



HAL
open science

Detection of CSF 14-3-3 Protein in Sporadic Creutzfeldt-Jakob Disease Patients Using a New Automated Capillary Western Assay

Anthony Fourier, Aline Dorey, Armand Perret-Liaudet, Isabelle Quadrio

► **To cite this version:**

Anthony Fourier, Aline Dorey, Armand Perret-Liaudet, Isabelle Quadrio. Detection of CSF 14-3-3 Protein in Sporadic Creutzfeldt-Jakob Disease Patients Using a New Automated Capillary Western Assay. *Molecular Neurobiology*, 2018, 55 (4), pp.3537-3545. 10.1007/s12035-017-0607-2 . hal-04796003

HAL Id: hal-04796003

<https://hal.science/hal-04796003v1>

Submitted on 23 Jan 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Detection of CSF 14-3-3 Protein in Sporadic Creutzfeldt-Jakob Disease Patients Using a New Automated Capillary Western Assay

A. Fourier^{1,2}  · A. Dorey^{1,3} · A. Perret-Liaudet^{1,2,3} · I. Quadrio^{1,2,3}

Abstract Sporadic Creutzfeldt-Jakob disease (sCJD) is a fatal rapidly progressive dementia. The detection of 14-3-3 protein in cerebrospinal fluid (CSF) is included in the WHO diagnostic criteria for the pre-mortem diagnosis of CJD. The aim of this study is to assess CSF 14-3-3 protein analytical and diagnostic performances using a new automated capillary Western technology (Simple Western technology-SW). For the validation of this assay, samples from a cohort of 268 patients suspected from sCJD were analyzed: 77 sCJD (including 40 definite sCJD) and 191 non-CJD samples were tested using both SW and the current Western Blot (WB) assays. Automated capillary Western determination provided better analytical performances than WB with a lower intra- and inter-assay variability. Analytical interferences such as hemolysis and high total protein concentration known to lead to false positive WB results were also assessed using SW assay: unfortunately, these interferences still remain confounders of CSF 14-3-3 protein determination. Finally, automated capillary Western assay's sensitivity and specificity were superior to those of WB assay (93.5 and 95.3%, respectively, compared to 92.2 and 84.8% for WB). In conclusion, with a shorter time of analysis than WB assays' (4 h versus 1.5 day), automated capillary Western assay is an excellent routine alternative method to the currently performed WB

assay for CSF 14-3-3 protein detection in patients suspected of sporadic Creutzfeldt-Jakob disease.

Keywords Creutzfeldt-Jakob disease · 14-3-3 protein · Immunoblotting · Automation · Sensitivity and specificity · Dementia

Introduction

Sporadic Creutzfeldt-Jakob disease (sCJD) is a fatal rapidly progressive dementia afflicting individuals with a mean age of onset around 65 [1, 2]. sCJD is a rare cause of dementia, whose annual incidence is approximately estimated at 1 to 2 cases per 1,000,000 persons [3, 4]. sCJD is difficult to diagnose: on one hand, clinical diagnosis is often problematic since sCJD cases may present non-specific psychiatric disturbances and, in many instances, sCJD diagnosis is hard to distinguish clinically from other dementias, including Alzheimer's disease and Lewy body dementia [5–8]; on the other hand, many pathologic conditions simulate the clinical pattern of sCJD including lymphomas, cerebrovascular amyloidosis, or cerebral hypoxic lesions [9].

The determination of 14-3-3 protein, a biochemical biomarker in Cerebrospinal Fluid (CSF), is a highly sensitive and specific assay for the pre-mortem diagnosis of Creutzfeldt-Jakob disease (CJD) [10–12]. The positivity of CSF 14-3-3 protein associated with rapidly progressive dementia, clinical features (i.e., myoclonus, visual or cerebellar symptoms, pyramidal/extrapyramidal syndromes, or akinetic mutism), and the absence of alternative diagnosis defines a probable sCJD, according to the World Health Organization [13] and the Centers for Disease Control and Prevention (CDC) diagnostic criteria for CJD (<https://www.cdc.gov/prions/cjd/diagnostic-criteria.html>). These guidelines

✉ A. Fourier
anthony.fourier@chu-lyon.fr

- ¹ Neurochemistry Laboratory, Biochemistry Department, Hospices Civils de Lyon, Groupement Hospitalier Est, Bron, France
- ² BIORAN Team, Lyon Neuroscience Research Center, CNRS UMR 5292, INSERM U1028, Lyon 1 University, Bron, France
- ³ Center for Memory Resources and Research, Hospices Civils de Lyon, Charpennes Hospital, Lyon 1 University, Villeurbanne, France

also include other biomarkers such as suggestive electroencephalography (EEG presenting characteristic triphasic sharp wave complexes) and specific magnetic resonance imaging signal abnormalities [14, 15] (including high-signal intensity, on either FLAIR or DWI, in the basal ganglia or in at least two cortical regions from either the temporal, parietal, or occipital cortices).

The 14-3-3 protein is a 30-kDa protein abundant in the brain, particularly inside neuronal cytoplasm, glia, and synaptic membranes [16]. This protein plays an important role on signal transduction, neurotransmission, and cell differentiation and is released into CSF along with extensive neuronal damage [17]. To date, 7 isoforms of 14-3-3 protein are known. Over the years, some isoforms of this protein were described to increase in sCJD patients' CSF compared to CSF of patients without prion disease [9, 10, 18]. Concretely, the 14-3-3 protein β , γ , ϵ , and η isoforms are detectable in CSF of sCJD patients [19, 20].

14-3-3 detection in CSF of suspected sCJD patients is usually performed through Western Blot (WB) technique, the gold standard assay, allowing a dichotomous result, i.e., positive or negative [11, 20, 21]. This qualitative technique is time-consuming (1.5 day for our current assay) and is generally associated to inter-assay variability around 35% [22].

