Advances in Prion Diseases 2

Advances in biomarkers for diagnosis and prognosis of prion diseases

Inga Zerr, Peter Hermann*, Tze How Mok, Simon Mead*

Seeded amplification assays have improved the accuracy of the diagnosis of prion diseases, and blood-based diagnostic biomarkers are now close to clinical application. Although clinical diagnosis can be accurate, research on biomarkers is still crucial for therapeutic development and studies are addressing their use at preclinical stages in mutation carriers, focusing on non-invasive sampling. Some biomarkers have been tested in blood, tear fluids, and urine and could become easily accessible testing platforms. Emerging biomarkers can predict the onset or reflect the clinical course of prion disease, and might become crucial for the evaluation of new therapeutics. Furthermore, detection of misfolded prion protein at preclinical stages in healthy mutation carriers warrants a comprehensive discussion of associations with other molecular signals that might be also detectable before disease onset. A new disease staging system based on biomarkers should be evaluated in future clinical studies.

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This is the second in a **Series** of two papers about advances in prion diseases

*Contributed equally

National Reference Center for CJD Surveillance, Department of Neurology, University Medical Center, Georg August University, Göttingen, Germany (Prof I Zerr MD, P Hermann MD); **Medical Research Council Prion Unit at University College London, Institute of Prion Diseases, London, UK** (T H Mok MD, Prof S Mead PhD)

Correspondence to: Prof Inga Zerr, National Reference Center for CJD Surveillance, Department of Neurology, University Medical Center, Georg August University, 37075 Göttingen, Germany **ingazerr@med.uni-goettingen.de**

Introduction

The spectrum of prion disease comprises different aetiologies (eg, sporadic, hereditary, and acquired),[1–3] with distinct clinical and neuropathological features. Similar to other more common neurodegenerative diseases, such as Parkinson's disease or Alzheimer's disease, early indicators and prodromal symptoms of prion disease need to be characterised, with the aim of identifying prevention interventions.[4,5] However, only the prescription of selective serotonin reuptake inhibitors within 3 years preceding disease onset has been identified as a potential prodromal marker of prion disease so far.[6]

Protein-based biomarkers can be measured in various biofluids or tissues, but most biomarkers have been developed in CSF. Research is now focusing on biomarkers from peripheral tissues, such as blood, urine, olfactory mucosa, skin biopsies, tear fluids, or saliva, which could bring new possibilities for early screening and disease monitoring. These biomarkers are being explored in healthy mutation carriers before disease conversion.[7–10]

The first paper of this Series on Advances in Prion Diseases covers genetic causes and other modifiers of the risk of these diseases.[3] This second paper in the Series provides an update of recent developments in non-invasive and preclinical biomarker detection of patients affected by a prion disease and in people who carry a mutation in the *PRNP* gene, which encodes the prion protein (PrP). We provide an overview on established diagnostic tests (eg, CSF biomarkers and neuroimaging), followed by evidence on emerging tests in peripherally accessible tissue and fluids. We also cover new evidence from longitudinal studies and propose a staging system for disease monitoring.

CSF diagnostic biomarkers

Routine CSF tests, such as cell count, parameters of inflammation, and measures of blood-brain barrier function, are usually normal in people with prion diseases. These normal findings are an important prerequisite for further diagnostic analyses, as described later.

Detection of abnormally folded prion protein in CSF using amplification techniques, such as real time-quaking induced conversion (RT-QuIC), has become a standard for the classification of Creutzfeldt–Jakob disease (panel 1 and appendix p 1).

The CSF of patients with prion diseases shows altered concentrations of brain-derived proteins, including 14-3-3 protein, tau protein, neurofilament light chain (NfL), α-synuclein, glial fibrillary acidic protein (GFAP),

Panel 1: PrP RT-QuIC and PrP seeded-aggregation assay

The real time-quaking induced conversion (RT-QuIC) assay, a form of seeded-aggregation assay, is an ultrasensitive diagnostic test that exploits the ability of amyloidogenic prion protein (PrP) seeds to convert recombinant PrP to PrP amyloid, accelerated by repeated cycles of mechanical agitation.[11] The assay shares similarities with the protein misfolding amplification assay (PMCA), but uses mechanical agitation (ie, shaking) instead of sonication to disrupt aggregated fibrils, and thioflavin T fluorescence instead of the detection of the abnormal scrapie form of PrP (PrP^{Sc}) on Western blot as the readout. Crucially, while prion strain fidelity and infectivity are preserved with serial rounds of PMCA, the end-products from RT-QuIC reactions do not recapitulate the biological properties of the original prion seed. Nevertheless, CSF RT-QuIC has proven to be a highly sensitive and specific diagnostic assay for sporadic Creutzfeldt–Jakob disease and has been used in several surveillance laboratories around the world for the past 10 years.[12] Some key attributes of CSF RT-QuIC include consistent reproducibility, corroborated by at least two round robin tests, and high sensitivity.[12–15] After more than a decade of clinical application, the clinical criteria were validated by prospective clinical studies.[12,16,17] However, for clinical interpretation and application of diagnostic criteria, it is important to mention that the test specificity is not 100% and occasional false positive results have been reported.[18]

While the RT-QuIC CSF test is well established for sporadic Creutzfeldt–Jakob disease, sensitivity for some forms of genetic prion diseases, such as Gerstmann–Sträussler–Scheinker syndrome and fatal familial insomnia, remains low. Protocol modifications using various recombinant PrP substrates[19,20] might lead to greater test sensitivity and further possibilities need to be explored.[21]

See Online for appendix

and amyloid-β40 and amyloid-β42.[12] These alterations might reflect the damage of brain cells. However, some of these biomarkers might also reflect copathology, which has been reported for patients with various forms of prion diseases at autopsy.[22]

The clinical diagnosis of prion disease is supported by elevated concentrations of protein 14-3-3 and tau in CSF. The analysis of 14-3-3 and total tau (t-tau) in CSF, which are markers of neuronal damage, is part of the diagnostic work-up in patients who develop rapid progressive dementia, frequently accompanied by other neurological signs and symptoms (eg, ataxia, myoclonus, and pyramidal and extrapyramidal signs) within a few weeks after onset. The clinical syndrome and abnormal 14-3-3 and tau can justify a clinical diagnosis of Creutzfeldt–Jakob disease.

