Biomarkers and diagnostic guidelines for sporadic Creutzfeldt-Jakob disease

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Summary

Sporadic Creutzfeldt-Jakob disease is a fatal and potentially transmissible neurodegenerative disease caused by misfolded prion proteins (PrPSc). Effective therapeutics are currently not available and accurate diagnosis can be challenging. Clinical diagnostic criteria use a combination of characteristic neuropsychiatric symptoms, CSF proteins 14-3-3, MRI, and EEG. Supportive biomarkers, such as high CSF total Tau, could aid the diagnostic process. However, discordant studies have led to controversies about the clinical value of some established surrogate biomarkers. Since 2011, 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 sporadic Creutzfeldt-Jakob disease. Updated criteria for the diagnosis of sporadic Creutzfeldt-Jakob disease including RT-QuIC will improve early clinical confirmation, surveillance, assessment of PrPSc seeding activity in different tissues, and trial monitoring. Moreover, emerging blood-based, prognostic, and potentially pre-symptomatic biomarker candidates are under investigation.

Introduction

Sporadic Creutzfeldt-Jakob disease is a rapidly progressive neuropsychiatric syndrome that is fatal and characterised by aggregations of misfolded prion protein Scrapie (PrPSc) in the brain. Sporadic Creutzfeldt-Jakob disease is the most common form of human prion disease (~90% of cases) with an incidence of approximately 1·5–2·0 cases per million person-years.[1] Different phenotypes of sporadic Creutzfeldt-Jakob disease can vary symptom evolution, biomarker profile, and neuropathological characteristics. They are associated with the polymorphism (Met and Val) at codon 129 of the prion protein gene (PRNP) and with molecular mass of PrPSc (glycotype 1 and 2).[2] Definite diagnosis requires neuropathological confirmation. The spectrum of possible symptoms is highly heterogeneous and includes rapidly progressive dementia, cerebellar ataxia, and myoclonus, which means that high-performing biomarkers are important for making a confident clinical diagnosis. In 1998, WHO included a combination of particular symptoms, electroencephalogram (EEG), and detection of CSF 14-3-3 proteins in the standard diagnostic criteria.[3] Patterns of signal alteration on fluid attenuated inversion recovery (FLAIR) or diffusion weighted imaging (DWI) sequences, or both, of brain MRI were suggested in 2009.[4] Another CSF protein, total-Tau (t-Tau), is considered a valuable supportive biomarker.[5]

Although comparative data on imaging markers for sporadic Creutzfeldt-Jakob disease are scarce, numerous studies have evaluated the diag nostic performance of CSF biomarkers, with occasional discrepancies leading to controversy about their clinical utility.[6,7] Since 2011, development and clinical application of PrPSc amplification assays, such as protein misfolding cyclic amplification (PMCA) and real-time quaking induced conversion (RT-QuIC)[8] have constituted major breakthroughs as aids for an improved pre-mortem diagnosis of prion diseases. RT-QuIC has shown excellent diagnostic accuracy for sporadic Creutzfeldt-Jakob disease in retrospective studies, ring trials (consistency between laboratories),[9,10] and prospective studies,[11,12] which shows its high value for an early and accurate diagnosis. Consequently, RT-QuIC (using CSF or other tissue, such as olfactory mucosa) was included in diagnostic criteria for sporadic Creutzfeldt-Jakob disease of some surveillance centers.[12,13] However, an important discussion on its clinical utility is needed. Another unmet need is the identification of blood-based biomarkers for early diagnosis and disease progression,[14–16] particularly regarding potential new therapeutic strategies. The aim of this Review is to provide an overview of the biomarker-based diagnosis of sporadic Creutzfeldt-Jakob disease and to suggest guidelines for clinicians to use in the differential diagnosis of rapidly progressive dementias. Over the past 5 years, advances are discussed and put in the context of clinical relevance, established biomarkers, and epidemiology.

Investigating diagnostic tests for sporadic Creutzfeldt-Jakob disease

When estimates of diagnostic accuracy are being translated into clinical practice, potential selection biases of case and control groups should be considered. The selection of control groups can be particularly challenging. Healthy age-matched controls usually do not reflect the population in which a diagnostic biomarker is used. However, referral centres often use Creutzfeldt-Jakob disease mimics (eg, autoimmune encephalitis) that represent the diagnostic challenges but may not reflect the routine of a tertiary institution. An example of a biased control group was the evaluation of diagnostic criteria for sporadic Creutzfeldt-Jakob disease published in 2018.[12] The control group included many patients without Creutzfeldt-Jakob disease who had been further investigated because of positive CSF 14-3-3 tests, resulting in a weak specificity of this biomarker. In 2017, a study evaluating the use of olfactory mucosa and CSF samples in RT-QuIC[17] reported a relatively low sensitivity compared with some other studies that used RT-QuIC assays. In this study, the case group was partially selected from samples that had a previous negative first-generation RT-QuIC result, leading to a case group

selection bias. Both examples highlight the importance of 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 sensitivity and specificity are the most useful measures of diagnostic performance as they are not easy to interpret in a clinical setting. Predictive values might 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, which requires including disease prevalence (proportion) in the calculations.[18] In the context of an extremely rare disease, such as sporadic Creutzfeldt-Jakob disease, a sufficient number of controls cannot be achieved and applying Bayes' rule would always lead to extremely low positive and extremely high negative predictive values. Thus, predictive values are not considered in this Review. In studies of established biomarkers with defined cut-offs, we use test sensitivity and specificity as measures for diagnostic accuracy. For experimental biomarkers, we report the area under the curve (AUC) from receiver operator characteristics.

Current state and recent advances in CJD biomarker research

Neuropathological investigation and immunostaining of PrPSc allow a definite diagnosis of prion diseases.[19] For definite ante-mortem diagnosis, brain biopsy is required but is complicated by infection control concerns, the possibility of a false negative result due to sampling error in which typical pathology and PrPSc might not be present in all cortical regions (eg, sporadic or familial fatal insomnia), and issues with tissue quality. Acknowledging these considerations and being highly invasive, brain biopsy is usually only considered when the diagnosis is not clear and potentially treatable conditions (eg, encephalitis or lymphoma) are under strong consideration or a potential contamination of medical instruments requires a clear case definition. A less invasive procedure, tonsillar or adenoid biopsy, was established for the diagnosis of variant Creutzfeldt-Jakob disease (prion disease caused by consumption of beef from bovines affected by bovine spongiform encephalopathy), but is not helpful for other forms of prion disease.[20] The direct in vivo detection of PrPSc in sporadic Creutzfeldt-Jakob disease using routinely accessible biofluids is possible but a pilot study using urine reported a poor sensitivity of 40%.[21] Given the limitations of traditional methods (eg, biopsy and direct detection) and growing clinical evidence in support of novel PrPSc-seeded assays, a shift in

clinical diagnosis criteria for sporadic Creutzfeldt-Jakob disease is warranted. We describe the evidence for these novel assays and the current state of established and new diagnostic surrogate biomarkers (diagnostic tests that indirectly mark the disease process).

