JAMA Neurology | Original Investigation

Diagnosis of Human Prion Disease Using Real-Time Quaking-Induced Conversion Testing of Olfactory Mucosa and Cerebrospinal Fluid Samples

Matilde Bongianni, PhD; Christina Orrù, PhD; Bradley R. Groveman, PhD; Luca Sacchetto, MD; Michele Fiorini, PhD; Giovanni Tonoli, MD; Giorgio Triva, BS; Stefano Capaldi, PhD; Silvia Testi, PhD; Sergio Ferrari, MD; Annachiara Cagnin, MD, PhD; Anna Ladogana, MD; Anna Poleggi, PhD; Elisa Colaizzo, MD; Dorina Tiple, MD; Luana Vaianella, MD; Santina Castriciano, BS; Daniele Marchioni, MD; Andrew G. Hughson, MS; Daniele Imperiale, MD; Tatiana Cattaruzza, MD, PhD; Gian Maria Fabrizi, MD; Maurizio Pocchiari, MD; Salvatore Monaco, MD; Byron Caughey, PhD; Gianluigi Zanusso, MD, PhD

IMPORTANCE Early and accurate in vivo diagnosis of Creutzfeldt-Jakob disease (CJD) is necessary for quickly distinguishing treatable from untreatable rapidly progressive dementias and for future therapeutic trials. This early diagnosis is becoming possible using the real-time quaking-induced conversion (RT-QuIC) seeding assay, which detects minute amounts of the disease-specific pathologic prion protein in cerebrospinal fluid (CSF) or olfactory mucosa (OM) samples.

OBJECTIVE To develop an algorithm for accurate and early diagnosis of CJD by using the RT-QuIC assay on CSF samples, OM samples, or both.

DESIGN, SETTING, AND PARTICIPANTS In this case-control study, samples of CSF and OM were collected from 86 patients with a clinical diagnosis of probable (n = 51), possible (n = 24), or suspected (n = 11) CJD and 104 negative control samples (54 CSF and 50 OM). The CSF and OM samples were analyzed using conventional RT-QuIC. The CSF samples underwent further testing using improved RT-QuIC conditions. In addition, the diagnostic performance of a novel, easy-to-use, gentle flocked swab for sampling of OM was evaluated. Data were collected from January 1 to June 30, 2015.

MAIN OUTCOME AND MEASURES Correlations between RT-QuIC results and the final diagnosis of recruited patients.

RESULTS Among the 86 patients (37 men [43%] and 49 women [57%]; mean [SD] age, 65.7 [11.5] years) included for analysis, all 61 patients with sporadic CJD had positive RT-QuIC findings using OM or CSF samples or both for an overall RT-QuIC diagnostic sensitivity of 100% (95% CI, 93%-100%). All patients with a final diagnosis of non–prion disease (71 CSF and 67 OM samples) had negative RT-QuIC findings for 100% specificity (95% CI, 94%-100%). Of 8 symptomatic patients with various mutations causing CJD or Gerstmann-Sträussler-Scheinker syndrome, 6 had positive and 2 had negative RT-QuIC findings for a sensitivity of 75% (95% CI, 36%-96%).

CONCLUSIONS AND RELEVANCE A proposed diagnostic algorithm for sporadic CJD combines CSF and OM RT-QuIC testing to provide virtually 100% diagnostic sensitivity and specificity in the clinical phase of the disease.

Editorial page 144

Author Audio Interview

Supplemental content

CME Quiz at jamanetworkcme.com and CME Questions page 252

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Gianluigi Zanusso, MD, PhD, Department of Neurosciences, Biomedicine, and Movement Sciences, University of Verona, Policlinico G. B. Rossi, Piazzale L. A. Scuro 10, 37134 Verona, Italy (gianluigi.zanusso@univr.it).

JAMA Neurol. 2017;74(2):155-162. doi:10.1001/jamaneurol.2016.4614
Published online December 12, 2016.

Sporadic Creutzfeldt-Jakob disease (CJD) is the most common form of human transmissible spongiform encephalopathy or prion disease. Genetic CJD, Gerstmann-Sträussler-Scheinker syndrome, and fatal familial insomnia also belong to this group of fatal neurodegenerative disorders. Criteria for the diagnosis of probable sporadic CJD include the following: the presence of dementia; at least 2 clinical signs among visual disturbances, ataxia, extrapyramidal or pyramidal signs, myoclonus, and akinetic mutism; and the presence of periodic sharp and slow wave complexes in the electroencephalogram, a positive test result for 14-3-3 protein in the cerebrospinal fluid (CSF), or bilateral high-signal abnormalities in the caudate and/or putamen at diffusion-weighted imaging or fluid-attenuated inversion recovery sequences of magnetic resonance imaging of the brain.[1] Definite diagnosis still relies on neuropathologic examination or the detection of the CJD-specific abnormal prion protein (PrP^{CJD}) in the central nervous system tissue.[2]

However, patients with sporadic CJD show great variability in clinical signs and pathologic lesions, partially depending on the polymorphic methionine (M) or valine (V) at codon 129 and the glycotype of protease-resistant PrP (types 1 and 2) that accumulates in brain.[3-5] Because of this phenotypic variability, accurate and early intra vitam diagnosis of sporadic CJD is often challenging, requiring the combination of clinical signs and instrumental or biochemical findings that usually occur in the late stage of disease. The sensitivity of these diagnostic criteria for sporadic CJD based on the analysis of probable and definite cases has been 83%, with a specificity of 71%.[1]

The real-time quaking-induced conversion (RT-QuIC) assay detects femtograms of PrP^{CJD} from all subtypes of sporadic CJD,[6-12] and previous applications of RT-QuIC to CSF (eg, PQ-CSF) have given diagnostic sensitivities of 77% to 91% and specificities of 98.5% to 100%.[7,9,13-15] A second-generation improved RT-QuIC assay for CSF samples (IQ-CSF) has increased sensitivity to 96%.[9] Finally, the application of RT-QuIC to brushings from the olfactory mucosa (OM) has shown a sensitivity of at least 97% and a specificity of 100%.[16,17]

In the present study, we used RT-QuIC to analyze CSF and OM samples from 86 patients referred to the Italian CJD surveillance system to determine the accuracy of the test at the time of CJD diagnosis. In addition, we propose a diagnostic algorithm for optimal diagnosis of suspected CJD based on RT-QuIC analysis of CSF and OM.