Elevated concentrations of 14-3-3 protein was the first fluid biomarker included in diagnostic criteria and subsequently, t-tau emerged as an additional alternative and is even discussed as a primary biomarker for the detection of neurodegeneration in patients with Creutzfeldt–Jakob disease.[12,23–24] Both biomarkers show good diagnostic utility in sporadic and familial Creutzfeldt–Jakob disease. Importantly, they are less accurate in very rare genetic prion diseases, such as fatal familial insomnia and Gerstmann–Sträussler–Scheinker syndrome.[25,26]

The relative utility of CSF 14-3-3 versus t-tau remains debated. The performances of these biomarkers varied in different studies, possibly due to differently selected case or control groups, inclusion and exclusion criteria, and investigated assays. Substantial differences in assay sensitivity have been reported between Western blot protocols for 14-3-3 detection, and better performance with respect to better sensitivity and specificity of the 14-3-3 ELISA assay over that of the Western blot. Some studies have favoured t-tau because of a slightly better diagnostic performance[27] or because of the fact that the test has better general accessibility, but meta-analyses could not validate an advantage of its diagnostic performance over 14-3-3 protein assays.[28] Moreover, the widespread availability of t-tau testing and its common application in Alzheimer's diagnostics can abet the misinterpretation of a neurodegeneration marker as a general screening tool for Creutzfeldt–Jakob disease. If used as a clinical screening test, the predictive accuracy would drop considerably due to the low prevalence of prion diseases.[12] In cohorts that encompass unselected patients recruited in a diagnostic department, non-prion diseases account for 63%[29] to 75%[30] of tests with highly elevated CSF t-tau concentrations. The phosphorylated-tau to t-tau ratio might improve the diagnostic accuracy of tau biomarkers for Creutzfeldt–Jakob disease,[28] especially in distinguishing people with prion disease from those with Alzheimer's disease,[27,30] and has been suggested as an alert marker in the prevention of iatrogenic transmission of prion disease.[31] However, tau biomarkers are not specific to prion disease and abnormal t-tau and tau ratio values occur in various more prevalent differential diagnoses, such as Alzheimer's disease (t-tau), inflammatory CNS diseases, or seizures (tau ratio).[30]

In addition to their diagnostic use, the concentration of these CSF biomarkers has been associated with survival, with higher tau concentrations indicative of lower survival. Whereas the potential of 14-3-3 protein in prognosis has not yet been clarified, and another candidate, S100-b, was not a significant predictor either,[32] t-tau[33] was included in a promising model for prognosis in people with sporadic Creutzfeldt–Jakob disease.

Recent studies have explored alternative CSF biomarkers, focusing on NfL,[12] α-synuclein,[34] β-synuclein,[35] neurogranin,[36] or synaptosomal-associated protein-25 (SNAP-25).[37] In CSF, NfL and α-synuclein concentrations were significantly higher in patients with Creutzfeldt–Jakob disease than in controls with other neurological and neurodegenerative diseases[12,34,38] and achieved a sensitivity for diagnosis between 89% and 98%, and a specificity of 92–97%.[34,39,40] These biomarkers have been also suggested as a prognostic indicator.[38] CSF neurogranin concentrations in people with Creutzfeldt–Jakob disease show a 4·75-fold increase compared with people with other neurological diseases,[36,37] while the synaptic biomarker SNAP-25 has greater CSF concentrations in patients with Creutzfeldt–Jakob disease compared with those with other rapid progressive dementias of both neurodegenerative and non-neurodegenerative origin,[37] revealing similar diagnostic performance as tau and 14-3-3 protein. Furthermore, CSF SNAP-25 concentrations are associated with survival time being shorter in those with higher levels.[4,37]

A study of 302 symptomatic patients with hereditary prion disease, recruited in 11 diagnostic centres and encompassing 36 different *PRNP* mutations, evaluated the diagnostic accuracy of CSF biomarkers.[25] High sensitivity of 14-3-3 protein, t-tau, and α-synuclein assays in CSF were detected for hereditary Creutzfeldt–Jakob disease associated with the E200K and V210I mutations, but low sensitivity was observed for mutations associated with familial fatal insomnia or Gerstmann–Sträussler–Scheinker syndrome.[25] CSF t-tau concentrations correlated with the degree of cortical atrophy, disease severity, and cognitive decline in patients with genetic Creutzfeldt–Jakob disease with E200K mutation.[41,42] NfL CSF concentrations were increased in people with sporadic or familial fatal insomnia.[43]

