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An automated alert system based on the p-Tau/Tau ratio to quickly inform health professionals upon a suspected case of sporadic Creutzfeldt-Jakob disease.

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Abstract

Introduction

Knowing the risk of potential sCJD instrument-contamination is essential in hospitals. We examined the relevance of the p-Tau/Tau ratio to exclude a probable case of sCJD in clinical practice, and we established an alert system to quickly inform health professionals in case of positivity.

Methods

This retrospective study was conducted on 143 CSF samples from patients suspected for sCJD. The distinction between probable cases of sCJD and other patients was based on clinical, paraclinical and biological (14-3-3, Tau, p-Tau, A β ₁₋₄₂) data. From this experience, the health professionals developed an alert system to be implemented upon a suspected case of sCJD.

Results

A significant decrease in p-Tau/Tau ratio between sCJD and the other diseases was observed ($p < 0.001$). The combined Tau test presented a sensitivity higher than 14-3-3 (100% versus 92.3%, $p = 0.006$) and an equivalent specificity (90% versus 96.1%). The time required for obtaining results was higher for 14-3-3 due to the centralization of investigations in some laboratories (3 weeks versus 2 hours). In the presence of these elements, the triggering of the alert system was based on the p-Tau/Tau ratio. This system involves sending an automatic mail to the hospital department involved in the patient's care and the hospital hygiene team, which oversees the application of the procedures.

Conclusion

The p-Tau/Tau concentrations present the desired criteria for use in current medical practice to fight against iatrogenic transmission. The alert system confirms a probable case of sCJD instantly to health professionals. Hygiene and sterilization measures can be applied immediately.

1. Introduction

Creutzfeldt-Jakob disease (CJD) is characterized by rapid neurodegeneration leading to death in a few months to a few years. CJD belongs to the category of prion diseases, also known as transmissible subacute spongiform encephalopathies. These rare neurodegenerative diseases are characterized by the accumulation in the central nervous system (CNS) of the prion protein, at the origin of the transmission of the disease [1]. CJD can be of genetic, infectious or sporadic origin. The latter case represents 85% of all CJD diagnosed each year [2] and its median survival is only 6 months [3].

The diagnosis of sCJD is based on clinical signs suggestive of recent onset and a rapid and progressive evolution (fast worsening dementia, myoclonus, vision or cerebellar disorders, pyramidal/extrapyramidal disorders, akinetic mutism). However, this diagnosis may not be possible early in the illness based on diagnostic surveillance criteria. Indeed, heterogeneity of symptoms, particularly in the early in the course of the disease, can be associated with another progressive, rapidly irreversible form of dementia. Diagnosis should be supported by electroencephalogram (EEG), magnetic resonance imaging (MRI) or biomarker testing in cerebrospinal fluid (CSF) [4–6]. The definitive diagnosis of CJD is based on post-mortem neuropathological examination of the brain with positive identification of pathological prion protein [7].

The 14-3-3 protein is widely used as a surrogate marker for the premortal diagnosis of sCJD [8–10]. This biomarker has been included in the diagnostic criteria of sCJD by the World Health Organization (WHO) [6]. Neuronal lysis observed during sCJD is associated with the leakage of this protein into the CSF [8]. The investigation of the 14-3-3 protein being carried

out in a few specialized laboratories spread over the territory, the time required for the obtaining of results is several weeks.

Updated criteria for prion diseases suggest RT-QuIC (Real-Time Quaking-Induced Conversion) [2]. The performance of this technique was compared to the performance of the 14-3-3 protein detection. RT-QuIC is as sensitive as 14-3-3 test but its undeniable advantage is its excellent specificity which can be considered at 100%, while 14-3-3 protein is only a lysis marker [11]. However, in addition to the requirement of specialised equipment and a significant cost, this reference technique presents three major disadvantages in general hospitals. First, this technique is based on amplification of the supposed pathogen. Therefore, RT-QuIC must be performed in a P3 laboratory in France [12]. Secondly, this experimentation used recombinant prion protein. Its quality is essential and is a condition to the success of the experimentation. Thirdly, RT-QuIC analysis takes time (36h in the optimized versions) and is in plate (serial analysis).