Key Points

Question How can diagnosis of Creutzfeldt-Jakob disease be optimized using cerebrospinal fluid and nasal swabbing samples?

Findings In this case-control laboratory analysis, a diagnostic algorithm had 100% sensitivity and 100% specificity for 61 cases of sporadic Creutzfeldt-Jakob disease relative to 71 non–prion disease cases using real-time quaking-induced conversion analysis of cerebrospinal fluid and/or olfactory mucosa samples. The sensitivity for genetic prion diseases was 75%, and gentler nasal swabs worked as well as cytobrushes for olfactory mucosa sampling.

Meaning Real-time quaking-induced conversion testing can provide rapid and accurate intra vitam diagnosis of Creutzfeldt-Jakob disease.

Methods

Study Participants

This study included 86 patients referred to the Italian surveillance system because of clinical suspicion of CJD. Final diagnosis of these patients was established at death or when a definite alternative diagnosis was available. Diagnosis of human prion disease was made according to internationally established criteria, including magnetic resonance imaging as a supportive investigation (eTable 1 in the Supplement).[1,2] Sixty-nine patients had a final diagnosis of sporadic CJD or genetic prion disease, whereas 17 had non-CJD. Negative control CSF samples were collected from 54 patients with clinical diagnoses of other neurologic disorders. Olfactory mucosa samples were collected from 27 patients with other neurologic disorders and 23 patients without neurologic disorders.

This study was approved by the ethical committees of the Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy (UV), and the Istituto Superiore di Sanità, Rome, Italy (ISS), which is recognized by the Office for Human Research Protections of the US Department of Health and Human Services. Written informed consent for participation in research was obtained in accordance with the Declaration of Helsinki (1964-2008)[18] and the Additional Protocol on the Convention of Human Rights and Biomedicine Concerning Biomedical Research (2005).[19] All OM samples were obtained after written informed consent from each patient or their representatives. We performed RT-QuIC testing of these specimens at Rocky Mountain Laboratories, Hamilton, Montana (RML), under exemption 11517 provided to one of us (B.C.) by the Office of Human Subjects Research of the National Institutes of Health.

OM Brushing Procedure and Sample Analyses

Data were collected from January 1 to June 30, 2015. A step-by-step tutorial video of the nasal brushing procedure is available at https://www.youtube.com/watch?v=wYb9W3u6uMY. This procedure was performed as previously described[16] using flocked swabs (FLOQSwabs; Copan Italia), a cytobrush (Kitobrush; Kaltek), or both. In addition, to reduce the potential risk for prion contamination, patients and medical staff wore adequate personal protection equipment for the procedure (which is not fully depicted in the tutorial video), including a disposable shirt and surgical cap, leaving visible only the access to the patient's nostrils. An absorbent cloth was used to cover the patient's mouth to avoid dispersion of oral secretions in case of sneezing after OM sample collection. Olfactory mucosa samples were taken by 19 trained otolaryngologists (including L.S. and G. Tonoli) or by 67 otolaryngologists instructed by the tutorial video and were stored at −80°C until assayed.

We performed cytologic and immunocytochemical analyses to evaluate the quality of OM sampling and the relative amounts of olfactory neurons collected. Cytocentrifuged preparations of OM samples were processed as previously described.[16]

Immunocytochemical studies were performed using a rabbit polyclonal antibody to olfactory marker protein (dilution, 1:100) (Abcam).

For Western blot analysis, OM cells were placed in lysis buffer, and proteins were separated in 12% sodium dodecyl sulfate – polyacrylamide gel electrophoresis.[16] Membranes were probed with antibodies to anti-glyceraldehyde 3-phosphate dehydrogenase (dilution, 1:5000) (Sigma-Aldrich) and βIII-tubulin (dilution, 1:1000) (Chemicon), followed by appropriate secondary antibodies and revealed by chemiluminescence (Amersham, ECL; GE Healthcare).

RT-QuIC Analysis

Recombinant PrP substrates (see below) were prepared as previously described.[20] We performed RT-QuIC assays as reported previously for CSF[9] and OM[16] except where indicated. For CSF analysis, reactions were run as previously described with hamster recombinant PrP^{Sen} 23-231 (PQ-CSF) or 90-231 (IQ-CSF). Twenty microliters of undiluted CSF was used per reaction well at RML and UV and 15 or 30 µL of undiluted CSF at ISS. For PQ-CSF, positive and negative assessments were made at the 90-hour point. For IQ-CSF, positive and negative results were scored based on the highest peak value before 50 hours to account for the signal degradation over time at higher temperatures.

Olfactory mucosa analyses at RML and UV were performed as described,[16] whereas at ISS, plates were incubated in a plate reader (FLUOstar Omega; BMG LABTECH) at 42°C for 70 to 90 hours with cycles of 90 seconds of shaking (900 rpm, double-orbital) and 30 seconds of rest throughout the incubation. Thioflavin T (ThT) fluorescence measurements (mean [SD] excitation, 450 [10] nm; mean emission, 480 [10] nm [bottom read]) were taken every 45 minutes at RML and UV and every 15 minutes at ISS.