Neuroimaging biomarkers

MRI is both accurate and indispensable in the differential diagnosis of Creutzfeldt–Jakob disease. Although the MRI signal patterns are not specific per se, the diagnostic sensitivity can be extremely high when images are assessed by experienced raters.[44] A combination of MRI and CSF biomarkers can identify Creutzfeldt–Jakob disease with a sensitivity of up to 100%.[18] In 2009, a

multinational consortium included MRI criteria in the diagnostic guidelines.[45] A typical pattern for Creutzfeldt–Jakob disease was defined as restricted diffusion with increased signal intensity in diffusion-weighted images (so-called ribboning) and corresponding decreased signal intensity on apparent diffusion coefficient maps in either two cortical regions (ie, temporal, parietal, or occipital), the caudate nucleus, or both. The putamen, the thalamus, and the frontal cortical region can additionally be affected. These criteria are recommended by multinational consensus guidelines.[12] Recently, modified MRI criteria added high signal intensity of the cortex in a single cortical lobe, including the frontal lobe, to enhance the sensitivity.[46] However, a subsequent Australian study found similar sensitivity and specificity between both sets of criteria.[47] Applying the 2009 diagnostic criteria, several surveillance and MRI studies have reported the sensitivity and specificity to range between 80% and 98% for both measures for the diagnosis of Creutzfeldt–Jakob disease.[12] A UK study showed the importance of expertise, reporting 99% sensitivity of the 2009 diagnostic criteria in specialised centres compared with 70% in non-specialised settings.[48] In addition to its excellent diagnostic utility, MRI can also be useful to estimate the prognosis of patients who are symptomatic. An algorithm combining DWI lesion patterns and codon 129 *PRNP* genotyping has allowed for the identification of distinct molecular subtypes of sporadic Creutzfeldt–Jakob disease with an overall accuracy of 89%.[49,50] Such ante-mortem subtyping will not only improve diagnosis and prognosis, but might also be crucial for future clinical trials, particularly regarding patient selection, stratification, and historical data classification for single-arm trials (ie, single arm studies without a control arm would compare historical data and subtype classification would be crucial for the validity of the historical data).

Although [^{18}F]fluorodeoxyglucose (FDG)-PET can display cortical and subcortical hypometabolism in prion diseases, no specific patterns have been established.[51] Conversely, this imaging technique can facilitate early diagnosis of genetic prion diseases[52,53] and improve detection of the rare, typically MRI-negative MM2T-type (sporadic fatal insomnia) by the early identification of characteristic thalamic hypometabolism.[54]

Peripheral biomarkers in development

Improved analytical assays for brain-derived proteins in plasma are being developed for diagnosis and prognosis in patients with prion disease. Various biomarkers have been tested in plasma and serum, and might function as diagnostic, dynamic, or prognostic biomarkers.[55] In Creutzfeldt–Jakob disease, numerous publications have reported abnormal concentrations of tau in plasma samples, which correlate with CSF concentrations and are increased across most prion disease types. CSF tau levels might discriminate Creutzfeldt–Jakob disease from other diseases with moderate (eg, Alzheimer's disease) to excellent (eg, healthy controls) accuracy. Of note, high plasma t-tau and CSF t-tau concentrations are significantly associated with the likelihood of reduced survival after controlling for the codon 129 genotype and Barthel Index.[56] When tested in comparison with other CSF and blood-based biomarkers,[56] plasma t-tau showed the strongest effect size with a hazard ratio that was more than 40% larger than that of any other biomarkers. Plasma t-tau is independently associated with rate of clinical progression in sporadic Creutzfeldt–Jakob disease.[57] High plasma concentrations of brain-derived tau were associated with reduced survival ($p<0·001$). Blood phosphorylated tau 217 was revealed as a potential marker of prion-specific tauopathy and might improve diagnostic accuracy,[58] and the concentration of N-terminal tau in plasma could have diagnostic potential.[59]

NfL has been extensively studied for many neurological disorders, proving particularly valuable as a dynamic biomarker in motor neuron diseases for tracking disease progression.[60,61] As summarised in table 1 and the appendix (pp 2–5), NfL concentration in plasma is substantially elevated in Creutzfeldt–Jakob disease compared with healthy controls, including at early disease stages in people with minimal functional impairment.[62–65] Concentrations increase in follow-up samples of patients, and high plasma NfL concentrations

	Tau†	Neurofilament light chain	Glial fibrillary acidic protein	S100B	β-synuclein	YKL-40	Cellular prion protein
Diagnostic accuracy *vs* healthy controls	+	+	..	..	+	(+)	+
Diagnostic accuracy *vs* neurodegenerative dementia	+	+	(+)	(+)	+	(+)	(+)
Diagnostic accuracy *vs* CJD mimics*	+	–	..	..	+	..	..
Dynamic	+	+	+	..	–	..	–
Prognostic	+	(+)	(+)	..	+	..	(+)
Preclinical	–	(+)	–	..	–	..	..

The table provides a non-structured interpretation of currently available data on new biomarkers explored in prion diseases and controls. A detailed overview is given in the appendix (p 1). +=good accuracy or association. (+)=moderate accuracy or few or discrepant data on association. –=insufficient accuracy or no association. ..=insufficient data. CJD=Creutzfeldt-Jakob disease. *Rapidly progressive dementia syndromes, mostly non-primarily neurodegenerative encephalopathies. †Includes various forms phosphorylation, ratios, and brain-derived tau.