PrPSc-seeded aggregation assays

PMCA

In 2001, PMCA was developed to reproduce and amplify PrPSc in microtubes. Brain homogenate provided normal prion protein (PrPC) substrate for the reaction and sonication fragmented growing PrPSc particles to increase their concentration.[22] Subsequently, a modified protocol introduced the use of recombinant hamster PrPC as substrate to accelerate the reaction and increase its sensitivity to detect PrPSc in the CSF of scrapie-infected hamsters.[23] PMCA protocols showed excellent sensitivity for the detection of PrPSc in CSF (100%),[24] plasma (100%)[25,26] and urine (93%)[27] of patients with variant Creutzfeldt-Jakob disease but high sensitivity could not be shown in sporadic Creutzfeldt-Jakob disease or other prion diseases seen in current clinical practice.

RT-QuIC

A modified multiwell plate-based PrPSc amplification technology using quaking to energise the misfolding of prion protein coupled to a fluorescent readout was named RT-QuIC.[8,28] RT-QuIC has shown to be a potential tool not only in the diagnosis of prion diseases but also in drug screening, prion strain discrimination, and detection of other protein misfolding diseases (eg, tauopathies and synocleinopathies).[29,30] Different protocols concerning substrate (eg, recombinant hamster PrP, hamster–sheep chimeric PrP, or bank vole PrP), reaction conditions, and the definition of test positivity have been reported.[8,29,30] In general, each sample is analysed in quadruplicates[9] and positivity is confirmed when at least two of four replicates cross a fluorescence signal cut-off value, similarly, samples can be analysed as triplates[29] whereby positivity is confirmed when at least two of three replicates cross the fluorescence threshold. In 2015, the original protocol (first generation RT-QuIC) was technically modified by increasing reaction temperature and using N-terminally truncated PrPSen (second generation RT-QuIC) to shorten the assay time and to improve the sensitivity.[31] CSF RT-QuIC represents a disease-specific biomarker and retrospective studies have investigated its diagnostic accuracy with test specificity of 99–100%.[8–11,17,31–37]

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

	Cases		Controls		Sensitivity	Specificity	Protocol
	n	type	n	type			
Atarashi et al. 2011[8*]	34	definite sCJD	49	OND+	85%	100%	1st Gen
McGuire et al. 2012[9]	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[31]	48	probable + definite sCJD	39	OND+	96%	100%	2nd Gen
Cramm et al. 2016[10]	110	definite sCJD + gCJD	400	OND+	85%	99%	1st Gen°
Groveman et al. 2016[33†]	113	probable + definite sCJD	64	OND+	73%	100%	1st Gen
Groveman et al. 2016[33†]	113	probable + definite sCJD	64	OND+	94%	100%	2nd Gen
Park et al. 2016[34]	81	probable + definite sCJD	100	non-CJD	77%	100%	1st Gen
Franceschini et al. 2017[35]	145	probable + definite sCJD + gCJD	42	RPD	97%	100%	2nd Gen
Bongianni et al. 2017[17†]	49	probable + definite sCJD	71	OND+	73%	100%	1st Gen
Bongianni et al. 2017[17†]	22	probable + definite sCJD	71	OND+	86%	100%	2nd Gen
Lattanzio et al. 2017[36]	225	definite sCJD	348	RPD	84%	99%	1st Gen
Foutz et al. 2017[11]	126	definite sCJD + gCJD	67	RPD	92%	99%	2nd Gen
Rudge et al. 2018[37]	171	definite sCJD	47	RPD	89%	100%	1st Gen
Foutz et al. 2017[11]	65	definite sCJD + gCJD	14	RPD	95%	100%	2nd Gen
Hermann et al. 2018[12]	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[42]	102	probable + definite sCJD	80	RPD	96%	100%	2nd Gen
Mammana et al. 2020[43]	24	probable + definite sCJD	12	RPD	88%	100%	1nd Gen
Rhoads et al. 2020[39]	439	definite sCJD	69	RPD	93%	99%	2nd Gen

1st paragraph (Atarashio et al. to Rudge et al.): retrospective studies; 2nd paragraph (Foutz et al. to Rhoads et al.): prospective studies. Abbreviations: definite sCJD: neuropathological confirmed diagnosis of sporadic Creutzfeldt-Jakob disease; probable sCJD: clinical diagnose of sporadic Creutzfeldt-Jakob disease based on syndrome and biomarkers;[4] 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;[8] 2nd Gen: second generation test[31]

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

†These studies performed two different protocols and used the same control group for both investigations.

°This protocol used hamster-sheep chimeric recombinant PrP as substrate (instead of hamster PrP) and test positivity was indicated by two out of three positive replicates (instead of two out of four)[29]

Most studies, however, did not use control groups consisting primarily of cases with rapidly progressive dementias in whom Creutzfeldt-Jakob disease was considered as a potential diagnosis during the disease course. Some false positive cases in retrospective studies were speculated to possibly represent unrecognised prion diseases.[10] Nonetheless, two cases of autopsy-verified non-Creutzfeldt-Jakob disease showing positive CSF RT-QuIC during the diagnostic process have been reported.[38,39] One of these patients had convulsions caused by steroid-responsive encephalitis, which is a potential clinical sporadic Creutzfeldt-Jakob disease mimic.[38] Prospective studies using rapidly progressive dementias as controls and mostly neuropathological confirmed sporadic Creutzfeldt-Jakob disease cases, were published since 2017 and the specificity was also reported as 99–100% (table).[11,12,39–42] Because of its reliability and high diagnostic accuracy, CSF RT-QuIC was incorporated in the diagnostic criteria for sporadic Creutzfeldt-Jakob disease of several surveillance centers.[13,14] Regarding the test sensitivity, figures range from 73%[19,33] to 89%[9,12,37] using first generation RT-QuIC, and 92%[11] to 97%[35] using second generation RT-QuIC.

Molecular subtypes of sporadic Creutzfeldt-Jakob disease are defined by codon 129 polymorphism (M and V) and PrPSc glycotype (1 and 2),[2] resulting in different subtypes (eg, MM1, MV1). The sensitivity is very high in MM1/MV1 and VV2 cases, the most common subtypes among sporadic Creutzfeldt-Jakob disease patients, whereas it is slightly lower in MV2 cases (75–93%).[11,35,36,39,40] Regarding rare subtypes, there are few reported cases, hampering the validity of results but sensitivity has been reported to be substantially lower in VV1 (range 0–100%)[37–39] and MM2 cases (range 44–78%).[36,39] The MM2 subtype is further differentiated into a cortical type (MM2C) and a very rare thalamic type (MM2T) that shows a distinct clinical syndrome called sporadic fatal insomnia. Only few known cases of sporadic fatal insomnia implicate that classic sporadic Creutzfeldt-Jakob disease biomarkers and RT-QuIC show poor sensitivity in this condition.[39] CSF RT-QuIC showed high sensitivity for genetic prion diseases with E200K and V210I mutations but being low for fatal familial insomnia (D178N-129M).[10,11,39,40] However, supporting data are based on small case numbers.

RT-QuIC might also aid in the differentiation of distinct prion diseases such as sporadic Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, and fatal familial insomnia and sporadic Creutzfeldt-Jakob disease subtypes.[30,41] Regarding other tissues, promising studies that applied RT-QuIC to olfactory mucosa[17,32,42] and skin biopsies[43,44] showed high sensitivities of 89% to 100% suggesting even better diagnostic accuracy than using CSF.

