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REVIEW ARTICLE

Biomarkers for sporadic Creutzfeldt–Jakob disease

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Abstract

Sporadic Creutzfeldt–Jakob disease (sCJD) is a rare but fatal type of spongiform encephalopathy with unknown cause. Unfortunately, definitive diagnosis of this disease can only be done by examination of postmortem brain tissue. Presumptive diagnosis is done through a combination of clinical manifestations, radiology results, and cerebrospinal fluid (CSF) testing for CSF 14-3-3. Even with these guidelines, premortem diagnosis of sCJD can be unreliable with high rates of misdiagnosis. This calls for more reliable biomarkers of the disease, allowing for better diagnosis as well as understanding the pathogenesis of sCJD. This review compiles potential genetic, protein, biomolecular, and imaging biomarker studies for sCJD since 2010, highlighting the promise of proteins, cytokines, and composite biomarkers for improving the diagnosis as well as understanding the pathogenesis of this mysterious ailment.

Introduction

Creutzfeldt–Jakob disease (CJD) is a rare but fatal neurodegenerative prion disease that occurs with symptoms of rapidly progressive dementia, myoclonus, visual or cerebellar symptoms, pyramidal or extrapyramidal signs, and akineteic mutism.[1] CJD can be classified as sporadic (sCJD), genetic (gCJD), iatrogenic (iCJD), or variant (vCJD) with sCJD being the most common. sCJD is diagnosed using the WHO guidelines with a combination of clinical manifestations, electroencephalogram (EEG), and a laboratory measure of CSF 14-3-3 that categorizes the patient as having probable or possible CJD. More recent criteria include magnetic resonance imaging (MRI) manifestations to diagnose probable CJD, but it has been shown that this addition decreases the sensitivity of diagnosis.[2,3] Definite diagnosis is only possible through neuropathological examination of the pathological isoform of prion protein (PrP^{Sc}) in the central nervous system through a biopsy or autopsy. Despite the inclusion of CSF 14-3-3 and MRI in the diagnostic criteria, cases of CJD are often misdiagnosed; more importantly, patients with other causes of dementia, including Alzheimer's disease (AD), are diagnosed as CJD and managed as such.[4,5] Since definitive diagnosis is often made postmortem, it is critical to identify antemortem biomarkers for CJD that are specific and sensitive enough to distinguish CJD from other dementias. Attempts at identifying biomarkers are often done using CSF, as it is closest to the site of pathology in the brain. Till now, several protein biomarkers, including 14-3-3, t-tau, and PrP^{Sc} have been extensively researched. A review was published in 2010 covering the background and development of antemortem diagnosis of CJD including the review of some candidate protein markers.[6] The current review will present an overview of progress made since then in identifying genetic and protein markers for sCJD. It has been shown that some marker levels are similar in sCDJ and gCDJ.[7] Hence, markers discussed in this review may potentially be applicable in assessing the diagnosis or pathogenesis of multiple other types of CJD.

Genetic Markers of sCJD

Studies have shown that the APOE e4 allele and homozygosity at codon 129 in the PRNP gene are major genetic risk factors for AD and human prion diseases including CJD. The role of these genes was studied by Calero et al. who reported that the APOE e4 allele is related to a higher risk of developing AD, while homozygosity at the

PRNP gene establishes a risk for sCJD. More interestingly after dividing patient populations according to their respective risk genes, an age dependent interaction of other gene was noted: an increased prevalence of the PRNP gene was seen in AD patients with early-onset disease, whereas increased APOE e4 allele was seen in patients with late-onset sCJD. Although this contrasts with previous finding of no risk of AD with PRNP, Calero cites genetic differences as a major factor.[8]

Tian et al. have similarly reported shared as well as unique gene expression profiles in patients with sCJD, AD, and fatal familial insomnia (FFI) indicating, as Calero found, overlapping neouropathogenesis mechanisms between sCJD and AD. Various signal transduction, synaptic transmission, and neuropeptide signaling pathways were commonly activated in sCJD, AD, as well as FFI, whereas mitogen-activated protein kinase (MAPK) signaling, and oxidative phosphorylation pathways were differentially activated in these diseases.[9] These differing pathways may provide insight into the differing pathogenic mechanisms underlying each disease and allude to the need to uncover biomarkers differentiating these three diseases.