Table 1: **Blood-based biomarkers of neurodegeneration in patients with prion diseases**

are associated with higher severity of functional impairment.[57] A specific situation arises in patients with familial fatal insomnia.[66] In patients with symptomatic familial fatal insomnia, there is a significant elevation of plasma NfL concentrations.[67] In healthy people who carry *PRNP* mutations, elevated NfL concentrations can be detected in CSF and plasma up to 2 years before conversion.[8,57] However, NfL concentrations need to be carefully interpreted in the clinical context, given the limited understanding of its clearance from biofluids.[68]

S100b, an astrocytic protein, was reported to be elevated in the plasma of patients with sporadic CJD.[69] Plasma β-synuclein is markedly elevated in cases of symptomatic Creutzfeldt–Jakob disease compared with other diagnostic groups,[35,70,71] and elevated concentrations of YKL-40 and GFAP have also been documented in people with Creutzfeldt–Jakob disease.[37,72]

PrP^{Sc} detection

Although primarily associated with the CNS (ie, brain and spinal cord), the abnormal scrapie form of PrP (PrP^{Sc}) has been also identified in various peripheral tissues and fluids, including the olfactory mucosa, internal organs (eg, lung, spleen, and liver), other tissues (eg, digestive system and skin), and various body fluids (eg, CSF, tears, urine, and blood). Widespread distribution of PrP^{Sc} across multiple tissue types and body fluids suggests its systemic involvement in prion diseases.

Olfactory mucosa

Olfactory mucosa is easily collected by a minimally-invasive procedure that uses nasal brushing. Prion seeding activity can be detected in olfactory mucosa in patients with sporadic Creutzfeldt–Jakob disease and genetic prion diseases by RT-QuIC and protein misfolding amplification assays (PMCA).[73–77] The overall sensitivity of these assays using the olfactory mucosa from patients with sporadic Creutzfeldt–Jakob disease was 95%, showing a diagnostic accuracy similar to that of CSF.[74] In contrast to CSF or RT-QuIC products, nasal brushing samples and PMCA products from patients with Creutzfeldt–Jakob disease or fatal familial insomnia transmitted the disease to mice by intracerebral inoculation.[78,79]

Tears

Tear fluid collection using paper strips offers a minimally invasive sampling method, although quantitative analysis can be complicated by variable fluid volumes. Tear fluid testing has revealed abnormal prion protein seeding activity detected by RT-QuIC in more than 80% of sporadic and genetic cases with P102L (in Gerstmann–Sträussler–Scheinker syndrome), D178N–129M (in fatal familial insomnia), octapeptide repeat insertion, and other mutations. Seeding activity is typically lower than that obtained in CSF, possibly due to lower PrP^{Sc} concentration in tear fluid.[20]

Skin biopsies and hair roots

First reported in 21 deceased cases of sporadic Creutzfeldt–Jakob disease and two cases with variant Creutzfeldt–Jakob disease,[80] and then in 30 postmortem and five antemortem cases of sporadic Creutzfeldt–Jakob disease,[81] prion seeding activity in skin biopsies showed diagnostic sensitivity ranging from 87% to 95%.[82–85] The diagnostic performance of skin PrP^{Sc}-seeding assay is influenced by PrP^{Sc} types, *PRNP* 129 polymorphisms, dermatome location, and disease duration.[58] The skin area next to the ear gave the highest sensitivity, followed by skin biopsies from the lower back and apex of the head.[82–85] Of note, PrP RT-QuIC of the skin might show potential as a dynamic biomarker for clinical trials, as the seeding activity decreases after treatment in skin samples from animal models.[83] New data report PrP^{Sc} activity in hair roots.[86]

Urine

PrP^{Sc} detection in urine was first reported in patients with variant Creutzfeldt–Jakob disease, and was positive in 13 (92·9%) of 14 urine samples.[87,88] In sporadic Creutzfeldt–Jakob disease, aggregation assays in urine have been negative using RT-QuIC, but became positive in 29 (35·8%) of 81 patients by PMCA testing, which suggests the diagnostic potential of using this easily accessible biofluid.[89]

Blood

Compared with patients with sporadic Creutzfeldt–Jakob disease, in whom PrP^{Sc} could not be detected, PrP^{Sc} detection in blood was reported in three of four patients with confirmed variant Creutzfeldt–Jakob disease.[90] The optimisation of PMCA, by use of pre-processing blood samples with sarkosyl and high-speed centrifugation,[91] had 100% sensitivity and specificity in a study, detecting prions in all 14 patients with variant Creutzfeldt–Jakob disease. Plasminogen-coated magnetic nanobeads for PrP^{Sc} capture led to 100% diagnostic sensitivity and specificity in plasma samples from 18 patients with variant Creutzfeldt–Jakob disease.[92] Of note, in non-human primates inoculated with variant Creutzfeldt–Jakob disease prions, the prions could be amplified in preclinical blood samples 2 months post-inoculation and PrP^{Sc} was detected throughout the entire preclinical stage.[93]

These findings underscore the efficacy of blood PMCA for the diagnosis of variant Creutzfeldt–Jakob disease, but also highlight the challenge of detecting sporadic Creutzfeldt–Jakob disease prions in blood. Although the protein is likely to be present,[94] so far PrP^{Sc} detection in blood of patients with sporadic Creutzfeldt–Jakob has not been consistently successful.[95]

Neuroimaging biomarkers in development

Functional MRI can be used to assess the evolution of Alzheimer's disease and can detect abnormalities even before symptoms onset.[96] Growing evidence suggests that functional MRI might detect abnormalities at the

***Panel 2*: Recommendations for the use of neuroimaging in prion diseases**

Diagnostic imaging

- Brain MRI is part of the diagnostic criteria for sporadic Creutzfeldt–Jakob disease and indicated in all cases of suspected prion disease to confirm or rule out the diagnosis.[12,44]
- The sensitivity of MRI is very high; areas of restricted diffusion (eg, cortex or striatum) are the most important pathological finding in people with CJD.[12]
- In cases of negative or ambiguous results, raters with experience in prion disease diagnosis should be consulted.[48]
- [^{18}F]fluorodeoxyglucose (FDG)-PET imaging is useful to detect neurodegeneration in typically MRI-negative prion diseases, such as sporadic fatal insomnia and fatal familial insomnia.[52,54]

Prognostic imaging

The molecular disease subtype is the most important prognostic factor in sporadic Creutzfeldt–Jakob disease. In combination with codon 129 *PRNP* genotyping and assessment of the clinical phenotype, the lesion patterns can enable ante-mortem identification of the subtype.[49,50,103]

Preclinical imaging

Disease-typical lesions in clinically healthy people with *PRNP* mutations or other healthy individuals can point to impending prion disease onset. However, the specification of the time to onset that could be up to years, is not feasible yet. As long as no disease-modifying interventions are available, we do not recommended imaging in mutation carriers outside of specific clinical trials.