Multiple components of the eye have tested positive by RT-QuIC[45] post mortem but the diagnostic value of analysis of routinely accessible eye tissue or fluid remains to be determined.

CSF surrogate 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, plasma membranes, and organelles. Involvement in various functions (eg, cell signalling, growth, apoptosis) has been identified but not completely clarified.[46] Since 14-3-3 protein detection by western blot became part of commonly used clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease,[3] numerous studies evaluated its diagnostic performance. In 2012, a structured meta-analysis reported a sensitivity of 92% and a specificity of 80%[47] but it was reported that the test sensitivity is lower than 92% in early disease stages and differs across the spectrum of molecular subtypes. The MV2 and MM2 subtypes showed lower test sensitivities of between 60% to 70%.[48] Reported specificity ranges between 40%[49] and 92%.[50] Such discrepancies might be explained, at least partially, by different characteristics of the control groups. Over the past 5 years, several studies reported a high specificity in the discrimination of sporadic Creutzfeldt-Jakob disease and neurodegenerative diseases such as Alzheimer's disease, dementia with Lewy bodies, and fronto-temporal lobar degeneration (appendix table 1).[36,50–53]

By contrast, the specificity of CSF 14-3-3 was lowered when control groups included acute neuronal injury events and inflammatory and infiltrative neoplastic CNS diseases.[36,50] Another factor possibly affecting specificity could be the execution and rating of 14-3-3 western blot. Intermediate results (ie, weak or trace readouts) can be difficult to interpret. Comparative evaluations of a new 14-3-3γ isoform ELISA assay showed a superior diagnostic performance by comparison with 14-3-3 western blot.[40,54,55] One study reported a sensitivity of 97% and a specificity of 94% with an AUC of 0·982 (optimal cut-off >14552 AU/mL),[54] whereas a larger study (including ring trials) reported a sensitivity of 88% and a specificity of 96% (cut off >20·000 AU/mL).[55]

Tau protein

Tau, a microtubule-associated protein, is expressed in neuronal and glial cells.[56] Extremely elevated CSF t-Tau was proposed as a diagnostic biomarker for sporadic Creutzfeldt-Jakob

disease5 and most studies reported good test sensitivity and specificity, each around 90%.[36,48,49,57] However, currently, CSF t-Tau is not formally accepted as part of case definition criteria.[4] Similar to 14-3-3, reduced sensitivity has been shown in MM2 and MV2 subtypes[48,58] and early disease stages.[59] Some studies reported relatively poor specificities of 67%49 or lower than 50% at varying optimal diagnostic cut-offs.[51–53] Specificity of lower than 50% was observed when patients with atypical Alzheimer's disease were used as controls (appendix table 1). Some studies, however, have found t-Tau to be a better diagnostic marker than 14-3-3,[49,60] leading to an ongoing discussion and controversy over which biomarker should be used primarily. Besides Alzheimer's disease, inflammatory and neoplastic CNS diseases are important differential diagnoses of elevated t-Tau concentrations.[61] Unfortunately, there is no general consensus regarding the best t-Tau ELISA assay or cut-off that should be used to support sporadic Creutzfeldt-Jakob disease (eg, studies have suggested either >1072 pg/mL, >1250 pg/mL, >1300 pg/mL, or >1400 pg/mL).[5,35,62–64] CSF t-Tau can also be a predictor of survival time.[65,66] The p-Tau/t-Tau (or t-Tau/p-Tau) ratio is an important alternative biomarker for sporadic Creutzfeldt-Jakob disease.[67] It showed a very high diagnostic accuracy in the differentiation of sporadic Creutzfeldt-Jakob disease from other neurological diseases (AUC 0·98), Alzheimer's disease (AUC 0·99),[64] and rapidly progressive Alzheimer's disease (AUC 0·99).[68] Several studies that investigated large cohorts reported a superior diagnostic performance compared with t-Tau alone.[51,64,68]

Neurofilaments

Neurofilaments comprise three subunits: a light (NfL), a medium, and a heavy chain. As neuron-specific cytoskeleton proteins, their presence in body fluids represents neuroaxonal damage.[69] Several studies showed an excellent diagnostic accuracy in the discrimination of healthy individuals and patients with sporadic Creutzfeldt-Jakob disease (AUCs >0·99).[16,70,71] However, NfL might have insufficient specificity for sporadic Creutzfeldt-Jakob disease.[40] Concerning important differential diagnoses, reported AUCs were 0·95 versus patients with other neurological diseases (including dementia syndromes),[15] 0·77 versus Alzheimer's disease,16 0·4516 and 0·9070 versus other neurological diseases with dementia syndrome, 0·93 versus neurodegenerative dementias,[53] and 0·86 to 0·89 versus rapidly progressive dementia.[72] The notable differences between these studies might be explained by different group selection criteria but this requires further clarification. Additionally, different optimal cut-offs were identified (eg, >5016 pg/ml or >10 500 pg/ml).[53,70] By contrast to 14-3-3 and t-Tau, NfL was

shown to be markedly elevated in MV2 and VV2 subtypes when compared with the MM1 sporadic Creutzfeldt-Jakob disease subtype.[53]

Other CSF surrogate biomarkers

Several other CSF biomarkers for sporadic Creutzfeldt-Jakob disease have been identified over the past two decades. Herein, only those that have a high amount of supported evidence are considered. CSF S100b has been widely studied but comparative studies showed inferior diagnostic performance compared with 14-3-3 and t-Tau,[48,73] and S100b has not been widely used clinically. Total prion protein (t-PrP) is decreased in the CSF of patients with sporadic Creutzfeldt-Jakob disease, showing moderate diagnostic accuracy.[51,74] A study using targeted mass spectrometry (instead of the more routinely used ELISA) showed that all human PrP domains were reduced in the CSF of patients with sporadic Creutzfeldt-Jakob disease compared with patients with rapidly progressive dementia.[75] Additionally, it might have potential in trial monitoring[76] and constitute a valuable part of composite biomarker profiles.[51,53] Alpha-synuclein, a synaptic protein that aggregates in synucleinopathies was observed to be massively increased in patients with sporadic Creutzfeldt-Jakob disease, possibly related to rapid neurodegeneration. A multicentre study showed an excellent diagnostic accuracy (AUC >0·99, 98% sensitivity, 97% specificity) in the discrimination of sporadic Creutzfeldt-Jakob disease and other neurological diseases (including dementia syndromes) at an optimal cut-off of 820 pg/mL using a commercial ELISA.[77,78] Similar results were found in an inter-laboratory validation study.79 Advantages and disadvantages of common CSF biomarkers are summarized (appendix panel 1), more potential CSF biomarker candidates evaluated in the last 5 years are also noted (appendix table 2).