Another alternative to the 14-3-3 protein for a diagnosis of probable sCJD is the quantification in the CSF of Tau protein and its phosphorylated form (p-Tau) [13]. Unlike 14-3-3 protein, Tau protein is increased at the onset of clinical signs [9,10]. Its concentration is correlated with the intensity of neuronal degeneration [14]. A threshold concentration to guide the diagnosis to sCJD was defined as between 1200 and 1300 ng/L [9,15,16]. An increase of Tau protein, at a lower concentration, is also observed in Alzheimer's disease [17]. In contrast, a significant increase in p-Tau concentration is characteristic of Alzheimer's disease (> 60 pg/ml) due to an imbalance in cell kinases and phosphatases [10]. Nevertheless, moderate increases in p-Tau concentrations have also been detected in the CSF of sCJD cases compared to other types of dementia [18,19]. Overall, a p-Tau/Tau ratio lower than 0.0713

allows the distinguishing of sCJD from other neurological disorders [20]. The combination of increased Tau protein concentrations and decreased p-Tau/Tau ratio in patients with sCJD has high specificity and is a useful diagnostic test for sCJD [16]. The combined Tau test, i.e. Tau and p-Tau protein assays, offers the advantage of being able to be performed by automated methods in many laboratories [15]. Every day in healthcare facilities, patients with suggestive symptomatology of sCJD are hospitalized. It is essential to be able to provide a probable diagnosis of sCJDs in a very short time in suspected patients in order to put in place safety procedures to avoid the risk of iatrogenic transmission of the prion, especially during invasive procedures [21].

In this context, the objective of our study was to assess the relevance of the p-Tau/Tau ratio for the diagnosis of sCJD in routine hospital practice and to establish an alert system to quickly inform health professionals in case of positivity in order to immediately apply hygiene and sterilization measures to avoid transmission.

2. Materials and Methods

2.1 Study population

This retrospective study was conducted on 143 CSF samples collected between 2011 and 2019 as part of routine care in patients suspected for sCJD. The samples were analysed by our institution's hormonology Laboratory. For each patient, the presence of protein 14-3-3 was investigated and the proteins Tau, p-Tau as well as the amyloid peptide A β ₁₋₄₂ were measured in the CSF. The study population included 2 confirmed cases by post-mortem examination and 11 probable cases of sCJD. According to the European MRI-CJD Consortium criteria

[22], a patient is considered likely to have sCJD if at least 2 clinical criteria and 1 paraclinical criterion on the following list are present:

- Clinical signs: rapidly progressive dementia, myoclonus, cerebellar or visual disturbance, pyramidal or extrapyramidal dysfunction, akinetic mutism
- Tests:
 - o EEG: periodic sharp wave complexes
 - o 14.3.3 detection in CSF (in patients with a disease duration of less than 2 years)
 - o MRI: high signal abnormalities in caudate nucleus and putamen or at least two cortical regions (temporal-parietal-occipital) either on DWI or FLAIR
- Disease duration <2 years

The distinction between probable cases of sCJD (n=13) and other suspected patients (control group) was the subject of multidisciplinary expertise (neurologists, psychiatrists, geriatricians and biologists) (Fig. 1). Of the 130 patients in the control group, four subpopulations were distinguished based on clinical, paraclinical and biological data: Alzheimer's disease group (AD) (n=23), frontotemporal degeneration group (FTD) (n=22), Lewy body dementia group (LBD) (n=8), other neurological pathologies group (n=77, including in particular stroke, Parkinson's disease and toxic-metabolic disorders).