Protein Markers of sCJD

CSF 14-3-3

The only molecular marker included in WHO's diagnostic criteria for CJD is CSF 14-3-3. In normal physiology, 14-3-3 is involved in mitogenic signal transduction, apoptotic cell death, and cell cycle control.[10] It is commonly detected through immunoblots and a positive result is indicative of CJD. The diagnostic potential of CSF 14-3-3 has been continually challenged in the literature, showing low sensitivity and specificity compared to other potential markers. Misdiagnosis is common despite a CSF 14-3-3 test,[11] with sensitivities ranging from 61 to 96% and specificities ranging from 67 to 93%.[12–18] An area under the receiver operating characteristic (ROC) curve for 14-3-3 shows a value of only 0.67,[15] remarkably low compared to other markers in this review, but only autopsied patients were used in that specific study. The likelihood of a negative 14-3-3 increases the likelihood of the autopsy being performed which may bias the patient population.[19] In 2012, a meta-analysis of 9 independent studies and 1849 patients found that CSF 14-3-3 had a combined sensitivity and specificity of 92% and 80%.[20] It is to be noted that the sensitivity of 14-3-3 is highest in patients with the shortest disease duration and homozygosity at codon 129 of the PrP gene.[21] Further studies of CSF 14-3-3 are warranted in autopsy or histopathologically confirmed patients to further its correlation with definitive diagnosis.

CSF tau

Tau, a neuronal protein important in microtubule stability,[22] has been shown to be elevated in the CSF of patients with CJD. The sensitivities and specificities of CSF total-tau (t-tau) range from 75 to 98% and from 67 to 99%, respectively,[12–17,23,24] with the highest area under ROC reaching 0.949.[19] CSF tau positivity has been evaluated as having better diagnostic value compared to 14-3-3 in the early stages of the disease,[17] whereas CSF 14-3-3 tends to be more variable toward the end stage of CJD.[25] Another study supported these findings and found CSF t-tau to be the most sensitive in the early stages of the disease.[16] Although CSF tau has been implicated in other neurodegenerative diseases such as AD, CSF tau levels are comparatively high in sCJD (445–41,000 pg/mL) compared to AD (75–1200 pg/mL), possibly reflecting more rapid neurodegeneration in CJD versus Alzheimer's.[12]

Somewhat more promising is the use of CSF p-tau, a phosphorylated form of the same protein, since CSF t-tau/p-tau ratio exhibits sensitivities and specificities in the range 79–86% and 84–99%[12,24] and an area under ROC of 0.82%.[24] This ratio has been reported to increase just before death as noted in longitudinal samples, thus allowing it to be potentially used to monitor disease activity and predict mortality in CJD.[24]

CSF S100b

CSF S100b, a cytoplasmic protein that has been identified as a neurotrophic factor and neuronal survival protein during central nervous system development,[26] has also been assessed for use as a marker of CJD, though with less promise. Sensitivities and specificities of CSF S100b range from 65 to 98% and from 29 to 90%, with an area under the ROC of 0.98%. Alone it does not have better predictive potential than the already used clinical markers or CSF 14-3-3, but the combination of S100b with other markers including CSF 14-3-3 may improve diagnostic capability, as discussed below.

Composite markers

Because of the relatively low sensitivity and specificity of single markers, multiple markers have been evaluated in combination in an attempt to increase diagnostic accuracy. Different marker combinations have been tested, but the most promising combines the use of CSF p-tau/t-tau ratio with CSF 14-3-3, yielding a sensitivity and specificity of 100% and 96%, respectively.[12] The combination of CSF t-tau and 14-3-3 exhibits good diagnostic potential but the addition of p-tau/t-tau ratio increases this

potential even further. Other tested CSF biomarker combinations are listed in Table 1.

