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**The role of cerebrospinal fluid 14-3-3 and other proteins in the diagnosis of sporadic Creutzfeldt-Jakob disease in the United Kingdom: a 10 year review**

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## **ABSTRACT**

It is 10 years since the detection of cerebrospinal fluid (CSF) 14-3-3 was included in the diagnostic criteria for sporadic Creutzfeldt-Jakob disease (sCJD) by the World Health Organisation. Since that time other CSF proteins, such as S100b and tau protein have been proposed as surrogate markers for sCJD. We aimed to investigate the diagnostic value of each of these three proteins. CSF samples collected from patients who were referred to the National CJD Surveillance Unit as suspected cases of sCJD during the period 1997 – 2007 were analysed for 14-3-3, S100b and tau protein. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each of these markers either alone or in combination for the diagnosis of sCJD were assessed. The impact of CSF 14-3-3 analysis on the case classification of sCJD was investigated. CSF 14-3-3 had the greatest sensitivity (86%) when compared to tau protein (81%) and S100b (65%). The combination of a positive CSF 14-3-3 or an elevated tau protein with a raised S100b had the highest positive predictive power for sCJD. During the study period 100 patients were classified as probable sCJD solely on the basis of the clinical features and a positive CSF 14-3-3. The most sensitive marker for sCJD was a positive CSF 14-3-3. The analysis of CSF 14-3-3 plays a crucial role in the case classification of sCJD.

## INTRODUCTION

Creutzfeldt-Jakob disease belongs to a family of fatal neurodegenerative diseases collectively known as human transmissible spongiform encephalopathies. Sporadic CJD (sCJD) remains the most prevalent form worldwide with an annual incidence of 1.0-1.5/million/year.[1] Human spongiform encephalopathies are characterised by the accumulation of pathological prion protein (PrP) in the central nervous tissue. The pathological isoform is termed PrP<sup>Sc</sup> and this differs from the normal cellular isoform by its high content of  $\beta$ -sheet structure and partial resistance to protease digestion.[2] Additional histological changes identified include spongiosis, neuronal loss and gliosis. Neuropathological studies remain the only means of obtaining a definitive diagnosis; the initial diagnosis however is still dependent on the clinical phenotype, as defined by the WHO criteria.[3]. Marked phenotypic heterogeneity is well documented in all human prion diseases and in sCJD this observation is yet to be explained. Early experimental transmission studies on primates,[4] subsequent epidemiological studies [5,6] and isolated case reports have allowed diagnostic criteria to be refined over the years, however early diagnosis and in turn accurate surveillance remains a challenge. Atypical forms of sCJD are well recognized, it has been postulated that such variation is partially dependent on, or associated with, genetic and molecular factors, *PRNP* codon 129 genotype and prion protein isotype respectively. The typical short duration disease phenotype being linked to methionine homozygosity and PrP<sup>Sc</sup> type 1.[2] The atypical and rarer variants (those defined as having a long duration of illness, young age of onset and unusual clinical or pathological features) are linked to valine homozygosity or heterozygosity at codon 129 and PrP<sup>Sc</sup> type 2.

The advent of novel diagnostic tests, specifically the cerebrospinal fluid (CSF) 14-3-3 protein, has also allowed improvement in classification over the last decade. The 14-3-3 has better diagnostic utility than investigative tests such as the EEG.[7,8] However the sensitivity of CSF 14-3-3 has been shown to vary, partially dependent on the genetic and molecular influences described above.[9] The detection of CSF 14-3-3 remains a supportive tool in the appropriate clinical context but as a solitary test, independent of clinical phenotype, has little value.

The major differential diagnoses of sCJD remain those of other irreversible neurodegenerative conditions; however a small proportion of patients may have a

potentially treatable condition. Therefore CSF analyses, such as cell count and total protein, are an important early investigation in these patients and CSF 14-3-3 is often performed at this time. Many conditions associated with acute neuronal damage may result in a positive CSF 14-3-3 and thereby reduce the specificity of CSF 14-3-3 for sCJD.[8] Therefore other brain-specific proteins in the CSF may be of value as diagnostic markers. These additional markers include the CSF astrocytic marker S100b and the neuronal marker tau, protein in isolation or in combination.

In this study we aim to firstly review the sensitivity and specificity of each CSF protein in sCJD and also review the potential role of a combination of several markers to improve sensitivity and specificity in the clinical diagnosis of sCJD. Secondly, the impact of the CSF 14-3-3 on UK surveillance is of great importance and is therefore considered, specifically reviewing the number of cases of probable sCJD classified on the basis of CSF 14-3-3. In addition those cases classified as a probable case of sCJD on the basis of a positive 14-3-3 but found to have an alternative pathological diagnosis are investigated. The additional role of combining neuronal markers in order to potentially exclude sCJD is reviewed.

