic accuracy. The diagnosis of prion disease demands the incorporation of signs and symptoms, diagnostic tests, and imaging. A high level of suspicion is necessary so that the disease is not missed in critically ill patients,

and aerosol precautions should be employed when performing any aerosol-generating procedure for patients in whom there is any suspicion of prion disease.

Limitations

The statistical analysis was limited due to our small sample size. After 2016, our laboratory reported CSF total-Tau to a maximum of >4000, whereas previously there was no upper limit.

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References

1. Appleby BS, Appleby KK, Crain BJ, Onyike CU, Wallin MT, Rabins PV. Characteristics of established and proposed sporadic Creutzfeldt-Jakob disease variants. Arch Neurol **2009**; 66(2):208-15. doi: 10.1001/archneurol.2008.533. PMID: 19204157.

2. Fragoso DC, Gonçalves Filho AL, Pacheco FT, et al. Imaging of Creutzfeldt-Jakob Disease: Imaging Patterns and Their Differential Diagnosis. Radiographics **2017**; 37(1):234-57. doi: 10.1148/rg.2017160075. PMID: 28076012.

3. Geschwind MD. Prion Diseases. Continuum (Minneap Minn) **2015**; 21(6 Neuroinfectious Disease):1612-38. doi: 10.1212/con.0000000000000251. PMID: 26633779.

4. Holman RC, Belay ED, Christensen KY, et al. Human prion diseases in the United States. PLoS One **2010**; 5(1):e8521. doi: 10.1371/journal.pone.0008521. PMID: 20049325.

5. Connor A, Wang H, Appleby BS, Rhoads DD. Clinical Laboratory Tests Used To Aid in Diagnosis of Human Prion Disease. J Clin Microbiol **2019**; 57(10). doi: 10.1128/jcm.00769-19. PMID: 31366689.

6. Ford L, Rudge P, Robinson K, Collinge J, Gorham M, Mead S. The most problematic symptoms of prion disease - an analysis of carer experiences. Int Psychogeriatr **2019**; 31(8):1181-90. doi: 10.1017/s1041610218001588. PMID: 30353798.

7. Spiropoulos J, Lockey R, Beck KE, et al. Incomplete inactivation of atypical scrapie following recommended autoclave decontamination procedures. Transbound Emerg Dis **2019**; 66(5):1993-2001. doi: 10.1111/tbed.13247. PMID: 31111687.

8. World Health Organization. WHO infection control guidelines for transmissible spongiform encephalophathies. Report of a WHO consultation, Geneva Switcherland, 23-26 March 1999. Available at: https://www.who.int/csr/resources/publications/bse/WHO_CDS_CSR_APH_2000_3/en/. Accessed 5 June 2021.

9. Lemmer K, Mielke M, Pauli G, Beekes M. Decontamination of surgical instruments from prion proteins: in vitro studies on the detachment, destabilization and degradation of PrPSc bound to steel surfaces. J Gen Virol **2004**; 85(Pt 12):3805-16. doi: 10.1099/vir.0.80346-0. PMID: 15557254.

10. Stitz L, Aguzzi A. Aerosols: an underestimated vehicle for transmission of prion diseases? Prion **2011**; 5(3):138-41. doi: 10.4161/pri.5.3.16851. PMID: 21778819.

11. Zerr I, Kallenberg K, Summers DM, et al. Updated clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease. Brain **2009**; 132(Pt 10):2659-68. doi: 10.1093/brain/awp191. PMID: 19773352.

12. Manix M, Kalakoti P, Henry M, et al. Creutzfeldt-Jakob disease: updated diagnostic criteria, treatment algorithm, and the utility of brain biopsy. Neurosurg Focus **2015**; 39(5):E2. doi: 10.3171/2015.8.Focus15328. PMID: 26646926.

13. Centers for Disease Control and Prevention. Variant Creutzfeldt-Jakob Disease (vCJD). Available at: https://www.cdc.gov/prions/vcjd/diagnostic-criteria.html. Accessed 5 June 2021.

14. Centers for Disease Control and Prevention. Creutzfeldt-Jakob Disease, Classic (CJD). Available at: https://www.cdc.gov/prions/cjd/diagnostic-criteria.html. Accessed 5 June 2021.

15. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of software platform partners. J Biomed Inform **2019**; 95:103208. doi: 10.1016/j.jbi.2019.103208. PMID: 31078660.

16. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform **2009**; 42(2):377-81. doi: 10.1016/j.jbi.2008.08.010. PMID: 18929686.

17. Stata Statistical Software. 16 ed. College Station, TX: StataCorp LLC, **2019**.

18. Llorens F, Villar-Piqué A, Hermann P, et al. Diagnostic Accuracy of Prion Disease Biomarkers in Iatrogenic Creutzfeldt-Jakob Disease. Biomolecules **2020**; 10(2). doi: 10.3390/biom10020290. PMID: 32059611.

19. Hamlin C, Puoti G, Berri S, et al. A comparison of tau and 14-3-3 protein in the diagnosis of Creutzfeldt-Jakob disease. Neurology **2012**; 79(6):547-52. doi: 10.1212/WNL.0b013e318263565f. PMID: 22843257.

20. Bahl JM, Heegaard NH, Falkenhorst G, et al. The diagnostic efficiency of biomarkers in sporadic Creutzfeldt-Jakob disease compared to Alzheimer's disease. Neurobiol Aging **2009**; 30(11):1834-41. doi: 10.1016/j.neurobiolaging.2008.01.013. PMID: 18339451.

21. McGuire LI, Peden AH, Orrú CD, et al. Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. Ann Neurol **2012**; 72(2):278-85. doi: 10.1002/ana.23589. PMID: 22926858.

22. Bongianni M, Orrù C, Groveman BR, et al. Diagnosis of Human Prion Disease Using Real-Time Quaking-Induced Conversion Testing of Olfactory Mucosa and Cerebrospinal Fluid Samples. JAMA Neurol **2017**; 74(2):155-62. doi: 10.1001/jamaneurol.2016.4614. PMID: 27942718.

23. Nestor CC, Wang S, Irwin MG. Are tracheal intubation and extubation aerosol-generating procedures? Anaesthesia **2021**; 76(2):151-5. doi: 10.1111/anae.15328. PMID: 33274761.