Facing an increase in 14-3-3 test referrals in CJD reference laboratories [23], the aim of this study was to develop and assess a new automated capillary Simple Western (SW) technology, drastically reducing the time of CSF 14-3-3 protein analysis to 4 h. The assessment of SW assay analytical and diagnostic performances was compared to those obtained with the current WB assay in a population of 268 patients suspected of sCJD.

Material and Methods

Study Population

This retrospective study included a population of 268 patients undergoing a lumbar puncture for the evaluation of CSF 14-3-3 protein in the frame of CJD diagnosis. Lumbar puncture was addressed to Neurochemistry Laboratory (Hospices Civils de Lyon, France) to support a suspicion of CJD or a differential diagnosis between CJD and other rapidly progressive dementias. Samples were collected between 2008 and 2016. After centrifugation, CSF supernatants were frozen at -80°C until further analysis.

The population of 268 patients included 77 sCJD with unambiguous CSF 14-3-3 protein results. According to the WHO diagnostic criteria [13, 15], 37 fulfilled the diagnosis of "probable sCJD" and 40 were "definite sCJD" confirmed by neuropathological examination after autopsy driven in our P3 laboratory. Amongst the 77 sCJD patients, 71 patients had

a positive WB for CSF 14-3-3 protein and 6 patients had a negative WB (sensitivity established at 92.2% for WB).

Concerning the non-CJD patients, CJD diagnosis was excluded by neurologists and the French surveillance network of CJD [24]. The clinical probable diagnosis was proposed by neurologists regarding clinical data, imaging/biological markers, and disease evolution. The non-CJD population was composed of 191 patients with unambiguous CSF 14-3-3 protein result: 131 other neurodegenerative dementias, 9 Normal Pressure Hydrocephalus, 7 metabolic encephalopathies, 14 psychiatric conditions, 6 epilepsies, 1 neoplastic syndrome, 2 infectious CNS syndromes, 2 autoimmune diseases, and 19 patients with other causes. Amongst these 191 non-CJD patients, 29 had a positive WB for CSF 14-3-3 protein, and 162 had a negative WB (specificity established at 84.8% for WB).

This study, based on biological samples collected for routine diagnosis, was not considered as "biomedical research" under French regulations and therefore did not require supplemental informed consent. Samples were stored in a bio bank with authorization from the French Ministry of Health (Declaration Number DC-2008-304). Collected data included age, gender, date of lumbar puncture, and CJD clinical/neuropathological diagnosis. Authorization for handling personal data was granted by the French data protection commission [*Commission Nationale de l'Informatique et des Libertés* (CNIL)].

CSF Analysis

CSF Total Protein Concentration Assay

CSF total proteins were measured using a benzethonium chloride assay on Architect c16000 analyzer (Abbott Diagnostics®, Santa Clara, CA, USA).

Western Blot Assay

14-3-3 protein immunoassay was performed by Western blot analysis as described before [25]. Briefly, 75 microliters of CSF samples were denatured with a solution containing Tris 0.05 M pH 7.4, SDS 10%, DTT 1%, and glycerol heated at 98°C for 5 min. The proteins were separated on an ExcelGel® SDS Homogeneous 15 for SDS-PAGE (GE Healthcare®, Little Chalfont, Buckinghamshire, UK), transferred to polyvinylidene fluoride (PVDF) membrane and blocked overnight with 1% nonfat-dried milk in Tris Buffer Saline (TBS). The membranes were incubated for 2 h with the primary anti-14.3.3 antibody 1/400th (β K19: sc-629, Santa Cruz Biotechnology®, Dallas, TX, USA). After incubation, three washes were performed in TBS-0.1% nonfat-dried milk. Then membranes were saturated with alkaline phosphatase conjugated anti-rabbit second antibody 1/1000th (sc-2057, Santa

Cruz Biotechnology®, Dallas, TX, USA) during 1 h and washed three more times with TBS-0.1% nonfat-dried milk and once with TBS. Finally, the colorimetric revelation was performed incubating 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium (Thermo Scientific®, Waltham, MA, USA) during 5 min. The 14-3-3 protein band in CSF samples was optically observed and compared to known specimen to permit a characterization as negative or positive sample.

Automated Capillary Western Assay

Peggy Sue® 12–230 k Dalton (kDa) size assays were carried out: each analysis cycle was composed of 12 capillaries including a ladder and 11 CSF samples. Six microliters of CSF samples were denatured with a Fluorescent Master Mix® containing fluorescent molecular weight markers and 200 mM dithiothreitol, according to Protein Simple® recommendations. The mixtures were heated at 95 °C during 5 min. Primary anti-14-3-3 antibody (γ C-16: sc-731, Santa Cruz Biotechnology®, Dallas, TX, USA) and 10× System Control (10xSC) primary rabbit antibody were diluted with antibody diluent to obtain a final dilution of 1/100th and 1/40th, respectively. Samples, blocking reagent, mix of primary antibodies, HRP conjugated second antibodies, chemiluminescent substrate, separation, and stacking matrices were dispensed to designated wells in a 384-well plate. The plate was then loaded on the Peggy Sue® platform to perform the different steps of separation electrophoresis and immunodetection automatically. Briefly, the proteins were separated inside a capillary per sample during 45 min and fixed on the surface of these capillaries. The incubations of primary antibodies and second antibody lasted 1 h and 30 min, respectively. Finally, the chemiluminescent revelation was performed incubating capillaries with a mix of luminol and peroxide. The determination of the size, areas, heights, and signal to noise (S/N) ratios of 14-3-3 protein and 10× System Control protein (used as internal standard) was automatically calculated on Compass for Simple Western® software, version 3.0.9. A composite criterion, called areas ratio, was also calculated to introduce the use of 10xSC protein as an internal standard. The formula of areas ratio was (area of 14-3-3 protein/area of 10× SC protein) × 10,000.