## **MATERIALS AND METHODS**

### **Patients**

The National CJD Surveillance Unit (NCJDSU) was established in May 1990 to prospectively identify and record all suspected cases of sporadic CJD in the UK. The primary aim of the programme was to detect any change in the epidemiology of the disease that might be attributable to bovine spongiform encephalopathy, and a distinct clinical-pathological phenotype was described in 1996 (variant CJD). Global surveillance of CJD has continued within the UK and active surveillance has improved due to strong collaborations with the neuroscience community. Patients are referred and where possible visited in life. A detailed history of the current illness and past medical history, including potential risk exposure is undertaken. Each case is further investigated by clinical examination and review of clinical investigations. Investigative tests which are potentially supportive for a diagnosis of sCJD such as an EEG and MRI are reviewed by a member of the NCJDSU. The NCJDSU acts as a referral centre for CSF 14-3-3 analysis throughout the UK

During the period 1997 and 2007 inclusive, 245 cases of neuropathologically confirmed sCJD[10] (117 female, 128 male aged 27-87 years (mean  $65.8 \pm 9.7$  years) at notification), 163 cases of clinically probable sCJD[3] (82 female, 81 male aged 41-92 years (mean  $68.0 \pm 9.8$  years) at notification) and 171 disease control cases (86 female, 85 male aged 28-89 years (mean  $66.4 \pm 11.4$  years) at notification) who had CSF 14-3-3 analysis were identified for this study. Cases classified as not suffering from CJD (disease control cases) included those with a pathologically proven alternative diagnosis or those provided with an alternative clinical diagnosis by either the clinical team or by a member of the NCJDSU (Table 1).

**Table 1** Disease control cases (pathologically proven alternative diagnosis or alternative clinical diagnosis)

<b>Alternative pathological proven diagnosis</b>	<b>Number of cases</b>
Alzheimer's disease	14
Biopsy/post-mortem showed no evidence of CJD	12*
Malignancy/Paraneoplastic syndrome	10**
Lewy body dementia	4
Cerebral lymphoma	4
Encephalitis	4
Parkinson's disease	4
Cerebrovascular disease	3
Alzheimer's disease and ischaemic change	3
Alzheimer's disease and Lewy body dementia	3
Progressive multifocal leucoencephalopathy	1
Normal pressure hydrocephalus	1
Fronto-temporal dementia	1
Corticostriatal degenerative disease	1
Demyelination	1
<b>Alternative Clinical Diagnosis</b>	<b>Number of cases</b>
Dementia (unknown etiology) or clinically not CJD	22
Clinically improved with or without steroids	22
Alzheimer's disease	18
Cerebrovascular disease/cerebral vasculitis	9
Fronto-temporal dementia	7
Lewy body dementia	6
Paraneoplastic syndrome	6
Psychiatric disorder	4

Huntington's disease	3
Hashimoto's encephalitis	2
Corticobasal degeneration	2
Multi-system atrophy	1
Granulomatous disease	1
Central pontine myelinolysis	1
Serotonin syndrome	1

\*1 patient clinically not thought to have had CJD and post mortem conducted but brain tissue not examined

\*\* 1 patient had a western blot for PrP<sup>Sc</sup> which was negative, clinically this patient is thought to have a paraneoplastic process

### **CSF protein analysis**

CSF samples are sent to the laboratory on dry ice and stored at -80°C prior to analysis. For this study CSF 14-3-3, S100b and tau protein were analysed. Protein 14-3-3 in CSF was detected by western blotting after SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with chemiluminescent visualization.[11-13] A positive, negative and two weak positive 14-3-3 controls were included on each run. These were controls from patients with neuropathologically confirmed sporadic CJD (positive control) or from patients with either a clinical or pathological diagnosis of an alternative disease (negative and weak positive controls). The relative immunoreactivity of positive, negative and weak positive 14-3-3 CSF samples is given in fig 1. The blots were independently assessed by two people (AJEG, MA, GC or CP) and only positive CSF 14-3-3 results were used for case classification. CSF S100b was measured using a previously reported sandwich enzyme linked immunosorbant assay (ELISA).[11] A concentration of <0.5ng/ml was considered to be normal, whilst a concentration of >1.0ng/ml was considered to be diagnostic. CSF tau protein was measured using an enzyme immunoassay (Innotest hTAU-Ag, Innogenetics, Ghent, Belgium), according to the manufacturer's recommendations. A concentration of >1260pg/ml was considered to be diagnostic.[14] This assay measures total tau protein concentrations and as such measures both normally phosphorylated tau and hyper-phosphorylated tau. Some CSF samples had insufficient volume for all three analytes to be measured.