Imaging in research settings

Neuroimaging is a promising tool for risk assessment and disease monitoring, but evidence is limited.

- Serial MRI should be implemented in the protocols of all interventional studies and in observational studies in preclinical cohorts of *PRNP* mutation carriers.
- Ante-mortem subtyping with MRI and codon 129 *PRNP* analyses might be useful as inclusion criteria, subgroup stratification, and classification of historical data in clinical trials.
- The minimum MRI dataset should include diffusion-weighted imaging with apparent diffusion coefficient and T2-fluid attenuated inversion recovery, and T1-weighted images (to monitor typical lesions and atrophy, respectively).[103]
- Diffusion tensor imaging and FDG-PET abnormalities can precede MRI restricted diffusion in people with prion diseases and should be explored in specific studies with preclinical cohorts.

preclinical stage also in prion disease. By nature, preclinical studies in a rare sporadic disease, such as Creutzfeldt–Jakob disease, are unfeasible, but several case studies have reported incidentally detected preclinical abnormalities of high cortical or basal ganglia signal intensities in people who then developed Creutzfeldt–Jakob disease.[97–99] These abnormalities were detected preceding clinical disease onset by 3 months to 3 years.[97–100] Recent studies on asymptomatic carriers of *PRNP* mutations reported that MRI diffusion tensor imaging[101] and FDG-PET[53,84] show brain hypometabolism and white matter abnormalities in E200K or G114V *PRNP* mutation carriers, respectively. MRI DTI abnormalities were associated with increased CSF tau concentrations in asymptomatic E200K *PRNP* mutation carriers,[102] while earlier research identified reduced thalamic metabolism in asymptomatic D178N *PRNP* mutation carriers.[52] Nonetheless, the use of preclinical neuroimaging remains restricted by the lack of therapeutic interventions to stop or delay prion disease, and insufficient data regarding the temporal relationship between imaging abnormalities and clinical onset, which might extend beyond 3 years in some cases.[101]

Restricted diffusion on MRI,[103,104] DTI abnormality along the perivascular space,[101] brain atrophy,[105] and hypometabolism on FDG-PET[106] might be associated with disease progression.[50,107] However, large-scale data supporting neuroimaging as a dynamic biomarker in clinical trials is restricted to DWI. These data indicate that DWI abnormalities progress over time in terms of intensity and spatial extent.[103] Our recommendations for the use of neuroimaging are presented in panel 2.

Biomarkers to monitor people at risk

The identification of stage-specific biomarker profiles and the preclinical characterisation of pathology in *PRNP* mutation carriers are crucial for understanding the disease process and for the development of therapeutic interventions. The sensitivity of biomarkers, like that of a conventional CSF RT-QuIC test, might be low in preclinical at-risk stages, and in people with fatal familial insomnia or Gerstmann–Sträussler–Scheinker syndrome. Similarly, preclinical markers of neurodegeneration for prion diseases have not been validated yet on a large scale.

Although only scarce data are available for the preclinical stages, few studies have reported an increase in plasma NfL concentrations at or close to the onset of clinical disease. In the asymptomatic stage, in mutation carriers, plasma NfL concentrations start becoming higher than normal within 2 years before symptom onset.[8,9,57] A subsequent follow-up study by the same research group observed an increase in NfL plasma concentration around the clinical onset of the disease.[8] NfL concentrations in plasma were negative during the preclinical stage in a D178N–129M mutation carrier for 3 years, and started to increase at onset of familial fatal insomnia, at the same time as the patient developed depression and sleep disturbances.[67] No such effect was

observed for CSF t-tau, GFAP, UCH-L1, β-synuclein, plasma tau, or plasma UCH-L1 concentrations.[8,9,57] For plasma GFAP, significant differences were observed between the preclinical and clinical stage,[8,9] and concentrations were elevated in a E200K mutation carrier more than 2 years before disease onset.[9]

Findings from studies investigating the detection by RT-QuIC of the misfolded prion protein in CSF in relation to clinical conversion, are summarised in table 2 and the appendix (pp 6–15). Detection was positive in a subset of patients up to 3 years or more before disease onset. So far, data are available from healthy mutation carriers with E200K, D178N, or P102L *PRNP* mutations. Seven (9·9%) of 71 E200K mutations carriers tested positive for PrP^{Sc}, with five (71·4%) of these people converting to clinical disease within 2–36 months, while two (28·6%) remained asymptomatic for more than 3 years. One healthy carrier of the E200K mutation who tested negative at enrolment became positive 1 year before disease onset. In people at risk of familial fatal insomnia (seven mutation carriers) or Gerstmann–Sträussler–Scheinker syndrome (six mutation carriers), none tested positive by use of CSF RT-QuIC, but one (16·7%) mutation carrier subsequently developed Gerstmann–Sträussler–Scheinker syndrome during follow-up. These findings suggest that, in E200K carriers, CSF RT-QuIC of misfolded prion protein might become positive at least 3 years before disease onset.