CSF PrP^{Sc}

A form of the prion protein, PrP^{Sc} is one of the biomarkers most closely linked to the pathogenesis of the CJD. This prion protein is a misfolded form of the normal prion protein, PrP^{c}, which accumulates in the brain as the disease progresses. Detection of PrP^{Sc} in biopsy or autopsy confirms the diagnosis of CJD, but direct detection of the protein in CSF is not easily accomplished because of its low levels. Atrashi's development of a method of amplifying PrP^{Sc} by RT-QUIC (real-time quake induced conversion) has allowed for improved detection in CSF with a sensitivity of 80% and specificity of 90%.[27] In this method, soluble recombinant PrP is used as a substrate. PrP^{Sc} is then seeded and the reaction is then subjected to automated shaking. McGuire has also reported that this method yields a sensitivity and specificity of 89% and 99%, with positive and negative predictive values of 99% and 88% in a histopathologically confirmed CJD patient population. This allows for better diagnostic potential compared to other reported markers.[28] Rubenstein et al. have successfully detected PrP^{Sc} in spleens, lymph nodes, and tonsils of both sCJD and vCJD patients but were unsuccessful in detecting the protein in blood and urine.[29] This agrees with Glatzel et al.'s study that showed PrP^{Sc} was concentrated and detected in the spleen and skeletal tissue of sCJD patients,[30] but contrasts with findings by Hill et al. who had shown the ability to use tonsil biopsies to detect PrP^{Sc} in vCJD but not sCJD.[31] Further research on the detection of this pathogenic protein in peripheral tissue of sCJD is warranted, as its noninvasive detection could render it an ideal biomarker. A study by Torres et al. indicated that there was a decrease in the total prion protein levels in the CSF of sCJD patients compared to healthy controls, as well as a change in the glycosylation pattern of PrP that reflects disease progression in CJD. This decrease also involves a lower expression of the normal prion mRNA and its translated protein in CJD CSF.[32–34] PrP is distinct from others in that it is pathogenically disease specific, but further validation needs to be done in larger patient cohorts. Although the normal form of the prion protein can be evaluated using an enzyme-linked immunosorbent assay (ELISA), the detection of PrP^{Sc} is more complex because of the additional step involving proteinase K, a protease that can destroy PrP^{c} but not PrP^{Sc}. PrP^{Sc} can be assayed quantitatively using RT-QUIC, but the detection process may require up to 30 h and advanced diagnostic equipment. One promising aspect of RT-QUIC is its use in detecting PrP^{Sc} in nasal brushings from the olfactory epithelium of patients with sCJD. When compared to PrP^{Sc} detection in the CSF of these same patients, nasal brushing PrP^{Sc} has yielded increased sensitivity – 97% versus 77%, respectively.[35] This holds promise for the diagnostic use of PrP^{Sc} as a biomarker because obtaining nasal brushings is much less invasive than obtaining CSF from patients.

CSF ERK1/2

Promising CSF markers of sCJD also include extracellular-signal-regulated kinases 1 and 2 (ERK1/2). In normal cells, ERK1 and ERK2 participate in the Ras-Raf-MEK-ERK signal transduction cascade that is involved in a wide variety of processes including cell adhesion, cell cycle progression, cell migration, cell survival, differentiation, metabolism, proliferation, and transcription.[36] Steinacker et al. have reported the use of CSF ERK2 as a potential biomarker for CJD. Testing the levels of ERK1/2 with an electrochemiluminescence assay in the CSF of 19 patients with CJD, 23 patients with other dementias including Alzheimer's, and 12 patients with other neurological disorders, they reported that elevated levels of ERK1/2 had a sensitivity of 87% and specificity of 100%, comparable to the sensitivity and specify of CSF tau. The area under the ROC determines the diagnostic potency of CSF ERK1/2 in discriminating CJD versus other disease controls to be 0.94 and in discriminating CJD versus other dementias to be 0.97.[37] Some correlation between CSF ERK1/2 and CSF tau has been noted in other neurodegenerative diseases but not in CJD, and it has been suggested that the underlying mechanisms by which these proteins may contribute to neurodegenerative disease may be different in different diseases. Also, the cellular source of ERK1/2 in CSF and its phosphorylation status warrant further exploration.