### ***PRNP* codon 129 genotype and PrP isotyping**

Prion protein isotyping was performed on all suspected cases of prion disease where fresh brain tissue was received by the NCJDSU. Small quantities of cerebral cortex were homogenized, treated with proteases and the size and abundance of the three PrP<sup>Sc</sup> glycoforms was determined by Western blot analysis.[15] Genotyping for polymorphism at codon 129 of the *PRNP* gene was carried out on all available blood specimens. DNA was extracted from blood using standard techniques and analysed using the Helsinki method.[16]

### **Statistical analysis**

Comparison of age by Mann Whitney tests was carried out. Descriptive statistics were calculated for the sCJD and control patients. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and efficiency of each marker and for combinations of markers were obtained. To investigate the influence of stage of disease on the sensitivity of CSF 14-3-3 and tau protein, we divided the disease duration for each patient into equal thirds. The stage in which the LP was performed was noted and the sensitivity of each marker for sCJD in each of the three stages was calculated.

### **RESULTS**

The sensitivity, specificity, PPV, NPV and efficiency of each neuronal marker for the diagnosis of neuropathologically confirmed sCJD cases are shown in Table 2. CSF 14-3-3 is the most sensitive marker and has a higher sensitivity than CSF tau, 86% and 81% respectively. CSF S100b does not have adequate sensitivity (65%) to be used as an isolated marker of sCJD. The difference in sensitivity between CSF 14-3-3 and tau protein is not influenced by the different numbers of cases investigated for each analyte. The sensitivity of CSF 14-3-3 and tau protein calculated using those samples where both analytes were measured is 85% and 81% respectively. The specificity of CSF 14-3-3 and tau protein in those samples where both analytes were measured is 74% and 85% respectively.



**Table 2** The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and efficiency for each marker and combination of CSF markers in neuropathologically confirmed sCJD. Figures in parentheses are 95% confidence limits.

	<b>14-3-3</b>	<b>Tau</b>	<b>S100b</b>	<b>14-3-3 and S100b</b>	<b>Tau and S100b</b>	<b>14-3-3 and Tau</b>	<b>14-3-3, Tau and S100b</b>
<b>sCJD<sup>1</sup></b>	210/245	175/216	158/243	151/242	127/216	162/216	123/216
<b>Not sCJD<sup>1</sup></b>	44/171	20/135	17/169	9/169	7/135	16/135	6/135
<b>Sensitivity</b>	86% (81, 90)	81% (75, 86)	65% (59, 71)	62% (57, 69)	59% (52, 65)	75% (69, 81)	57% (50, 64)
<b>Specificity</b>	74% (67, 81)	84% (78, 91)	90% (84, 94)	95% (90, 98)	95% (90, 98)	88% (81, 93)	96% (91, 98)
<b>PPV</b>	83% (77, 87)	90% (85, 94)	90% (85, 94)	94% (90, 97)	95% (90, 98)	91% (86, 95)	95% (90, 98)
<b>NPV</b>	78% (71, 84)	74% (66, 80)	64% (58, 70)	64% (58, 70)	59% (52, 66)	69% (61, 76)	58% (51, 65)
<b>Efficiency</b>	81% (77, 85)	83% (78, 86)	75% (71, 79)	76% (72, 80)	73% (68, 77)	80% (75, 84)	72% (67, 76)

<sup>1</sup>The figures given are the number of positive or negative results over the total number of samples investigated, Efficiency was defined as:  $\frac{\text{True positives} + \text{True negatives}}{\text{Total number tested}}$

The combination of a positive CSF 14-3-3 with either an elevated S100b or an elevated tau protein increased the PPV of 14-3-3 from 83% to 94% or 91%, respectively (Table 2 and fig 2a,b). Likewise the combination of an elevated tau protein and an elevated S100b resulted in a higher PPV (95%) for sCJD when compared to using each marker alone (Table 2, fig 2c). However combining the tests either in pairs or taking all three markers together resulted in a reduction in sensitivity and a reduction in the efficiency of each marker. That is to say the ability of each marker to distinguish between those patients with sCJD and those that present with symptoms similar to sCJD but turn out to have an alternative diagnosis is reduced if they are combined (Table 2).