Analytical Performances Evaluation

Intra-assay and inter-assay variability of both techniques were assessed using 2 CSF positive quality controls (QC) previously analyzed with WB assay: the first one with a strong 14-3-3 band intensity and the second one with a faint 14-3-3 band intensity. The determination of the 14-3-3 protein signals was quantified calculating area 14-3-3 and areas ratio for automated capillary Western assay. To perform a quantitative evaluation of Western blot variability, some membranes were read

out using ChemiDoc XRS (BioRad®, Hercules, CA, USA) and Quantity One® software to calculate the band intensity signal correlated to the amount of 14-3-3 protein. Results were expressed as percentage coefficient of variation for CSF 14-3-3 protein determination.

Human recombinant histidine-tagged 14-3-3 γ protein (Sigma-Aldrich®, St. Louis, MO, USA) was spiked into negative 14-3-3 protein CSF to assess 14-3-3 protein detection and size using SW assay. Other spiking assays were performed to analyze the influence of hemolysis and total protein concentration into CSF samples, according to an adapted protocol [26]. A red blood cell lysate containing 71 g/L hemoglobin was prepared: hemoglobin was measured on ABL800 Flex (Radiometer®, Copenhagen, Denmark). This lysate was spiked into a limpid negative 14-3-3 CSF sample to create a likely visible hemolyzed sample (0.28 g/L hemoglobin, by dilution of red blood cell lysate 1/250th) and a strongly visible hemolyzed sample (1.42 g/L hemoglobin, by dilution of red blood cell lysate 1/50th). Plasma containing 80 g/L total proteins (Biuret protocol on Architect c16000 analyzer) was diluted and spiked (5% volume/volume) into the same negative 14-3-3 CSF sample with proteins at 0.43 g/L to increase its total protein final concentration (from 1 to 4 g/L). Finally, the neat CSF sample, the likely visible and strongly visible hemolyzed samples and the increased total protein concentrations samples were analyzed in the same automated capillary assay.

Statistical Analysis

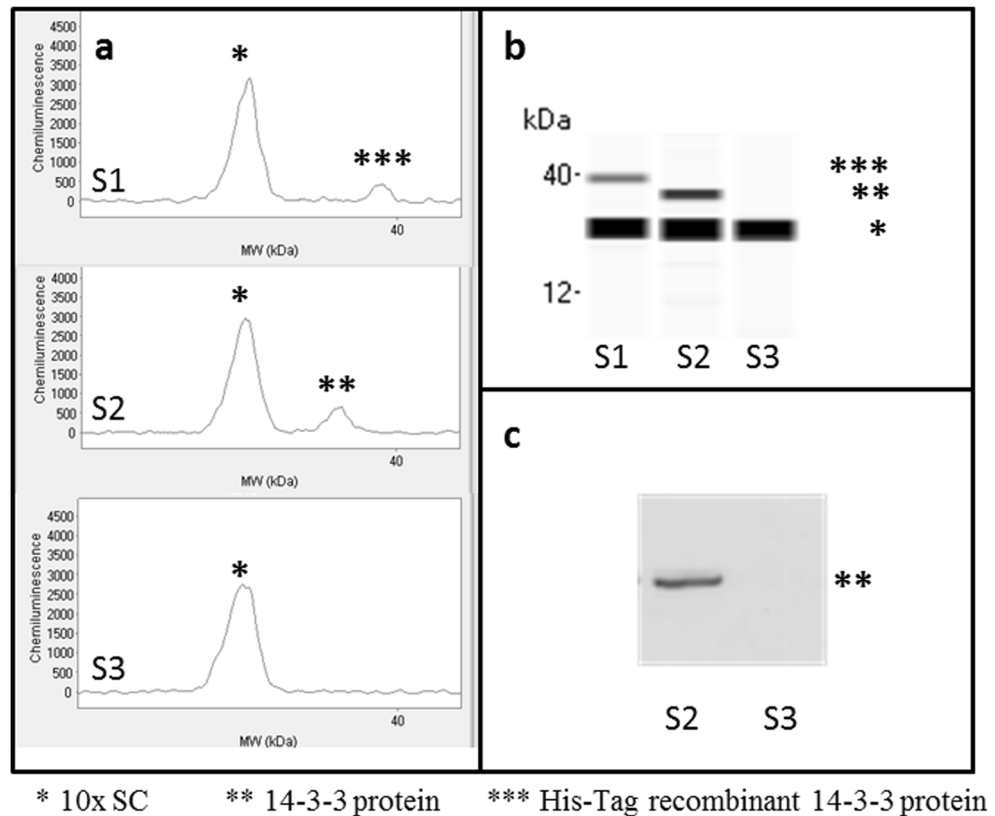
Mann-Whitney test and chi-square test were performed to assess differences between continuous and categorical variables respectively. Receiver operating characteristics (ROC) curves were performed to assess the diagnostic performances of CSF 14-3-3 protein regarding CJD status. Optimal cutoff values, sensitivity, and specificity were calculated using the Youden index [Se + (1–Sp)]. All the statistics were performed on MedCalc® software, version 15.11.4.

Results

Detection of CSF 14-3-3 Protein Using Automated Capillary Western Assay

Figure 1 represents the detection of 14-3-3 protein and recombinant histidine-tagged 14-3-3 protein, with both automated capillary Western (Fig. 1a, b) and Western Blot assays (Fig. 1c). Recombinant 14-3-3 protein was spiked into a negative 14-3-3 protein non-CJD CSF sample (S1). A representation of a positive CSF 14-3-3 protein sCJD sample (S2) and a negative CSF 14-3-3 protein non-CJD sample (S3) are also shown.