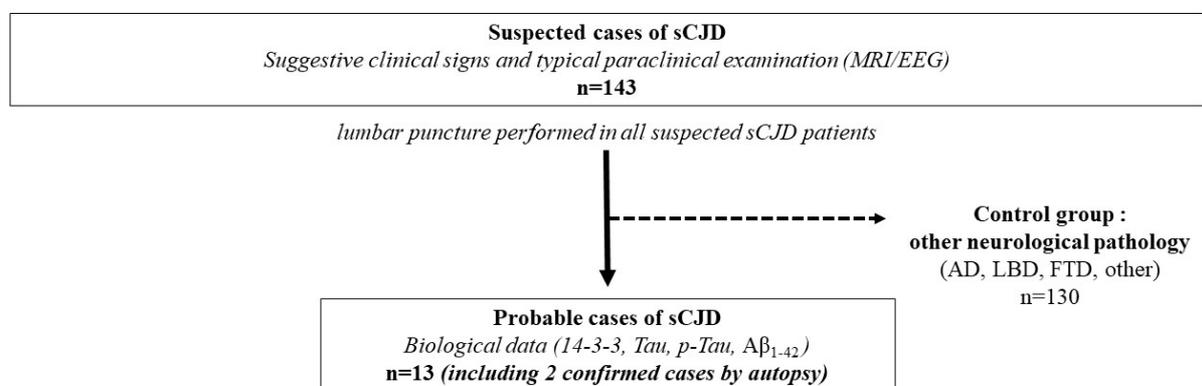


Figure 1: Patient distribution included in the study. The figure represents the stratification of patients included in the study, suspected/probable/confirmed case of sporadic Creutzfeldt-Jakob disease (sCJD), according to clinical signs, paraclinical examinations and biological data. *In the absence of the patient's and/or his family's agreement, the other 11 probable cases did not receive an autopsy. AD: Alzheimer's disease, EEG: electroencephalogram, FTD: fronto-temporal degeneration, LBD: Lewy body dementia, MRI: magnetic resonance imaging

2.2 Biomarker analysis in CSF

Lumbar punctures were performed according to the standardized procedure for routine diagnosis. The CSF samples were collected into polypropylene test tubes (Sarstedt) and centrifuged (4000g, 10min, 4°C). The supernatants were aliquoted into polypropylene test tubes (Sorenson Bioscience) and frozen (-80°C) within 2 hours of puncture. Western blot immunoassay for protein 14-3-3 was performed in the Neurochemistry Unit, Hospices Civils de Lyon according to the protocol previously described [23]. Positive and negative CSF control samples with trace test results were used in all experiments as internal quality control. Biomarker analyses (Tau, p-Tau, A β ₁₋₄₂) were performed in our hospital with commercially available ELISA kits (Innotest hTau Ag, Innotest phospho-Tau₁₈₁, Innotest β -amyloid₁₋₄₂, Fujirebio®, Belgium) according to the manufacturer's instructions. Optical densities were measured by a photometer (Appliskan®, ThermoFisher Scientific, Germany). Each analysis was performed in duplicate. Longitudinal stability in the biomarker measurements was monitored, including controls at the beginning and end of each measurement series. Inter-assay coefficients of variation were 7.6% for Tau, 5.4% for p-Tau and 8.7% for A β ₁₋₄₂. All samples were obtained and analysed as part of routine care and in accordance with French legislation.

2.3 Development and implementation of an alert system to inform health professionals

Multidisciplinary meetings involving the biologists, neurologists, hygienists and the information technology department of our institution took place in order to determine the computer programs to be used and the data transmission circuit depending on the result of the ratio p-Tau/Tau (i.e, higher or lower than 0.0713). The role of each health professional of the circuit was clearly identified. Trials with fictitious patients (hospitalized in geriatrics or neurology, with ratio p-Tau/Tau $>$ or \leq to 0.0713) were carried out several times to verify this computer alert system. It was an essential preliminary step before its application in medical practice.

2.4 Data analysis

The results were analyzed using the GraphPad Prism v.7 software, California, USA. Comparisons were adjusted for age and sex with a logistic regression. The Benjamini-Hochberg post-test was applied following the Kruskal-Wallis test in the event of significant differences between the groups. The sensitivity, specificity, positive and negative predictive values of the tests were compared with the Fisher's exact test.

3. Results

3.1.1 Diagnosis of sCJD in hospital practice

The demographic characteristics of the study population (median age 71 years old, sex 50/50) and CSF biomarker data are described in Table 1.

	n	Age (median in years)	Sex (F/M)	Tau (ng/L) [#]	p-Tau (ng/L) [#]	ratio p- Tau/Tau [#]	A β ₁₋₄₂ (ng/L) [#]	14-3-3 protein (P-T-N)
Dementia by transmissible agents (sCJD)	13	74	5/8	7175 (4879-10910)	68 (54-77)	0.0089 (0.0067-0.0138)	922 (685-1434)	12-0-1
Alzheimer's disease (AD)	23	68	13/10	774 (468-1339)	87 (69-138)	0.1272 (0.0965-0.1508)	581 (431-676)	0-1-22
Fronto- temporal degeneration (FTD)	22	66	14/8	342 (176-527)	51 (31-55)	0.1505 (0.1015-0.2033)	915 (591-1167)	0-1-21
Lewy body dementia (LBD)	8	83	1/7	410 (268-450)	55 (40-63)	0.1453 (0.1114-0.1641)	711 (516-840)	0-0-8
Other neurological pathologies	77	70	38/39	265 (168-429)	39 (32-55)	0.1520 (0.1070-0.1918)	876 (565-1156)	1-2-74

