Use of 14-3-3 in the diagnosis of Creutzfeldt–Jakob disease
A. J. E. Green
The National Creutzfeldt–Jakob Disease Surveillance Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, U.K.

Abstract
The transmissible spongiform encephalopathies include human diseases such as Creutzfeldt–Jakob disease (CJD) and kuru as well as animal diseases such as scrapie and bovine spongiform encephalopathy (BSE). The emergence of variant CJD, which is causally related to BSE, has generated much interest in the development of rapid and sensitive diagnostic tests for the pre-mortem diagnosis of CJD. In 1986 two proteins were detected in the cerebrospinal fluid (CSF) of patients with sporadic CJD. These proteins were later demonstrated to be members of the 14-3-3 family, and tests for the detection of CSF 14-3-3 were developed. A number of studies have shown that the detection of CSF 14-3-3 is an accurate test for sporadic CJD, although the results with variant CJD are less promising.

Introduction
Creutzfeldt–Jakob disease (CJD) belongs to a family of rare fatal neurodegenerative diseases known as transmissible spongiform encephalopathies, that also include animal diseases such as scrapie and bovine spongiform encephalopathy (BSE). These diseases are characterized by neuro-pathological evidence of neuronal loss, astrocytosis and spongiform change. The brains of these patients also show deposition of a partially proteinase K-resistant isoform (PrPSc) of a sialoglycoprotein called the host-encoded prion protein (PrPc). PrPSc is coded for by the prion protein gene (PRNP) located on chromosome 20 and is expressed throughout the body but in particularly high levels in neuronal cells, where it is found as a glycosylphosphatidylinositol-anchored cell surface protein [1]. These diseases can be transmitted to chimpanzees and other laboratory animals in brain tissue from infected patients by either intracerebral or peripheral inoculation, although much larger doses are required for peripheral transmission [2]. The transmissible agent has been shown to co-localize with PrPSc and is resistant to most agents that inactivate nucleic acid and viruses. For a review of transmissible spongiform encephalopathies see Johnson and Gibbs [3].

The majority of cases of CJD (85%) occur sporadically and 10–15% arise due to mutations in the PRNP gene. The remaining cases are iatrogenic, occurring following the administration of contaminated growth hormone [4], or in the recipients of human dura mater [5] or corneal grafts [6], or very rarely via contaminated neurosurgical instruments [7] or intracerebral electrodes [8]. Patients with sporadic CJD (spCJD) present with a rapidly progressing dementia, often with neurological features such as myoclonus and ataxia. The age at onset is usually in the 45–75 year age group, with the peak age at onset being 60–65 years. The disease course is rapid and most patients die within 6 months. A genetic polymorphism at codon 129 of the PRNP gene can influence the clinical phenotype of CJD. In Caucasians, 38% of the population are homozygous for the allele with a methionine at this position, 51% are heterozygous for methionine and valine and the remaining 11% are homozygous for valine. The majority of patients with spCJD are homozygous for methionine. Those patients who are heterozygous for methionine/valine or homozygous for valine tend to have longer disease duration, often present at an earlier age or have other clinical and pathological variations that may correlate with PRNP codon 129 genotype [9,10].

A variant form of CJD (vCJD) was described in 1996, which affects younger patients and has a more prolonged clinical course when compared to spCJD [11,12]. Subsequent evidence has provided strong support for the hypothesis that vCJD is causally linked to BSE [13,14]. These patients tend to present with early and persistent psychiatric symptoms and definitive neurological features such as ataxia and cognitive impairment develop at a relatively late stage [15,16].
Physiology of 14-3-3

14-3-3 was first described by Moore and Perez in 1967 as an abundant acidic brain protein [17]. The name is derived from the combination of its fraction number on DEAE-cellulose chromatography and its migration position in the subsequent starch-gel electrophoresis. In mammalian tissues there are seven isoforms (β, ε, γ, η, σ, τ and ζ) which are encoded by separate genes and are highly homologous. Of these isoforms, five (β, ε, γ, η and ζ) are known to be present in neuronal cells [18]. The 14-3-3 proteins exist mainly as dimers with a monomeric mass of approx. 30 kDa and have an isoelectric point of 4-5 [19]. The 14-3-3 proteins are expressed in all eukaryotic cells [20] and are a group of multifunctional proteins that bind to and modulate the function of a wide array of cellular proteins; including kinases, phosphatases and transmembrane receptors. Through these interactions, 14-3-3 participates in the regulation of diverse biological processes, including neuronal development, cell growth control and cell cycling [19,20].