Sample findings were judged to be RT-QuIC positive using criteria similar to those previously described for RT-QuIC analyses of OM and CSF specimens[13,16] using baseline-adjusted fluorescence values normalized to the maximum ThT fluorescence of the plate readers (260 000 relative fluorescence units) and suitably adjusted cutoff values.[16] For OM sampling, we used a cutoff of 50 (RML and UV) or 70 (ISS) hours.

Results

Patients

At the time of CSF or OM sampling, the 86 patients (37 men [43%] and 49 women [57%]; mean [SD] age, 65.7 [11.5] years) referred to the CJD surveillance system were classified as having probable (n = 51), possible (n = 24), or suspected (ie, not fully meeting the CJD diagnostic criteria) (n = 11) CJD. These patients were followed up until death or until an alternative diagnosis became available, when they were reclassified according to the internationally recognized diagnostic criteria without taking into account the results of the RT-QuIC test. Final diagnosis among these patients yielded 69 patients with CJD classified as definite CJD (n = 30), probable CJD (n = 31), or genetic prion disease (n = 8) (**Table 1** and eTables 1-3 in the Supplement). Seventeen patients were reclassified as having non-CJD (Table 1 and eTable 3 in the Supplement).

CSF Analyses Using PQ- and IQ-CSF Conditions in Patients With Prion Diseases

We tested CSF samples using the PQ- and/or the IQ-CSF assays (**Table 2**). Results of PQ-CSF testing were positive for 36 of 49 patients with definite or probable CJD for 73% (95% CI, 59%-85%) diagnostic sensitivity. Results of IQ-CSF were positive for 19 of 22 patients with definite or probable CJD for 86% (95% CI, 62%-96%) diagnostic sensitivity; however, we note that 13 of the 21 IQ-CSF samples were selected because they previously had negative PQ-CSF findings, thus biasing this patient group toward those with lower seeding activity in the CSF. Overall, CSF RT-QuIC assays were positive in 54 of 57 patients with sporadic CJD, with an overall sensitivity of 95% (95% CI, 84%-99%). Three patients with definite sporadic CJD had negative RT-QuIC findings of both CSF tests (Table 2). Six of 8 patients with genetic prion disease had positive CSF RT-QuIC findings (sensitivity, 75%; 95% CI, 36%-96%) (Table 2). One patient with genetic CJD (V180I) and 1 patient with Gerstmann-Sträussler-Scheinker syndrome continued to have negative findings. All non-prion disease control CSF samples, including those originally with suspected prion disease (n = 71), had negative findings for a specificity of 100% (95% CI, 94%-100%) (eTable 2 in the Supplement). Our findings confirm that IQ-CSF RT-QuIC is the more sensitive RT-QuIC protocol for analysis of CSF from patients with sporadic CJD.

RT-QuIC Results Using OM Samples Taken by Flocked Swab or Brushes

We also tested OM samples and compared the RT-QuIC results from OM sampling using flocked swabs and cytobrushes (**Figure 1**A and B). Flocked swabs significantly reduced discomfort for the patients during the sampling procedure by decreasing abrasions of the nasal mucosa compared with the cytobrush. Swabs and brushes collected similar numbers of olfactory neurons as judged by microscopy and Western blot analysis (eFigure, C-H in the Supplement). Red blood cells were more frequently observed in OM samples collected with brushes than swabs (eFigure, A, B, E, and F in the Supplement).

The relative efficiencies of OM sampling with swabs and brushes were evaluated for the 86 patients referred to the surveillance system (Table 1 and eTables 2 and 3 in the Supplement), 27 patients with other neurologic disorders, and 23 individuals without neurologic disorders (eTables 2 and 3 in the Supplement). The data for cytobrush samples from controls with other neurologic disorders and without neurologic disorders have been previously reported[16] and are included herein for comparison with the newer swab data.

Mean data from the swab and cytobrush samples show close overlap of the RT-QuIC fluorescence traces (Figure 1C), suggesting that the level of seeding activity in these samples was comparable using the 2 distinct tools. Analysis of the peak mean ThT fluorescence readings within 50 hours for each

Table 1. Demographic Characteristics and Diagnostic Investigations of Patients With Prion Disease

Final Diagnosis by Genotype	Mean (SD)		PCWCs in EEG, No. of Patients With Positive Finding/No. Tested	CSF Findings, No. of Patients With Positive Finding/No. Tested		Typical MRI, No. of Patients With Positive Finding/No. Tested
	Age, y	Disease Duration, mo		Positive 14-3-3 Test Result	Tau Levels >1300 pg/mL	
Definite CJD (n = 30)						
MM (n = 16)[a]	70 (9)	4 (2.3)	9/16	14/14	9/16	14/16
MV (n = 5)[b]	68 (9)	10.8 (8.1)	1/5	3/5	5/5	2/5
ND (n = 9)[c]	69 (9)	4.7 (1.9)	3/9	9/9	9/9	7/9
Probable CJD (n = 31)						
MM (n = 9)	59 (14)	9 (11.8)	6/9	7/9	4/5	6/8
MV (n = 12)	65 (9)	21 (10.6)	2/12	8/12	8/9	11/12
VV (n = 3)	70 (3)	13 (6.2)	1/3	3/3	2/2	2/3
ND (n = 7)	67 (9)	9 (6.0)	3/7	5/6	5/6	6/7
Genetic CJD (n = 8)						
V210I (n = 2)	52 and 71	2.5 (0.7)	1/2	2/2	1/1	2/2
E200K (n = 3)	56 (10)	18.7 (14.6)	1/3	1/3	NP	3/3
V180I (n = 1)	74	35	0/1	0/1	NP	1/1
P102L (n = 2)	51 and 62	50 (48)	0/2	2/2	1/2	2/2
Non-CJD (n = 17)						
MM (n = 2)	73 and 81	17 (18.4)	0/2	0/2	NP	2/2
MV (n = 4)	66 (13)	24 (25.1)	0/4	0/4	NP	0/4
VV (n = 2)	64 and 67	4.5 (0.7)	0/2	0/2	NP	0/2
ND (n = 9)	62 (20)	17.7 (11.3)	2/9	7/9	6/9	2/9