Blood-based biomarker candidates

Several potential roles might feasibly be fulfilled by blood-based biomarkers. Currently, there is no immediate prospect of a highly specific diagnostic blood test comparable to RT-QuIC in CSF samples. Blood assays, however, 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 prioritisation. One potential candidate is the t-Tau concentration in plasma or serum. Studies showed elevated concentrations in sporadic Creutzfeldt-Jakob disease compared with healthy controls and other neurological diseases.[15,16,80] The diagnostic accuracy ranged from an AUC of 0·94 versus healthy controls to 0·72 versus other neurological diseases that included dementia syndromes (appendix table 3). Another investigation showed that the plasma t-Tau

concentration is a better predictor of survival time in sporadic Creutzfeldt-Jakob disease than CSF t-Tau concentration or other fluid biomarkers.[66] Another promising candidate for a blood-based biomarker is NfL, the most soluble subunit of neurofilament. NfL was shown to be an effective therapeutic biomarker in CNS disease during trials for multiple sclerosis.81 NfL showed similar or even better diagnostic accuracy compared with t-Tau in the discrimination of sporadic Creutzfeldt-Jakob disease from a healthy control population.[16,80] By contrast, a study that investigated a large cohort of prion diseases and used rapidly progressive dementias as controls showed that plasma t-Tau had better diagnostic accuracy than NfL (appendix table 3). Similar to the CSF counterpart, both plasma t-Tau and NfL concentrations were signifi cantly associated with the sporadic Creutzfeldt-Jakob disease subtype.[82]

More potential blood-based biomarkers for sporadic Creutzfeldt-Jakob disease, such as S100b and others (appendix table 3) were elevated in serum or plasma, but few available data display inferior diagnostic accuracy compared with t-Tau and NfL or still have to be validated by other groups. PrP was reported to be decreased in the CSF of sporadic Creutzfeldt-Jakob disease cases[51,52] although it was reported in another study to be increased in plasma.[83] The explanation for this dissociation has not yet been clarified.

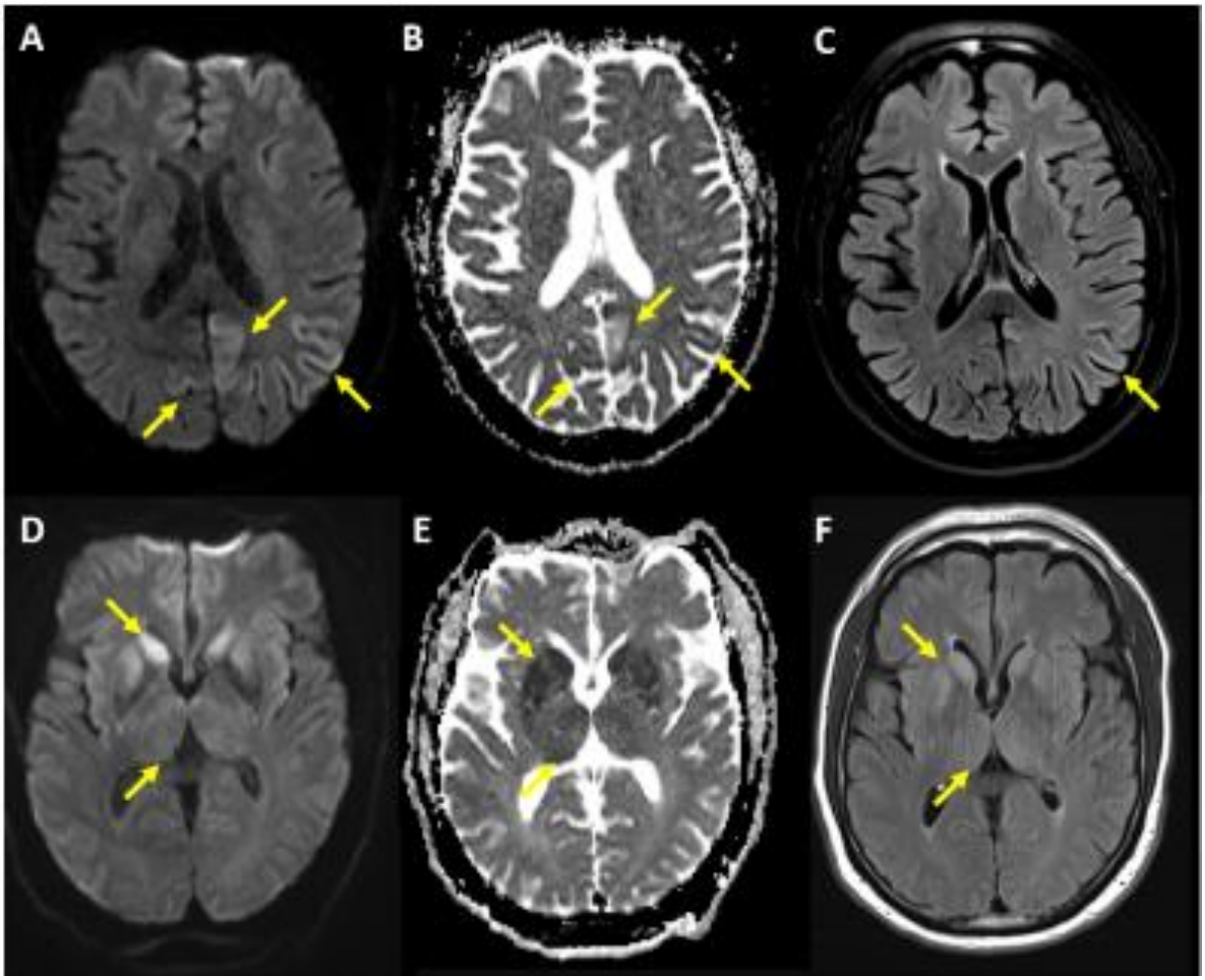
Imaging markers

MRI

MRI is an essential tool in the diagnosis of sporadic Creutzfeldt-Jakob disease. It allows the identification of important differential diagnoses such as ischaemia, encephalitis, and neoplasia. In Creutzfeldt-Jakob disease, typical patterns of restricted diffusion on DWI and hyper-intensities in FLAIR images were suggested to be included in the WHO diagnostic criteria in 2009.[4] Another widely used protocol recommends the use of DWI and apparent diffusion coefficient (ADC) maps only.[84,85] Typical Creutzfeldt-Jakob disease MRI displays restricted diffusion in at least two cortical regions (ribboning) or restricted diffusion predominantly in the caudate nucleus, or both, followed by putamen and thalamus (figure 1). Involvement of the subcortical white matter cannot be observed in visual assessments (DWI, ADC, or FLAIR)[4,84] but was detected by quantitative diffusion tensor imaging.[86] Cortical ribboning and involvement of the caudate nucleus (of one or both hemispheres, rarely perfectly symmetric) is typically seen in the most common MM1 subtype. Involvement of the thalamus (aside from the caudate nucleus and putamen) is more common in VV2 and MV2 subtypes.[87] High signal only on

FLAIR and DWI in the posterior thalamus brighter than in anterior putamen (pulvinar sign) is a strong indicator of variant Creutzfeldt-Jakob disease.[20]

Figure 1: Typical patterns of restricted diffusion on Creutzfeldt-Jakob disease MRI



MRI scans were provided by the National Prion Disease Pathology Surveillance Center, Case Western Reserve University, Cleveland, OH, USA. (A–C) Brain MRI of a patient with sporadic Creutzfeldt-Jakob Disease (MM1 subtype). (A) Restricted diffusion in occipital and parietal lobes, green arrows show associated hyperintensities on DWI. (B) Hypointensities on ADC maps. (C) Less clear hyperintensities on FLAIR images than on DWI. Other patients with MM1 subtypes can present additional restricted diffusion in caudate nucleus and putamen, a similar pattern (with caudate nucleus and putamen less likely involved) can be seen in MM2 and VV1 subtypes. (D–F) Brain MRI of a patient with sporadic Creutzfeldt-Jakob Disease (VV2 subtype), restricted diffusion in caudate nucleus, putamen and thalamus, predominantly in the pulvinar (less clear than in caudate nucleus and putamen) in both hemispheres. (D) Associated hyperintensities on DWI. (E) Associated hypointensities on ADC maps. (F) Associated hyperintensities on FLAIR images. A similar pattern (with additional cortical involvement) can be seen in the MV2 subtype. ADC=apparent diffusion coefficient. DWI=diffusion weighted images. FLAIR=fluid attenuated inversion recovery.