The use of CSF 14-3-3 in the diagnosis of CJD

The development of an early reliable and reproducible clinical test for CJD is a high priority for the surveillance of CJD and for obtaining timely data to predict the total numbers of vCJD cases using prediction models. Recently, there has been much interest in the use of quinacrine and chlorpromazine [21] in the treatment of vCJD and this increases the need for early diagnostic markers. It is also important to diagnose CJD early to limit the potential risk of secondary infection by surgical instruments and blood donation.

The use of CSF p130/p131 in the diagnosis of spCJD

In 1986 two proteins, designated p130 and p131, were detected in the cerebrospinal fluid (CSF) of 21 patients with spCJD using two-dimensional electrophoresis [22,23]. Protein p130 was found to have a molecular mass of 26 kDa and a pI of 5.2, whereas p131 had a molecular mass of 29 kDa and a pI of 5.1. These proteins were not found in patients with other forms of dementia, such as Alzheimer’s disease, Parkinsonian-dementia complex of Guam or AIDS-related dementia complex, or in other neurological diseases such as multiple sclerosis, Parkinson’s disease or Huntington’s disease. However, half of the patients with herpes simplex encephalitis were found to be positive for both p130 and p131. A larger study investigating 182 patients with suspected CJD found that p130/p131 were found in 81% of patients with spCJD but in none of the patients subsequently found not to have spCJD. These studies suggested that p130/p131 were useful diagnostic markers for spCJD, but two-dimensional gel electrophoresis was not a practical technique for routine clinical use. It was not until p130 was isolated from healthy adult brain and digested to give a series of peptide fragments and identified as a member of the 14-3-3 family that a SDS/PAGE and immunoblotting technique became available for routine diagnostic use [24].

Table I

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningoencephalitis</td>
<td>8</td>
</tr>
<tr>
<td>Multi-infarct dementia</td>
<td>6</td>
</tr>
<tr>
<td>Intracerebral malignancy</td>
<td>3</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>2</td>
</tr>
<tr>
<td>Hypoxic brain damage</td>
<td>2</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>1</td>
</tr>
<tr>
<td>Lewy body dementia</td>
<td>1</td>
</tr>
<tr>
<td>Down’s syndrome</td>
<td>1</td>
</tr>
<tr>
<td>Progressive dementia of unknown cause</td>
<td>1</td>
</tr>
<tr>
<td>Delirium in Down’s syndrome</td>
<td>1</td>
</tr>
</tbody>
</table>
ditions that would give rise to a false-positive 14-3-3 were excluded on clinical grounds. The high autopsy rate and subsequent neuropathological examination of patients with suspected CJD have confirmed the accuracy of CSF 14-3-3. This has lead the World Health Organization to revise their clinical criteria for the diagnosis of probable spCJD to include a positive 14-3-3 in patients who have a progressive dementia of less than 2 years duration [29]. A recent report has shown that the inclusion of CSF 14-3-3 in the diagnostic criteria has increased the number of cases of spCJD identified [30]. A small percentage of spCJD cases are negative for 14-3-3 and these patients tend to be those with longer disease durations and/or atypical clinical presentations [10].

There have been a number of reports of CSF 14-3-3 being detected in conditions such as paraneoplastic syndrome [31], transverse myelitis [32] and Hashimoto’s encephalitis [33]. Both Hashimoto’s encephalitis and paraneoplastic syndrome are conditions that can be difficult to distinguish from spCJD in the early stages. A number of approaches have been used to try to increase the specificity of CSF 14-3-3. Some studies have suggested performing a second lumbar puncture after 1-2 weeks, as many conditions giving a false-positive CSF 14-3-3 will have undetectable CSF 14-3-3 in the second sample [28]. An alternative approach has been to look at the isoforms of 14-3-3 present in the CSF. Most studies have used a commercially available polyclonal antiserum that detects all isoforms. The predominant isoform present in spCJD is \( \gamma \), although smaller amounts of \( \beta, \varepsilon \) and \( \eta \) are also present [34,35]. There is evidence that \( \eta \) is the major isoform present in non-CJD dementia [35]. Thus the development of commercially available isoform-specific antisera that can be used to identify the 14-3-3 isoforms present in CSF may help to increase specificity.