Table 1: Demographic characteristics of the study population and corresponding cerebral spinal fluid biomarker data. For each disease represented in the population, the total number of cases, the demographic characteristics (age, sex) and the corresponding cerebral spinal fluid biomarker data (Tau, p-Tau, A β ₁₋₄₂ concentrations, p-Tau/Tau ratio, 14-3-3 protein) are reported. [#]median (25% - 75% percentile). *F: female, M: male, N: negative, P: positive, T: trace.*

Tau protein concentration was higher in sCJD than in AD, LBD, FTD and ‘other disorders’ groups but not significantly different after adjusting for age and sex. A significant increase in Tau protein concentration was observed in AD group *versus* LBD, FTD and ‘other disorders’ groups (Fig. 2a). Concerning p-Tau protein concentration, a significant increase was observed in AD group *versus* FTD and other neurological disorders groups. p-Tau protein concentration was also higher in sCJD group compared to all the other groups (Fig. 2b). There was a significant decrease ($p < 0.001$) in p-Tau/Tau ratio values between sCJD and all the other groups (Fig. 2c). Concerning $A\beta_{1-42}$ concentration, a significant decrease was observed in AD group *versus* sCJD, FTD and other neurological disorders (Fig. 2d). The 14-3-3 protein was positive in 13 patients (12 sCJD and 1 other neurological pathology) and doubtful in 4 cases (1 AD, 1 FTD, 2 other neurological pathologies).

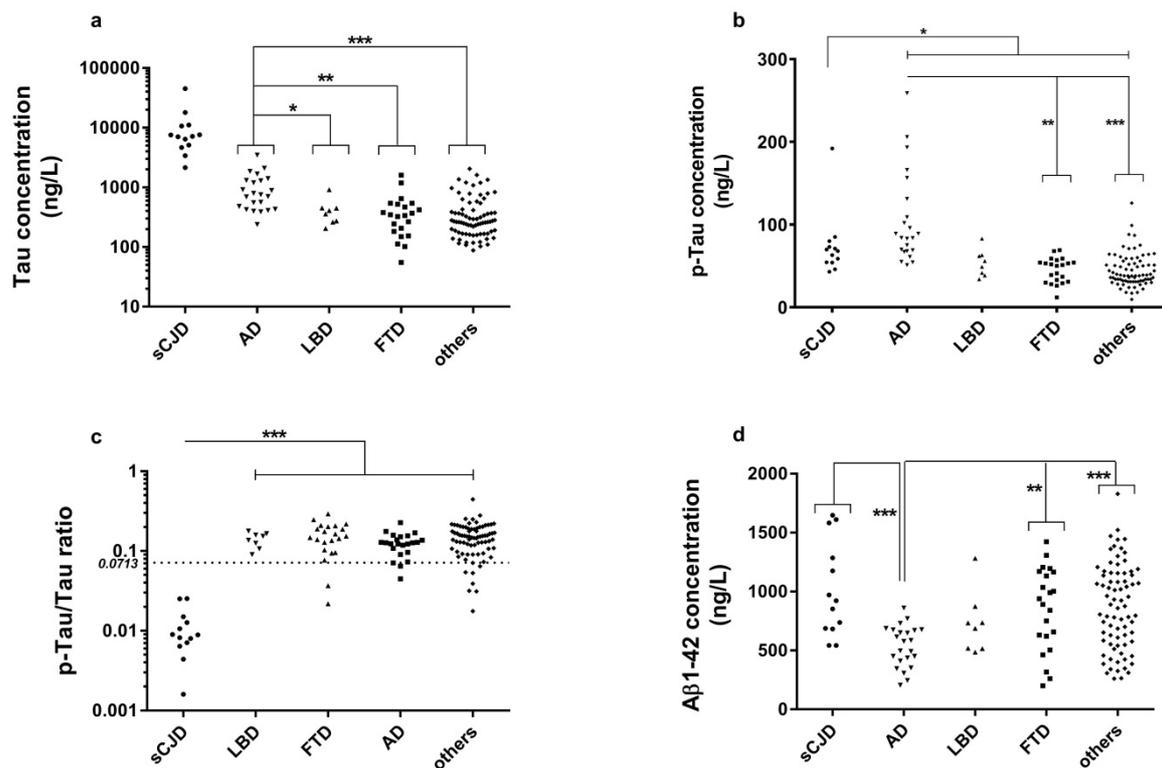


Figure 2: Cerebrospinal fluid biomarkers in subpopulations of patients. Comparative analysis of CSF-Tau concentration (a), p-Tau concentration (b), p-Tau/Tau ratio (c) and $A\beta_{1-42}$ concentrations (d) in sporadic Creutzfeldt-Jakob disease (sCJD), Alzheimer's disease