The overall diagnostic accuracy of MRI is possibly even superior to CSF 14-3-3 and t-Tau[88,89] but extensive comparison data with CSF biomarkers is scarce. Some studies showed a sensitivity of around 80%,[12,35,40,42] others reported 92% to 98%.[37,88,89] Similarly, specificity ranges from 74%[40] to 98%.[12] In 2020, a study investigating a large cohort with 770 patients with definite sporadic Creutzfeldt-Jakob disease applied an improved diagnostic index showing 92% sensitivity and 97% specificity.[89] The discrepancies disease, such changes were observed more than one year before symptom onset in case reports. [94]

Positron Emission Tomography

Positron emission tomography using ^{18}F-fluoro-2-deoxy-d-glucose (FDG-PET) as tracer is able to detect decreased glucose metabolism in cortical regions of patients with sporadic Creutzfeldt-Jakob disease. However, the value of FDG-PET in the differential diagnosis is limited by the absence of specific patterns. No specific patterns have been identified. FDG-PET has potential as a marker of early sporadic Creutzfeldt-Jakob disease and showed a correlation with clinical symptoms.[95] In the rare MM2T subtype (sporadic fatal insomnia), an early-reduced thalamic glucose metabolism is a distinctive feature.[96]

EEG

Periodic sharp-wave complexes (PSWCs) with a frequency of 1 Hz are considered as an EEG pattern typical of Creutzfeldt-Jakob disease and have shown a sensitivity of 64% and a specificity of 91%.[97] The non-convulsive status epilepticus is the most frequent clinical condition with Creutzfeldt-Jakob disease EEG.[98,99] Over the past 5 years, CSF biomarker studies reported a substantially lower sensitivity (39% to 45%) for EEG.[12,34,37,40] Most likely, the decreasing sensitivity of EEG is a result of improved early recognition of sporadic Creutzfeldt-Jakob disease cases. Typical PSWCs occur in late disease stages and are less frequent in MV2, VV2, and MM2 cases. However, the method is less invasive than CSF sampling and non-specific periodic rhythm abnormalities[100] and quantitative analysis of frequency alterations[101] might have the potential to aid diagnosis in early stages and to predict disease progression.

Genetic markers

PRNP mutations account for between 10–15% of all human prion diseases.[1] Some cause specific clinical syndromes (eg, GSS or FFI), others can mimic clinical presentation and biomarker profiles of sporadic Creutzfeldt-Jakob disease (eg, E200K).[102] Thus, the sequencing

of PRNP is an important biomarker that should be considered in the differential diagnosis of prion diseases and is crucial in atypical cases, and in cases with positive or uninformed family history of rapidly progressive dementias. In some sporadic Creutzfeldt-Jakob disease subtypes, reduced sensitivity of surrogate biomarkers has been observed, especially in patients with the MV2 and MM2-types.[48,58] The identification of the PrPSc type is only possible in brain tissue but the analysis of codon 129 PRNP might help to interpret inconclusive biomarker results.[103]

Panel 1. Historical case studies from the German CJD surveillance center

Case A (typical CJD)

A 63-year-old woman complained about language disturbance (mild amnestic aphasia) that had started two weeks before hospital admission.

Neurological and neuropsychiatric examination showed cognitive deficits and ataxia. The EEG showed continuous focal epileptiform patterns but enforced antiepileptic medication showed no clinical benefit. In the CSF, 14-3-3 proteins (64455 AU/mL, cut-off > 20000 AU/mL) and t-Tau (12460 pg/mL, cut-off >1300 pg/mL) were both highly increased and RT-QuIC was positive. No signs of CNS inflammation were present. MRI showed restricted diffusion in frontal, temporal, and parietal regions, as well as in caudate nucleus and left putamen. The clinical condition of the patient worsened within one week. Clinical examination showed severe dementia, pyramidal and extrapyramidal signs, as well as myoclonus. Follow-up EEGs showed CJD-typical PSWCs. *PRNP* sequencing revealed no pathogenic mutation and homozygosity for Methionine at Codon 129. The patient was diagnosed with probable sCJD according to common criteria,[4] supported by positivity of CSF RT-QuIC.

The patient passed after 2 months of disease duration. Brain autopsy revealed PrP^{Sc} depositions with neuropathological characteristics of the most frequent MM/MV1 sCJD subtype.

Case B (atypical sCJD)

Family members recognized personality changes and mild cognitive deficits in a 54-year-old woman, and suspected an affective disorder.

After 5 months of symptom duration, a neurology specialist observed rapidly progressive dementia with apraxia. MRI showed restricted diffusion in parietal, occipital, and temporal regions with very subtle involvement of caudate nucleus (no other pathological findings); EEG showed sporadic triphasic complexes but no PSWCs. The CJD surveillance center was consulted and recommended further clinical investigations including CSF analyses. The CSF showed no evidence for inflammatory CNS diseases, positive 14-3-3 proteins at a rather low level (21527 AU/mL, cut-off >20000 AU/mL), and positive RT-QuIC. *PRNP* sequencing revealed no pathogenic mutation and homozygosity for Methionine at Codon 129. Although clinical diagnostic criteria[4] for sCJD were not fulfilled at that time (the patient showed only rapidly progressive dementia), the biomarker signature was highly suggestive and no alternative diagnoses were revealed.

The case was classified as probable sCJD according to amended surveillance center criteria[12] based on RT-QuIC positivity. Disease course (rather slow progression), MRI results (predominant cortical involvement), and Codon 129 were suggestive for the rare MM2C ("cortical") sCJD subtype. The patient passed after 11 months of disease duration. Brain autopsy revealed PrP^{Sc} depositions with neuropathological characteristics of the MM/MV2C sCJD subtype.