**Use of 14-3-3 in the diagnosis of variant CJD**

As CSF 14-3-3 has been shown to be very useful in the pre-mortem diagnosis of spCJD there has been much interest in whether it has the same value in vCJD. The initial results suggested that CSF 14-3-3 might not be as useful in vCJD as in spCJD [16]. A more recent study which involved a larger number of patients showed that CSF 14-3-3 was detected in 50% of the patients with histologically confirmed vCJD, but was only found in 9% of control cases [36]. Therefore CSF 14-3-3 has a lower sensitivity for the diagnosis of vCJD than for the diagnosis of spCJD, but has a comparable specificity. This means that the detection of 14-3-3 in this group of patients has a high positive predictive value (86%), although the failure to detect 14-3-3 cannot be used to exclude the diagnosis of vCJD. There was no difference between those vCJD patients who were positive for CSF 14-3-3 and those who were negative in terms of age at onset of illness, disease duration and the timing of the lumbar puncture [36]. CSF 14-3-3 may be detected in a second CSF sample taken 3-4 weeks after the first. In our experience two out of three vCJD patients who were initially negative for CSF 14-3-3 had detectable levels in subsequent samples. In addition to being present in only half of the vCJD patients investigated, the amount of 14-3-3 present in the CSF is less than that found in spCJD patients (Figure 1).

**The use of CSF 14-3-3 in the diagnosis of iatrogenic CJD secondary to human growth hormone administration**

Human cadaveric pituitary-derived growth hormone (h-GH) was first used to treat hypopituitary dwarfism in 1958 [37]. The hormone was manufactured in batches, and each batch used up to 2000 human pituitary glands. The hormone was administered by either intramuscular or subcutaneous injection. The first reports of CJD occurring in h-GH recipients were published in 1985 [38-40]. Since then nearly 140 further cases have been documented, most of which have been in France [41]. These patients usually present with ataxia and visual disorders and dementia develops at a later stage [42]. CSF 14-3-3 is detectable in these patients, although the proportion of patients with detectable levels depends on when the sample was taken. If the CSF sample...
is taken in the early stages of disease, when ataxia is the main clinical feature, then only 20% of patients will have detectable CSF 14-3-3, but this figure rises to 100% in the later stages of disease when dementia becomes the more prominent feature [43]. Interestingly the amount of CSF 14-3-3 detected in these patients using SDS/PAGE and immunoblotting is similar to that in spCJD patients [44].

Use of CSF 14-3-3 in the diagnosis of familial CJD

The sensitivity of CSF 14-3-3 in the detection of familial CJD cases depends on the mutation involved. CSF 14-3-3 was detected in 97% of codon E200K mutation cases [25,26,45,46], in 100% of codon V2101 mutation cases [46], in 40% of P102L mutation cases [45,46] but in none of the familial fatal insomnia cases investigated [46].

Alternative methods for CSF 14-3-3 analysis

The analysis of CSF 14-3-3 using SDS/PAGE and immunoblotting is subject to a number of analytical and interpretative problems. The most serious of these is the difficulty in assay standardization. There is no recognized international standard against which all patient samples can be compared. This means that the detection of low concentrations of CSF 14-3-3, where the Western blot only shows faint bands, can be difficult. The assessment of the significance of these bands is also subjective, as many patients without CJD can also have small amounts of 14-3-3 present in the CSF. This is an important issue as vCJD patients (Figure 1) [36]. To try to overcome these problems quantitative assays have been developed [47,48]. Kenney et al. [47] described an ELISA that uses bovine 14-3-3 as a standard and an in-house monoclonal antibody as a capture antibody. Using this assay it was shown that patients with spCJD had significantly raised CSF 14-3-3 concentrations when compared with patients without spCJD (38.1 ± 3.0 versus 3.1 ± 2.9 ng/ml, P < 0.001; Student’s t test). However, comparison of the ELISA and Western blot using the same cohort of patients showed that although the ELISA had comparable specificity with the Western blot it had lower sensitivity. An alternative approach has been used by Leuck et al. [47], who have developed a protein-capture assay that exploits the protein-binding properties of 14-3-3. A chemically synthesized phosphoserine peptide is covalently linked to a microtiter plate and the 14-3-3 present in CSF or standard binds to the peptide with a high affinity. The captured 14-3-3 is then detected using a monoclonal antibody. Using this assay it was shown that although the mean CSF 14-3-3 concentration in vCJD patients was significantly raised when compared with that of control patients, the range of concentrations in these two groups of patients showed considerable overlap and this may limit the diagnostic use of the assay. This study also showed that the mean concentration of CSF 14-3-3 in vCJD patients was half that found in spCJD patients.

Conclusions

CSF 14-3-3 is a sensitive and specific test for spCJD, provided that it is used as a diagnostic test in patients who have a high degree of clinical suspicion. It is less sensitive in patients with vCJD but the high specificity means that the detection of CSF 14-3-3 in a patient with suspected vCJD has a high positive predictive value. The value of CSF 14-3-3 in GH-related iatrogenic CJD depends on the clinical stage of the disease, being highly sensitive in the latter stages.

References