(AD), Lewy body dementia (LBD), fronto-temporal degeneration (FTD) and other neurological diseases group. For these comparisons, age and sex were included as covariate. Representation in log-scale for figures a and c. For p-Tau/Tau group, the broken line represents the normal cut-off (i.e., 0.0713). *p<0.05; ** p<0.01; ***p<0.001

The clinical characteristics of patients with sCJD and the main results of the studies are shown in Table 2.

F/M	Age (years)	EEG	MRI	Time from first symptoms to lumbar puncture (weeks)	Time from first symptoms to death (weeks)	p-Tau (ng/L)	Tau (ng/L)	ratio p-Tau/Tau	14-3-3	Time from lumbar puncture to results of the protein 14-3-3 test (days)
M	72	T	T	9	16	59	7175	0.0082	P	12
M	78	NT	CI	17	20	70	4648	0.015	P	12
M	74	T	T	3	10	43	3399	0.0127	P	9
M	83	NT	NT	3	7	71	11131	0.0064	P	11
M	80	T	T	6	10	192	7617	0.0252	P	10
M	68	T	T	4	10	85	10689	0.008	P	20
F	77	T	T	22	37	63	7072	0.0089	P	13
M	74	NT	NT	10	17	54	7574	0.0071	N	12
F	74	NT	T	2	5	80	18050	0.0044	P	28
F	86	D	NR	5	10	68	6430	0.0106	P	31
F	64	T	T	5	10	46	5109	0.009	P	17
M	83	T	D	6	8	73	45021	0.0016	P	15
F	48	T	T	7	8	55	2150	0.0253	P	54

Table 2: Clinical features of sporadic Creutzfeldt-Jakob disease patients and principal investigation results. * *CI*, contra indication, *D*: doubtful, *F*: female, *M*: male, *N*: negative, *NT*: not typical, *NR*: no realized, *P*: positive, *T*: typical

Among these patients, the diagnosis of sCJD was confirmed by post mortem anatomopathological examination in two cases. These did not present typical paraclinical examination (EEG/MRI atypical for one case, EEG typical but MRI doubtful in the other case). A typical EEG and MRI were both observed in half of the cases. The average time from first symptoms to lumbar puncture and sCJD patient's death were respectively 8 (\pm 3) and 13 (\pm 6) weeks. Tau concentrations were dramatically increased for all probable cases of sCJD with an average at 10 467 ng/L (normal cut-off is 500 ng/L) since the first symptoms. Similarly, p-Tau/Tau ratio was decreased in these patients with an average at 0.0110 (cut-off is 0.0713). The average time from lumbar puncture to results of the 14-3-3 immunoassay on CSF was about 3 weeks (19 days). In the context of our study, when the 14-3-3 test was doubtful, we considered that a case of sCJD could not be excluded. Hence, a doubtful test result was considered as a positive result. The sensitivity of the p-Tau/Tau test was significantly higher ($p=0.006$) than the 14-3-3 test (100% versus 92.3%). However, the positive predictive value of the combined Tau test was significantly lower ($p<0.0001$) than the 14-3-3 test (50% versus 70.6%) No statistical difference was observed for specificity (90% versus 96.1%), and negative predictive value (100% versus 99.2%) between these two tests (Table 3, Table 4).

a	Combined TAU test	
	Positive (≤ 0.0713)	Negative (> 0.0713)
Probable cases of sCJD	13	0
Other neurological pathology	13	117

b	14-3-3 test	
	Positive	Negative
Probable cases of sCJD	12	1
Other neurological pathology	5	125

Table 3: Contingency tables with the values of true positives, true negatives, false positives, false negatives for combined Tau test (a) and 14-3-3 test (b). The 14-3-3 protein test was doubtful in 4 cases.