Abbreviations: CJD, Creutzfeldt-Jakob disease; CSF, cerebrospinal fluid; EEG, electroencephalogram; M, methionine; MRI, magnetic resonance imaging; ND, not determined; NP, not performed; PCWCs, periodic sharp and slow wave complexes; V, valine.

[a] Includes MM/1 (n = 13) and MM/1 and 2 (n = 3) Creutzfeldt-Jakob disease–specific abnormal prion protein (PrP^{CJD}) glycotypes.

[b] Includes MV/1 (n = 1) and MV/2 (n = 4) PrP^{CJD} glycotypes.

[c] Denotes codon 129 ND, ND type 1.

Table 2. Clinical Data and Results of RT-QuIC Analyses of CSF and OM Samples Collected From Patients With Probable, Possible, or Suspected CJD

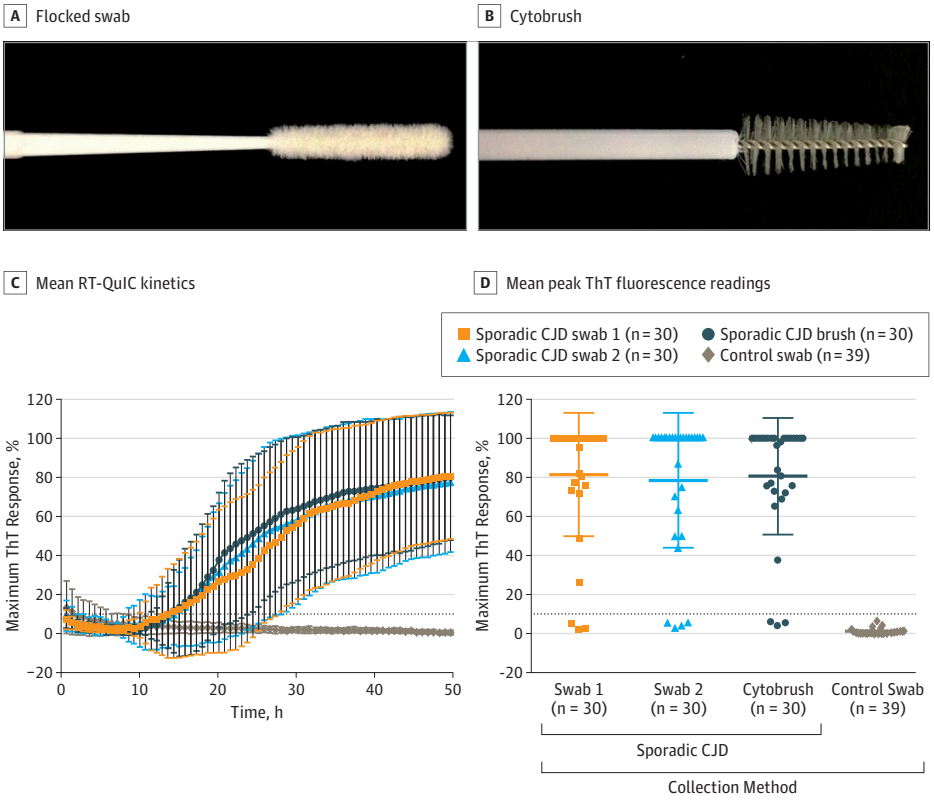
CJD Diagnosis at Time of Sampling Sorted by Final Diagnosis	CSF				OM				Overall Outcome, No. of Patients With Positive Finding/No. Tested (Accuracy, %)
	Time From Disease Onset to Spinal Tap, Mean (SD), mo	No. of Patients With Positive Finding/Tested			Time From Disease Onset to Sample, Mean (SD), mo	No. of Patients With Positive Finding/Tested			
		RT-QuIC Results		Final Outcome		Sampling Method		Final Outcome	
		PQ	IQ			Swab	Brush		
Probable CJD (n = 51)									
Definite CJD (n = 21)[a]	2.4 (1.2)	14/18	4/6	18/20	2.6 (1.2)	20/20	15/15	21/21	21/21 (100)
Probable CJD (n = 25)[b]	5.9 (5.3)	14/22	10/10	23/23	8.2 (8.1)	21/23	15/16	24/25	25/25 (100)
Genetic CJD (n = 4)	3.5 (2.4)	3/4	1/1	4/4	4.8 (4.5)	3/3	3/3	4/4	4/4 (100)
Non-CJD (n = 1)	4	NP	0/1	0/1	4	0/1	0/1	0/1	0/1 (100)
Possible CJD (n = 24)									
Definite CJD (n = 9)[a]	5.6 (5.9)	5/6	3/4	8/9	5.8 (5.9)	7/7	5/6	9/9	9/9 (100)
Probable CJD (n = 6)[b]	7.0 (4.0)	3/4	2/2	5/5	8.3 (3.8)	5/6	3/5	5/6	6/6 (100)
Genetic CJD (n = 2)	4.0 (4.3)	1/2	0/1	1/2	12.0 (7.1)	1/2	0/1	1/2	1/2 (50)
Non-CJD (n = 7)	3.7 (3.0)	NP	0/7	0/7	4.0 (3.2)	0/7	0/7	0/7	0/7 (100)
Suspected CJD (n = 11)									
GSS (n = 2)	12	0/1	NP	0/1	31.0 (26.9)	1/2	1/1	1/2	1/2 (50)
Non-CJD (n = 9)	8.7 (9.1)	NP	0/9	0/9	8.1 (9.1)	0/9	0/9	0/9	0/9 (100)