In the atypical subgroups, although the numbers are small, CSF 14-3-3 is more sensitive than an elevated tau (Table 3). This finding is particularly noticeable in those patients

who are 50 years or less at the onset of the disease. However, in those sCJD cases that were negative for either CSF 14-3-3 or tau protein there did not seem to be a difference in the demographics of the patients or any obvious effect of codon 129 and PrP<sup>Sc</sup> isotype. In the 14-3-3 negative group there were 35 sCJD cases (27-81 years (mean 62.0 ± 10.9 years) at notification) and in the tau negative group there were 41 sCJD cases (44-81 years (mean 63.3 ± 9.9 years) at notification). The disease duration was also comparable 2-54 months (mean 15.1 ± 11.7 months) and 2-54 months (mean 14.4 ± 12.2 months) respectively. These disease durations are much longer than those in sCJD cases who are positive for CSF 14-3-3 (mean 6.6 ± 6.8 months) or CSF tau protein (mean 6.5 ± 6.6 months).

**Table 3** *Effect of age at onset of disease, disease duration and PRNP-codon 129 genotype on the sensitivity of CSF 14-3-3 and Tau protein.*

	<b>14-3-3 (%)</b>	<b>Tau protein (%)</b>
<b>&lt; 50 years at onset disease</b>	12/17 (71%) (44, 90)	10/16 (62%) (35, 84)
<b>≥ 50 years at onset disease</b>	198/228 (87%) (82, 91)	165/200 (83%) (77, 87)
<b>&gt; 12 months disease duration</b>	23/41 (56%) (40, 72)	21/39 (54%) (37, 70)
<b>≤ 12 months disease duration</b>	184/199 (92%) (88, 96)	151/175 (86%) (80, 91)
<b>PRNP Codon 129-MM</b>	106/121 (88%) (80, 93)	92/108 (85%) (77, 91)
<b>PRNP Codon 129-MV</b>	32/44 (73%) (57, 85)	26/39 (67%) (50, 81)
<b>PRNP Codon 129-VV</b>	27/29 (93%) (77, 99)	24/27 (89%) (71, 98)

Results expressed as number of positives over total number investigated. Figures in parentheses are the sensitivity of the individual marker in each subset of sCJD. The 95% confidence limits are given on the second row.

The effect of stage of disease on the sensitivity of CSF 14-3-3 and tau protein is shown in Table 4. The time of the LP and the date of the onset of disease were only available in 209 patients. Only 7% of patients had an LP within the first stage of disease, whilst 42% and 51% of patients had CSF samples taken in the second and third stages of disease

respectively. Both CSF 14-3-3 and tau protein show comparable sensitivity in the first two stages of disease, however in the final stage of the disease CSF 14-3-3 is more sensitive than CSF tau protein.

**Table 4** *Influence of time of CSF sampling on CSF 14-3-3 and tau protein positive results.*

<b>Stage of disease</b>	<b>14-3-3 (%)</b>	<b>Tau protein (%)</b>
<b>First stage (0-33% disease duration)</b>	9/14 (64%) (35, 87)	10/14 (71%) (42, 92)
<b>Second stage (34-66% disease duration)</b>	73/88 (83%) (73, 90)	72/88 (82%) (72, 89)
<b>Third stage (67-100% of disease duration)</b>	97/107 (91%) (83, 95)	89/107 (83%) (75, 90)

The time of the LP was calculated by expressing the time of LP from disease onset as a percentage of the total disease duration. The patients were classified into 3 groups depending on whether they had CSF samples taken in the first, second or third stage of the disease. Results expressed as number of positives over total number investigated. The 95% confidence limits are given on the second row.

During the study period 21 patients had a weak positive CSF 14-3-3. Of the 21 patients with weak positive CSF 14-3-3 results, 9 patients had neuropathologically confirmed sCJD whilst the remaining 12 patients had Alzheimer' disease (3), Lewy body disease (2), no neuropathological evidence of CJD (2), frontal lobe degeneration (1), epilepsy (1), multi-focal leukoencephalopathy (1), angiotrophic lymphoma (1) and no further information could be obtained on the final diagnosis in the remaining patient.

The most specific individual marker was CSF S100b however it also had the poorest sensitivity which limits its use as an isolated marker. CSF tau protein had a greater specificity than CSF 14-3-3 protein (85% vs 74%). Analysis of the control cases that had a positive CSF 14-3-3 and/or an elevated CSF tau protein showed that the most prevalent diagnoses included Alzheimer's disease, paraneoplastic syndrome and patients who clinically improved without an alternative diagnosis (Table 5).