Clinical value of RT-QuIC and CSF surrogate biomarkers

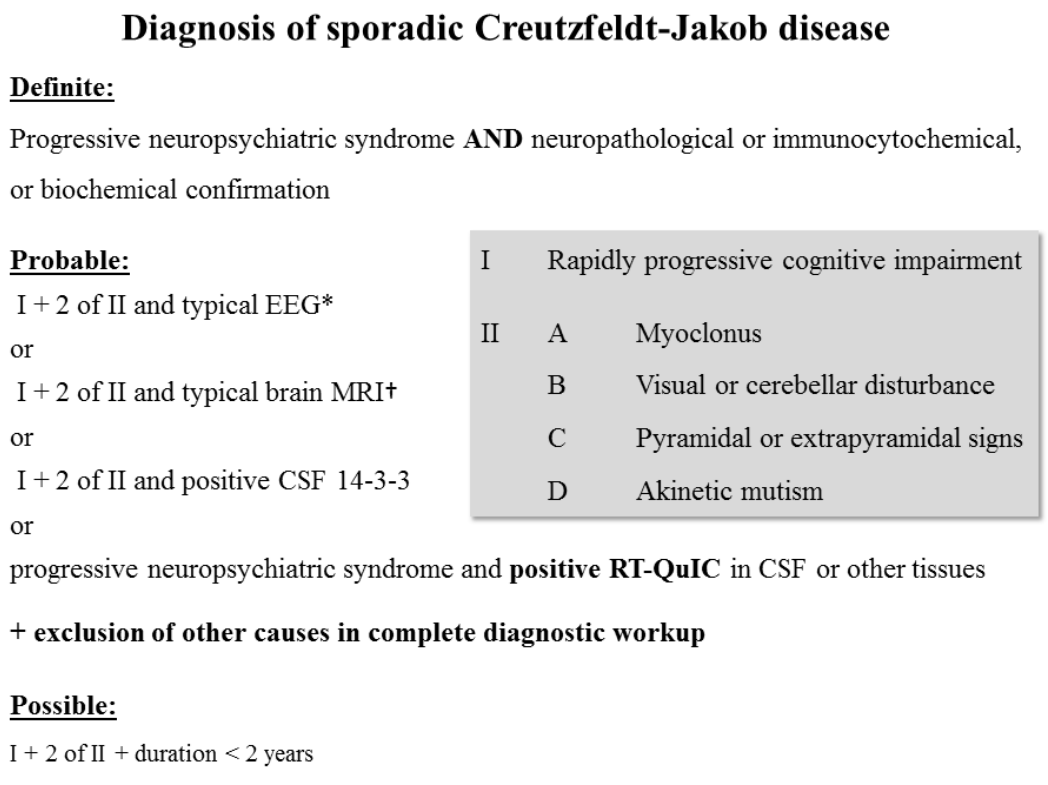
Over the last 9 years, the evidence suggesting CSF RT-QuIC as a major improvement in the clinical diagnosis of sporadic Creutzfeldt-Jakob disease has reached a substantial point. The test sensitivity is similar to the best available surrogate biomarkers and the data display superior specificity (table). By contrast to all established biomarkers for sporadic Creutzfeldt-Jakob disease and other neurodegenerative diseases, RT-QuIC is able to detect the protein that was consensually identified to be primarily pathogenic (PrPSc). Although different protocols and definitions of test positivity have been proposed,[9,29,30] reproducibility of test results has been shown in ring trials.[10,104] However, RT-QuIC is rather expensive regarding its substrate (recombinant PrP) and, although the test is less reliant on specialised equipment when compared with MRI and PET, the method still has to be established in more centers to provide all-encompassing availability. There is an ongoing debate on infectivity of the aggregates produced by PrPSc amplification assays. Although infectivity was shown in PMCA-replicated PrPSc from patients with variant Creutzfeldt-Jakob disease,[105] mouse models could not show infectivity of the RT-QuIC product from sporadic Creutzfeldt-Jakob disease samples so far.[106]

Surrogate CSF biomarkers of sporadic Creutzfeldt-Jakob disease are reliable diagnostics but the accuracy might differ with respect to the clinical context in which these markers are used. They are not disease specific by their very nature. Thus, physicians should interpret test results with caution. CSF 14-3-3 protein is highly sensitive and well validated, but acute brain injury events might cause false positive results. CSF 14-3-3 protein is part of a widely used clinical diagnostic gold standard[3,4] and estimates of the diagnostic accuracy, especially in comparative analyses, might be influenced by verification bias.[107] A problem with the 14-3-3 western blot method is its complex interpretation and the presence of borderline results (traces). 14-3-3 ELISAs might resolve this problem but they have not been widely established. The most commonly used alternative CSF biomarker, t-Tau, showed improved (but still only moderate) specificity in the differentiation of sporadic Creutzfeldt-Jakob disease and acute brain injury events or encephalitis,[36,50] but there is some evidence that t-Tau might not have sufficient specificity in the discrimination of rapidly progressive or atypical Alzheimer's disease and sporadic Creutzfeldt-Jakob disease (appendix table 1). In a large cohort representing the full clinical spectrum of a non-specialised neurochemical laboratory, sporadic Creutzfeldt-Jakob disease accounted for only 18% of patients with highly elevated (>1200 pg/mL) CSF t-Tau concentration;[61] therefore, as with other biomarkers, it should not be used in general screening but in the proper clinical context when suspecting prion disease. Evidence-based consensus cut-

offs for CSF t-Tau, at best considering different assays, differential diagnoses, and supportive information on sporadic Creutzfeldt-Jakob disease cases (eg, codon 129 *PRNP* polymorphism), would be most helpful and should be evaluated through a structured analysis. In conclusion, both markers (t-Tau and 14-3-3) share several characteristics, advantages, and dis advantages (appendix panel 1). The clinical use must be assessed in the light of suspected differential diagnoses and can be improved by stratification of demographic and genetic factors.[103]

An upcoming issue in the biomarker-based diagnosis of sporadic Creutzfeldt-Jakob disease is the use of composites. Regarding this issue, the best evidence is available for the p-Tau/t-Tau ratio, which was shown to be of superior diagnostic accuracy compared with t-Tau alone, especially in the differentiation of sporadic Creutzfeldt-Jakob disease from Alzheimer's disease.[51,64,68] Proposed ratios combining t-Tau, p-Tau, 14-3-3, S100b, t-PrP, or beta amyloid showed high diagnostic accuracy[48,52,73,108] but have not been established in the clinical setting.

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



The figure has been adapted from NCJDRSU criteria[13] that were based on the WHO criteria[3,4] and amended by RT-QuIC as an additional biomarker. Here, imaging criteria were refined and the need for a thorough diagnostic work-up in suspected probable sCJD is emphasized.
*Generalised periodic sharp/wave complexes (PSWCs); †Restricted diffusion in caudate or caudate/putamen or caudate/putamen/thalamus, or at least two cortical regions (temporal, parietal, occipital) on MRI brain scan,[4] no subcortical white matter involvement, no isolated restricted diffusion in the thalamus. Characteristic hyperintensities may be seen on fluid attenuated inversion recovery (FLAIR) images, but diffusion weighted (DWI) sequences are required to confirm CJD-typical restricted diffusion.[84,85]

Guidelines for the biomarker-based diagnosis

Based on WHO criteria,[3,4] the studies presented here, and previous suggestions that include RT-QuIC,[12,13] the majority of the authors recommend amended criteria for the clinical diagnosis of sporadic Creutzfeldt-Jakob disease (figure 2).