Other Biomolecules

CSF Frx and t-Tf

Haldar et al. have reported the utility of CSF transferrin (t-Tf), a glycoproteins that controls the level of free iron,[38] and nonprotein ferroxidase (Frx), involved in iron homeostasis,[39] as diagnostic biomarkers in distinguishing sCJD from other dementias including AD with single marker sensitivities and specificities of 86% and 49% for Frx and 88% and 72% for t-Tf, respectively. When used together, the combination of CSF Frx and t-Tf shows a sensitivity of 86%, specificity of 93%, and area under ROC of 0.94.[23] Frx and t-Tf take part in iron metabolism and these findings suggest that there may be a characteristic iron imbalance in CJD that sets it apart from other

Table 1. CSF protein biomarkers in Creutzfeldt–Jakob disease (CJD).

Biomolecule	Reference	Subjects	Methods	Sensitivity	Specificity	Notes
14-3-3 positivity	Bahl 2009	21 sCJD 49 AD 164 NC	Immunoblot	95%	78%	
	Chohan 2010	245 sCJD 163 p-sCJD 171 DC	Immunoblot	86%	74%	PPV = 83% NPV = 78%
	Coulthart 2011	127 sCJD (autopsy) 873 non-CJD	Immunoblot	88%	72%	
	Hamlin 2012	420 CJD (autopsy)	Immunoblot	90%		ROC = 0.67
	Meiner 2011	60 sCJD 70 gCJD 560 non-CJD	Immunoblot	77%	93%	
	Pennington 2009	47 sCJD<6 wks 21 non-sCJD<6 wks 206 sCJD>6 wks 166 non-sCJD>6 wks	Immunoblot	96%	67%	PPV = 87% NPV = 88%
	Stoeck 2012	3556 CJD 7175 NC 18291 DC	Immunoblot	61–82%	91–95%	PPV = 47–83% NPV = 86–97%
Increased t-tau	Bahl 2009	21 sCJD 49 AD 164 NC	ELISA	75%	85%	
	Chohan 2010	245 sCJD 163 p-sCJD 171 DC	ELISA	81%	84%	PPV = 90% NPV = 74%
	Coulthart 2011	127 sCJD (autopsy) 873 non-CJD	ELISA	91%	88%	ROC = 0.947
	Haldar 2013	98 sCJD 192 DM 52 ND	ELISA	86%	73%	PPV = 77% NPV = 83% ROC = 0.84
	Hamlin 2012	420 CJD (autopsy)	ELISA	87%	67%	ROC = 0.82
	Meiner 2011	60 sCJD 70 gCJD 560 non-CJD	ELISA	77%	83%	
	Pennington 2009	47 sCJD<6 wks 21 non-sCJD<6 wks 206 sCJD>6 wks 166 non-sCJD>6 wks	ELISA	98%	82%	PPV = 93% NPV = 93%
	Skillback 2014	93 CJD (52 autopsy) 9672 DC	ELISA	79%	99%	ROC = 0.949
S100b	Chohan 2010	245 sCJD 163 p-sCJD 171 DC	ELISA	65%	90%	PPV = 64% NPV = 75%
	Coulthart 2011	127 sCJD (autopsy) 873 non-CJD	ELISA	87%	87%	ROC = 0.908
	Pennington 2009	47 sCJD<6 wks 21 non-sCJD<6 wks 206 sCJD>6 wks 166 non-sCJD>6 wks	ELISA	98%	29%	PPV = 75% NPV = 86%
Increased t-tau/p-tau	Bahl 2009	21 sCJD 49 AD 164 NC	–	86%	94%	
	Skillback 2014	93 CJD (52 autopsy) 9672 DC	–	79%	99%	ROC = 0.982
14-3-3 & S100b	Chohan 2010		–	62%	95%	PPV = 64%

(*Continued*)

Table 1. Continued.