**Table 5** Diagnoses in disease controls with positive CSF 14-3-3 and elevated tau protein cases

<b>Diagnosis (14-3-3 positive)</b>	<b>Pathological (26)</b>	<b>Clinical (18)</b>
Alzheimer's disease	7*	2
Malignancy/Paraneoplastic	5	1
Improved/ no evidence of CJD	3**	7
Encephalitis/limbic encephalitis	3	2
Lewy Body Dementia	1	0
Parkinson's disease	1	0
Cerebrovascular disease	0	2***
B cell Lymphoma/cerebral lymphoma	3	0
Fronto-temporal Dementia	0	1
Vasculitis	0	1
Central pontine myelinolysis	0	1
Multifocal demyelination	1	0
Anoxic brain injury	1	0
Normal pressure hydrocephalus	1	0
Corticobasal degeneration	0	1
<b>Diagnosis (tau protein &gt;1260pg/ml)</b>	<b>Pathological (12)</b>	<b>Clinical (6)</b>
Alzheimer's disease	3	1
Malignancy/paraneoplastic	3	0
Improved or no evidence of CJD	1	1
Fronto-temporal Dementia	0	1
Corticobasal degeneration	0	1
Encephalitis	0	1
Lymphoma	1	0
Cerebrovascular disease	1***	1
Multi focal demyelination	1	0
Lewy body disease	1	0
Anoxic brain damage	1	0

\*three had evidence of additional cerebrovascular disease and two also had evidence of LBD

\*\* one did not have brain examined

\*\*\*one had evidence of Alzheimer's disease

Out of a total of 242 cases of sCJD which had both CSF 14-3-3 and S100b measured only 10 had a negative 14-3-3 and a normal S100b concentration of <0.5ng/ml. Likewise out of 216 sCJD cases that had both CSF tau protein and S100b measured only 14 had concentrations of both markers within the normal range (fig 3a,b)

The impact of CSF 14-3-3 on UK surveillance over the last decade was assessed by examining the number of clinically probable cases classified as a result of a positive CSF alone. 163 probable cases that died without post mortem examination were identified. No information regarding whether an EEG had been performed was available in 25 cases. Of the remaining 138 cases, 3 had no EEG performed and 135 had an EEG reviewed by a senior member of the NCJDSU. Of these 135 patients, 38 had an EEG classified as highly suggestive or typical and hence appropriate for use in classification. The remaining 97 were classified as probable with the aid of a positive CSF 14-3-3 (72%). Therefore over the study period at least 100 patients were classified as having probable sCJD on the basis of CSF 14-3-3 alone. Sixty-nine patients initially classified as probable sCJD on the basis of a positive 14-3-3 alone, with either an unresponsive or unobtainable EEG, had subsequent post-mortem examination. Neuropathological confirmation of sCJD was obtained in 66 of these cases. The remaining three cases were misclassified as probable sCJD but in fact had pathological confirmation of carcinomatosis of the meninges (14-3-3 positive, S100b 0.57ng/ml, tau protein 3201pg/ml) and Lewy body dementia (14-3-3 positive, S100b 0.57ng/ml, tau protein 1170pg/ml) and Alzheimer's disease (14-3-3 positive, S100b 0.94ng/ml, tau protein 1294pg/ml). This gives a misclassification rate of 4%.

## **DISCUSSION**

It is ten years since a positive CSF 14-3-3 was added to the WHO diagnostic criteria for classifying probable sCJD. Since that time many studies have reported poorer sensitivity and specificity of CSF 14-3-3 than initially described, and have suggested that other markers of neuronal damage such as tau protein perform better. We have examined the diagnostic utility of CSF 14-3-3, tau protein and S100b analysis in the investigation of patients with suspected sCJD over a ten year period. In addition we have examined the overall impact that the inclusion of a positive CSF 14-3-3 has made on the diagnosis sCJD since it was introduced in 1997.

The sensitivity of a positive 14-3-3 in our study of sCJD is 86%, which is higher than that of tau protein (81%). The difference in sensitivity is more marked in the atypical forms of sCJD such as those who are younger than 50 years old at the onset of disease. In these cases the sensitivity of CSF 14-3-3 is 71% compared to 62% for CSF tau protein. This supports the findings of a larger European-wide study that found a positive CSF 14-3-3

was more sensitive than CSF tau protein in atypical sCJD cases.[9] CSF 14-3-3 is also more sensitive than CSF tau protein in the final stage of disease. This is important as this is the time at which the majority of CSF samples are taken.