	Combined Tau test (%) $p\text{-Tau/Tau ratio} \leq 0.0713$	14-3-3 test (%)	p-value
Sensitivity	100	92.3	**
Specificity	90	96.1	NS
Positive predictive value	50	70.6	***
Negative predictive value	100	99.2	NS

NS: not significant, ** $p < 0.01$, *** $p < 0.001$

Table 4: Sensitivity, specificity and predictive values for the 14-3-3 and combined Tau tests.

3.2 An alert system to save time in setting up safety procedures (Fig. 3)

Work collaboration led to the hospital of a warning system aimed at alerting the hospital hygiene unit as soon as possible about the confirmation of a probable sCJD case. Hence, in order to save time in setting up security procedures, an alert email is automatically sent to the hospital hygiene team and the care service if a suspected patient of sCJD has a p-Tau/Tau ratio lower than the minimum threshold for the diagnosis of CJD. These alert emails mention the patient identification number and the ratio p-Tau/Tau value. This information is directly

extracted from the computerized patient record that includes the biological data entered by the biochemistry laboratory and is sent by automatic email from the laboratory computer system (Millenium® Cerner) to the hospital hygiene unit. On receipt of the alert, the hospital hygiene unit contacts the care service where the patient is hospitalized, then the teams of clinicians proceed with the declaration of the disease to the competent authorities. Any suspicion requires an approach to ascertain that previously the patient has not undergone any examination or intervention likely to involve a risk for another patient, including any invasive acts during the 6 months preceding the onset of symptoms. Other actions are carried out in the event of a probable case of sCJD: (i) Manage the patient with Standard Hygiene Precautions. (ii) Remind healthcare teams that the use of single-use medical devices is to be preferred. (iii) At the same time, the hospital hygiene unit, in collaboration with the pharmacy (sterilization department), ensures the management of any medical devices used and the adaptation of the treatment procedures involving equipment used if further investigations are to be carried out. When prescribing, the prescriber reports the suspicion of sCJD so as to adapt the cleaning of medical devices to a prionocidal protocol and to limit acts according to benefit/risk assessment. (iiii) Schedule an autopsy to confirm the diagnosis.

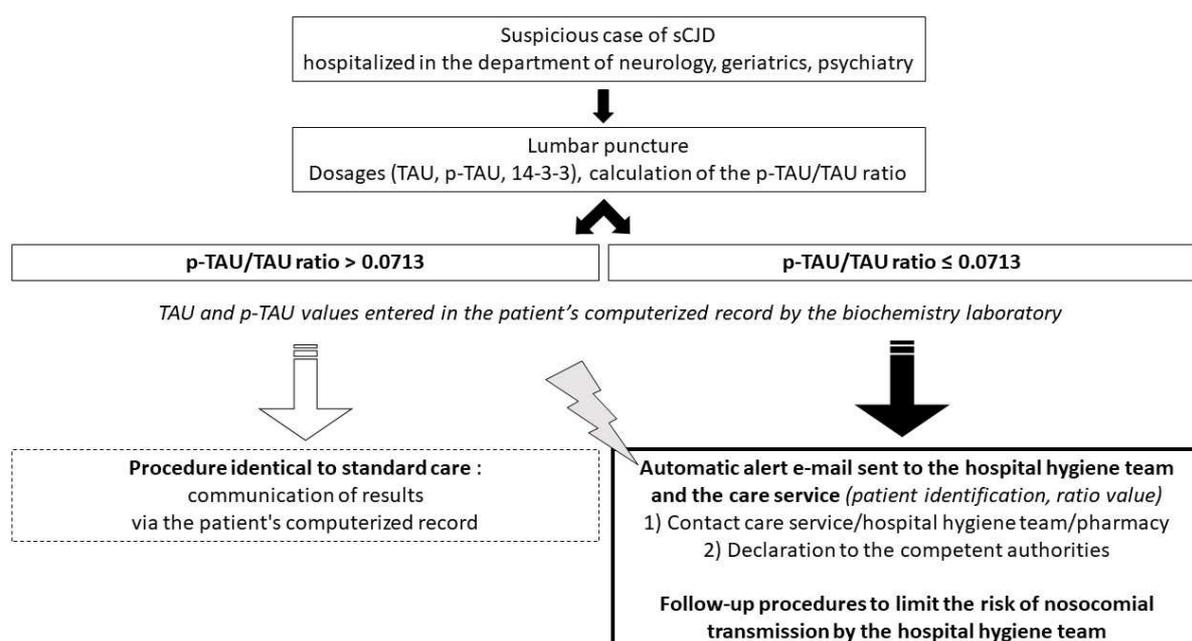


Figure 3: An alert system for probable sporadic Creutzfeldt-Jakob disease case (sCJD). Description of an early warning system based on p-Tau/Tau ratio values to save time in setting up CJD security procedures in the event of probable sCJD.