Biomolecule	Reference	Subjects	Methods	Sensitivity	Specificity	Notes
tau & S100b	Chohan 2010		–	59%	95%	PPV = 95%
14-3-3 & tau	Bahl 2009		–	84%	96%	
p-tau/t-tau & 14-3-3	Bahl 2009		–	100%	96%	PPV = 95%
14-3-3 & tau & S100b	Chohan 2010		–	57%	96%	
PrP^{Sc} positivity	Atarashi 2011	59 CJD 179 non-CJD	RT-QUIC	80%	100%	
	McGuire 2012	123 sCJD 103 DC	RT-QUIC	89%	99%	PPV = 99% NPV = 88%
Frx	Haldar 2013	98 sCJD 192 DM 52 ND	In house assays (see methods of paper)	86%	49%	PPV = 74% NPV = 66% ROC = 0.75
t-Tf	Haldar 2013	98 sCJD 192 DM 52 NC	In house assays (see methods of paper)	88%	72%	PPV = 84% NPV = 78% ROC = 0.89
Frx and t-Tf	Haldar 2013	98 sCJD 192 DM 52 ND	In house assays (see methods of paper)	86.00%	93.00%	PPV = 99% NPV = 52% ROC = 0.94
cAMP	Oeckle 2012	15 CJD 11 DC	LC-MS/MS	100%	64%	
cGMP	Oeckle 2012	15 CJD 11 DC	LC-MS/MS	67%	100%	
t-tau/cAMP	Oeckle 2012	15 CJD 11 DC	–	93%	100%	
ERK1/2	Steinacker 2010	10 CJD 23 DM 12 NC	Electrochemi-luminescence assay	87%	100%	ROC = 0.936
IL-8	Stoeck 2005	23 CJD 76 iCNS 31 DC 18 ES 111 DC	ELISA	70%	82%	
	Stoeck 2014	12 sCJD 35 AD 12 DC	Cytokine assay	Not given	Not given	
TGF-β-2	Stoeck 2005	23 CJD 76 iCNS 31 DC 18 ES 111 DC	ELISA	83%	89%	

dementias including AD. As of now, the ability of these biomarkers to distinguish CJD from Alzheimer's holds promise, but further validation in independent cohorts is warranted.

CSF cAMP and cGMP

The cyclic nucleotides cyclic adenosine-39,59-monophosphate (cAMP) and cyclic guanosine-39,59-monophosphate (cGMP), both involved in numerous protein-ligand interactions and cell signaling,[40] have been shown by Oeckl et al. to be promising biomarkers for CJD. By measuring cAMP and cGMP with liquid chromatography/tandem mass spectrometry in the CSF of histopathologically confirmed cases of CJD, they found a reduction in CSF cAMP and cGMP in patients with CJD ($P = 0.002$). The specificities and sensitivities of these markers were 100% and 63% for cAMP and 67% and 100% for cGMP, respectively. The levels of cAMP were correlated with CSF tau, and t-tau/cAMP ratios attained sensitivity and specificity of 93% and 100%, respectively.[41] Oeckl has hypothesized that the decreased levels of cAMP and cGMP may be because of decreased synthesis of nucleotides in the degenerating brain. The diagnostic potency of using CSF cAMP, especially coupled with CSF t-tau, seems promising, due to the stability of nucleotides in CSF compared to protein biomarkers, but further independent validation is needed. Also,

mass-spectrometry independent assay methods for assaying these nucleotides conveniently are needed.