A CSF S100b concentration of greater than 1.0ng/ml increases the PPV of a positive CSF 14-3-3 from 83% to 94%, and an elevated CSF tau protein from 90% to 95%. CSF S100b is routinely analysed by the NCJDSU for this reason. CSF S100b is also useful in excluding disease, with a negative CSF 14-3-3 and CSF S100b concentration of less than 0.5ng/ml having a NPV of 88%. Normal concentrations of CSF tau protein and CSF S100b concentration of less than 0.5ng/ml have a NPV of 84%.

There are no differences in the age of onset of disease, disease duration or codon 129 status of sCJD patients who are negative for CSF 14-3-3 and those that are negative for CSF tau. Therefore it is unlikely that the additional measurement of CSF tau will help improve the identification of CSF 14-3-3 negative sCJD cases.

Less than half the patients with weak positive CSF 14-3-3 results have sCJD and this suggests that the diagnostic utility of weak positive 14-3-3 results is limited. However, a repeat CSF 14-3-3 analysis in this group of patients may be of value if the clinical circumstances warrant it.[9]

Elevated CSF tau protein has a better specificity for sCJD than CSF 14-3-3 (84% vs 74%). This is a similar finding to a recently published study where tau protein was felt to be the single best marker for sCJD with a specificity of 90%.[17] It is however important to note that the diagnostic test accuracy and the differences reported by these various studies is partially dependent on the cut-off concentration of tau protein used. A universally agreed level for tau protein is not currently available. The diagnoses in the non CJD cases were similar for both tests. It is unclear why CSF tau protein should be of greater specificity than 14-3-3. The factors influencing the release of neuronal proteins in CJD and other conditions are not fully understood. Many studies have investigated the sensitivity of these two markers for the diagnosis of sCJD but very few have compared their specificity. Indeed specificity is highly dependent on the population investigated and therefore it is very difficult to compare individual studies. However the population

investigated in this study is highly selected and consists of patients where the preceding pre-test probability of sCJD is high.

During the ten years since its introduction CSF 14-3-3 analysis has enabled 100 patients in the UK with suspected sCJD who died without a post-mortem and without supportive EEG data to be classified as probable sCJD. During this time only three patients have been mis-classified as probable sCJD on the basis of a positive CSF 14-3-3.

We conclude that within the UK population referred with a clinical suspicion of sCJD to the NCJDSU over the last decade, brain derived proteins such as 14-3-3, S100b and tau have immense diagnostic value (see conclusion Table 6). In our experience, the combination of 14-3-3 and S100b remains the best predictor of supporting or excluding sCJD as a diagnosis when employed in an algorithmic manner using 14-3-3 detection as the primary screening marker before utilizing the S100b result. The importance of interpreting these results in the appropriate clinical context however is vital, especially as the phenotypic heterogeneity of sCJD remains wide.

**Table 6** *Conclusions regarding the use of CSF 14-3-3 and other markers in the diagnosis of sCJD*

CSF 14-3-3 as a sole marker has the highest sensitivity, particularly in the final stage of disease. As a sole marker CSF tau has the greatest specificity.
A combination of CSF 14-3-3 and elevated S100b or elevated tau protein and S100b has a greater PPV than CSF 14-3-3 alone
The combination of CSF 14-3-3 and tau protein in CSF 14-3-3 negative sCJD cases has not been shown to be of any additional value, even in phenotypically atypical cases
The combination of a negative CSF 14-3-3 and S100b is of value as a potential means of excluding sCJD
The differential diagnosis of sCJD in the 14-3-3 positive non-CJD cases remains as documented in previous studies