Because of the outstanding specificity of RT-QuIC, positive cases can be classified as probable sporadic Creutzfeldt-Jakob disease in early clinical stages, even when only one cardinal symptom is present, which will improve the early identification of sporadic Creutzfeldt-Jakob disease.[12,39,108] Similar to other diagnostics, as the test becomes widely applied, even a false positive rate below 1% will lead to some incorrect diagnoses. This likelihood becomes particularly concerning if treatable conditions are missed. Ability to rely solely on RT-QuIC is further compromised by the test's inability to distinguish accurately between different forms of human prion disease and test sensitivities that vary from 73% to 97%. Additionally, RT-QuIC is mostly unavailable in countries without major surveillance programmes. Therefore, we recommend that clinicians contact national Creutzfeldt-Jakob disease surveillance units or referral centres to get information on the availability of RT-QuIC and general clinical guidance for the diagnosis and management of suspected prion disease cases (appendix panel 2).

Readily available, economical, and field-tested CSF biomarkers such as 14-3-3 and t-Tau, and EEG and MRI (preferably DWI including ADC sequences) are still of major importance and should be used as routine diagnostic tests in cases of suspected sporadic Creutzfeldt-Jakob disease. These tools have been shown to be effective and accurate in the differential diagnosis of sporadic Creutzfeldt-Jakob disease, when they are applied and interpreted in a reasonable context. In case of ambiguous results or uncertain differential diagnoses, the p-Tau/t-Tau (or t-Tau/p-Tau) ratio might be considered as a supportive biomarker.[63,64,68] Genetic analysis of PRNP should be considered in all cases of suspected Creutzfeldt-Jakob disease to determine the codon 129 polymorphism and to exclude pathogenic mutations, which can be present in patients with a negative family history.[102] Importantly, routine blood, CSF, and imaging diagnostics should always be done to rule out the most common differential diagnoses (appendix table 4, panel 1, panel 2).

Panel 2. 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 amending the established WHO criteria for the clinical diagnosis of "probable" 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;* CSF t-Tau and the p-Tau/t-tau (or t-Tau/p-Tau) ratio are valuable supportive biomarkers. All markers have to be performed in experienced and certified laboratories. MRI and EEG are highly specific but require experienced raters. MRI sequences should include T1 weighted images with contrast agent sequences (for differential diagnosis), FLAIR, and DWI with ADC maps. CJD-typical MRI findings are clearly visible on DWI rather than other sequences. 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. Diagnosis of "possible" sCJD (absence of suggestive biomarkers, figure 2) should only be made if extensive diagnostics had not revealed alternative explanations for the clinical condition.

Important differential diagnoses

Genetic 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.

*In some centers, CSF t-Tau is considered as primary CSF surrogate biomarker.

Future challenges and perspectives

Despite improvements of diagnostic measures for sporadic Creutzfeldt-Jakob disease over the past 25 years, there are still plenty of challenges. The value of established and new biomarkers in the differential diagnosis of sporadic Creutzfeldt-Jakob disease subtypes and other human prion diseases (eg, iatrogenic Creutzfeldt-Jakob disease, variant Creutzfeldt-Jakob disease, and genetic Creutzfeldt-Jakob disease) has to be clarified. RT-QuIC has to be 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 olfactory mucosa,[106] might be reappraised.

More potential diagnostic biomarkers are currently under investigation. We recommend that new biomarkers could be considered in future diagnostic criteria under particular conditions. Besides strong clinical evidence (ie, validation of cut-offs in independent cohorts, appropriate controls), such a biomarker should substantially improve the clinical diagnosis of sporadic Creutzfeldt-Jakob disease. This improvement can be a biomarker that shows superior diagnostic accuracy compared with established markers or equal accuracy with reduction of test invasiveness (eg, blood-based tests). Although analysis of codon 129 PRNP polymorphism, clinical observations, and biomarker profiles (especially MRI DWI lesion patterns[87]) already allow conclusions on the sporadic Creutzfeldt-Jakob disease subtype, new biomarkers should be able to go beyond phenotypical variability and disease stage, or at least be evaluated in this respect.

Over the last 5 years, some investigations have opened the field of prodromal, prognostic, and predictive biomarkers for sporadic Creutzfeldt-Jakob disease. One of the challenges for clinical trials in sporadic Creutzfeldt-Jakob disease is that clinical features are highly heterogeneous and it has been difficult to find a suitable single continuous measure as an outcome. Therefore, specific Creutzfeldt-Jakob disease tests, such as RT-QuIC, might be used at trial enrolment and blood-based biomarkers might be used repeatedly during a trial to track axonal damage in the course of experimental treatment. Further work is required to establish variability of biomarkers in the natural history of Creutzfeldt-Jakob disease and if biomarkers of neurodegeneration can contribute to prognostic or trial models. Finally, blood-based biomarkers could have a role in preventive trials as a prodromal biomarker for healthy individuals who are at-risk of Creutzfeldt-Jakob disease because of iatrogenic prion exposure or PRNP mutation. Available published work suggests a prodromal biomarker window is small or rare in at-risk individuals with pathogenic PRNP mutations[109,110] but this concept is currently the focus of many prion researchers.

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", "imaging", "MRI", "EEG", and "RT-QuIC". We included articles published between Jan 1, 2015, and Nov 15, 2020, written in English or German on the basis of the scientific merit and contribution to developments in biomarker research for sporadic Creutzfeldt-Jakob disease (ie, the biomarkers have shown potential for a clinical utilization and results were independently validated). However, comprehensive lists of articles (after 2015, not mentioned in the main text) presenting altered biomarkers in sporadic Creutzfeldt-Jakob disease were noted (appendix table 2). Older articles (ie, articles published before 2015) were selected on the basis of author expertise to substantiate basic information and evidence of biomarkers that are currently being investigated.

Contributors

PH designed the Review outline, wrote the manuscript, did the literature search, and designed and prepared illustrations, including processing of the MRI scans. IZ designed the Review outline, performed literature search, and revised the manuscript. BA contributed to the literature search, provided the MRI scans, and revised the manuscript. All other authors contributed to the literature search and revised the manuscript.

Declaration of interests

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Appendix

Appendix 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%
		37	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: The method gives positive or negative signals, and possibly traces that were rated negative; AD: Alzheimer's disease; atypical AD: Alzheimer's disease with rapid cognitive decline or patients with additional motor signs; DLB: Dementia with Lewy bodies; FTLD: fronto-temporal lobar degeneration; t-Tau: total Tau protein in the CSF

Appendix panel 1. Advantages, disadvantages, and perspectives of important CSF surrogate biomarkers for sCJD

14-3-3 proteins

- The CSF 14-3-3 proteins are part of a diagnostic gold standard,[6,7] highly sensitive for sCJD and specific in the differential diagnosis of neurodegenerative dementias.[1,8]
- The sensitivity is moderate for certain sCJD subtypes and the specificity is low in the discrimination from acute neuronal injury events.[1,9]
- Western Blot may produce ambiguous results ("weak", "traces").
- New 14-3-3γ ELISA showed superior diagnostic performance[10] but is not widely established.