Fig. 1 Detection of His-Tag recombinant 14-3-3 protein or endogenous 14-3-3 protein in CSF samples. **a** Graph view and **b** lane view of Compass for Simple Western® software representing electropherograms with automated capillary Western assay (10× SC protein, used as internal standard at ≈26 kDa, 14-3-3 protein at ≈35 kDa and His-Tag recombinant 14-3-3 protein at ≈39 kDa). **c** Western Blot results of CSF 14-3-3 protein, a band can be seen for positive samples at ≈35 kDa. *S1* His-Tag recombinant protein spiked in a negative 14-3-3 CSF sample, *S2* a positive 14-3-3 sCJD CSF sample, *S3* a negative 14-3-3 non-CJD CSF sample



Diagnostic Performances of Automated Capillary Western Assay

Table 1 presents the comparison between the sCJD and non-CJD populations, regarding demography, total proteins of CSF samples and automated capillary Western results.

No difference was observed between the two populations concerning gender, age, or CSF total protein concentrations. 14-3-3 protein expression using automated capillary Western assay was statistically different between sCJD and non-CJD populations, whatever its mode of quantification (area 14-3-3, height 14-3-3, S/N 14-3-3 and the composite criterion areas ratio). The diagnostic performances of the four different SW expressions of CSF 14-3-3 protein are illustrated in Table 2.

All the four SW quantifications of CSF 14-3-3 protein (area 14-3-3, height 14-3-3, S/N 14-3-3 and areas ratio) gave coherent results with an AUC higher than 0.900 (p value < 0.0001). Regarding Youden index, the criterion area 14-3-3, and the composite criterion areas ratio were the best to classify patients (Youden index at 0.8775 and 0.8879, respectively). Using the cutoff value at 1956 for the criterion area 14-3-3, the sensitivity and specificity were at 93.5 and 94.2%, respectively (5 sCJD CSF samples were classified as negative and 11 non-CJD CSF samples were classified as positive by SW assay). Using the cutoff value at 235 for the composite criterion areas ratio, the sensitivity was the same—93.5%—and the specificity was slightly increased at 95.3% (five sCJD CSF

samples were classified as negative and nine non-CJD CSF samples were classified as positive by SW assay).

Analytical Performances of Automated Capillary Western Assay

The results obtained for variability using SW and WB assays are presented in Table 3. Two positive CSF quality controls

Table 1 Comparison of sCJD and non-CJD populations regarding demography, CSF total protein concentrations, and automated capillary Western results

	sCJD ($n = 77$)	Non-CJD ($n = 191$)
Demography		
Gender, M/F	31/46	96/95
Age, years	71.0 (52.4–86.7)	72.0 (54.1–86.0)
CSF samples		
Total proteins, g/L	0.39 (0.26–0.70)	0.40 (0.23–0.68)
Automated capillary Western results		
Area 14-3-3	7259 (1723–26,744)*	411 (0–2040)
Height 14-3-3	512 (99–1977)*	32 (0–199)
S/N 14-3-3	13.2 (2.8–40.0)*	2.9 (1.1–7.3)
Areas ratio	872 (195–3149)*	60 (0–234)

Data expressed as median (5th–95th percentile), * $p < 0.0001$ versus non-CJD

Table 2 Diagnostic performances of the 4 Simple Western expressions of CSF 14-3-3 protein

	AUC (95% CI)	Youden index	Criterion	Sensitivity (%)	Specificity (%)
Area 14-3-3	0.973 (0.946–0.989)	0.8775	>1956	93.5	94.2
Height 14-3-3	0.971 (0.943–0.987)	0.8488	>147	92.2	92.7
S/N 14-3-3	0.913 (0.873–0.944)	0.7420	>5.2	85.7	88.5
Areas ratio	0.968 (0.939–0.985)	0.8879	>235	93.5	95.3

(QC) were analyzed in the same assay (intra-assay variability) or in different assays (inter-assay variability). The variability of SW assay was assessed using both area 14-3-3 and areas ratio since these two quantification modes showed the best diagnostic performances for SW assay (see Table 2).

Globally, SW assay provided a better intra-assay and inter-assay variability than WB assay, whatever the intensity of the 14-3-3 protein signal (strong positive or faint positive 14-3-3 protein band on CSF QC samples). Comparing SW assay quantification modes (area 14-3-3 and areas ratio), intra-assay variability was considered equal for both quantification modes. However, a difference in the inter-assay variability could be seen, the lowest was obtained for areas ratio expression. Indeed, the inter-assay variability decreased by 5 and 4 percentage points regarding strong positive or faint positive 14-3-3 protein band respectively on CSF QC samples using areas ratio instead of area 14-3-3 (Table 3). Consequently, the composite criterion areas ratio was chosen to compare SW and WB assays in subsequent assays regarding both its analytical and diagnostic performances.

Table 4 gives the distribution of the 268 CSF samples regarding both SW and WB assays:

A correct classification of CSF samples, considered as the addition of the populations of SW positive/WB positive samples (77/268) and SW negative/WB negative samples (164/268) was obtained for 241 samples (89.9% of the cohort of 268 patients).

The 27 CSF samples with a different classification between SW and WB assays were characterized regarding their belonging to sCJD or non-CJD populations. Amongst the 23 SW negative/WB positive samples, 22 were non-CJD patients: automated capillary western assay provided a better

specificity than Western Blot assay. Amongst the 4 SW positive/WB negative samples, 2 were definite sCJD patients with areas ratio at 330 and 448, respectively. Finally, 4 sCJD samples remained negative using both assays: these patients were all definite sCJD (areas ratio at 34, 73, 155, and 194, respectively).

Table 5 indicates the results obtained during hemolysis and protein spiking assays. The size of the detected 14-3-3 peak using Compass for Simple Western software®, the values calculated for the composite criterion areas ratio and the interpretation of areas ratio are shown.

Hemolysis and total protein concentration in CSF were found in our study as confounders leading to a misinterpretation of 14-3-3 protein results. Indeed, a strongly visible hemolysis and/or CSF total proteins ≥ 1.5 g/L could lead to positive 14-3-3 protein results.

