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The diagnostic efficiency of biomarkers in sporadic Creutzfeldt-Jakob disease compared to Alzheimer's disease

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Abstract

Laboratory markers have a prominent place among the diagnostic criteria for sporadic Creutzfeldt-Jakob disease (sCJD). Here we investigate the capability of protein 14-3-3, total-tau (t-tau), threonin-181-phosphorylated tau (p-tau), and neuron-specific enolase (NSE) in cerebrospinal fluid (CSF) together with the prion protein gene genotype to discriminate patients with sCJD ($n = 21$) from neurological controls ($n = 164$) and Alzheimer's disease (AD) patients ($n = 49$).

Low p-tau/t-tau ratio was the best single marker for sCJD with 90% specificity against neurological controls at 86% sensitivity whilst NSE was the least accurate with 79% sensitivity at 90% specificity. Many of the sCJD patients had extremely elevated t-tau values but normal values of the AD-marker p-tau. Protein 14-3-3 was very sensitive (95%) although the specificity was relatively low (75%). A combination of elevated t-tau concentration with the presence of 14-3-3 protein in CSF gave the best test specificity of 96% at 84% sensitivity.

We conclude that the combination of more than one CSF marker for neurodegeneration can improve the diagnostic test accuracy for sCJD against neurological controls including patients with other dementias.

Keywords: Creutzfeldt-Jakob disease; Alzheimer's disease; Tau; Phosphorylated tau; 14-3-3 protein; Neuron-specific enolase; Cerebrospinal fluid; Biomarker

1. Introduction

Creutzfeldt-Jakob disease (CJD) is a fatal neurodegenerative disease in which post-mortem brain autopsy or biopsy is necessary for a definite diagnosis. The pre-mortem diagnosis of sporadic CJD (sCJD) in patients is established by clinical criteria for probable or possible sCJD and is complicated by being dependent on relatively non-specific clinical symptoms and signs such as rapid cognitive decline, myoclonus, ataxia, and visual disturbances as well as neurophysiological changes with typical EEG changes with triphasic sharp wave pattern or a more non-specific burst-suppression pattern (Brown et al., 2003). Because progressive dementia is an important clinical criterion for possible and probable sCJD, the differential diagnosis against other dementia disorders and especially Alzheimer's disease (AD) is important, particularly in the early phase. Signal abnormalities in magnetic resonance imaging (MRI) patterns can be very useful in subtyping sCJD cases but there are no sCJD-specific MRI

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patterns (Kallenberg et al., 2006; Meissner et al., 2004; Tschampa et al., 2005). Thus, the ability of different laboratory tests and new biomarkers to differentiate CJD patients from patients with other neurodegenerative diseases is of interest.

Regarding CSF markers, the presence of neuronal protein 14-3-3 by immunoblotting is used as a laboratory marker for sCJD (Beaudry et al., 1999; Geschwind et al., 2003; Hsich et al., 1996; Zerr et al., 1998). The release of protein 14-3-3 into CSF reflects non-specific neuronal damage in the brain. Accordingly, the presence of 14-3-3 in CSF is also seen in other neurological disorders such as stroke, viral encephalitides, and in patients with other types of dementia (Cuadrado-Corrales et al., 2006; Hsich et al., 1996; Huang et al., 2003; Windl et al., 1999; Zerr et al., 1998). Neuron-specific enolase (NSE) is another marker for neurodegenerative processes and it has been found to be elevated in CSF from CJD patients (Aksamit et al., 2001). Recently, the tau protein was also found to be highly elevated in many patients with sCJD (Kapaki et al., 2001; Otto et al., 1997). Total-tau (t-tau) protein is an indicator of neuronal damage and elevated tau levels in CSF are typical of AD patients (Andreasen et al., 2003; Hulstaert et al., 1999). In typical AD both t-tau levels in CSF as well as levels of specifically hyperphosphorylated tau (p-tau) are elevated (Andreasen et al., 2003; Hampel et al., 2004; Hulstaert et al., 1999). CSF levels of t-tau are also elevated in other disorders with neuronal damage including frontotemporal dementia, vascular dementia, dementia with Lewy bodies, and stroke. However, we found that the magnitude of increase in CSF t-tau in sCJD cases was much more pronounced than in other chronic neurodegenerative disorders and that this abnormality, in contrast to what is found in AD patients, occurred without p-tau levels being affected. This pattern thus is potentially diagnostically helpful.

We here report on the diagnostic performance of established markers and CSF t-tau and p-tau levels in patients with definite and probable sCJD compared to AD patients and to controls with initially suspected, but unconfirmed sCJD.

2. Materials and methods

2.1. Patients

In Denmark, patients suspected of CJD are to be notified to the National Surveillance Unit for Infectious Diseases. All notifications are reviewed by a national expert committee. Patients are classified as definite, probable, possible or non-CJD according to WHO's classification criteria of sporadic CJD (Table 1) (Brown et al., 2003). The study population consisted of patients with suspected CJD reported during the period 1998–2004 as well as patients clinically diagnosed with AD which underwent lumbar puncture and CSF biomarker analysis during 2003 and 2004.

Patients with a clinical diagnosis of probable AD were recruited from the Copenhagen Memory Clinic at Rigshospitalet. The diagnosis was established after thorough clinical examination and paraclinical investigations including as a minimum neurological exam, cranial CT or MRI, blood screening test and cognitive testing. CSF from these patients was analysed for t-tau and p-tau in the present study.

The total number of samples and the distribution according to sex and diagnosis are given in Table 2. In total, 234 patients (117 male, 117 female) were included in the study, 185 were patients initially suspected of having CJD of which 21 had been diagnosed with definite or probable sCJD. Possible sCJD cases were excluded from this study. Forty-nine patients had the clinical diagnosis of probable AD. Samples from 169 patients were assessed for 14-3-3, 167 underwent NSE analysis, 158 patient samples were *PRNP* genotyped, and 186 were analysed for total-tau (t-tau) and Thr-181 phospho-tau (p-tau) levels in CSF (Table 2).

We compared three groups of patients: patients with a definite or probable diagnosis of sporadic CJD ("sCJD group"), patients initially reported as suspected sCJD, but subsequently classified as not CJD ("non-CJD group") and patients diagnosed as Alzheimer's disease ("AD group"). The non-CJD group was characterised by having been clin-

Table 1
WHO's classification criteria of sporadic Creutzfeldt-Jakob disease (Brown et al., 2003)

Definite CJD	Probable CJD (in the absence of an alternative diagnosis from routine investigation)	Possible CJD
Neuropathological confirmation and/or confirmation of protease resistant prion protein and/or presence of scrapie-associated fibrils	Progressive dementia. A typical EEG, whatever the clinical duration of the disease and/or a positive 14-3-3 assay for CSF and a clinical duration to death <2 years	Progressive dementia. EEG atypical or not known. Duration <2 years
	At least two of following	At least two of following
	Myoclonus	Myoclonus
	Visual or cerebellar disturbance	Visual or cerebellar disturbance
	Pyramidal/extrapyramidal dysfunction	Pyramidal/extrapyramidal dysfunction
	Akinetic mutism	Akinetic mutism