A guide to the cerebrospinal fluid test panel for sporadic Creutzfeldt-Jakob Disease

Preamble

This guide provides a brief description of the assays, and suggestions for interpretation of results. For further information, please contact the Prion Diseases Section at 204-789-6083 or phac.cjd.aspc@canada.ca.

Background

Sub-acute encephalopathies, including Creutzfeldt-Jakob disease (CJD), constitute a large and heterogeneous group of brain diseases that can be diagnostically challenging[1]. Among these diseases CJD has a special urgency, as this disease can be transmitted between individuals under certain circumstances by an infection-like mechanism. Additional diagnostic difficulties arise due to the fact that the infectious agents are not conventional viruses or bacteria, but rather are composed of a disease-associated, misfolded isoform (PrPd) of the host prion protein (PrPc). This key biological fact precludes the use of conventional technologies, such as PCR and serology that are commonly applied to the direct detection of other infectious agents.

The diagnostic approach for the most common form of CJD, sporadic CJD (sCJD), has to date relied upon a standardized clinical presentation and supporting investigations such as the presence of increased amounts of indirect protein markers of disease found in patients' cerebrospinal fluid (CSF).The most commonly used markers are 14-3-3[2] and microtubule-associated tau[3]. Although neither of these indirect markers is fully diagnostic, both have useful sensitivities and specificities for sCJD[4]. Recent advances in technology have allowed for the elaboration of a new test, known as real-time quaking-induced conversion (RT-QuIC)[5]. To allow for robust clinical testing, the Prion Diseases Section developed a version of this new test called end-point quaking-induced conversion (EP-QuIC)[6]. By contrast with the previous approaches which focused on indirect markers, EP-QuIC allows the detection of the pathogenic protein more directly. EP-QuIC performance was demonstrated as equivalent to RT-QuIC in an international ring trial[7].

Test Availability

The Public Health Agency of Canada's Prion Diseases Section offers a CSF test panel consisting of immunoassays for 14-3-3 and tau proteins as well as detection of the disease-associated form of the prion protein by EP-QuIC. All three assays are accredited under Can-P4E (ISO/IEC 17025).

Principles of the Assays

The 14-3-3 and tau assays are conducted using commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits to detect 14-3-3 (CycLex Co.) and tau (Fujirebio). In a sandwich ELISA, the protein antigen to be measured is bound between the capture antibody (bound to the plate) and the detection antibody (linked to an enzyme). Addition of the

enzyme substrate results in the production of a coloured end product. The concentration of the coloured end product correlates, within a characteristic linear range, with the concentration of the target protein present in the original sample.

The EP-QuIC test exploits the natural ability of the disease-associated, misfolded isoform, PrPd, to induce conversion of the normal cellular form of the prion protein (PrPc) into a misfolded form *in vivo*. In EP-QuIC, the CSF samples of patients with suspected CJD are added to wells containing recombinant PrP (rPrP). The mixture is incubated at 42°C with intermittent shaking over a period of 66 hours. Patient CSF containing sufficient PrPd will convert the rPrP into a misfolded form. The resulting insoluble rPrP aggregates generated by this molecular templating process then bind a fluorescent dye (thioflavin T), causing a change in the dye's fluorescence emission spectrum that can be measured with a spectrofluorometer at the outset and completion of the assay.

Test Performance

Definitive diagnoses require pathological examination of brain tissue post-mortem. The EP-QuIC performance characteristics were estimated in a prospective study population of 186 Canadian CSF samples submitted to the PDS for testing. In all cases sCJD was included in the differential diagnosis and subsequently definitive diagnosis was available.

Using a study-specific intermediate optimal cut-off of 976 pg/mL, the diagnostic sensitivity and specificity of the tau ELISA test in this cohort of patients were 93% and 84%, respectively. Comparable diagnostic accuracy estimates have been obtained for this marker in other studies[8].

The performance characteristics of the 14-3-3 ELISA kit were estimated on the same prospectively recruited patients described above. Using 20,000 arbitrary units per mL (AU/mL; 1 AU ≈ 1 pg/mL) as the cut-off for a positive test, sensitivity and specificity estimates of 82% and 90% respectively were obtained. Despite differences in the composition of the study populations, similar results were obtained in a retrospective study conducted by an international consortium[9].

The same 186 prospectively recruited Canadian CSF samples were used to estimate the performance characteristics of the EP-QuIC assay. An individual well of a 96 well plate (3 replicate wells run for each sample) was scored as positive when the final fluorescent signal was determined to have increased at least 4-fold relative to its initial fluorescent signal. A CSF sample was considered positive when at least 2 of the 3 assay replicates for a sample were positive. In instances where 1 of the 3 assay replicates for a sample was positive, the sample was reanalysed. Samples consistently exhibiting 1 of the 3 assay replicate wells as positive were scored as indeterminate. The one sample in this cohort scored as indeterminate by the QuIC assay was definitively diagnosed as frontotemporal dementia. The same sample resulted in positive ELISA tests for 14-3-3 and tau. In the table below, this sample has been

scored as a false positive for all three tests. The diagnostic sensitivity and specificity of the EP-QuIC test for this set of samples were 96% and 99% respectively. These results indicate that in a clinical setting the PDS is achieving the expected level of performance for the QuIC assay[7, 10].

	CJD		non-CJD		Sensitivity	Specificity
	TP (a)	FN (b)	FP (c)	TN (d)	a/(a+b)	d/(c+d)
EP-QuIC	43	2	2	139	96%	99%
					85% to 99%	96% to 100%
14-3-3	37	8	14	127	82%	90%
					85% to 95%	68% to 92%
Tau	42	3	23	118	93%	84%
					82% to 99%	77% to 90%

	Positive Predictive Value	Negative Predictive Value
	a/(a+c)	d/(b+d)
EP-QuIC	96%	99%
	86% to 100%	95% to 100%
14-3-3	73%	94%
	63% to 83%	89% to 97%
Tau	65%	98%
	56% to 74%	93% to 99%

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