Abbreviations: CJD, Creutzfeldt-Jakob disease; CSF, cerebrospinal fluid; GSS, Gerstmann-Sträussler-Scheinker syndrome; IQ, improved QuIC; NP, not performed; OM, olfactory mucosa; PQ, previous QuIC; RT-QuIC, real-time quaking-induced conversion.

[a] In 1 patient, spinal tap was not performed; 5 samples were tested with IQ only.

[b] In 3 patients, spinal tap was not performed; 3 samples were tested with IQ only.

Figure 1. Olfactory Mucosa (OM) Sample Collection and Results of Real-Time Quaking-Induced Conversion (RT-QuIC) Assays



A subset of 30 patients with sporadic Creutzfeldt-Jakob disease (CJD) underwent OM collection with 2 swabs and 1 cytobrush; 39 non-CJD controls underwent OM collection with swabs. The mean RT-QuIC kinetics for each of these samples from these patients is shown in part C. The results from 43 non-CJD controls undergoing OM collection with the cytobrush have been reported previously with 100% specificity (95% CI, 90%-100%)[15] and therefore are not shown. Traces represent the percentage of thioflavin T (ThT) fluorescence from 4 replicate reactions (normalized as described in the Methods section) with the means (thick lines) and SDs (thin lines) shown as a function of RT-QuIC reaction time. The peak mean ThT fluorescence readings are depicted within 50 hours for each patient (data points) undergoing sampling with swabs or a cytobrush as shown in part D. RT-QuIC testing for 10 cytobrush samples have already been reported previously[15] and are included in these analyses for comparative purposes. Dashed lines indicate the 10% maximum ThT fluorescence threshold.

patient undergoing sampling with swabs and a cytobrush (n = 30) showed similar ThT maximum levels and numbers of patients with positive RT-QuIC findings for each sampling (Figure 1D). When broken down into individual samples for all 61 patients with sporadic CJD by the sampling instrument used, we obtained sensitivities for probable and definite CJD of 95% (95% CI, 84%-99%) when using swab 1, 90% (95% CI, 75%-97%) using swab 2, and 91% (95% CI, 77%-97%) using the cytobrush (eTable 2 in the Supplement). In 4 patients, no seeding activity was detected in at least 1 of the OM samples but was detected in another sample from the same patient, indicating that multiple sample collections from an individual patient can improve the chance of detection. Patients 37 and 41 had negative RT-QuIC findings for all 3 of their OM samplings (eTable 2 in the Supplement).

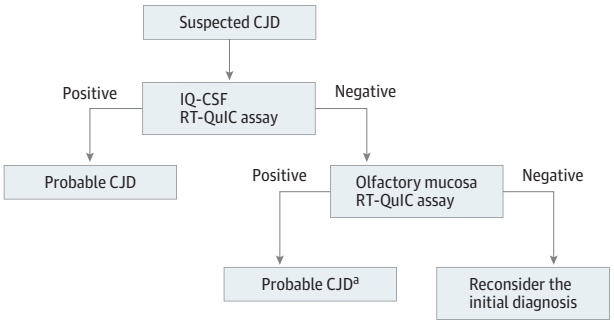
In patient 41, 1 of the 2 patients with CJD and a false-negative OM test result, the procedure was compromised by severe deviation of the nasal septum (eTable 2 in the Supplement). When RT-QuIC data from the OM or the CSF specimens or both were considered, all 61 patients with definite and probable sporadic CJD had positive findings, giving an overall diagnostic sensitivity of 100% (95% CI, 93%-100%) (eTable 2 in the Supplement). Furthermore, all OM samples from the 17 non-prion disease controls had negative RT-QuIC findings, resulting in a diagnostic specificity of 100% (95% CI, 91%-100%).

Comparison of Accuracy of RT-QuIC With the Combination of Other In Vivo Diagnostic Criteria

In this study, the initial diagnosis was formulated without considering the RT-QuIC results. However, when a retrospective follow-up of the patients was performed using the current diagnostic criteria paired with the RT-QuIC results, the accuracy of the diagnosis for patients with sporadic CJD dramatically improved (Table 2). In particular, of the 51 patients who were classified as having probable CJD (sporadic or genetic) at the time of sampling, 50 (98%) had positive RT-QuIC findings. When the final diagnosis was determined for these patients, 46 had definite or probable sporadic CJD, revealing 100% RT-QuIC accuracy for sporadic CJD. Four of the patients initially classified with probable CJD and who had positive RT-QuIC findings were classified with genetic CJD, again giving 100% accuracy. One patient was initially classified with probable CJD but finally diagnosed with non-CJD with negative RT-QuIC findings.

Of the 24 patients initially diagnosed with possible CJD (Table 2) at the time of sampling, 16 (67%) had positive RT-QuIC findings. Their final diagnosis showed that 15 of these 24 patients (63%) had definite or probable sporadic CJD, again demonstrating 100% RT-QuIC accuracy for sporadic CJD. Within this group, 2 patients were finally classified with genetic CJD; one had positive (E200K) and the other had negative (V180I) RT-QuIC findings. Seven of 24

Figure 2. Algorithm for In Vivo Diagnosis of Probable Creutzfeldt-Jakob Disease (CJD)



IQ-CSF indicates second-generation real-time quaking-induced conversion (RT-QuIC) assay for cerebrospinal fluid (CSF) samples.