Discussion

For the first time, a new automated capillary Western assay was developed using Peggy Sue® platform (Protein Simple©) in order to detect CSF 14-3-3 protein, providing a format adapted to the routine biological diagnosis of CJD. Endogenous 14-3-3 protein and recombinant histidine-tagged 14-3-3 protein were correctly detected using automated capillary Western technology (Fig. 1). The four different modes of quantification of 14-3-3 protein signal (i.e., area 14-3-3, height 14-3-3, S/N 14-3-3, and the composite criterion areas ratio) gave all excellent results to discriminate between sCJD and non-CJD patients (Table 1). The criterion area 14-3-3 and the composite criterion areas ratio presented the best

Table 3 SW and WB variability analyzing 2 CSF positive quality controls

	WB assay (%)	SW assay Area 14-3-3(%)	SW assay Areas ratio (%)
Intra-assay variability (<i>n</i> = 5 repeats)			
Positive CSF QC1 (strong 14-3-3 band)	10	6	7
Positive CSF QC2 (faint 14-3-3 band)	30	13	12
Inter-assay variability (<i>n</i> = 20 repeats)			
Positive CSF QC1 (strong 14-3-3 band)	34	22	17
Positive CSF QC2 (faint 14-3-3 band)	39	29	25

QC1 with a strong 14-3-3 protein band and QC2 with a faint 14-3-3 protein band using WB

Table 4 Distribution of the 268 sCJD and non-CJD samples according to their 14-3-3 protein results using both SW and WB assays

	sCJD	Non-CJD	Total
Concordant results			
SW+/WB+	70	7	77
SW-/WB-	4	160	164
Discordant results			
SW+/WB-	2	2	4
SW-/WB+	1	22	23
Total	77	191	268

An SW assay was considered positive if areas ratio > 235

diagnostic performances with Youden indexes at 0.878 and 0.888, respectively. According to the variability assay results, areas ratio was the criterion chosen in our study to discriminate sCJD and non-CJD patients. Indeed, variability was lower with this mode of quantification using SW assay, especially focusing on inter-assay variability (Table 2). In addition, the use of this composite criterion ensured a supplemental analytical control, with the presence of one peak at least (10× SC protein) and two peaks for positive samples (10× SC and 14-3-3 proteins) in each capillary.

Areas ratio criterion calculated in SW assay provided better analytical performances than WB assay with lower intra- and inter-assay variability for a strong positive 14-3-3 protein QC sample at 7 and 17%, respectively (Table 3). Analyzing the same sample, intra- and inter-assay variability of WB assay were 10 and 34%, respectively, which is consistent with data from the literature [22]. Variability was higher with a faint positive QC sample, which is a commonly observed for the majority of immunoassays (Table 3). These good performances of SW assay were partly explained by the automation of the assay, improving protocols reproducibility (incubation time with antibodies, standardized revelation).

Miniaturization also provided a decrease of antibodies uptake, allowing a less frequent variation of antibodies batches, at the origin of a great variability [27]. The automation of CSF 14-3-3 protein assay including an easier waste management also enhanced safety for technical staff manipulating potentially infectious CSF. Finally, CSF 14-3-3 protein detection using SW assay provided a shorter deadline to obtain results (4 h for the first samples) than WB assay, a method known for its time consumption (currently 1.5 day including an overnight saturation). The rapidity of this assay conjugated to its automation is helpful to deliver a result quickly. Indeed, in case of suspicion of sCJD in a patient with rapid cognitive and neurologic degradation, a negative CSF 14-3-3 could reorient diagnosis to other neurological disorders, some of them being curable [28, 29].

As for WB assay, CSF intrinsic properties such as hemolysis or total protein concentration were crucial confounders that should be determined before analysis [23]. Indeed, a strongly visible hemolysis led to a positive 14-3-3 result. This observation is explained by the physiological presence of 14-3-3 inside red blood cells [30]. Total protein concentrations in CSF higher than 1.5 g/L also led to apparent positive 14-3-3 protein results. However, the size of protein detected with SW assay was below the expected size of endogenous 14-3-3 protein (33 versus 35 kDa, respectively). For high total protein concentration samples, a careful observation of graph views was required in addition to the calculation of areas ratio for a correct interpretation of results. In accordance with the red blood cell lysate and proteins spiking assays, the detection of CSF 14-3-3 protein should not be performed for strongly visible hemolyzed or high total protein CSF samples (Table 5). Unfortunately, SW assay does not therefore bring any analytical improvement comparing to WB assay concerning these particular samples.

In the retrospective sCJD and non-CJD cohorts chosen for the study, automated capillary Western assay's sensitivity and specificity were superior to those of Western Blot assay (93.5

Table 5 CSF hemolysis and protein spiking assays

CSF sample	Size (kDa)	Areas ratio	Interpretation ^a
Hemolysis			
Neat CSF (limpid)	35	118	Negative
Likely visible hemolysis (hemoglobin 0.28 g/L)	35	138	Negative
Strongly visible hemolysis (hemoglobin 1.42 g/L)	35	919	Positive
Total protein concentration			
Neat CSF (0.43 g/L)	35	118	Negative
Protein-spiked CSF (1 g/L final)	35	102	Negative
Protein-spiked CSF (1.5 g/L final)	33	1109	Positive
Protein-spiked CSF (2.5 g/L final)	33	1560	Positive
Protein-spiked CSF (4 g/L final)	33	3751	Positive

