Title:

Biomarker-based diagnosis of sporadic Creutzfeldt-Jakob disease

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Summary

Sporadic Creutzfeldt-Jakob disease (sCJD) is a fatal and potentially transmissible neurodegenerative disease caused by misfolded prion proteins. To date, effective therapeutics are not available and accurate diagnosis can be challenging. Clinical diagnostic criteria employ a combination of characteristic cerebrospinal fluid (CSF) 14-3-3 proteins, MRI findings, EEG changes, and neuropsychiatric symptoms. Supportive biomarkers such as high CSF total Tau may aid the diagnostic process. However, discordant results of studies have led to controversies about the clinical value of established surrogate biomarkers. The recent development and clinical application of disease-specific protein aggregation and amplification assays such as Real-time Quaking Induced Conversion (RT-QuIC) have constituted major breakthroughs for the confident pre-mortem diagnosis of sCJD. Updated criteria for the biomarker-based diagnosis of sCJD including RT-QuIC will improve early clinical confirmation, disease surveillance, assessment of potential tissue infectivity, and trial monitoring. Further, potential pre-symptomatic, prognostic, and blood-based biomarker candidates have been identified in recent years.

Introduction

Sporadic Creutzfeldt-Jakob disease (sCJD) is a rapidly progressive neuropsychiatric syndrome with fatal outcome, characterized by aggregations of pathological Prion Protein Scrapie (PrP^{SC}) in the brain. Sporadic CJD is the most common form of human prion disease (about 90%) with an incidence around 1.5 to 2.0 per million person-years.[1] The clinical phenotype correlates with the molecular subtype[2] and definite diagnosis requires neuropathological examination. Clinical diagnostic criteria were suggested 40 years ago, including a combination of distinctive clinical features and best available auxiliary paraclinical investigations, which at that time was electroencephalography (EEG).[3,4] In 1998, the World Health Organization (WHO) included detection of cerebrospinal fluid (CSF) 14-3-3 proteins in the standard diagnostic criteria.[5] Patterns of signal alteration on fluid attenuated inversion recovery and diffusion weighted sequences of magnetic resonance images (MRI) were included in 2009.[6] Another CSF protein, total-Tau (t-Tau), is considered a valuable supportive biomarker.[7] While comparative data on imaging markers for sCJD are scarce, numerous studies have evaluated the diagnostic performance of CSF biomarkers, with occasional discrepancy leading to controversy about their clinical utility.[8,9] The recent development and clinical application of PrP^{SC} amplification assays such as Protein Misfolding Cyclic Amplification (PMCA) and Real-time Quaking Induced Conversion (RT-QuIC)[10] have constituted major breakthroughs as aids for a confident pre-mortem diagnosis of sCJD. RT-QuIC has shown excellent diagnostic accuracy in retrospective and observational studies, as well as ring trials[11,12] with prospective studies[15,16] also indicating its high value for an early and accurate diagnosis. Based on expert consensus, RT-QuIC was included in several diagnostic criteria for sCJD.[13,14] The current unmet need is the identification of blood-based biomarkers for early diagnosis and as surrogate markers of disease progression,[17–19] especially in view of new therapeutic strategies.

The aim of this review is to provide an overview of the biomarker-based diagnosis of sCJD and to suggest guidelines for clinicians to utilize in the differential diagnosis with other rapidly progressive dementias (RPD). Recent advances are critically discussed and put in the context of clinical relevance, established biomarkers, and epidemiology.

Search strategy and selection criteria

We searched Google Scholar and PubMed using the terms “prion” and “Creutzfeldt-Jakob disease”, each in combination with “diagnosis”, “criteria”, “biomarker”, “MRI”, “EEG”, and “RT-QuIC”. Studies and articles published between January 1, 2015 and March 15, 2020

written in English, German or Spanish language were included based on the scientific merit and contribution to recent developments in biomarker research for human prion diseases. Some earlier articles were selected to substantiate general information and basic evidence.

Investigating the performance of diagnostic tests for sCJD

When estimates of diagnostic accuracy are being translated into clinical practice, potential selection biases of case and control groups should be considered. An example of a biased control group was the evaluation of the diagnostic criteria for sCJD reported in 2018.[16] The control group was selected on the basis of a (false) positive CSF 14-3-3 test, resulting in an extremely weak specificity of this biomarker (27%). In 2017, a study evaluating the utility of olfactory mucosa and CSF samples in second-generation RT-QuIC[20] reported a rather low sensitivity of 86% compared to other reports employing second-generation RT-QuIC assays: the authors stated that the case group was partially selected from samples that had prior negative first-generation RT-QuIC result. Both examples underline the importance of the interpreting all biomarker test results in an adequate clinical context.

Most biomarker studies report the sensitivity and the specificity of diagnostic tests. It is debatable whether these are the most useful measures of diagnostic performance since they are not easy to interpret in a clinical setting. Predictive values may be more accurate to determine the likelihood of a disease but they are associated with disease prevalence. To calculate predictive values, the rate of cases and controls in a study has to reflect the respective rate in the population, or Bayes' rule has to be applied.[21,22] The first condition cannot be achieved in the context of an extremely rare disease like sCJD and the latter would always result in extremely low values. Thus, predictive values are not considered in this review. In case of established biomarkers with defined cut-offs, we indicate test sensitivity and specificity as measures for diagnostic accuracy. For experimental biomarkers, we indicate the area under the curve (AUC) from receiver operator characteristics.

Current state and recent advances in CJD biomarker research

Neuropathological investigation and immunostaining of PrP^{SC} allow a definite diagnosis of prion diseases.[23] For ante-mortem diagnosis of definite sCJD, brain biopsy is required, but is complicated by the possibility of a false negative result in some types of prion disease (e.g., fatal insomnia) and is reliant upon tissue quality. Being highly invasive, this procedure is considered when the diagnosis is not clear and potentially treatable conditions (e.g. encephalitis or cerebral lymphoma) are assumed,[24] or the risk of transmission has to be

determined. A less invasive procedure, tonsillar biopsy, can be established for the diagnosis of vCJD, but is not helpful for other forms of prion disease.[25] The direct in vivo detection of PrP^{SC} in sCJD seems possible but a pilot study using urine reported a poor sensitivity of 40%.[26] Thus, clinical diagnostic criteria of sCJD were initially based on surrogate markers. On the other hand, growing clinical evidence enforces the use of new PrP^{SC} aggregation assays. Below, we describe the evidence for these novel assays as well as the current state of established and new surrogate biomarkers.

Table 1. Diagnostic accuracy of CSF RT-QuIC in retrospective studies

	Cases		**Controls**		**Sensitivity**	**Specificity**	**Protocol**
	n	type	n	type			
Atarashi et al. 2011[10*]	34	definite sCJD	49	OND+	85%	100%	1st Gen
McGuire et al. 2012[11]	123	definite sCJD	103	RPD	89%	99%	1st Gen
Orrú et al. 2014[32]	30	probable + definite sCJD	46	non-CJD	77%	100%	1st Gen
Orrú et al. 2015[33]	48	probable + definite sCJD	39	OND+	96%	100%	2nd Gen
Cramm et al. 2016[12]	110	definite sCJD + gCJD	400	OND+	85%	99%	1st Gen
Groveman et al. 2016[34°]	113	probable + definite sCJD	64	OND+	73%	100%	1st Gen
Groveman et al. 2016[34°]	113	probable + definite sCJD	64	OND+	94%	100%	2nd Gen
Park et al. 2016[35]	81	probable + definite sCJD	100	non-CJD	77%	100%	1st Gen
Franceschini et al. 2017[36]	145	probable + definite sCJD + gCJD	42	OND+	97%	100%	2nd Gen
Bongianni et al. 2017[20°]	49	probable + definite sCJD	71	OND+	73%	100%	1st Gen
Bongianni et al. 2017[20°]	22	probable + definite sCJD	71	OND+	86%	100%	2nd Gen
Lattanzio et al. 2017[37]	225	definite sCJD	348	OND+	84%	99%	1st Gen
Foutz et al. 2017[15]	126	definite sCJD + gCJD	67	RPD	92%	99%	2nd Gen
Rudge et al. 2018[38°]	171	definite sCJD	47	RPD	89%	100%	1st Gen

sCJD: sporadic Creutzfeldt-Jakob disease; gCJD: genetic Creutzfeldt-Jakob disease; OND+: other neurological diseases including dementia syndromes; RPD: rapidly progressive dementia, clinically suspicious for CJD; non-CJD: including non-neurologic disorders, neurologic disorders and dementia syndromes; 1st Gen: first generation tests[12]; 2nd Gen: second generation test[32]

* This study investigated two different cohorts. Overall sensitivity and specificity were summarized for this table.

°These study performed 2 different protocols and used the same control group for both investigations.