In tear fluids, the situation differs from CSF assays. Tear fluid samples tested positive in seven (87·5%) of eight healthy people carrying P102L, D178N-129M, octapeptide repeat insertion, or the E200K mutation. Remarkably, these mutation carriers remained asymptomatic for more than 5 years after initial tear fluid collection. Also, olfactory mucosa swabs collected from

	Preclinical	Before onset	Clinical phase
E200K*[8,19,107-109]			
CSF	+	+†	+
Olfactory mucosa	–	nd	+
Tear fluids	–	nd	nd
D178N*[19,109]			
CSF	–	nd	+
Tear fluids	+‡	nd	+
P102L*[8,19,109]			
CSF	–	nd	+
Tear fluids	+§	nd	+

PrP^{Sc}=scrapie form of the prion protein. nd=not done. *Only limited data are available (see appendix pp 6–15). †2–37 months before clinical conversion. ‡59–63 months without clinical conversion. §36–53 months without clinical conversion.

Table 2: **Detection of PrP^{Sc} seeding activity in preclinical stages of prion diseases**

	E200K familial CJD	D178N FFI	P102L GSS	Sporadic CJD	AD	NC	HC	Notes on cellular PrP concentrations
CSF in HMC								
Villar-Piqué et al (2019)[111]	100–500* (++)	~100 (+)	<200 (+)	..	..	230* ± 140 (++)	..	E200K was similar to controls; lower in FFI than in other mutations
Vallabh et al (2020)[112]	Similar to controls† (++)	Lower than other mutations (+)	Similar to controls† (++)	..	..	..	..	E200K was similar to controls; lower in FFI than in other mutations; concentrations stable over for 10–20 months after baseline; variation of 7% in 2–4 months of observation
Vallabh et al (2024)[9]	54 ± 23 (+)	21 ± 5 (+)	45 ± 14·5 (+)	..	..	..	71 ± 24 (27–120; ++)	Lower in all people with mutations than in controls; lower in FFI than in other mutations
CSF after clinical onset								
Villar-Piqué et al (2019)[111]	98 ± 77 (+)	119 ± 94 (+)	200 ± 148 (++)	120 ± 83 (+)	..	230* ± 140 (++)	..	Lower in all prion diseases; decreases with disease stage
Meyne et al (2009)[113]	..	..	..	160 ± 76 (+)	177 ± 80 (+)	..	262 ± 70 (185 ± 48; ++)‡	Lower than HC in prion disease and in AD; gradual decline with disease severity
Rumeileh et al (2017)[114]	..	..	..	173 (103–261; +)	335 (232–455; ++)	..	327 (264–453; ++)	Lower in prion disease than in HC
Plasma								
Llorens et al (2020)[115]	69 ± 34 (+++)	38 ± 23 (+++)	..	54 ± 25 (+++)	40 ± 29 (+++)	28 ± 34 (++)	22 ± 11 (++)	Higher in all prion diseases; no correlation with age in HC
Völkel et al (2001)[116]	..	..	..	15 (6–26; +++)	19 (18–20; +++)	..	6 (3–9; ++)	Higher in all prion diseases; no correlation with age in CJD and HC

All cellular PrP concentrations are in ng/mL, ± indicates standard deviations and in other studies, IQR is given in brackets. +++=higher concentration than reference level. ++=concentration similar to reference level. +=lower concentration than reference level. AD=Alzheimer's disease. CJD=Creutzfeldt–Jakob disease. FFI=fatal familial insomnia. GSS=Gerstmann–Sträussler–Scheinker syndrome. HC=healthy controls and controls lacking evidence for a neurological disease. HMC=healthy people with PRNP mutations. NC=neurological controls excluding neurodegenerative diseases. PrP=prion protein. *Referred to controls. †No numerical data are available, information taken from figure 1 of the publication. ‡=In this study, higher medians were measured in patients older than age 40 years than in those younger than age 40 years (in brackets).

Table 3: **Prion protein concentrations across stages in patients with prion diseases**

Panel 3: Expert clinical advice and support networks for prion disease

National surveillance programmes and referral centres
Creutzfeldt–Jakob disease surveillance units and referral centres have been established in several countries. The International Creutzfeldt–Jakob disease Surveillance Network, the US National Prion Disease Pathology Surveillance Center, the PrionAtRisk consortium, and the Creutzfeldt–Jakob disease International Support Alliance (CJDISA) can provide information on cooperating centres in their own and other global regions. In countries without dedicated referral centres, clinicians can contact public health institutions or refer to other countries' centres.

PrionAtRisk
Members of the PrionAtRisk consortium do research on individuals at risk, offer individual counselling, and do diagnostics in cases of suspected disease onset.

Further support for clinicians, patients, and families
In many countries, non-profit associations offer information and help for people who are affected by human prion diseases, particularly in terms of practical and social needs. The CJDISA is the umbrella organisation and a list of associated groups are listed on their website.

Request for prion disease biomarkers
CSF analyses for total-tau, 14-3-3 protein, and neurofilament light chain are broadly available in reference centres and in laboratories that specialise in markers of neurodegeneration. CSF real time-quaking induced conversion (RT-QuIC) analyses are provided by most prion disease surveillance centres, many of them accepting samples from other countries for analyses. Peripheral diagnostic biomarkers are not yet broadly established, but some centres provide RT-QuIC testing in nasal brushing, skin biopsy, and tear fluids upon request. We recommend checking individual availability of specific tests with the aforementioned centres and associations. If there are no therapeutic options, we advise against routine biomarker analyses in people who have mutations.