^a Positive 14-3-3 protein result if areas ratio > 235; negative 14-3-3 protein result if areas ratio ≤ 235

and 95.3% respectively for areas ratio, compared to 92.2 and 84.8% for the current WB assay). The results obtained for both assays were consistent to a meta-analysis published by Muayqil et al., reporting an average sensitivity and specificity of CSF 14-3-3 protein assay at 92 and 80%, respectively [31]. More precisely, SW assay's diagnostic performances were similar to CSF 14-3-3 protein determination performed with an ELISA assay [23, 32–34], supposed to yield more consistent results than WB assay [34]. However, ELISA assays are known to provide lower intra- and inter-assay variability. In our cohort of patients, four definite sCJD patients had a negative CSF 14-3-3 protein result using both SW and WB assays. Sensitivity of CSF 14-3-3 protein is not perfect and some sCJD patients may present negative CSF 14-3-3 results, especially in some specific clinical onsets [2]. The absence of CSF 14-3-3 protein could also be explained by the time when lumbar puncture was performed regarding disease onset, the sensitivity of CSF 14-3-3 determination increasing with disease course [25]. If clinical features persist, a second detection of CSF 14-3-3 protein should be performed on another lumbar puncture. Likewise, samples with area ratio next to the cutoff value at 235 should be carefully interpreted and re-analyzed on another run since inter-assay variability of SW assay was 25% for a faint positive CSF sample. On the other side, CSF 14-3-3 protein is a surrogate marker of sCJD [10] increasing with extensive brain damage [35], explaining its potential lack of specificity [11, 36, 37]. To counteract CSF 14-3-3 determination drawbacks, a lot of efforts have been done to develop other surrogate markers of CJD. In CJD patients, increases of total tau protein—another marker of neuronal lysis—and alpha-synuclein—a marker of synaptic degeneration—were observed [38, 39]. Diagnostic performances of these markers are similar or better than those of 14-3-3 protein, with sensitivity and specificity at 94 and 90% respectively for total tau protein and at 98 and 97% respectively for alpha-synuclein using ELISA assays [40, 41]. Furthermore, assays detecting prion protein, the etiological marker of CJD, are also now performed using ELISA or RT-QuIC methods [42, 43]. Pending the complete validation of these different assays before use in daily clinical practice, improving 14-3-3 protein determination is essential for CJD diagnosis since it remains nowadays the sole biological marker included in the WHO CJD diagnostic criteria.

Finally, the automation of WB and isoelectric focusing (IEF) assays on the Peggy Sue® platform enables the emergence of many applications in fundamental or applied research [44, 45]. Concerning the development of neurodegenerative marker automated assays, Halgebauer et al. developed an IEF assay to detect oligoclonal bands in CSF [46] and proposed it for clinical practice. Using automated capillary technology will certainly open the way to the adaptation and routine use of many markers of neurodegenerative proteinopathies [47].

Conclusion

CSF 14-3-3 protein remains presently the biological marker recommended by international consensus to improve the probability of sCJD diagnosis, despite its nature of surrogate sCJD marker. Using the new automated capillary Western assay, analytical and diagnostic performances of CSF 14-3-3 protein detection were improved compared to the classical Western Blot assay. With a shorter time of analysis (4 h versus 1.5 day), this method would rapidly replace WB assay for the detection of CSF 14-3-3 protein in the frame of routine CJD diagnosis. Moreover, the automated Western platform has the potential to implement others biomarkers in the field of neurodegenerative disorders.

Acknowledgements We would to thank the French surveillance network of CJD for the support of CJD or non-CJD diagnosis. Particular thanks to Elisabeth Barbey, AD, and Geneviève Gentil-Perret, AD, Hospices Civils de Lyon, Groupement Hospitalier Est, Department of Biochemistry, Lyon, France.

References

1. Will RG, Ironside JW (2017) Sporadic and infectious human prion diseases. *Cold Spring Harbor perspectives in medicine* 7 (1). doi:10.1101/cshperspect.a024364
2. Collins SJ, Sanchez-Juan P, Masters CL, Klug GM, van Duijn C, Poggi A, Pocchiarini M, Almonti S et al (2006) Determinants of diagnostic investigation sensitivities across the clinical spectrum of sporadic Creutzfeldt-Jakob disease. *Brain J Neurol* 129(Pt 9):2278–2287. doi:10.1093/brain/awl159
3. Brown P, Cathala F, Raubertas RF, Gajdusek DC, Castaigne P (1987) The epidemiology of Creutzfeldt-Jakob disease: conclusion of a 15-year investigation in France and review of the world literature. *Neurology* 37(6):895–904
4. Holman RC, Belay ED, Christensen KY, Maddox RA, Minino AM, Folkema AM, Haberling DL, Hammett TA et al (2010) Human prion diseases in the United States. *PLoS One* 5(1):e8521. doi:10.1371/journal.pone.0008521
5. Van Everbroeck B, Dobbeleir I, De Waele M, De Deyn P, Martin JJ, Cras P (2004) Differential diagnosis of 201 possible Creutzfeldt-Jakob disease patients. *J Neurol* 251(3):298–304. doi:10.1007/s00415-004-0311-9
6. Paterson RW, Torres-Chae CC, Kuo AL, Ando T, Nguyen EA, Wong K, DeArmond SJ, Haman A et al (2012) Differential diagnosis of Jakob-Creutzfeldt disease. *Arch Neurol* 69(12):1578–1582. doi:10.1001/2013.jamaneurol.79
7. Jayaratnam S, Khoo AK, Basic D (2008) Rapidly progressive Alzheimer's disease and elevated 14-3-3 proteins in cerebrospinal fluid. *Age Ageing* 37(4):467–469. doi:10.1093/ageing/afn094
8. Reinwald S, Westner IM, Niedermaier N (2004) Rapidly progressive Alzheimer's disease mimicking Creutzfeldt-Jakob disease. *J Neurol* 251(8):1020–1022. doi:10.1007/s00415-004-0480-6