RT-QuIC as a Biomarker for sCJD

CSF RT-QuIC represents a disease-specific biomarker. Since 2011, retrospective studies investigated its diagnostic accuracy. Most of these studies used control groups that included cases with important differential diagnoses of sCJD. Nonetheless, the test specificity in all of these studies was 99%-100% (Table 1).[10–12,15,20, 32–38] Some false positive cases in retrospective studies were speculated to be previously unrecognized prion diseases.[12] However, single cases of definite non-CJD showing false positive CSF RT-QuIC have been reported.[38] Prospective studies were published since 2017 and the specificity was 99%-100% in all of them (Table 2).[15,16,40–43]

Table 2. Diagnostic accuracy of CSF RT-QuIC in prospective studies

Reference	Cases		Controls		Sensitivity	Specificity	Protocol
	n	Type	n	type			
Foutz et al. 2017[15]	65	definite sCJD + gCJD	14	RPD	95%	100%	2nd Gen
Hermann et al. 2018[16]	65	definite sCJD	118	RPD	89%	100%	1st Gen
Abu-Rumeileh et al. 2019*[40]	65	definite sCJD + gCJD	62	RPD	82%	100%	1st Gen
Abu-Rumeileh et al. 2019*[40]	65	definite sCJD + gCJD	62	RPD	96%	100%	2nd Gen
Fiorini et al. 2020[41]	102	probable + definite sCJD	80	RPD	96%	100%	2nd Gen
Mammana et al. 2020[42]	24	probable + definite sCJD	12	OND+	88%	100%	2nd Gen
Rhoads et al. 2020[43]	430	definite sCJD	69	RPD	93%	99%	2nd Gen

sCJD: sporadic Creutzfeldt-Jakob disease; gCJD: genetic Creutzfeldt-Jakob disease; OND+: other neurological diseases including dementia syndromes; RPD: rapidly progressive dementia, clinically suspicious for CJD; 1st Gen: first generation tests[12]; 2nd Gen: second generation test referring[32]
*This study performed 2 different protocols and used the same control group for both investigations.

Due to its reliability and high diagnostic accuracy, CSF RT-QuIC was incorporated in the diagnostic criteria for sCJD of several surveillance centers.[13,14,16] Regarding the test sensitivity, figures range from 73%[20,34] to 89%[11,16,38] using first generation RT-QuIC, and 92%[15] to 97%[36] using second generation RT-QuIC. Across the spectrum of molecular subtypes of sCJD[2], the sensitivity is very high in MM1/MV1 and VV2 cases while being slightly lower in MV2 cases.[15,36,37,40] These subtypes are most common among sCJD patients.

Regarding rare subtypes, small case numbers hamper the validity of the known results, but sensitivity has been reported to be substantially lower in VV1 and MM2 cases. [36,37,43] CSF RT-QuIC showed also high sensitivity for genetic prion diseases with E200K and V210I mutations while being low for fatal familial insomnia (FFI, D178N-129M).[12,15,40] However, supporting data are based on small case numbers. Although most of the RT-QuIC have focused on sCJD, it may also aid in the diagnosis of vCJD and the differentiation of prion strains.[31,44]

Regarding other tissues and body fluids, recent promising studies that applied RT-QuIC to olfactory mucosa[20,31,41] and skin biopsies[42,45] showed high sensitivities of 89% to 100% suggesting even better diagnostic accuracy than CSF RT-QuIC. Multiple components of the eyes have tested positive by RT-QuIC[46] but the diagnostic value of analysis of any routinely accessible eye tissue or fluid remains to be determined. Studies that were able to demonstrate the diagnostic value of PrP^{SC} aggregation assays (in this case, PMCA) using blood or urine are only available for vCJD.[47–48]

CSF surrogates biomarkers

14-3-3 proteins

The 14-3-3 proteins are abundantly but not solely expressed in the brain. They are located in the cytoplasm, cell plasma membranes, and organelles. Involvement in various functions such as cell signaling, growth, apoptosis etc. has been identified but not completely clarified.[49] Since 14-3-3 protein detection by western blot (WB) became part of commonly used clinical diagnostic criteria for sCJD,[5] numerous studies evaluated its diagnostic performance. In 2012, a structured meta-analysis reported a sensitivity of 92% and a specificity of 80%[50] but it was also reported that the test sensitivity is lower in early disease stages and differs across the spectrum of molecular subtypes of sCJD. The MV2 and MM2 subtypes displayed lower test sensitivities of around 60% to 70%.[51] Reported specificity ranges between 40%[52] and 92%.[53] Such discrepancies might be explained, at least partially, by different characteristics of the control groups. In recent years, several studies reported a high specificity in the discrimination of sCJD and neurodegenerative diseases such Alzheimer's disease (AD), dementia with Lewy bodies, and fronto-temporal lobar degeneration (supplementary table 2).[37,53–56] In contrast, the specificity of CSF 14-3-3 was lower when the control groups included acute neuronal injury events as well as inflammatory and infiltrative neoplastic CNS diseases.[37,53] Another factor possibly influencing specificity may be the execution and rating of 14-3-3 WB: intermediate results ("weak" or "trace") can be difficult to interpret. A problem might be the execution and

rating of 14-3-3 WB. The method might show intermediate results ("weak" or "traces") that can be difficult to interpret. Comparative evaluations of a new 14-3-3γ isoform ELISA assay showed a superior diagnostic performance compared to 14-3-3 WB.[40,57,58] One smaller study reported a sensitivity of 97% and a specificity of 94% with an AUC of 0·982 (optimal cut-off >14,552 AU/mL),[57] while a larger study (including ring trials) reported a sensitivity of 88% and a specificity of 96% (cut off >20.000 AU/mL).[58]

Tau protein

Tau is a microtubule-associated protein that is expressed in neuronal and glial cells.[59] Extremely elevated CSF t-Tau was proposed as a diagnostic biomarker for sCJD[7] and most studies reported good test sensitivity and specificity, each around 90%.[37,51,52,60] At present however, CSF t-Tau is not formally accepted as part of case definition criteria. Similar to 14-3-3, reduced sensitivity has been shown in MM2 and MV2 subtypes[51,61] as well as early disease stages.[62] Although the overall discrepancy of reported specificity was lower than that observed for 14-3-3, some studies reported a specificity of 67%[52] or lower than 50% at varying optimal diagnostic cut-offs.[54–56] The latter was observed in cases with atypical AD (supplementary table 1). Besides AD, inflammatory and neoplastic CNS diseases are important differential diagnoses of elevated t-Tau levels.[63] Unfortunately, there is no general consensus regarding the cut-off that should be used to support sCJD. Recent studies suggested either >1072 pg/ml[64] >1250 pg/mL,[36] >1300 pg/mL,[65] or >1400 pg/mL.[66] CSF t-Tau has also become a candidate as predictor of survival time.[67] The p-Tau/t-Tau (or t-Tau/p-Tau) ratio is a very important alternative biomarker for sCJD.[66] It showed a very high diagnostic accuracy in the differentiation of sCJD from other neurological diseases (OND, AUC: 0·98), AD (AUC 0·99),[68] and rapidly progressive AD (AUC 0·99).[69] Several studies that investigated large cohorts reported a superior diagnostic performance compared to t-Tau alone.[54,68,69]

Neurofilaments

Neurofilaments comprise three subunits: a light (NFL), a medium, and a heavy chain (NFH). As neuron-specific cytoskeleton proteins, their presence in body fluids represents neuroaxonal damage.[70] Several studies showed an excellent diagnostic accuracy in the discrimination of healthy controls (HC) and sCJD. The AUCs ranged from 0·992 to 0·998.[19,71,72] NFL however may lack sufficient specificity for sCJD.[40] Concerning important differential diagnoses, reported AUCs were 0·95 versus demented and non-demented OND,[18] 0·77 versus AD,[19] 0·45[19] and 0·90[71] versus OND with dementia syndrome, 0·93 versus neurodegenerative

dementias,[56] and 0·86 to 0·89 versus RPD.[73] The substantial differences between the studies might be explained by different group selection criteria but this requires further clarification. In addition, different optimal cut-offs were identified, e.g. >5016 pg/ml[56] or >10500 pg/ml.[71] In contrast to 14-3-3 and t-Tau, NFL was shown to be markedly elevated in MV2 and VV2 compared to the MM1 sCJD subtype.[56]

Other CSF surrogate biomarkers

Several other CSF biomarkers for sCJD have been identified over the past two decades. Here, we concentrate on those that have a high level of supported evidence as well as recently discovered candidates. CSF S100b has been evaluated multiple times but comparative studies showed inferior diagnostic performance compared to 14-3-3 and t-Tau[51,74] and it was not widely used clinically. The total prion protein (t-PrP) represents a special case. It is decreased in the CSF of patients with sCJD, showing a moderate diagnostic accuracy.[54,75] A study using targeted mass spectrometry instead of the more routinely used ELISA showed that all human PrP was reduced in the CSF of sCJD compared to other RPD cases.[76] In addition, it might be valuable as part of a composite biomarker profile.[54,55] Alpha-Synuclein, a synaptic protein that aggregates in synucleinopathies (Parkinson's Disease and related disorders) was observed to be massively increased in sCJD, probably related to rapid neurodegeneration. A multi-center study showed an excellent diagnostic accuracy (AUC = 0·997, 98% sensitivity, 97% specificity) in the discrimination of sCJD and OND (including dementia syndromes) at an optimal cut-off of 820 pg/mL using commercial ELISA.[77,78] Similar results were found in an inter-laboratory validation study.[79] In 2019, it was shown that CSF Neurogranin, a neuronal calmodulin-binding protein, discriminated CJD from OND (excluding neurodegenerative diseases) with an AUC of 0·96 and CJD from AD with an AUC of 0·85.[80] Another study from 2020 validated the findings.[72] Advantages and disadvantages of the most common CSF surrogate biomarkers are summarized in Table 3.