[a] Because the olfactory mucosa RT-QuIC assay is performed on central nervous system tissue, a positive finding might allow a definite diagnosis of CJD according to the World Health Organization diagnostic criteria (http://www.who.int/csr/resources/publications/bse/whoemczdi989.pdf).

patients (29%) were finally classified with non-CJD and none had positive RT-QuIC findings.

Of the 11 patients who were classified with only suspected CJD (Table 2), only 1 patient had positive RT-QuIC findings. This patient had Gerstmann-Sträussler-Scheinker syndrome P102L mutation. A second patient in this group had the P102L mutation but no positive RT-QuIC finding. The remaining 9 patients were finally diagnosed with non-CJD and had negative RT-QuIC findings. Overall, these results indicate that RT-QuIC testing can markedly improve the accuracy of CJD diagnosis, especially in cases designated by other criteria as having only possible or suspected CJD.

Discussion

Our data from a large cohort of patients with suspected CJD reveal that combined results of RT-QuIC assays on CSF and OM samples allow antemortem diagnosis of sporadic CJD with 100% specificity and sensitivity. The sensitivity of RT-QuIC in detecting cases of genetic prion disease was lower than that in detecting sporadic CJD. The small number of patients with genetic forms of prion disease undergoing testing in this study does not allow for any conclusions about relative sensitivities, but genetic CJD cases would ordinarily be identified by results of genetic testing for mutations in the prion protein (*PRNP* gene [GenBank U29185]). Also, we did not test any samples from patients with variant CJD, but clinical cases of this category of CJD have almost disappeared.

Our results suggest that the application of RT-QuIC testing will improve the accuracy and speed of sporadic CJD diagnosis compared with internationally recognized antemortem diagnostic criteria.[1,2] For patients with rapidly evolving dementia, routine CSF analyses should include the IQ-CSF analysis to confirm or exclude prion diseases.[21] Because RT-QuIC analysis of CSF is not 100% sensitive (Table 2 and eTable 2 in the Supplement),[9] we suggest RT-QuIC testing of OM in all patients in whom CJD is still suspected after a negative CSF result (**Figure 2**). Cerebrospinal fluid samples are routinely collected in patients with rapidly progressive dementias to exclude potentially treatable diseases[22,23]; therefore, CSF is usually the primary tissue for initial RT-QuIC analysis. A positive RT-QuIC finding in the CSF of patients with progressive neurologic signs should be sufficient to establish a diagnosis of probable CJD[21] and would make OM sampling unnecessary. However, when the RT-QuIC CSF finding is negative or lumbar puncture is not feasible (eTable 2 in the Supplement),[24] OM sampling would become necessary to confirm prion disease or to divert to alternative diagnoses (Figure 2). One patient who was diagnosed as having probable sporadic CJD at the time of referral but had negative RT-QuIC findings using CSF and OM samples was finally diagnosed with encephalitis rather than prion disease.

Patients 5 and 9, who had the MV/2 PrP^{CJD} glycotype of sporadic CJD (eTable 2 in the Supplement), had negative CSF RT-QuIC findings using both PQ-CSF and IQ-CSF conditions, whereas OM testing had positive findings. This result suggests that in the sporadic CJD MV/2 PrP^{CJD} glycotype, where the diagnostic sensitivity of supportive investigations is relatively low, OM brushing may be the only premortem diagnostic test with positive findings.[3,6]

Olfactory mucosa sampling is a simple procedure and can be performed without special training by otolaryngologists guided by a tutorial video. Our comparison of 2 OM sample collection tools shows that the efficacy of the gentle and soft nasal swab is comparable to that of the previously used cytobrush. A few samples from patients with sporadic CJD had negative RT-QuIC findings, which suggests that the collection of OM from the walls of the superior nasal cavity was not always successful. Consequently, our findings indicate that multiple OM samplings improve diagnostic sensitivity.

For patients 41 and 37, who had sporadic CJD, the OM RT-QuIC findings were negative. Patient 41 (eTable 2 in the Supplement) had anatomical abnormalities that likely impeded efficient OM sampling. Patient 37 (eTable 2 in the Supplement) had a diagnosis of probable CJD and died after a long disease course of 30 months with no autopsy (eTable 1 in the Supplement). The reason that these patients have negative OM RT-QuIC findings and positive CSF findings is not yet clear. However, these cases indicate that in a small subset of patients with CJD, prion seeding activity might not be detectable in nasal samples because prion seeding activity in the OM does not always reach detectable levels, or sampling is impeded by anatomical abnormalities, such as a deviated septum, or due to inflammatory conditions, such as rhinitis or nasal polyposis. Alternatively, in a very few cases, the repeated collection of the OM may occur in an area of the olfactory neuroepithelium without PrP^{CJD}. This possibility would confirm the uneven distribution of PrP^{CJD} in the layers of the olfactory mucosa.[25] The reduced RT-QuIC sensitivity observed in cases with genetic prion disease might be explained by differences in prion tissue distribution in these patients or by different prion conformations.[26]

Finally, the fiberscopes used in this study can be safely reused because the fiberscope was always covered by a

disposable sheath and inserted into the lower turbinate of the nose without touching the OM. Furthermore, no infectivity has yet been detected in nasal secretions or saliva of patients with CJD,[27] and PrP^{CJD} is not detectable in respiratory mucosa.[25]

Limitations

A limitation of this study is that all patients with CJD were symptomatic at the time of testing; therefore, it is still uncertain whether RT-QuIC in the CSF or in the OM is able to detect prion seeding activity during the preclinical stage of disease.