9. Stoeck K, Sanchez-Juan P, Gawinecka J, Green A, Ladogana A, Pocchiari M, Sanchez-Valle R, Mitrova E et al (2012) Cerebrospinal fluid biomarker supported diagnosis of Creutzfeldt-Jakob disease and rapid dementias: a longitudinal multicentre study over 10 years. *Brain J Neurol* 135(Pt 10):3051–3061. doi:10.1093/brain/awv238
10. Hsich G, Kenney K, Gibbs CJ, Lee KH, Harrington MG (1996) The 14-3-3 brain protein in cerebrospinal fluid as a marker for transmissible spongiform encephalopathies. *N Engl J Med* 335(13):924–930
11. Zerr I, Bodemer M, Gefeller O, Otto M, Poser S, Wiltfang J, Windl O, Kretschmar HA et al (1998) Detection of 14-3-3 protein in the cerebrospinal fluid supports the diagnosis of Creutzfeldt-Jakob disease. *Ann Neurol* 43(1):32–40. doi:10.1002/ana.410430109
12. Collins S, Boyd A, Fletcher A, Gonzales M, McLean CA, Byron K, Masters CL (2000) Creutzfeldt-Jakob disease: diagnostic utility of 14-3-3 protein immunodetection in cerebrospinal fluid. *Journal of clinical neuroscience: official journal of the Neurosurgical Society of Australasia* 7(3):203–208. doi:10.1054/jocn.1999.0193
13. WHO (2003) WHO manual for surveillance of human transmissible spongiform encephalopathies, including variant Creutzfeldt-Jakob disease. World Health Organization, Geneva
14. Shiga Y, Miyazawa K, Sato S, Fukushima R, Shibuya S, Sato Y, Konno H, Doh-ura K et al (2004) Diffusion-weighted MRI abnormalities as an early diagnostic marker for Creutzfeldt-Jakob disease. *Neurology* 63(3):443–449
15. Zerr I, Kallenberg K, Summers DM, Romero C, Taratuto A, Heinemann U, Breithaupt M, Vargas D et al (2009) Updated clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease. *Brain J Neurol* 132(Pt 10):2659–2668. doi:10.1093/brain/awp191
16. Sanchez-Valle R, Saiz A, Graus F (2002) 14-3-3 protein isoforms and atypical patterns of the 14-3-3 assay in the diagnosis of Creutzfeldt-Jakob disease. *Neurosci Lett* 320(1–2):69–72
17. Shimada T, Fournier AE, Yamagata K (2013) Neuroprotective function of 14-3-3 proteins in neurodegeneration. *Biomed Res Int* 2013:564534. doi:10.1155/2013/564534
18. Shiga Y, Wakabayashi H, Miyazawa K, Kido H, Itoyama Y (2006) 14-3-3 protein levels and isoform patterns in the cerebrospinal fluid of Creutzfeldt-Jakob disease patients in the progressive and terminal stages. *Journal of clinical neuroscience: official journal of the Neurosurgical Society of Australasia* 13(6):661–665. doi:10.1016/j.jocn.2005.09.004
19. Wiltfang J, Otto M, Baxter HC, Bodemer M, Steinacker P, Bahn E, Zerr I, Kornhuber J et al (1999) Isoform pattern of 14-3-3 proteins in the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. *J Neurochem* 73(6):2485–2490
20. Satoh K, Tobiume M, Matsui Y, Mutsukura K, Nishida N, Shiga Y, Eguchi K, Shirabe S et al (2010) Establishment of a standard 14-3-3 protein assay of cerebrospinal fluid as a diagnostic tool for Creutzfeldt-Jakob disease. *Laboratory investigation; a journal of technical methods and pathology* 90(11):1637–1644. doi:10.1038/labinvest.2009.68
21. Green AJ, Thompson EJ, Stewart GE, Zeidler M, McKenzie JM, MacLeod MA, Ironside JW, Will RG et al (2001) Use of 14-3-3 and other brain-specific proteins in CSF in the diagnosis of variant Creutzfeldt-Jakob disease. *J Neurol Neurosurg Psychiatry* 70(6):744–748
22. Koller A, Watzig H (2005) Precision and variance components in quantitative gel electrophoresis. *Electrophoresis* 26(12):2470–2475. doi:10.1002/elps.200500024
23. Schmitz M, Ebert E, Stoeck K, Karch A, Collins S, Calero M, Sklaviadis T, Laplanche JL et al (2016) Validation of 14-3-3 protein as a marker in sporadic Creutzfeldt-Jakob disease diagnostic. *Mol Neurobiol* 53(4):2189–2199. doi:10.1007/s12035-015-9167-5
24. Brandel JP, Peckeu L, Haik S (2013) The French surveillance network of Creutzfeldt-Jakob disease. *Epidemiological data in France and worldwide. Transfusion clinique et biologique: journal de la Societe francaise de transfusion sanguine* 20(4):395–397. doi:10.1016/j.tracli.2013.02.029
25. Giraud P, Biacabe AG, Chazot G, Later R, Joyeux O, Moene Y, Perret-Liaudet A (2002) Increased detection of 14-3-3 protein in cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease during the disease course. *Eur Neurol* 48(4):218–221
26. Dimeski G (2008) Interference testing. *The Clinical biochemist Reviews* 29(Suppl 1):S43–S48
27. Baker M (2015) Reproducibility crisis: blame it on the antibodies. *Nature* 521(7552):274–276. doi:10.1038/521274a
28. Chitravas N, Jung RS, Kofskey DM, Blevins JE, Gambetti P, Leigh RJ, Cohen ML (2011) Treatable neurological disorders misdiagnosed as Creutzfeldt-Jakob disease. *Ann Neurol* 70(3):437–444. doi:10.1002/ana.22454
29. Geschwind MD, Shu H, Haman A, Sejvar JJ, Miller BL (2008) Rapidly progressive dementia. *Ann Neurol* 64(1):97–108. doi:10.1002/ana.21430
30. Day IN, Thompson RJ (1984) Levels of immunoreactive aldolase C, creatine kinase-BB, neuronal and non-neuronal enolase, and 14-3-3 protein in circulating human blood cells. *Clinica chimica acta; international journal of clinical chemistry* 136(2–3):219–228
31. Muayqil T, Gronseth G, Camicioli R (2012) Evidence-based guideline: diagnostic accuracy of CSF 14-3-3 protein in sporadic Creutzfeldt-Jakob disease: report of the guideline development subcommittee of the American Academy of Neurology. *Neurology* 79(14):1499–1506. doi:10.1212/WNL.0b013e31826d5fc3
32. Van Everbroeck BR, Boons J, Cras P (2005) 14-3-3 [1]-isoform detection distinguishes sporadic Creutzfeldt-Jakob disease from other dementias. *J Neurol Neurosurg Psychiatry* 76(1):100–102. doi:10.1136/jnnp.2003.032037
33. Matsui Y, Satoh K, Miyazaki T, Shirabe S, Atarashi R, Mutsukura K, Satoh A, Kataoka Y et al (2011) High sensitivity of an ELISA kit for detection of the gamma-isoform of 14-3-3 proteins: usefulness in laboratory diagnosis of human prion disease. *BMC Neurol* 11:120. doi:10.1186/1471-2377-11-120
34. Leitao MJ, Baldeiras I, Almeida MR, Ribeiro MH, Santos AC, Ribeiro M, Tomas J, Rocha S et al (2016) Sporadic Creutzfeldt-Jakob disease diagnostic accuracy is improved by a new CSF ELISA 14-3-3gamma assay. *Neuroscience* 322:398–407. doi:10.1016/j.neuroscience.2016.02.057
35. Satoh J, Kurohara K, Yukitake M, Kuroda Y (1999) The 14-3-3 protein detectable in the cerebrospinal fluid of patients with prion-unrelated neurological diseases is expressed constitutively in neurons and glial cells in culture. *Eur Neurol* 41(4):216–225
36. Kenney K, Brechtel C, Takahashi H, Kurohara K, Anderson P, Gibbs CJ Jr (2000) An enzyme-linked immunosorbent assay to quantify 14-3-3 proteins in the cerebrospinal fluid of suspected Creutzfeldt-Jakob disease patients. *Ann Neurol* 48(3):395–398
37. Beaudry P, Cohen P, Brandel JP, Delasnerie-Laupretre N, Richard S, Launay JM, Laplanche JL (1999) 14-3-3 protein, neuron-specific enolase, and S-100 protein in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. *Dement Geriatr Cogn Disord* 10(1):40–46
38. Riemenschneider M, Wagenpfeil S, Vanderstichele H, Otto M, Wiltfang J, Kretschmar H, Vanmechelen E, Forstl H et al (2003) Phospho-tau/total tau ratio in cerebrospinal fluid discriminates Creutzfeldt-Jakob disease from other dementias. *Mol Psychiatry* 8(3):343–347. doi:10.1038/sj.mp.4001220
39. Kasai T, Tokuda T, Ishii R, Ishigami N, Tsuboi Y, Nakagawa M, Mizuno T, El-Agnaf OM (2014) Increased alpha-synuclein levels in the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. *J Neurol* 261(6):1203–1209. doi:10.1007/s00415-014-7334-7
40. Otto M, Wiltfang J, Cepek L, Neumann M, Mollenhauer B, Steinacker P, Ciesielczyk B, Schulz-Schaeffer W et al (2002) Tau