Table 3. Advantages, disadvantages, and perspectives of important CSF biomarkers for sCJD

	Pros	Cons	Perspectives
14-3-3	• clinical gold standard[5,6] • high sensitivity[49] • high specificity versus neurodegenerative dementias[37,53–56]	• low specificity versus acute events and encephalitis[37,53] • moderate sensitivity for certain sCJD types[51] • "traces" in Western blot (ambiguous	• improved accuracy (14-3-3γ ELISA)[40,57,58] • potential prognostic marker (14-3-3γ ELISA)

		test results)	
Tau	• high sensitivity[37,51,52,60] • high specificity versus most neurodegenerative dementias[37,53,54]	• moderate specificity versus acute events and encephalitis[63] • low specificity versus atypical AD[54-56] • moderate sensitivity for certain sCJD types[51,61] • different cut-offs in the literature[36,64–66]	• improved accuracy (p-Tau/t-Tau ratio)[65,67] • prognostic marker[66] • peripheral marker (blood-based)[18,19]
S100b	• good sensitivity	• moderate specificity • overall accuracy inferior to 14-3-3 and t-Tau in most comparative studies[51,74]	• peripheral marker (blood-based)[18]
NFL	• high sensitivity[19,71,72]	• grade of evidence is lower than for 14-3-3 and t-Tau • lack of specificity versus other neurological diseases[40]	• further validation as diagnostic and potential prognostic marker • peripheral marker (blood-based)[19]

sCJD: sporadic Creutzfeldt-Jakob disease; AD: Alzheimer's Disease; t-Tau: total Tau; p-Tau: phosphorylated Tau; NFL: Neurofilament light chain

Blood-based biomarker candidates

Blood-based surrogate biomarkers for neurodegenerative diseases, in particular for prion diseases, have come into focus in recent years. To date, few data from a limited number of studies are available. However, they open new perspectives and possibilities. One of the potential candidates is the t-Tau concentration in plasma or serum. Studies demonstrated elevated levels in sCJD compared to HC and OND.[18,19,81] The diagnostic accuracy ranged from an AUC of 0·94 versus HC to 0·72 versus ONDs that included dementia syndromes (supplementary Table 2). Another investigation showed that the plasma t-Tau level is a predictor of survival time in sCJD, rather than CSF levels or other fluid biomarkers.[82] Another promising candidate for a blood-based biomarker is NFL, the smallest and most soluble subunit of neurofilament. In blood, it reflects unspecific neuronal and axonal damage.[70] Studies that investigated its potential in the diagnosis of sCJD showed similar diagnostic accuracy compared to t-Tau but an almost perfect accuracy in the discrimination from HC.[19,81] Other proteins that were considered as potential CSF biomarkers for sCJD, such as S100b and YKL-40[83] were elevated in serum or plasma, but few available data display inferior diagnostic accuracy compared to t-Tau and NFL (supplementary Table 3). Interestingly, PrP was

reported to be to be decreased in the CSF of sCJD cases[54,55] while it was increased in plasma.[84] This dissociation has not been clarified, yet.

There are several potential roles that might feasibly be fulfilled by blood biomarkers. At present there is no immediate prospect of a highly specific diagnostic blood test comparable to RT-QuIC in CSF, however blood assays might offer an accessible triage test in primary care or first specialist assessment that flags the possibility of rapid neuronal damage and could be useful in case prioritization. A further opportunity is markers of ongoing neuronal damage. NfL for example has been shown to be an effective therapeutic biomarker in CNS disease during trials for multiple sclerosis. One of the challenges for clinical trials in sCJD is that clinical features are highly heterogeneous, and it has been difficult to find a suitable single continuous measure as an outcome. In this circumstance, specific CJD tests like RT-QuIC might be used at trial enrollment and blood biomarkers might be used repeatedly during a trial to track neuro-axonal damage in the course of experimental treatment. Further work is required to establish variability of biomarkers in the natural history of CJD and if blood biomarkers of neurodegeneration can contribute to prognostic or trial models. Finally, blood biomarkers may have a role in preventive trials as a prodromal biomarker for individuals healthy but at-risk of CJD because of iatrogenic prion exposure or *PRNP* mutation. Present published work suggests a prodromal biomarker window is small or rare in at risk individuals, but this is an area of active research.

Imaging markers

Magnetic Resonance Imaging

MRI is an essential tool in the diagnosis of sCJD. It allows the identification of important differential diagnoses such as ischemia, encephalitis, and neoplasia. CJD-typical patterns of restricted diffusion were included in the diagnostic criteria in 2009.[6] A similar and widely used protocol was published two years later.[85] The CJD-typical MRI displays restricted diffusion in cortical regions ("ribboning") and/ or restricted diffusion predominantly in the caudate nucleus, followed by putamen and thalamus (Figure 1). The subcortical white matter is not involved.[6,85] Cortical ribboning and involvement of the caudate nucleus (on one or both hemispheres, rarely perfectly symmetric) is typically seen in the most common MM1 subtype. Involvement of the thalamus (besides caudate nucleus and putamen) is a hallmark for VV2 and MV2 subtypes. Restricted diffusion only in the thalamus ("pulvinar sign" without other basal ganglia involvement) is an indicator of vCJD.[25]

Figure 1. CJD-typical patterns of restricted diffusion on MRI *(outline)*

A MM1 case <u>DWI</u> Hyperintensities: >1 cortical region and caudate (+putamen) on one hemisphere or both (asymmetric)	**B** <u>ADC map</u> associated hypointensities	**C** <u>FLAIR</u> Hyperintensities (less impressive)
D MV2 (or VV2) case <u>DWI</u> Hyperintensities: Caudate Putamen Thalamus	**E** <u>ADC map</u> associated hypointensities	**F** <u>FLAIR</u> Hyperintensities (less impressive)

The overall diagnostic accuracy of MRI is possibly even superior to CSF 14-3-3 and t-Tau,[86] but comparison data is scarce. Recent studies showed a sensitivity of around 80%,[16,35,39,40] with others reporting up to 98%.[84] Recent studies showed a sensitivity of around 80%,[16,36,40,41] others reported up to 98%.[85] Similarly, specificity ranges from 74%[40] to 98%.[16] In 2018, a study investigated MRI results in a cohort of 171 definite sCJD cases and 47 controls (all clinical CJD mimics) and revealed a sensitivity of 92% with a specificity of 96%.[38] The discrepancies may be caused by different scanners, imaging and rating protocols, or a study focus on other biomarkers. The diagnostic accuracy depends on the individual experience image interpreters.[87] In addition, apparent diffusion coefficient (ADC-) map is prone to movement artifacts. The future possibilities of brain MRI include its application as a prognostic marker[88] and as a potential marker in trial monitoring.[89,90] Interestingly, restricted diffusion can occur in very early disease stages. Although prospective data is not available, it was illustrated that it can be observed in pre-symptomatic patients with familial CJD (E200K mutation) and sCJD.[91]

Positron Emission Tomography

Positron emission tomography using [18F]fluoro-2-deoxy-D-glucose as tracer (FDG-PET) is able to detected decreased glucose metabolism in cortical regions of sCJD patients. The value of FDG-PET in the differential diagnosis is limited, though. No specific patterns have been

identified. However, FDG-PET has potential as a marker of early sCJD and showed a correlation with clinical symptoms.[92] In the rare MM2-thalamic subtype (sporadic fatal insomnia) as well as in FFI, a massively reduced thalamic glucose metabolism is a distinctive feature and may even precede the clinical onset.[93]

Electroencephalography

More than 20 years ago, periodic sharp-wave complexes (PSWC) with a frequency of 1 Hz were identified as CJD-typical EEG pattern with a sensitivity of 67% and a specificity of 86%.[4] The non-convulsive status epilepticus is the most frequent clinical condition with CJD-like EEG.[94] Large-scale prospective evaluations focusing on this biomarker are not available but CSF biomarker comparison studies from the last 2 years reported a substantially lower sensitivity (39% to 45%) for EEG.[16,35,38,40] Most likely, the decreasing sensitivity of EEG is a result of improved early recognition of sCJD cases. Typical PSWCs occur in late disease stages and are less frequent in MV2, VV2, and MM2 cases. Nonetheless, the method is less invasive than CSF sampling and non-specific periodic patterns of rhythm abnormalities[95] as well as quantitative analysis of frequency alterations[96] may have the potential to aid the diagnosis in early stages and to predict disease progression.