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## CONFLICTS OF INTEREST

We have no conflicts of interest

## LEGENDS

### Figure 1

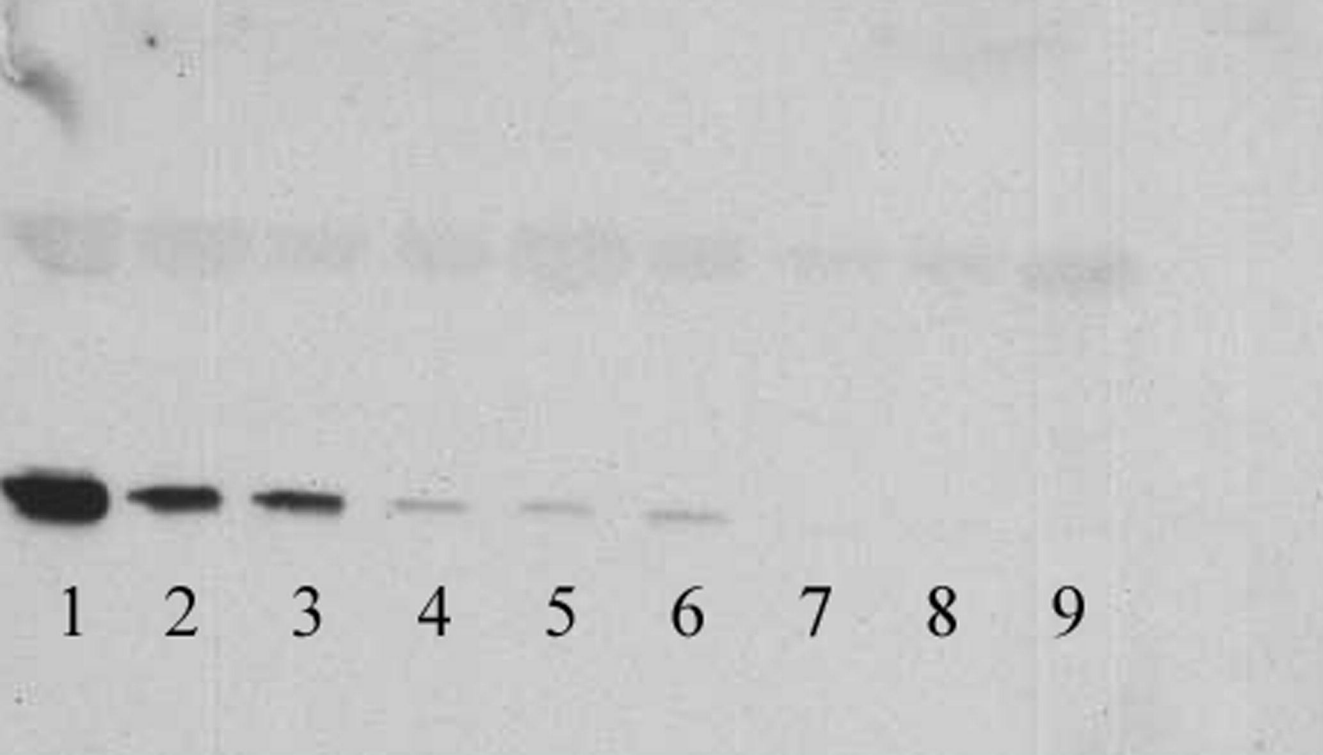
A western blotting illustrating the presence of positive, weak positive and negative CSF 14-3-3. Lanes 1, 2, 3 are positive for CSF 14-3-3, lanes 4, 5, 6 are weakly positive for CSF 14-3-3 and lanes 7, 8 and 9 are negative for CSF 14-3-3. Lanes 1 and 2 are from two patients with sporadic Creutzfeldt-Jakob disease (CJD), lanes 3 and 4 are from patients who have had a stroke and the remaining lanes contain CSF samples from patients who do not have CJD.