- protein and 14-3-3 protein in the differential diagnosis of Creutzfeldt-Jakob disease. *Neurology* 58(2):192–197
41. Llorens F, Kruse N, Karch A, Schmitz M, Zafar S, Gotzmann N, Sun T, Kochy S et al (2017) Validation of alpha-Synuclein as a CSF biomarker for sporadic Creutzfeldt-Jakob disease. *Mol Neurobiol*. doi:[10.1007/s12035-017-0479-5](https://doi.org/10.1007/s12035-017-0479-5)
 42. Dorey A, Tholance Y, Vighetto A, Perret-Liaudet A, Lachman I, Krolak-Salmon P, Wagner U, Struyfs H et al (2015) Association of cerebrospinal fluid prion protein levels and the distinction between Alzheimer disease and Creutzfeldt-Jakob disease. *JAMA neurology* 72(3):267–275. doi:[10.1001/jamaneuro.2014.4068](https://doi.org/10.1001/jamaneuro.2014.4068)
 43. McGuire LI, Peden AH, Orru CD, Wilham JM, Appleford NE, Mallinson G, Andrews M, Head MW et al (2012) Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. *Ann Neurol* 72(2):278–285. doi:[10.1002/ana.23589](https://doi.org/10.1002/ana.23589)
 44. Wang J, Valdez A, Chen Y (2016) Evaluation of automated Wes system as an analytical and characterization tool to support monoclonal antibody drug product development. *J Pharm Biomed Anal*. doi:[10.1016/j.jpba.2016.12.024](https://doi.org/10.1016/j.jpba.2016.12.024)
 45. Padhan N, Nordling TE, Sundstrom M, Akerud P, Birgisson H, Nygren P, Nelander S, Claesson-Welsh L (2016) High sensitivity isoelectric focusing to establish a signaling biomarker for the diagnosis of human colorectal cancer. *BMC Cancer* 16(1):683. doi:[10.1186/s12885-016-2725-z](https://doi.org/10.1186/s12885-016-2725-z)
 46. Halbgebauer S, Haussmann U, Klafki H, Tumani H, Wiltfang J, Otto M (2015) Capillary isoelectric focusing immunoassay as a new nanoscale approach for the detection of oligoclonal bands. *Electrophoresis* 36(2):355–362. doi:[10.1002/elps.201400339](https://doi.org/10.1002/elps.201400339)
 47. Kovacs GG (2016) Molecular pathological classification of neurodegenerative diseases: turning towards precision medicine. *Int J Mol Sci* 17(2). doi:[10.3390/ijms17020189](https://doi.org/10.3390/ijms17020189)