For the **International Creutzfeldt–Jakob disease Surveillance Network** see https://www.eurocjd.ed.ac.uk/

For the **US National Prion Disease Pathology Surveillance Center** see https://case.edu/medicine/pathology/divisions/prion-center

For the **PrionAtRisk consortium** see https://www.prionatrisk.de

For the **CJD International Support Alliance** see https://cjdisa.com

24 people with the E200K mutation at a pre-symptomatic stage did not show seeding reactions.[110]

Low CSF PrP concentration is a characteristic feature common to all types of prion diseases, regardless of their aetiology (table 3),[111,113–115] and concentration gradually declines with disease progression.[111,113] In clinical cohorts, the highest total-PrP concentrations in plasma were detected in patients with sporadic Creutzfeldt–Jakob disease and were associated with codon 129 *PRNP* genotype, followed by other dementia types. Plasma total-PrP concentrations correlate with CSF markers of neuronal–axonal damage, but not with PrP concentrations in CSF.[115]

In genetic prion disease, CSF PrP concentrations display a mutation-specific pattern. People with the D178N mutation (associated with risk for fatal familial insomnia) show significantly lower PrP concentrations compared with those with E200K or P102L, or other mutations.[111,112] This observation requires consideration of age-related effects, as PrP concentrations are significantly lower in individuals younger than age 40 years.[112,113] At the symptomatic stage, CSF PrP concentrations remain low in people with fatal familial insomnia[111] or other prion diseases, without any significant changes.[111,112] A different pattern has been observed for the E200K mutation; in E200K mutation carriers, CSF PrP concentrations are similar to those of healthy or other neurological controls,[111–113] but concentrations decline by approximately 50% at the clinical stage, reaching ranges similar to those in patients with sporadic Creutzfeldt–Jakob disease.[111] For the P102L mutation, a similar range of CSF PrP concentrations to those of healthy controls have been reported at preclinical and clinical stages.[111,112] Plasma PrP concentrations are lower at the clinical stage in patients with fatal familial insomnia than in carriers of other mutations or patients with sporadic Creutzfeldt–Jakob disease, in whom an increase has been observed compared with healthy controls.[115]

Conclusions and future directions

Progress in the development of anti-prion targeted therapies and the availability of non-invasive biomarkers is leading to innovative clinical trial designs for people at-risk. For instance, a pioneered study of doxycycline in members of families with fatal familial insomnia in Italy established the feasibility of early preclinical intervention and innovative trial design, which allowed participation without awareness of genetic status.[7] With the emergence of antisense oligonucleotide-based interventions, early preclinical identification is needed. Preventive trials in healthy mutation carriers are challenging and require the identification of biomarkers that can serve as surrogate endpoints.[108] Cohorts have been established in various countries, which might allow a timely enrolment of individuals at risk and at early disease stages for pharmacological trials (see, for instance, the PrionAtRisk initiative).[8,9,102]

For the **PrionAtRisk initiative** see https://www.prionatrisk.de and panel 3

In genetic and iatrogenic Creutzfeldt–Jakob disease, establishing cohorts for monitoring individuals who are at genetic or iatrogenic risk might help with the identification of early preclinical indicators of disease pathology. In sporadic CJD, rapid disease progression, relative rarity of the disease, and a lack of specific clinical features create a need for other approaches; for example, the identification of biomarkers in peripheral biofluids, which will be a less invasive procedure than lumbar puncture.

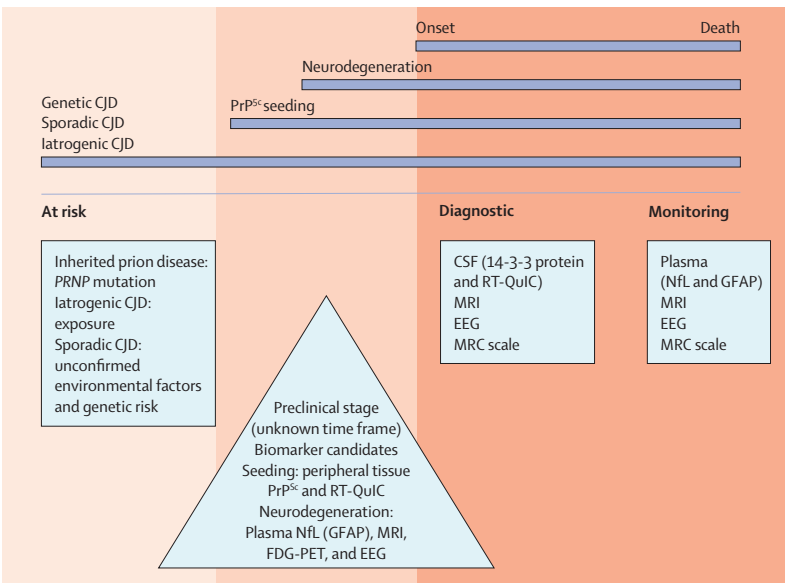

Figure: **Stages of prion diseases and proposed clinical workup**
A proposed model of preclinical stages and the onset of prion diseases, analogous to staging models of Alzheimer's disease and synucleinopathies. Whereas the model is considered to be valid for sporadic Creutzfeldt-Jakob disease iatrogenic and inherited prion diseases, application of the suggested biomarkers will only be feasible when a risk factor, such as iatrogenic risk or a *PRNP* mutation, has been identified. Furthermore, an application of preclinical biomarkers in a clinical setting will only be ethically reasonable if it has positive consequences for clinical management (eg, if disease-modifying drugs become available). Also, the sensitivity of proposed biomarkers varies considerably between different forms of prion disease. CJD=Creutzfeldt–Jakob disease. EEG=electroencephalography. FDG-PET=[^{18}F]fluorodeoxyglucose PET. GFAP=glial fibrillary acidic protein. MRC=Medical Research Council. NfL=neurofilament light chain. PrP^{Sc}=scrapie form of prion protein. RT-QuIC=real time-quaking induced conversion.