Genetic markers of human prion disease

Mutations of the PrP gene (*PRNP*) account for about 10-15% of all human prion diseases.[1] Some cause specific clinical syndromes like Gerstmann-Sträussler-Scheinker (GSS) or FFI, others may mimic the clinical presentation of sCJD (e.g. E200K).[97] Thus, the sequencing of *PRNP* is an important biomarker that should be considered in the differential diagnosis of prion diseases and is vital in atypical cases, as well as in cases with positive or uninformed family history of RPD. In sCJD, the combination of the codon 129 polymorphism (Methionine and Valine) with molecular mass of PrP^{SC} (glycotype 1 and 2) forms molecular subtypes that are associated with distinct neuropathological, clinical, and biomarker profiles.[2] Reduced sensitivity of surrogate biomarkers has been observed, especially in MV2 and MM2 subtypes.[51,60] The identification of the PrP^{SC} type is only possible in brain tissue but the analysis of codon 129 *PRNP* might help to interpret inconclusive biomarker results.[98]

Clinical value of RT-QuIC and CSF surrogate biomarkers

Surrogate CSF biomarkers of sCJD are reliable diagnostics but the accuracy may differ with respect to the clinical context in which these markers are utilized. They are not disease

specific by their very nature. Thus, physicians should interpret results of surrogate biomarker measurements with caution.

Over the last nine years, the evidence indicating CSF RT-QuIC as a major improvement in the clinical diagnosis of sCJD has reached a significant level. The test sensitivity is similar to the best available surrogate biomarkers but the data display superior specificity (Table 1, Table 2) and its reproducibility has been demonstrated in ring trials.[12,99] However, RT-QuIC is rather complex and costly. Different protocols and definitions of test positivity were proposed.[11,29,33] The fact that RT-QuIC assays can amplify PrP^{SC} aggregates has raised questions about whether the amplified CJD-seeded reaction products are themselves infectious and, therefore, biohazardous.[100,101]

CSF 14-3-3 protein is highly sensitive and well-validated, but atypical forms of sCJD, such as the MV2 subtype, and early disease stages are associated with decreased sensitivity.[51,74] Acute neurologic events like stroke or encephalitis may cause false positive results. CSF 14-3-3 protein is part of a widely used clinical diagnostic gold standard[5,6] and estimates of the diagnostic accuracy, especially in comparative analyses, may be influenced by verification bias.[102] A problem in the utilization of the 14-3-3 WB method is its complex interpretation and the presence of borderline results (traces). New 14-3-3 ELISAs may resolve this problem but they have not been widely established. The most commonly used alternative CSF biomarker is t-Tau. Some studies showed that it is slightly less sensitive than 14-3-3 WB but its higher specificity may indicate a superior overall diagnostic accuracy.[52,74] Indeed, t-Tau showed a better specificity in the differentiation of sCJD and acute events (e.g. stroke, seizures) or encephalitis,[37,53] but there is some evidence that t-Tau may lack sufficient specificity in the discrimination of rapidly progressive or atypical AD and sCJD (supplementary Table 1). In a large cohort representing the full clinical spectrum of a non-specialized neurochemical laboratory, sCJD accounted for only 18% of patients with highly elevated (> 1200 pg/mL) CSF t-Tau levels.[63] In conclusion, both markers (CSF 14-3-3 and t-Tau) are useful in the diagnosis of sCJD. They share several characteristics, advantages, and disadvantages (Table 2). The clinical utility has to be assessed in the light of suspected differential diagnoses and can be improved by stratification of demographic and genetic factors.[98]

An upcoming issue in the biomarker-based diagnosis of sCJD is the use of composites. Concerning this, the best evidence is available for the p-Tau/t-Tau ratio, which was demonstrated to be of superior diagnostic accuracy compared to t-Tau alone, especially in the differentiation of sCJD from AD.[54,68,69] Proposed ratios combining t-Tau, p-Tau, 14-3-3,

S100b, t-PrP, and beta amyloid have shown high diagnostic accuracy[51,55,74,103] but have not been established in the clinical setting, yet.

Guidelines for the biomarker-based diagnosis of sCJD

Based on the WHO criteria,[5,6] the studies presented here, and previous suggestions that include RT-QuIC,[13,14] we recommend amended criteria for the clinical diagnosis of sCJD as displayed in Figure 2. Due to the outstanding specificity of RT-QuIC, positive cases can be classified as probable sCJD in early clinical stages, even when only one cardinal symptom is present. This will improve the overall diagnostic performance and the early identification of sCJD.[16,43,103]

However, the limited availability of RT-QuIC in countries without major surveillance programs and differing test sensitivities from 73% and 97% prevent CSF RT-QuIC from becoming a solitary diagnostic criterion. Some false positive cases have been reported,[38,43] RT-QuIC is not able to accurately distinguish between different forms of human prion disease, and definite diagnosis requires neuropathological examination. Readily available, economical, and field-tested CSF biomarkers like 14-3-3 and t-Tau are still of major importance. In addition to EEG and brain MRI, we suggest the use of the highly sensitive CSF 14-3-3 test (at best 14-3-3γ ELISA) as a routine clinical diagnostic test in cases of suspected sCJD. In case of ambiguous results or uncertain differential diagnoses, CSF t-Tau and the t-Tau/p-Tau ratio might be considered as supportive biomarkers.[64,68,69] Genetic analysis of *PRNP* should be considered in all cases of suspected CJD to determine the codon 129 polymorphism and to exclude pathogenic mutations, which might be present even in patients with a negative family history.[97] Most important, routine blood, CSF, and imaging diagnostics should always be performed to rule out the most common differential diagnoses. The supplementary Table 3 gives an overview on clinical CJD mimics. See Panel 1 for a guideline summary.

Figure 2. Criteria for the diagnosis of sporadic Creutzfeldt-Jakob disease

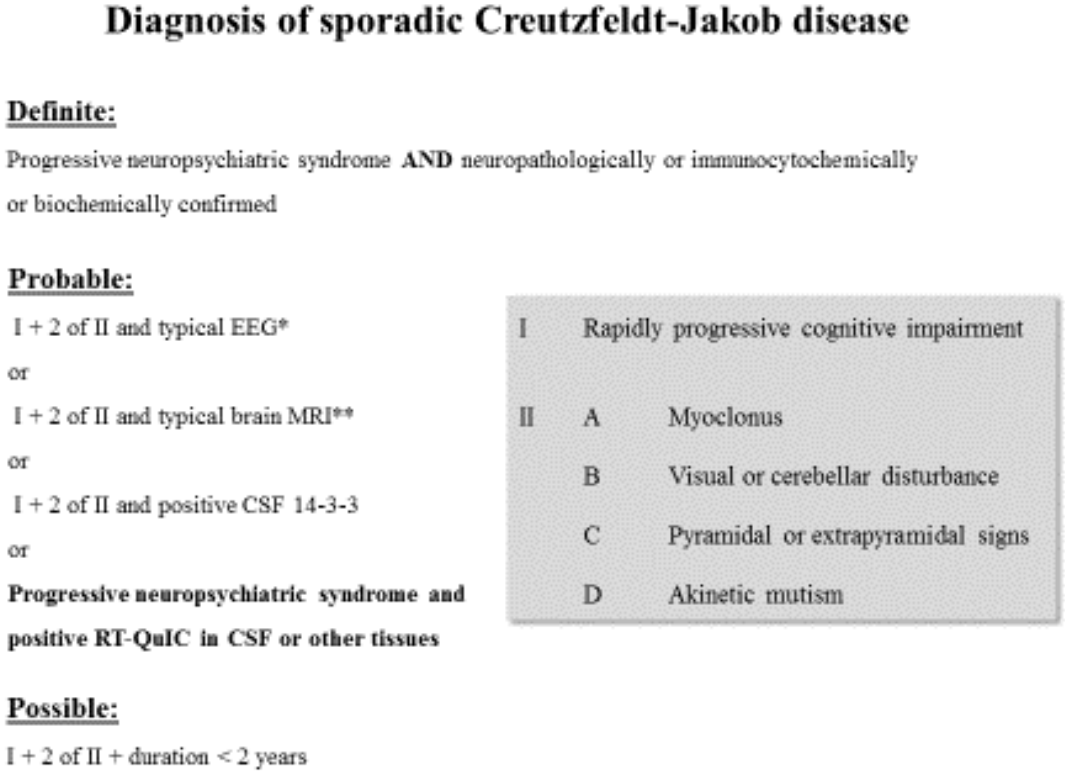

Figure Legend

The figure has been adapted from the NCJDRSU.[13] Here, imaging criteria were refined and restricted diffusion in cortical regions was included.[6]
* Generalised periodic spike/ wave complexes (PSWCs)
** Restricted diffusion in caudate/putamen or at least two cortical regions (temporal, parietal, occipital) on MRI brain scan, no subcortical white matter involvement, no isolated restricted diffusion in the thalamus

Future challenges and perspectives

Despite recent improvements of diagnostic measures for sCJD, there are still plenty of challenges. The value of established and new biomarkers in the differential diagnosis of sCJD subtypes and other human prion diseases (iatrogenic CJD, vCJD, and genetic CJD) has to be clarified. RT-QuIC has to be more widely distributed, protocols have to be unified, past studies on peripheral tissue have to be validated with regard to important differential diagnoses, and more candidate tissues have to be evaluated. In this context, the potential infectivity of RT-QuIC positive tissues and body fluids may be reappraised. Over the last five years, some investigations have opened the field of pre-symptomatic, prognostic, and probably predictive surrogate biomarkers for sCJD. Especially blood-based biomarkers have come into focus and may become new tools for diagnosis, case management, and trial

monitoring. This will become extremely important for the evaluation and the development of urgently needed new therapeutics.