Conclusions

We propose a diagnostic algorithm (Figure 2) for patients with clinically suspected sporadic CJD, which starts with RT-QuIC IQ-CSF analysis and, if the findings are negative, is followed by OM analysis. According to our results to date, this diagnostic flow provides a sensitivity and specificity of virtually 100%. Because RT-QuIC testing is now available in most western countries, Japan, and Australia,[28] the inclusion of this procedure in clinical practice seems feasible. In Italy, the mean cost ranges from the equivalent of US $100 to $200, and results are available in about 3 days for CSF tests and in a single day for OM tests.[9,16,29] The combination of RT-QuIC and genetic testing would quickly confirm or dismiss the diagnosis of virtually all patients with clinically suspected human prion diseases. In our study, 17 of 86 patients (20%) with potential CJD were promptly diverted to alternative diagnoses of non-CJD, and 5 of them received successful treatment (Tables 1 and 2 and eTables 2 and 3 in the Supplement). Such accurate discrimination of prion diseases from other neurodegenerative illnesses or treatable dementias will also improve the reliability of patient selection in any future therapeutic trials.[30,31]

ARTICLE INFORMATION

Accepted for Publication: September 22, 2016.

Published Online: December 12, 2016. doi:10.1001/jamaneurol.2016.4614

Author Affiliations: Department of Neurosciences, Biomedicine, and Movement Sciences, University of Verona, Policlinico G. B. Rossi, Verona, Italy (Bongianni, Fiorini, Testi, Ferrari, Fabrizi, Monaco, Zanusso); Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana (Orrù, Groveman, Hughson, Caughey); Department of Surgical Sciences, Dentistry, Gynecology, and Pediatrics, University of Verona, Verona, Italy (Sacchetto, Marchioni); Struttura Complessa di Otorinolaringoiatria, Ospedale Santa Maria della Misericordia, Rovigo, Italy (Tonoli); Copan Italia SpA, Brescia, Italy (Triva, Castriciano); Biocrystallography Laboratory, Department of Biotechnology, University of Verona, Italy (Capaldi); Department of Neuroscience, University of Padova, Padova, Italy (Cagnin); Istituto di Ricovero e Cura a Carattere Scientifico San Camillo Hospital, Venice, Italy (Cagnin); Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome, Italy (Ladogana, Poleggi, Colaizzo, Tiple, Vaianella, Pocchiari); Neurology Unit, Ospedale Maria Vittoria, Torino, Italy (Imperiale); Neurology Unit, Ospedale Cattinara, Trieste, Italy (Cattaruzza).

Author Contributions: Drs Bongianni, Orrù, and Groveman contributed equally to this work. Drs Zanusso and Caughey had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Pocchiari, Monaco, Caughey, Zanusso.
Acquisition, analysis, or interpretation of data: Bongianni, Orrù, Groveman, Sacchetto, Fiorini, Tonoli, Triva, Capaldi, Testi, Ferrari, Cagnin, Ladogana, Poleggi, Colaizzo, Tiple, Vaianella, Castriciano, Marchioni, Hughson, Imperiale, Cattaruzza, Fabrizi, Pocchiari, Monaco, Caughey.
Drafting of the manuscript: All authors.
Critical revision of the manuscript for important intellectual content: Bongianni, Orrù, Groveman, Pocchiari, Monaco, Caughey, Zanusso.
Statistical analysis: Groveman, Caughey.
Obtained funding: Orrù, Groveman, Hughson, Caughey, Zanusso.
Administrative, technical, or material support: Sacchetto, Fiorini, Tonoli, Triva, Capaldi, Castriciano, Hughson.
Study supervision: Pocchiari, Caughey, Zanusso.

Conflict of Interest Disclosures: None reported.

Funding/Support: This study was supported in part by grant FABS2013 "In vivo diagnosis of definite CJD" from Alliance Biosecure Foundation, joint projects OLFASSAY and Joint Programming Neurodegenerative Disease from the University of Verona (Dr Zanusso), and the Intramural Research Program of the National Institute of Allergy and Infectious Disease (Drs Orrù, Groveman, and Caughey and Mr Hughson).

Role of the Funder/Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: Gregory Raymond, PhD, Rocky Mountain Laboratories, Hamilton, Montana, orchestrated sample shipments and permits. Brent Race, DVM, Roger Moore, PhD, Matteo Manca, PhD, and Clayton Winkler, PhD, Rocky Mountain Laboratories, provided critical evaluation of this manuscript. Piero Parchi, MD, PhD, University of Bologna, Bologna, Italy, performed neuropathologic examinations. Cristiana Atzori, PhD, Neurology Unit, Ospedale Maria Vittoria, Torino, Italy, and Andrea Galassi, MD, Servizio Igene e Sanità pubblica ULSS 6, Vicenza, Italy, provided technical support. None of these contributors received compensation for their work. We thank the many colleagues and patients' families for their invaluable support of this project.

REFERENCES

1. Zerr I, Kallenberg K, Summers DM, et al. Updated clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease. *Brain*. 2009;132(pt 10):2659-2668.

2. Budka H, Aguzzi A, Brown P, et al. Neuropathological diagnostic criteria for Creutzfeldt-Jakob disease (CJD) and other human spongiform encephalopathies (prion diseases). *Brain Pathol*. 1995;5(4):459-466.