	Condition or syndrome	Medical Research Council scale	Disposition	Prion protein-seeding activity*	Neurodegeneration*
Before clinical onset					
0	Healthy	..	+	..	..
1s	At risk	..	+	+	..
1nd	Preclinical prion disease	..	+	+	+
After clinical onset					
2	Prodromal prion disease†	20	+	+	+
3e	Early prion disease	15–20	+	+	+
3m	Middle-stage prion disease	5–14	+	+	+
3l	Late-stage prion disease	<5	+	+	+

*The sensitivity of conventional CSF real time quaking induced conversion might be very low in preclinical or at-risk stages, in fatal familial insomnia, and in Gerstmann–Sträussler-Scheinker syndrome. Stage 1nd can be defined in carriers of the *PRNP* (pathogenic) mutation without detectable seeding activity, when other causes for neurodegeneration were excluded. Similar, preclinical markers for neurodegeneration in prion diseases have not been validated, yet. Current evidence and proposed markers are presented in the figure and discussed in the biomarkers to monitor people at risk section. †No functional disability or specific neurological signs (ie, prodromal signs can include non-specific symptoms, such as behavioural or mood changes, vertigo, weight loss, etc).

Table 4: **Staging system for human prion diseases proposed by the PrionAtRisk group**

An important aim of biomarker research is to fulfil the clinical need for screening, diagnosis, and disease monitoring tools. The same biomarker could work as a screening and a diagnostic biomarker, depending on the clinical context. A dynamic biomarker might reflect the onset of neurodegeneration, as reported for NfL. Therefore, a biomarker for disease monitoring might not be necessarily disease specific, if it reflects disease progression accurately. Biomarkers are needed to monitor clinical progression after disease onset, which might be also useful as prognostic biomarkers for survival. Tau and NfL in CSF or plasma might be useful in this regard. Despite major progress, there is still a lack of standardised protocols across laboratories. Whereas some round robin trials have been done to test validity and to make cross-laboratory comparisons of the aggregation assays,[13,14] novel peripheral biomarkers are not covered by these activities and comparisons between laboratories and methodologies have not been done yet. Detection of PrP^{Sc} in blood remains restricted to variant Creutzfeldt–Jakob disease, but is now feasible in other accessible matrices, such as tear fluids and olfactory mucosa, and to some degree, in urine. The low sensitivity of CSF RT-QuIC in some genetic cases, such as those with fatal familial insomnia or Gerstmann–Sträussler–Scheinker syndrome, needs to be addressed by improved detection protocols.

Emerging evidence might shift the way in which disease onset and disease risk are defined, and has opened new ways for trial designs, disease risk calculation, and disease monitoring. A staging system is needed that encompasses all aspects of the disease, such as disease risk, clinical parameters, and biomarkers. Such a staging system (figure, table 4) has been proposed by the PrionAtRisk group, and could help to define early and late diseases stages, identify patients at risk, and unify the clinical classification system for disease severity across the spectrum of prion diseases.

Search strategy and selection criteria

We searched PubMed for CSF-based and MRI-based studies from April 1, 2020 to May 1, 2025 using the terms "prion disease", "sporadic", "Creutzfeldt-Jakob disease", "Fatal Familial Insomnia", and "Gerstmann-Sträussler-Scheinker", each in combination with "RT-QuIC", "biomarker", "cerebrospinal fluid blood", "healthy mutation carrier", "blood", "plasma", and "serum". All articles addressing the aforementioned criteria and those reporting biomarker findings in peripherally accessible fluids and tissues were included. Articles published before 2019 were included when they were essential to substantiate important current scientific findings, based on the authors' ratings, or when no recent articles were found to report and discuss currently relevant findings and questions in the field. We included articles in English and German.

The PrionAtRisk Consortium members
Alice Anane, Noa Bregman, Nurit Omer (Israel), Brian S Appleby, Michael D Geschwind (USA), Simone Baiardi, Matilde Bongianni,

Sabina Capellari, Roberto Chiesa, Giuseppe Di Fede, Gianluigi Forloni, Anna Ladogana, Fabio Moda, Piero Parchi, Anna Poleggi, Gianluigi Zanusso (Italy), Stephanie Booth (Canada), Sergi Borrego-Ecija, Joaquín Castilla, Hasier Eraña, Izaro Kortazar-Zubizarreta, Raquel Sanchez-Valle, Guiomar Perez de Nanclares (Spain), Patricio Chrem Mendez (Argentina), Steven Collins, Genevieve Loftus-Hills, Suzanne Solvyns, Christiane Stehmann (Australia), Angela Patricia Da Silva Correia, Susana Margarida Da Silva Correia (Portugal), Anika Simonovska-Serra, Evelyn Berger Sieczkowski, Ellen Gelpi (Austria), Peter Hermann, Matthias Schmitz, Inga Zerr (Germany), Simon Mead, Tze How Mok (UK), Sezgi Canaslan (Türkiye), and Dana Žáková (Slovakia). Full affiliations can be found in the appendix (pp 16–20).

Contributors

All the authors wrote the manuscript, did the literature search, and designed illustrations.

Declaration of interests

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