Panel 1. Guidelines for the clinical diagnosis of sCJD

General
The clinical diagnosis of sCJD requires a thorough diagnostic work-up including clinical investigation, blood sampling, lumbar puncture, neuroimaging (MRI), and EEG at minimum. Further diagnostics (e.g. body CT, PET, specific CSF analyses) can be necessary depending on suspected differential diagnoses.
The diagnostic criteria and its measurements
We recommend amended criteria for the clinical diagnosis of sCJD (Figure 2). If available, RT-QuIC should be performed in every case of suspected prion disease. The 14-3-3 test is the primary CSF surrogate biomarker, which has to be performed in an experienced and certified laboratory. CSF t-Tau and the t-Tau/p-Tau ratio might be valuable supportive biomarkers. MRI and EEG are highly specific but require experienced raters. All mentioned biomarkers are less sensitive in early disease stage and in some molecular subtypes, follow-up investigations may be useful in case of negative results. The analysis of codon 129 PRNP polymorphism might assist in interpreting the results of other biomarker analyses.
Important differential diagnoses
Mutation analyses as well as clinical indicators of iCJD and vCJD should be considered in all cases with suspected prion disease. Rapidly progressive neurodegenerative diseases, (immune-mediated) encephalitis, status epilepticus, and cerebral ischemia, are frequent differential diagnoses. Among others, these conditions may mimic the clinical syndrome and most surrogate biomarkers of sCJD.
Brain biopsy
Brain biopsy is an invasive procedure that can be considered when non-invasive diagnostics remain inconclusive and a potentially treatable alternative diagnosis is suspected.

References

1. Ladogana A, Puopolo M, Croes EA, et al. Mortality from Creutzfeldt-Jakob disease and related disorders in Europe, Australia, and Canada. *Neurology* 2005; **64:** 1586–91.

2. Parchi P, Giese A, Capellari S, et al. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. *Ann Neurol* 1999; **46:** 224–33.
3. Masters CL, Harris JO, Gajdusek DC, Gibbs CJJ, Bernoulli C, Asher DM. Creutzfeldt-Jakob disease: patterns of worldwide occurrence and the significance of familial and sporadic clustering. *Ann Neurol* 1979; **5:** 177–88.
4. Steinhoff BJ, Racker S, Herrendorf G, et al. Accuracy and reliability of periodic sharp wave complexes in Creutzfeldt-Jakob disease. *Arch Neurol* 1996; **53:** 162–66.
5. WHO. Global Surveillance, diagnosis, and Therapy of Human Transmissible spongiform Encephalopathies: Report of WHO consultation, February 9-11, 1998, Geneva, Switzerland.
6. Zerr I, Kallenberg K, Summers DM et al. Updated clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease. *Brain* 2009; **132:** 2659–68.
7. Otto M, Wiltfang J, Tumani H, et al. Elevated levels of tau-protein in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. *Neurosci Lett* 1997; **225:** 210–12.
8. Collins SJ, Sanchez-Juan P, Masters CL, et al. Determinants of diagnostic investigation sensitivities across the clinical spectrum of sporadic Creutzfeldt-Jakob-disease. *Brain* 2006; **129:** 2278–87.
9. Geschwind MD, Martindale J, Miller D, et al. Challenging the clinical utility of the 14-3-3 protein for the diagnosis of sporadic Creutzfeldt-Jakob disease. *Arch Neurol* 2003; **60:** 813–16.
10. 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:** 175–78.
11. McGuire LI, Peden AH, Orrú CD et al. RT-QuIC analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. Ann Neurol 2012;72: 278–285, doi: 10.1002/ana.23589.
12. 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: 1896–904.
13. National Creutzfeldt-Jakob Disease Research & Surveillance Unit (NCJDRSU). Available online: https://www.cjd.ed.ac.uk/sites/default/files/criteria_0.pdf (accessed on 27 April 2020)
14. Centers for Disease Control and Prevention (CDC) Available online: https://www.cdc.gov/prions/cjd/diagnostic-criteria.html (accessed on 27 April 2020)
15. Foutz A, Appleby BS, Hamlin C, et al. Diagnostic and prognostic value of human prion detection in cerebrospinal fluid. Ann Neurol 2017; **81:** 79–92.
16. Hermann P, Laux M, Glatzel M, et al. Validation and utilization of amended diagnostic criteria in Creutzfeldt-Jakob disease surveillance. *Neurology* 2018; **91:** e331-e338.
17. Noguchi-Shinohara M, Hamaguchi T, Nozaki I, Sakai K, Yamada M. Serum tau protein as a marker for the diagnosis of Creutzfeldt-Jakob disease. *J Neurol* 2011; 258: 1464–8.
18. Steinacker P, Blennow K, Halbgebauer S, et al. Neurofilaments in blood and CSF for diagnosis and prediction of onset in Creutzfeldt-Jakob disease. *Sci Rep* 2016; **6:** 38737.
19. Kovacs GG, Andreasson U, Liman V, et al. Plasma and cerebrospinal fluid tau and neurofilament concentrations in rapidly progressive neurological syndromes: a neuropathology-based cohort. *Eur J Neurol* 2017; **24:** 1326–e77.
20. Bongianni M, Orrú 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:** 155–62.
21. Casscells W, Schoenberger A, Graboys TB. Interpretation by physicians of clinical laboratory results. *N Engl J Med* 1978; **299:** 999–1001.
22. Altman DG, Bland JM. Diagnostic tests 2: Predictive values. *BMJ* 1994; **309:** 102.
23. 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:** 459–66.
24. 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:** E2. doi: 10.3171/2015.8.FOCUS15328.
25. Heath CA, Cooper SA, Murray K, et al. Validation of diagnostic criteria for variant Creutzfeldt-Jakob disease. *Ann Neurol* 2010; 67: 761–70.
26. Luk C, Jones S, Thomas C, et al. Diagnosing Sporadic Creutzfeldt-Jakob Disease by the Detection of Abnormal Prion Protein in Patient Urine. *JAMA Neurol* 2016; **73:** 1454–60.

27. Atarashi R, Moore RA, Sim VL, et al. Ultrasensitive detection of scrapie prion protein using seeded conversion of recombinant prion protein. *Nat Methods* 2007; **4:** 645–50.
28. Wilham JM, Orrú CD, Bessen RA, et al. Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays. *PLoS Pathog* 2010. **6:** e1001217. doi:10.1371/journal.ppat.1001217
29. Schmitz, M., Cramm, M., Llorens, F. et al. The real-time quaking-induced conversion assay for detection of human prion disease and study of other protein misfolding diseases. *Nat Protoc* 2016; **11:** 2233–42.
30. Vallabh SM, Nobuhara CK, Llorens F, et al. Prion protein quantification in human cerebrospinal fluid as a tool for prion disease drug development. *Proc Natl Acad Sci U S A* 2019; **116:** 7793–98.
31. Orrú 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. 6: e1004983. doi: 10.1371/journal.ppat.1004983.
32. Orrú CD, Bongianni M, Tonoli G, et al. A test for Creutzfeldt-Jakob disease using nasal brushings. N Engl J Med 2014; **371:** 519–29.
33. Orrú 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:** e02451–14.
34. Groveman BR, Orrú CD, Hughson AG, et al. Extended and direct evaluation of RT-QuIC assays for Creutzfeldt-Jakob disease diagnosis. *Ann Clin Transl Neurol* 2017; **4:** 139–44.
35. Park JH, Choi YG, Lee YJ, et al. Real-Time Quaking-Induced Conversion Analysis for the Diagnosis of Sporadic Creutzfeldt-Jakob Disease in Korea. *J Clin Neurol* 2016; **12:** 101–6.
36. Franceschini A, Baiardi S, Hughson AG, et al. High diagnostic value of second generation CSF RT-QuIC across the wide spectrum of CJD prions. *Sci Rep* 2017; **7:** 10655.
37. Lattanzio F, Abu-Rumeileh S, Franceschini A, et al. Prion-specific and surrogate CSF biomarkers in Creutzfeldt-Jakob disease: diagnostic accuracy in relation to molecular subtypes and analysis of neuropathological correlates of p-tau and Aβ42 levels. *Acta Neuropathol* 2017; **133:** 559–78.
38. Rudge P, Hyare H, Green A, Collinge J, Mead S. Imaging and CSF analyses effectively distinguish CJD from its mimics. *J Neurol Neurosurg Psychiatry* 2018; **89:** 461–66.
39. Hayashi Y, Iwasaki Y, Yoshikura N, et al. An autopsy-verified case of steroid-responsive encephalopathy with convulsion and a false-positive result from the real-time quaking-induced conversion assay. *Prion* 2017; **11:** 284–92.
40. Abu-Rumeileh S, Baiardi S, Polischi B, et al. Diagnostic value of surrogate CSF biomarkers for Creutzfeldt-Jakob disease in the era of RT-QuIC. *J Neurol* 2019; **266:** 3136–43.
41. Fiorini M, Iselle G, Perra D, et al. High Diagnostic Accuracy of RT-QuIC Assay in a Prospective Study of Patients with Suspected sCJD. *Int J Mol Sci* 2020; **21:** pii: E880. doi: 10.3390/ijms21030880.
42. Mammana A, Baiardi S, Rossi M, et al. Detection of prions in skin punch biopsies of Creutzfeldt-Jakob disease patients. *Ann Clin Transl Neurol* 2020. doi: 10.1002/acn3.51000.
43. Rhoads D, Wrona A, Foutz A, et al. Diagnosis of Prion Diseases by RT-QuIC Results in Improved Surveillance. Neurology 2020. In press.
44. Zanusso G, Monaco S, Pocchiari M, Caughey B. Advanced tests for early and accurate diagnosis of Creutzfeldt-Jakob disease. *Nat Rev Neurol* 2016; **12:** 325–33.
45. Orrú CD, Yuan J, Appleby BS, et al. Prion seeding activity and infectivity in skin samples from patients with sporadic Creutzfeldt-Jakob disease. *Sci Transl Med* 2017; **9:** eaam7785.
46. Orrù CD, Soldau K, Cordano C, et al. Prion Seeds Distribute throughout the Eyes of Sporadic Creutzfeldt-Jakob Disease Patients.MBio 2018. 9: e02095-18. doi: 10.1128/mBio.02095-18
47. Bougard D, Brandel JP, Bélondrade M, et al. Detection of prions in the plasma of presymptomatic and symptomatic patients with variant Creutzfeldt-Jakob disease. *Sci Transl Med* 2016; **8:** 370ra182.
48. Concha-Marambio L, Pritzkow S, et al. Detection of prions in blood from patients with variant Creutzfeldt-Jakob disease. *Sci Transl Med* 2016; **8:** 370ra183.
49. Berg D, Holzmann C, Riess O. 14-3-3 proteins in the nervous system. *Nat Rev Neurosci* 2003; **4:** 752–62.
50. Muayqil T, Gronseth G, Camicioli R. Evidence-based guideline: diagnostic accuracy of CSF 14-3-3 protein in sporadic Creutzfeldt-Jakob disease: report of the guideline development subcommittee of the American Academy of Neurology. *Neurology* 2012, **79:** 1499–506.
51. Sanchez-Juan P, Green A, Ladogana A, et al. CSF tests in the differential diagnosis of Creutzfeldt-Jakob disease. *Neurology* 2006; **67:** 637–43.