Inflammatory cytokines in CSF

The role of proinflammatory cytokines in the pathogenesis of CJD has been alluded to in 2005 by Stoeck et al. when elevated levels of IL-8 and TGF-β-2 were first demonstrated in the CSF of CJD patients using ELISA.[42] Sensitivities and specificities were determined to be 70% and 82% for IL-8 and 83% and 89% for TGF-β-2, respectively. Stoeck et al. added elevated neopterin in a subtype of CJD to these two markers in 2011,[43] and they followed up these results with a cytokine assay in 2014 showing elevated IL-8 and MCP-1 in histopathologically confirmed cases of sCJD.[44] As these cytokines may constitute mediators of the inflammatory process during central nervous system (CNS) damage, it would be reasonable that these would be increased during neurodegeneration. Unfortunately inflammatory markers are usually not specific as they are often noted in other inflammatory brain diseases. Another drawback to using certain cytokines as biomarkers is their questionable stability in CSF. In this regard, Stoeck et al. suggest that cytokines can only be accurately measured in immediately frozen CSF, and improper handling can easily degrade the cytokine.[42]

MRI and sCJD

MRI has been shown to be a promising diagnostic tool for CJD especially because of its noninvasiveness. MRI is often under-looked in cases of sCJD because presentations may not be apparent in the first scan or in early disease states.[45] While screening CSF for selected biomarkers may not be as diagnostic in some cases, MRI has proven to be more useful in discerning late-onset CJD that is commonly misdiagnosed as other dementias.[19]

Diffusion-weighted imaging, a technique that measures the random diffusion of water through and around tissue, and fluid attenuated inversion recovery MRI, an approach that minimizes the effect of fluid from images allowing for the visualization of hemisphere boundaries and the periventricular region close to CSF, have also been diagnostically helpful in CJD, with sensitivity and specificity of over 90% when two readers reached a consensus on the diagnosis.[46,47] The magnetization transfer ratio (MTR), a measure of free and molecule bound protons, may also be a potential disease marker since a negative correlation of the MTR and spongiosis in the frontal gray matter of the CJD brain has been reported.[48] Because abnormal MTRs have also been observed in AD, MTRs may have greater potential as early predictors of neurodegenerative disease rather than being specific for CJD. These studies need to be evaluated further with larger sample sizes as well as longitudinally as have been done with other potential CJD markers.

Another promising use for the MRI may be in the detection and/or discrimination of amyloid and Kuru-type plaques, the latter having a unique morphological and histological appearance.[49] The presence of these plaques, which often test positive for PrP^{Sc}, in the cerebral cortex, subcortical white matter, and the cerebellum of sCJD patients[50–53] lends itself to the use of MRI for the detection of these lesions in sCJD. To date, there has not been research into the detection of these PrP^{Sc} positive plaques using MRI, but perhaps methodology similar to Viola et al.[54] can be used in which a sensitive molecular MRI contrast probe specific for PrP^{Sc} could be used as a method of noninvasive identification of these plaques, and this could potentially transform CJD diagnostics.

Conclusion

sCJD is a fatal neurodegenerative disease that can exhibit a variety of clinical manifestations and is often misdiagnosed. Accurate biomarkers are needed to correctly diagnose this disease and to prevent misdiagnosing a potentially preventable disorder. A spectrum of genetic, protein, biomolecular, and imaging biomarkers has been examined in CJD, as detailed in Table 1. Some like $PrP^{Sc,}$ inflammatory markers, and MRI may be relevant to the underlying pathogenesis, whereas others currently have no known connection to the pathogenesis. Because of their relevance to pathogenesis, biomarkers such as inflammatory cytokines also raise hope for targeted therapeutics. Regardless, biomarker research is really at its infancy in CJD. Multiple independent studies are warranted to validate currently reported biomarker candidates, especially in autopsy confirmed samples. More importantly, more powerful screening tools based on OMICS-based technologies that utilize unbiased protein biomarker discovery ought to be considered as we search for newer, more predictive and informative biomarkers for this dreadful neurological disease that still has uncertain pathogenic mechanisms.

Conflict of Interest

The authors declare no conflict of interest.

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