3. Krasnianski A, Schulz-Schaeffer WJ, Kallenberg K, et al. Clinical findings and diagnostic tests in the MV2 subtype of sporadic CJD. *Brain*. 2006;129(pt 9):2288-2296.

4. Puoti G, Bizzi A, Forloni G, Safar JG, Tagliavini F, Gambetti P. Sporadic human prion diseases: molecular insights and diagnosis. *Lancet Neurol*. 2012;11(7):618-628.

5. Parchi P, de Boni L, Saverioni D, et al. Consensus classification of human prion disease histotypes allows reliable identification of molecular subtypes: an inter-rater study among surveillance centres in Europe and USA. *Acta Neuropathol*. 2012;124(4):517-529.

6. Zanusso G, Monaco S, Pocchiari M, Caughey B. Advanced tests for early and accurate diagnosis of Creutzfeldt-Jakob disease. *Nat Rev Neurol*. 2016;12(6):325-333.

7. Atarashi R, Satoh K, Sano K, et al. Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nat Med*. 2011;17(2):175-178.

8. Peden AH, McGuire LI, Appleford NE, et al. Sensitive and specific detection of sporadic Creutzfeldt-Jakob disease brain prion protein using real-time quaking-induced conversion. *J Gen Virol*. 2012;93(pt 2):438-449.

9. Orrù CD, Groveman BR, Hughson AG, Zanusso G, Coulthart MB, Caughey B. Rapid and sensitive RT-QuIC detection of human Creutzfeldt-Jakob disease using cerebrospinal fluid. *MBio*. 2015;6(1):pii:e02451-14.

10. Orrù CD, Groveman BR, Raymond LD, et al. Bank vole prion protein as an apparently universal substrate for RT-QuIC–based detection and discrimination of prion strains. *PLoS Pathog*. 2015;11(6):e1004983.

11. Orrù CD, Wilham JM, Raymond LD, et al. Prion disease blood test using immunoprecipitation and improved quaking-induced conversion. *MBio*. 2011;2(3):e00078-e11.

12. Ladogana A, Puopolo M, Croes EA, et al. Mortality from Creutzfeldt-Jakob disease and related disorders in Europe, Australia, and Canada. *Neurology*. 2005;64(9):1586-1591.

13. McGuire LI, Peden AH, Orrù CD, et al. RT-QuIC analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. *Ann Neurol*. 2012;72(2):278-285.

14. Cramm M, Schmitz M, Karch A, et al. Characteristic CSF prion seeding efficiency in humans with prion diseases. *Mol Neurobiol*. 2015;51(1):396-405.

15. Cramm M, Schmitz M, Karch A, et al. Stability and reproducibility underscore utility of RT-QuIC for diagnosis of Creutzfeldt-Jakob disease. *Mol Neurobiol*. 2016;53(3):1896-1904.

16. Orrù CD, Bongianni M, Tonoli G, et al. A test for Creutzfeldt-Jakob disease using nasal brushings. *N Engl J Med*. 2014;371(6):519-529.

17. Zanusso G, Bongianni M, Caughey B. A test for Creutzfeldt-Jakob disease using nasal brushings. *N Engl J Med*. 2014;371(19):1842-1843.

18. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191-2194.

19. Council of Europe. Details of Treaty No. 195. https://www.coe.int/en/web/conventions/full-list/-/conventions/treaty/195. Published January 25, 2005. Accessed October 1, 2011.

20. Groveman BR, Kraus A, Raymond LD, et al. Charge neutralization of the central lysine cluster in prion protein (PrP) promotes PrP(Sc)-like folding of recombinant PrP amyloids. *J Biol Chem*. 2015;290(2):1119-1128.

21. 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.

22. Tagliapietra M, Zanusso G, Fiorini M, et al. Accuracy of diagnostic criteria for sporadic Creutzfeldt-Jakob disease among rapidly progressive dementia. *J Alzheimers Dis*. 2013;34(1):231-238.

23. Chitravas N, Jung RS, Kofskey DM, et al. Treatable neurological disorders misdiagnosed as Creutzfeldt-Jakob disease. *Ann Neurol*. 2011;70(3):437-444.

24. Edwards C, Leira EC, Gonzalez-Alegre P. Residency training: a failed lumbar puncture is more about obesity than lack of ability. *Neurology*. 2015;84(10):e69-e72.

25. Zanusso G, Ferrari S, Cardone F, et al. Detection of pathologic prion protein in the olfactory epithelium in sporadic Creutzfeldt-Jakob disease. *N Engl J Med*. 2003;348(8):711-719.

26. Sano K, Satoh K, Atarashi R, et al. Early detection of abnormal prion protein in genetic human prion diseases now possible using real-time QUIC assay. *PLoS One*. 2013;8(1):e54915.

27. Brown P, Gibbs CJ Jr, Rodgers-Johnson P, et al. Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. *Ann Neurol*. 1994;35(5):513-529.

28. McGuire LI, Poleggi A, Poggiolini I, et al. Cerebrospinal fluid real-time quaking-induced conversion is a robust and reliable test for sporadic Creutzfeldt-Jakob disease: an international study. *Ann Neurol*. 2016;80(1):160-165.

29. Orrù CD, Hughson AG, Groveman BR, et al. Factors that improve RT-QuIC detection of prion seeding activity. *Viruses*. 2016;8(5):pii:E140.

30. Brown P, Farrell M. A practical approach to avoiding iatrogenic Creutzfeldt-Jakob disease (CJD) from invasive instruments. *Infect Control Hosp Epidemiol*. 2015;36(7):844-848.

31. Brown P, Farrell M. A new practical diagnostic test for Creutzfeldt-Jakob disease. *Infect Control Hosp Epidemiol*. 2015;36(7):849.