52. 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:** 547–52.
53. Stoeck K, Sanchez-Juan P, Gawinecka J, et al. Cerebrospinal fluid biomarker supported diagnosis of Creutzfeldt-Jakob disease and rapid dementias: a longitudinal multicentre study over 10 years. *Brain* 2012; **135:** 3051–61
54. Dorey A, Tholance Y, Vighetto A, et al. Association of cerebrospinal fluid prion protein levels and the distinction between Alzheimer disease and Creutzfeldt-Jakob disease. *JAMA Neurol* 2015; **72:** 267–75.
55. Abu Rumeileh S, Lattanzio F, Stanzani Maserati M, Rizzi R, Capellari S, Parchi P. Diagnostic Accuracy of a Combined Analysis of Cerebrospinal Fluid t-PrP, t-tau, p-tau, and Aβ42 in the Differential Diagnosis of Creutzfeldt-Jakob Disease from Alzheimer's Disease with Emphasis on Atypical Disease Variants. *J Alzheimers Dis* 2017; **55:** 1471–80.
56. Abu-Rumeileh S, Capellari S, Stanzani-Maserati M, et al. The CSF neurofilament light signature in rapidly progressive neurodegenerative dementias. *Alzheimers Res Ther* 2018; **10:** 3.
57. Leitão MJ, Baldeiras I, Almeida MR, et al. Sporadic Creutzfeldt-Jakob disease diagnostic accuracy is improved by a new CSF ELISA 14-3-3γ assay. *Neuroscience* 2016; **322:** 398-407.
58. Schmitz M, Ebert E, Stoeck K, et al. Validation of 14-3-3 Protein as a Marker in Sporadic Creutzfeldt-Jakob Disease Diagnostic. *Mol Neurobiol* 2016; **53:** 2189–99.
59. Wang Y, Mandelkow E. Tau in physiology and pathology. *Nat Rev Neurosci* 2016; **17:** 5–21.
60. Koscova S, Zakova Slivarichova D, Tomeckova I, et al. Cerebrospinal Fluid Biomarkers in the Diagnosis of Creutzfeldt-Jakob Disease in Slovak Patients: over 10-Year Period Review. *Mol Neurobiol* 2017; **54:** 5919–27.
61. Karch A, Hermann P, Ponto C, et al. Cerebrospinal fluid tau levels are a marker for molecular subtype in sporadic Creutzfeldt-Jakob disease. *Neurobiol Aging* 2015; **36:** 1964–68.
62. Cohen OS, Chapman J, Korczyn AD, et al. CSF tau correlates with CJD disease severity and cognitive decline. *Acta Neurol Scand* 2016; **133:** 119–23.
63. Lehmann S, Paquet C, Malaplate-Armand C, et al. Diagnosis associated with Tau higher than 1200 pg/mL: Insights from the clinical and laboratory practice. *Clin Chim Acta* 2019; **495:** 451–56.
64. Li QX, Varghese S, Sarros S, et al. CSF Tau supplements 14-3-3 protein detection for sporadic Creutzfeldt-Jakob disease diagnosis while transitioning to next generation diagnostics. J Clin Neurosci 2018; 50: 292–293.
65. Llorens F, Karch A, Golanska E, et al. Cerebrospinal Fluid Biomarker-Based Diagnosis of Sporadic Creutzfeldt-Jakob Disease: A Validation Study for Previously Established Cutoffs. Dement. *Geriatr Cogn Disord* 2017; **43:** 71-80.
66. Blennow K, Johansson A, Zetterberg H. Diagnostic value of 14-3-3beta immunoblot and T-tau/P-tau ratio in clinically suspected Creutzfeldt-Jakob disease. *Int J Mol Med* 2005; **16:** 1147–9.
67. Llorens F, Rübsamen N, Hermann P, et al. A prognostic model for overall survival in sporadic Creutzfeldt-Jakob disease. Alzheimers Dement 2020. In press.
68. Skillbäck T, Rosén C, Asztely F, Mattsson N, Blennow K, Zetterberg H. Diagnostic performance of cerebrospinal fluid total tau and phosphorylated tau in Creutzfeldt-Jakob disease: results from the Swedish Mortality Registry. *JAMA Neurol* 2014; **71:** 476–83.
69. Llorens F, Schmitz M, Karch A, et al. Comparative analysis of cerebrospinal fluid biomarkers in the differential diagnosis of neurodegenerative dementia. *Alzheimers Dement* 2016; **12:** 577–89.
70. Petzold A. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. *J Neurol Sci* 2005; **233:** 183–98.
71. Zerr I, Schmitz M, Karch A, et al. Cerebrospinal fluid neurofilament light levels in neurodegenerative dementia: Evaluation of diagnostic accuracy in the differential diagnosis of prion diseases. *Alzheimers Dement* 2018; **14:** 751–63.
72. Antonell A, Tort-Merino A, Ríos J, et al. Synaptic, axonal damage and inflammatory cerebrospinal fluid biomarkers in neurodegenerative dementias. Alzheimers Dement 2020; 16: 262–72.
73. Kanata E, Golanska E, Villar-Piqué A, et al. Cerebrospinal fluid neurofilament light in suspected sporadic Creutzfeldt-Jakob disease. *J Clin Neurosci* 2019; **60:** 124–27.
74. Chohan G, Pennington C, Mackenzie JM, et al. The role of cerebrospinal fluid 14-3-3 and other proteins in the diagnosis of sporadic Creutzfeldt-Jakob disease in the UK: a 10-year review. *J Neurol Neurosurg Psychiatry* 2010; **81:** 1243–8.