Tau protein

- Very elevated CSF t-Tau is highly sensitive for sCJD and specific in the differential diagnosis of most neurodegenerative dementias.[1,4]
- The sensitivity is moderate for certain sCJD subtypes. The specificity is moderate in the discrimination from acute neuronal injury events and low in the discrimination from atypical AD.[1,4,11]
- A consensus cut-off has not been established. The p-Tau/t-Tau (or t-Tau/p-Tau ratio) showed superior diagnostic performance.[12]
- Tau protein is a potential blood-based and prognostic marker.[13]

S100b, NfL, t-PrP, and new candidates

- CSF S100b is a well-known biomarker but its diagnostic accuracy is inferior to 14-3-3 and t-Tau.[9]
- CSF NfL showed a very high accuracy in the discrimination of sCJD from healthy controls but lacks of specificity considering differential diagnoses. It may have potential as a prodromal, prognostic, and blood-based biomarker.[13,14]
- Decreased CSF t-PrP showed moderate accuracy and may be a valuable part of composite biomarkers.[2,3] In contrast, increased t-PrP was reported to be a potential blood-based biomarker for sCJD.[15]
- New candidate biomarkers such as CSF α-Synuclein and others were recently identified and require further investigation.[16]

Appendix table 2. Additional potential CSF biomarkers in the differential diagnosis of sCJD

Reference*	Biomarker	Result
Schmitz et al. 2016[17]	Malate Dehydrogenase 1 (MDH1)	MDH1 was increased in CSF of sCJD compared to OND+, showing a sensitivity of 83% and a specificity of 85%
Oeckl et al. 2016[18]	α-, β-, and γ-Synuclein	α-, β-, and γ-Synuclein were significantly increased in sCJD compared to neurodegenerative disease and non-neurodegenerative controls
Llorens et al. 2017[19]	YKL-40	YKL-40 was increased in sCJD compared to OND+ (AUC 0·92), AD, Vascular dementia, and Lewy body diseases
Ermann et al. 2018, [20] Llorens et al. 2020[21]	Non-phosphorylated Tau (non-P-Tau)	Increased non-P-Tau discriminated sCJD from AD (AUC 0·99)[20] and sCJD from OND+ (AUC 0·99)[21]
Blennow et al. 2019[22]	Neurogranin	Increased Neurogranin discriminated sCJD from OND+ (AUC 0·96) and AD (AUC 0·85)
Li et al. 2019[23]	Cell-free mitochondrial DNA (mtDNA)	Copy number of mtDNA was significantly increased in sCJD compared to OND+
Abu-Rumeileh et al. 2020[24]	Ubiquitin	Increased Ubiquitin discriminated prion diseases from healthy controls (AUC 0·95), AD (AUC 0·85), and frontotemporal dementia (AUC 0·95)
López-Pérez et al. 2020[25]	bone morphogenetic protein and activin membrane-bound inhibitor (BAMBI)	BAMBI protein was significantly increased in sCJD compared to OND+

*only articles published after January 1, 2015 showing significantly altered levels in CSF of human sCJD cases are considered

OND+: Other neurological diseaeses including dementia syndromes; AD: Alzheimer's disease

Appendix table 3. 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[26]	s-100b (s)	>213.0	108	probable + definite sCJD	74	OND+	78%	81%	··	··
Steinacker et al. 2016[14]	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[27]	t-Tau (p)	··	65	definite sCJD	21	healthy controls	··	··	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	healthy controls	··	··	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[28]	t-Tau (s)	>2.2	45	probable + definite sCJD	24	healthy controls	91%	83%	0·91	(0·83-0·98)
	NfL (s)	>44.7			24	healthy controls	100%	100%	1	··
Abu-Rumeileh et al. 2020[13]	t-Tau (p)	>5.89	336	probable + definite sCJD, gCJD	106	RPD	73%	82%	0·84	··
	NfL (p)	>87.9			106	RPD	70%	56%	0·62	··
Llorens et al. 2019[15]	t-PrP (p)	··	104	probable + definite sCJD	110	healthy controls	··	··	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[29]	YKL-40 (p)	··	78	probable+ definite sCJD	70	healthy controls	··	··	0·81	(0·74-0·88)
					44	OND+	··	··	0·72	(0·63-0·81)
Oeckl et al. 2020[30]	beta-synuclein (s)	··	25	definite sCJD	45	Controls*	96%	100%	0·99	(0·98-1.01)
					64	AD	96%	100%	0·98	(0·94-1.02)
					13	LBD	100%	96%	0·99	(0·98-1.01)
					16	bv FTD	96%	100%	1·00	(0·98-1.01)
					30	ALS	96%	100%	1·00	(0·99-1.01)
Norsworthy et al. 2020[31]	small RNA-seq read†	··	57 29	probable+ definite sCJD	48	healthy controls	86%	71%	0·79	··
					30	AD	72%	100%	0·92	··

AUC: area under the curve; gCJD: genetic prion diseases; (s): serum; (p): plasma; OND+: other neurological diseases including dementia syndromes; OND: other neurological diseases excluding neurodegenerative and vascular dementia syndromes; AD: Alzheimer's Disease; LBD: Lewy body diseases; bvFTD: behavioral variant fronto-temporal dementia; VD: vascular dementia; ALS: amyotrophic lateral sclerosis
*non-neurodegenerative diseases
†ratios of hsa-let-7i-5p, hsa-miR-16-5p and hsa-miR-93-5p measured relative to RNU6-2

Appendix panel 2. Supportive information on CJD surveillance, RT-QuIC availability, and further clinical support.

National surveillance programs and referral centers

Prion disease surveillance units, referral centers, and research groups are present in many countries all over the world, tasked with epidemiological surveillance, case classifications, and/or specific biomarker analyses. They may provide valuable help for clinicians. All authors of this article are affiliated with such centers/groups and a list of additional CJD centers can be found on the website of the CJD International Support Alliance (CJDISA).[32] The European Creutzfeldt-Jakob disease Surveillance Network (EuroCJD)[33] may also provide information on cooperating centers in Europe and other global regions. In countries that are not mentioned, clinicians should contact health authorities to get information on national or neighbor countries' referral centers.

Availability of RT-QuIC and other biomarkers

CSF analyses for t-Tau, p-Tau, and 14-3-3 are broadly available in CJD reference centers and in laboratories that are specialized on markers of neurodegeneration. CSF RT-QuIC analyses are currently provided by most CJD surveillance centers. We recommend that clinicians who seek RT-QuIC analyses contact their national surveillance unit (see above) and check for availability. Some centers may also provide analyses for patients from other countries.

Further support for clinicians, patients, and their families

In some countries, non-profit associations offer information and help for people that are affected by human prion diseases regarding practical and social needs. This includes patients and their families, as well as physicians, nurses, and other involved professions. The CJDISA is the umbrella organisation and a list of associated groups can be found on their website.[34]

Appendix table 4. 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
	Multiple-system atrophy, progressive supranuclear palsy, and other rare proteinopathies	• dementia and/or 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 (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.) as well as 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.) and atypical encephalitis caused by bacteria or other infectious agents (Whipples' disease, Lues, 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)
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	MELAS, NBIA 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[35-37]
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 virus; MELAS: mitochondrial encephalopathy, lactic acidosis and stroke-like episodes; NBIA: neurodegeneration with brain iron accumulation

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