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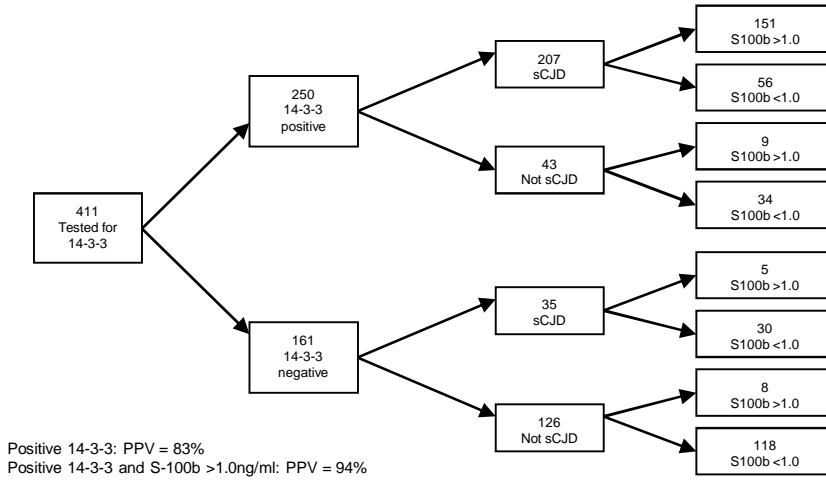
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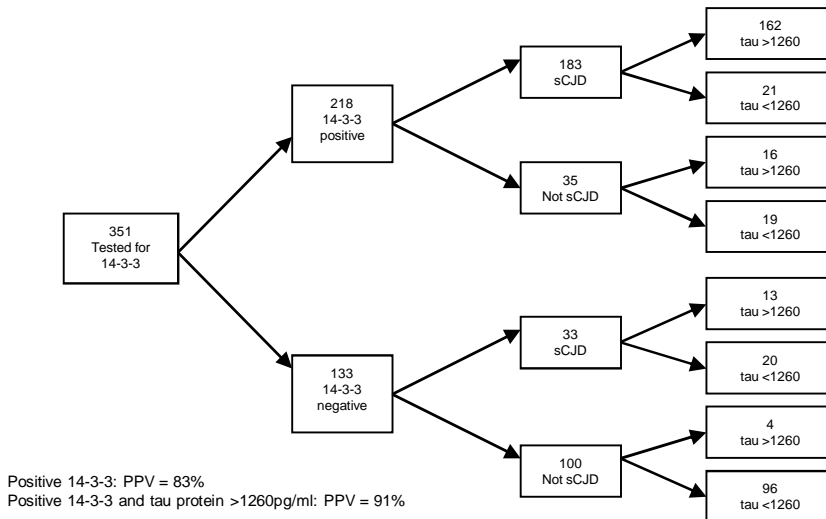
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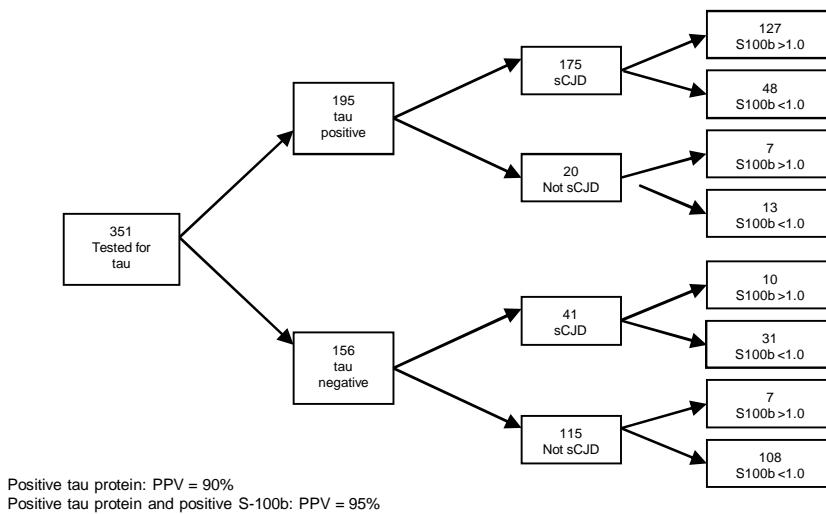
**Figure 2a** The role of a positive 14-3-3 and a diagnostic S100b in the diagnosis of sCJD



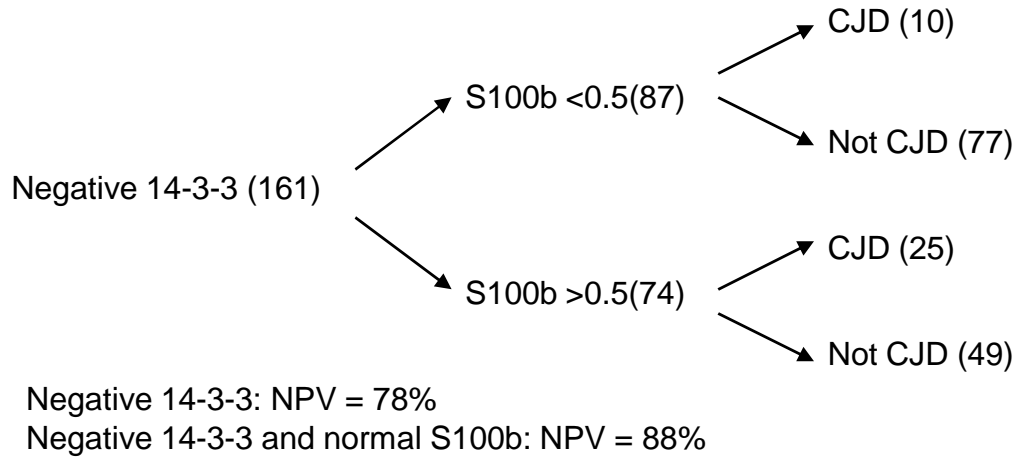
**Figure 2b** The role of a positive 14-3-3 and an elevated tau protein in the diagnosis of sCJD



**Figure 1c** The role of a positive tau protein and an elevated S100b in the diagnosis of sCJD



**Figure 3a** The role of a negative 14-3-3 and normal S100b in the diagnosis of sCJD



**Figure 3b** The role of a negative tau protein and a normal S100b in excluding sCJD