75. Villar-Piqué A, Schmitz M, Lachmann I, et al. Cerebrospinal Fluid Total Prion Protein in the Spectrum of Prion Diseases. *Mol Neurobiol* 2019; **56:** 2811–21.
76. Minikel EV, Kuhn E, Cocco A, et al. Domain-specific Quantification of Prion Protein in Cerebrospinal Fluid by Targeted Mass Spectrometry. *Mol Cell Proteomics* 2019; **18:** 2388–400.
77. Llorens F, Kruse N, Schmitz M, et al. Evaluation of α-synuclein as a novel cerebrospinal fluid biomarker in different forms of prion diseases. *Alzheimers Dement* 2017; **13:** 710–9.
78. Llorens F, Kruse N, Karch A, et al. Validation of α-Synuclein as a CSF Biomarker for Sporadic Creutzfeldt-Jakob Disease. *Mol Neurobiol* 2018; **55:** 2249–57.
79. Kruse N, Heslegrave A, Gupta V, et al. Interlaboratory validation of cerebrospinal fluid α-synuclein quantification in the diagnosis of sporadic Creutzfeldt-Jakob disease. *Alzheimers Dement (Amst)* 2018; **10:** 461–70.
80. Blennow K, Diaz-Lucena D, Zetterberg H, et al. CSF neurogranin as a neuronal damage marker in CJD: a comparative study with AD. *J Neurol Neurosurg Psychiatry* 2019; **90:** 846–53.
81. Thompson AGB, Luk C, Heslegrave AJ, et al. Neurofilament light chain and tau concentrations are markedly increased in the serum of patients with sporadic Creutzfeldt-Jakob disease, and tau correlates with rate of disease progression. *J Neurol Neurosurg Psychiatry* 2018; **89:** 955–61.
82. Staffaroni AM, Kramer AO, Casey M, et al. Association of Blood and Cerebrospinal Fluid Tau Level and Other Biomarkers With Survival Time in Sporadic Creutzfeldt-Jakob Disease. JAMA Neurol 2019; 76: 969–77.
83. Villar-Piqué A, Schmitz M, Hermann P, et al. Plasma YKL-40 in the spectrum of neurodegenerative dementia. *J Neuroinflammation* 2019; **16:** 145.
84. Llorens F, Villar-Piqué A, Schmitz M, et al. Plasma total prion protein as a potential biomarker for neurodegenerative dementia: diagnostic accuracy in the spectrum of prion diseases. *Neuropathol Appl Neurobiol* 2020; **46:** 240–54.
85. Vitali P, Maccagnano E, Caverzasi E, et al. Diffusion-weighted MRI hyperintensity patterns differentiate CJD from other rapid dementias. *Neurology* 2011; **76:** 1711–9.
86. Forner SA, Takada LT, Bettcher BM, et al. Comparing CSF biomarkers and brain MRI in the diagnosis of sporadic Creutzfeldt-Jakob disease. *Neurol Clin Pract* 2015; **5:** 116–25.
87. Carswell C, Thompson A, Lukic A, Stevens J, Rudge P, Mead S, Collinge J, Hyare H. MRI findings are often missed in the diagnosis of Creutzfeldt-Jakob disease. *BMC Neurol* 2012; **12:** 153.
88. Gao T, Lyu JH, Zhang JT, et al. Diffusion-weighted MRI findings and clinical correlations in sporadic Creutzfeldt-Jakob disease. *J Neurol* 2015; **262:** 1440–6.
89. Park SY, Wang M, Jang JW, et al. The clinical stages of Sporadic Creutzfeldt-Jakob disease with Met/Met genotype in Korean patients. *Eur Neurol* 2016; **75:** 213–22.
90. Eisenmenger L, Porter MC, Carswell CJ, et al. Evolution of Diffusion-Weighted Magnetic Resonance Imaging Signal Abnormality in Sporadic Creutzfeldt-Jakob Disease, With Histopathological Correlation. *JAMA Neurol* 2016; **73:** 76–84.
91. Zanusso G, Camporese G, Ferrari S, et al. Long-term preclinical magnetic resonance imaging alterations in sporadic Creutzfeldt–Jakob disease. *Ann Neurol* 2016; **80:** 629–32.
92. Renard D, Castelnovo G, Collombier L, Thouvenot E, Boudousq V. FDG-PET in Creutzfeldt-Jakob disease: Analysis of clinical-PET correlation. *Prion* 2017; **11:** 440–53.
93. Abu-Rumeileh S, Redaelli V, Baiardi S, et al. Sporadic Fatal Insomnia in Europe: Phenotypic Features and Diagnostic Challenges. *Ann Neurol* 2018; **84:** 347–60.
94. Marquetand J, Knake S, Strzelczyk A, et al. Periodic EEG patterns in sporadic Creutzfeld-Jakob-Disease can be benzodiazepine-responsive and be difficult to distinguish from non-convulsive status epilepticus. *Seizure* 2017; **53:** 47-50.
95. Shin JW, Byeongsoo Y, Seung HO, Kim NK, Lee SK, Kim OJ. Redefining periodic patterns on electroencephalograms of patients with sporadic Creutzfeldt-Jakob disease. *Neurophysiol Clin* 2017; **128:** 756–62.
96. Franko E, Wehner T, Joly O, et al. Quantitative EEG parameters correlate with the progression of human prion diseases. *J Neurol Neurosurg Psychiatry* 2016; **87:** 1061–7.
97. Ladogana A, Kovacs GG. Genetic Creutzfeldt-Jakob disease. *Handb Clin Neurol* 2018; **153:** 219–42.

98. Karch A, Llorens F, Schmitz M, et al. Stratification by Genetic and Demographic Characteristics Improves Diagnostic Accuracy of Cerebrospinal Fluid Biomarkers in Rapidly Progressive Dementia. *J Alzheimers Dis* 2016; **54:** 1385–93.
99. 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: 160–65.
100. Cali I, Lavrich J, Moda F, et al. PMCA-replicated PrPD in urine of vCJD patients maintains infectivity and strain characteristics of brain PrPD: Transmission study. Sci Rep 2019; 9: 5191.
101. Raymond GJ, Race B, Orru CD, et al. Transmission of CJD from nasal brushings but not spinal fluid or RT-QuIC product. Ann Clin Transl Neurol 2020. In press.
102. Karch A, Koch A, Zapf A, Zerr I, Karch A. Partial verification bias and incorporation bias affected accuracy estimates of diagnostic studies for biomarkers that were part of an existing composite gold standard. *J Clin Epidemiol* 2016, **78:** 73–82.
103. Peckeu L, Delasnerie-Lauprètre N, Brandel JP, et al. Accuracy of diagnosis criteria in patients with suspected diagnosis of sporadic Creutzfeldt-Jakob disease and detection of 14-3-3 protein, France, 1992 to 2009. *Euro Surveill* 2017; **22**. doi: 10.2807/1560-7917.ES.2017.22.41.16-00715.

Supplementary material

Table 1. CSF 14-3-3 and t-Tau in the differentiation of CJD and neurodegenerative dementias

Reference	Marker/ cut-off	Controls		Specificity
		n	type	
Stoeck et al. 2012[1]	14-3-3/ western blot*	878	AD	94%
		339	DLB	95%
		162	FTLD	93%
Dorey et al. 2015[2]	14-3-3/ western blot*	55	non-atypical AD	100%
		46	atypical AD	85%
Abu Rumeileh et al. 2017[3]	14-3-3/ western blot*	89	all AD	92%
		44	atypical AD	84%
Lattanzio et al. 2017[4]	14-3-3/ western blot*	101	AD	92%
		72	DLB	94%
		40	FTD	93%
Abu Rumeileh et al. 2018[5]	14-3-3/ western blot*	36	all AD	96%
			atypical AD	89%
		35	DLB	88%
		44	FTLD	98%
Stoeck et al. 2012[1]	t-Tau >1300 pg/mL	132	AD	92%
		55	DLB	98%
		28	FTLD	100%
Dorey et al. 2015[2]	t-Tau > 1128 pg/mL	55	non-atypical AD	93%
		46	atypical AD	35%
Abu Rumeileh et al. 2017[3]	t-Tau >1200 pg/mL	89	all AD	75%
		44	atypical AD	50%
Lattanzio et al. 2017[4]	t-Tau >1250 pg/mL	101	AD	84%
		72	DLB	93%
		40	FTD	93%
Abu Rumeileh et al. 2018[5]	t-Tau > 1100 pg/mL	36	all AD	70%
	t-Tau > 1100 pg/mL	37	atypical AD (vs. atypical prion diseases)	49%
	t-Tau > 1039 pg/mL	35	DLB	88%
	t-Tau > 741 pg/mL	44	FTLD	96%

*western blot: traces were rated negative; AD: Alzheimer's diseae; atypical AD: Alzheimer's disease with rapid cognitive decline or patients with additional motor signs; DLB: Dementia with Lewy body; FTLD: fronto-temporal lobar degeneration; t-Tau: total Tau protein in the CSF

Table 2. Diagnostic performance of serum or plasma markers for sCJD

Reference	Marker		Cases		Controls		Sensitivity	Specificity	AUC	(95% CI)
		cut-off (pg/ml)	n	type	n	type				
Otto et al. 1998[9]	s-100b (s)	>213.0	108	probable + definite sCJD	74	OND+	78%	81%	··	··
Steinacker et al. 2016[6]	t-Tau (s)	>2.2	43	probable + definite sCJD, gCJD	60	OND+	100%	86%	··	··
	NFL (s)	>44.7			60	OND+	85%	96%	··	··
	s-100b (s)	>64.0		probable + definite sCJD	60	OND+	84%	63%	··	··
Kovacs et al. 2017[7]	t-Tau (p)	··	65	definite sCJD	21	HC	··	··	0·94	(0·89-0·98)
					21	OND+	··	··	0·72	(0·60-0·83)
					25	AD	··	··	0·76	(0·63-0·87)
					18	gCJD	··	··	0·57	(0·43-0·71)
	NFL (p)	··			21	HC	··	··	0·99	(0·98-1·0)
					21	OND+	··	··	0·50	(0·30-0·69)
					25	AD	··	··	0·66	(0·48-0·83)
					18	gCJD	··	··	0·47	(0·33-0·60)
Thompson et al. 2018[8]	t-Tau (s)	>2.2	45	probable + definite sCJD	24	HC	91%	83%	0·91	(0·83-0·98)
	NFL (s)	>44.7			24	HC	100%	100%	1	··
Llorens et al. 2019[10]	t-Prp (p)	··	104	probable + definite sCJD	110	HC	··	··	0·92	(0·88-0·95)
					49	OND	··	··	0·85	(0·79-0·91)
					50	AD	··	··	0·66	(0·56-0·77)
					23	LBD	··	··	0·76	(0·66-0·87)
					12	bvFTD	··	··	0·64	(0·43-0·84)
					22	VD	··	··	0·71	(0·58-0·83)
Villar-Piqué et al. 2019[11]	YKL-40 (p)	··	78	probable+ definite sCJD	70	HC	··	··	0·81	(0·74-0·88)
					44	OND+	··	··	0·72	(0·63-0·81)

AUC: area under the curve; gCJD: genetic prion diseases; (s): serum; (p): plasma; HC: healthy controls; OND+: other neurological diseases including dementia syndromes; OND: other neurological diseases excluding neurodegenerative and vascular dementia syndromes; AD: Alzheimer's Diseae; LBD: Lewy body diseases; bvFTD: behavioral variant fronto-temporal dementia; VD: vascular dementia

Table 3. Clinical characteristics that may mimic sCJD in important differential diagnoses

	Diagnosis	Symptoms and biomarkers mimicking sCJD
Neurodegenerative diseases	Rapidly progressive and atypical Alzheimer's disease	• rapid disease progression • early occurrence of focal neurological signs • CSF: increased rate of highly elevated t-Tau (> 1300 pg/ml) and false positive 14-3-3
	Dementia with Lewy bodies	• fluctuating vigilance mimicking extremely rapid disease progression • early occurrence of extrapyramidal signs • myoclonus in late stages
	Multi-system atrophy, progressive supranuclear palsy, and other rare proteinopathies	• dementia and various focal neurological signs in early disease stages • rapid disease progression
Seizures and status epilepticus	Any etiology	• myoclonus and pyramidal signs • EEG: periodic spike-wave complexes in the (status epilepticus) • CSF: elevated t-Tau and 14-3-3 • MRI: restricted diffusion in single cortical regions and thalamus hyperintensities (T2/ FLAIR)
Vascular encephalopathy	Acute stroke, chronic vascular dementia, and cerebral vasculitis	• acute onset and/ or recurrent stroke mimicking rapid disease progression • various neuropsychiatric symptoms, seizures • CSF: elevated t-Tau and 14-3-3 (after acute events) • MRI: restricted diffusion may occur only in cortical regions
Immune-mediated encephalitis	Encephalitis caused by auto-antibodies (NMDA-R, LGI 1, thyroid antibodies in SREAT, etc.) and paraneoplastic antibodies (Hu, Ri, etc.) Post- and para-infectious encephalitis (e.g. post-influenza)	• subacute onset, ataxia, cognitive decline, myoclonus (seizures) • CSF: elevated t-Tau and 14-3-3 • MRI: restricted diffusion may occur in cortical regions, especially of the limbic system or in basal ganglia
Infectious encephalitis	Viral encephalitis (HSV, VZV, JC-virus, HIV, west nile virus, etc.)	• rapidly progressive cognitive decline and various focal neurological signs, myoclonus (seizures) • CSF: elevated t-Tau and 14-3-3 • MRI: restricted diffusion may occur in cortical regions during the disease course (e.g. temporal in HSV-encephalitis) as well as basal ganglia (e.g. west nile virus)
	Atypical encephalitis caused by bacteria and other infectious agents (Whipples' disease, Lues, etc.)	
Metabolic/ toxic encephalopathy	Wernicke encephalopathy, hepatic encephalopathy, extrapontine myelinolysis, hypoglycemia, etc.	• rapidly progressive cognitive decline and various focal neurological signs • CSF: elevated t-Tau and 14-3-3 • MRI: restricted diffusion in cortical regions (e.g. hepatic encephalopathy) or basal ganglia (e.g. Wernicke encephalopathy)
Storage diseases and mitochondrial cytopathies	NBIA, MELAS, etc.	• rapidly progressive cognitive decline and various focal neurological signs • CSF: elevated t-Tau and 14-3-3 • MRI: restricted diffusion in cortical regions (e.g. MELAS) or basal ganglia
Cerebral hypoxia		• Severely impaired cognition and various focal neurological signs • CSF: elevated t-Tau and 14-3-3 • MRI: restricted diffusion in cortex and/ or basal ganglia
Cerebral neoplasia	Lymphoma, glioma, metastatic	• rapidly progressive cognitive decline and various focal neurological signs • CSF: elevated t-Tau and 14-3-3 • MRI: basal ganglia hyperintensities (T2/FLAIR) may occur

This table is based on the clinical experience of the authors and recent publications on the differential diagnosis of sCJD and other rapidly progressive dementias[12,13,14,15]

NMDAR: n-methyl-D-aspartate receptor; LGI 1: leucine-rich glioma inactivated 1; SREAT: steroid-responsive encephalopathy, FLAIR: fluid-attenuated inversion recovery; HSV: herpes simplex virus; VZV: varicella zoster virus; HIV: human immunodeficiency viruse; MELAS: mitochondrial encephalopathy, lactic acidosis and stroke-like episodes; NBIA: neurodegeneration with brain iron accumulation

References

Stoeck K, Sanchez-Juan P, Gawinecka J, et al. Cerebrospinal fluid biomarker supported diagnosis of Creutzfeldt-Jakob disease and rapid dementias: a longitudinal multicentre study over 10 years. *Brain* 2012; **135:** 3051–61

Dorey A, Tholance Y, Vighetto A, et al. Association of cerebrospinal fluid prion protein levels and the distinction between Alzheimer disease and Creutzfeldt-Jakob disease. *JAMA Neurol* 2015; **72:** 267–75.

Abu Rumeileh S, Lattanzio F, Stanzani Maserati M, Rizzi R, Capellari S, Parchi P. Diagnostic Accuracy of a Combined Analysis of Cerebrospinal Fluid t-PrP, t-tau, p-tau, and Aβ42 in the Differential Diagnosis of Creutzfeldt-Jakob Disease from Alzheimer's Disease with Emphasis on Atypical Disease Variants. *J Alzheimers Dis* 2017; **55:** 1471–80.

Lattanzio F, Abu-Rumeileh S, Franceschini A, et al. Prion-specific and surrogate CSF biomarkers in Creutzfeldt-Jakob disease: diagnostic accuracy in relation to molecular subtypes and analysis of neuropathological correlates of p-tau and Aβ42 levels. *Acta Neuropathol* 2017; **133:** 559–78.

Abu-Rumeileh S, Capellari S, Stanzani-Maserati M, et al. The CSF neurofilament light signature in rapidly progressive neurodegenerative dementias. *Alzheimers Res Ther* 2018; **10:** 3.

Steinacker P, Blennow K, Halbgebauer S, et al. Neurofilaments in blood and CSF for diagnosis and prediction of onset in Creutzfeldt-Jakob disease. *Sci Rep* 2016; **6:** 38737.

Kovacs GG, Andreasson U, Liman V, et al. Plasma and cerebrospinal fluid tau and neurofilament concentrations in rapidly progressive neurological syndromes: a neuropathology-based cohort. *Eur J Neurol* 2017; **24:** 1326–e77.

Thompson AGB, Luk C, Heslegrave AJ, et al. Neurofilament light chain and tau concentrations are markedly increased in the serum of patients with sporadic Creutzfeldt-Jakob disease, and tau correlates with rate of disease progression. *J Neurol Neurosurg Psychiatry* 2018; **89:** 955–61.

Otto M, Wiltfang J, Schütz E, Zerr I, Otto A, Pfahlberg A, Gefeller O, Uhr M, Giese A, Weber T, Kretzschmar HA, Poser S. Diagnosis of Creutzfeldt-Jakob disease by measurement of S100 protein in serum: prospective case-control study. BMJ 1998; **316:** 577–582.

Llorens F, Villar-Piqué A, Schmitz M, et al. Plasma total prion protein as a potential biomarker for neurodegenerative dementia: diagnostic accuracy in the spectrum of prion diseases. Neuropathol Appl Neurobiol 2019. doi: 10.1111/nan.12573.

Villar-Piqué A, Schmitz M, Hermann P, et al. Plasma YKL-40 in the spectrum of neurodegenerative dementia. J Neuroinflammation 2019; **16:** 145.

Geschwind MD. Rapidly Progressive Dementia. *Continuum (Minneap Minn)* 2016; **22:** 510–37.

Mahajan S, Appleby BS. Comprehensive and Methodical: Diagnostic and Management Approaches to Rapidly Progressive Dementia. Curr Treat Options Neurol. 2017; **19:** 40.

Zerr I, Hermann P. Diagnostic challenges in rapidly progressive dementia. *Expert Rev Neurother* 2018; **18:**761–72

Geschwind MD, Murray K. Differential diagnosis with other rapid progressive dementias in human prion diseases. *Handb Clin Neurol* 2018; **153:** 371–97.