4. Conclusion

In this study, we aimed to implement an alert system to quickly and automatically inform health professionals in cases of positive probability diagnosis for sCJD in order to avoid iatrogenic transmission. With this objective in mind, we provide an investigation into the relevance of CSF biomarkers in hospital practice to quickly diagnose a probable case of sCJD. One of the study's strengths is the heterogeneity of the neurological diseases represented in our cohort study (especially, acute neurological disorders). Ethically, lumbar punctures are only performed if clinical signs suggest a neurological disorder. Hence, unlike a comparison “sCJD versus no neurological pathology”, our study cohort is representative of clinical practice, thus limiting bias.

Knowing the risk of sCJD before proceeding with invasive investigations or surgical interventions, and thus being able to implement infection control measures with single use equipment, or using prionocidal protocol medical device disinfection, is essential in healthcare facilities. The probability diagnosis of sCJD remains challenging because symptoms and paraclinical examinations only become suggestive after a certain time in the course of the disease [24,25]. Reliable and early biomarkers for diagnosis of sCJD would be very useful in medical practice.

The 14-3-3 protein is the reference biological marker for the probability diagnosis of sCJD according to the WHO [6]. The duration of the disease influences its sensitivity [10,26]. Neuronal lesions in other conditions, such as encephalitis, AD and other dementia disorders can produce false positive results [27,28]. Traumatic lumbar puncture can also lead to false positive since the 14-3-3 protein is present in large amounts in red blood cells [29]. A combination of elevated Tau protein concentration with the presence of 14-3-3 protein in CSF gives considerable specificity [13]. Hence, several research teams were interested in the value of the p-Tau/Tau ratio to discriminate sCJD from other neurological disorders. They highlighted a p-Tau/Tau sensitivity higher than 14-3-3 test (94% versus 44-95%) [13,25,30] and confirmed the robustness of this surrogate biomarker in sCJD reflecting the intensity of neuronal damage [20,31,32].

In our study, the p-Tau/Tau ratio test presented a higher sensitivity than 14-3-3. This result confirms the data published in previous studies [13,25,30]. Remarkably, although not significantly, the negative predictive value of the combined Tau test was higher than that of the 14-3-3 test, which is a considerable asset in limiting the risk of wrongly excluding a probable case of sCJD. Hence, this data attests that the use of the p-Tau/Tau ratio is clinically very relevant in confirming a probable case of sCJD. However, it should be noted that these encouraging values can be influenced in part by the small size of our cohort. In addition, the p-Tau/Tau ratio tends to be lower in the early stages of the disease, when the risk of doubtful paraclinical examinations is highest. Interestingly, one sCJD patient without typical EEG/IRM and a negative 14-3-3 protein 10 weeks after the first symptoms, but a ratio p-Tau/Tau positive, died after 17 weeks of disease progression. In this case, the patient's clinical evolution probably confirms the diagnosis of sCJD but a post-mortem diagnosis would have been helpful to confirm or deny with certainty the presence of this disease. Unfortunately,

autopsy is often refused by the family. Another advantage of the p-Tau/Tau test is the possibility of carrying it out using automated chemiluminescent rapid methods and using commercial immunoassays, which are standardized and used worldwide in many laboratories in clinical routine because of the use of these biomarkers for the diagnosis of Alzheimer's disease [31]. The duration of the analysis is a couple of minutes guaranteeing the possibility to inform the clinicians in less than 2 hours. In our study, the time from lumbar puncture to the return of the 14-3-3 test results was approximately 3 weeks. This delay is incompatible with clinical practice. The clinical evolution of the patient, or even his death, make the diagnosis highly probable before obtaining the result. It is worth noting the total time necessary to obtain the 14-3-3 test result, which includes: first, the time necessary to send the CSF sample to the reference laboratory, then, the time necessary to perform the analysis. In France, as elsewhere in Europe, the detection of the 14-3-3 protein for diagnostic purposes is centralized in reference laboratories. These prion disease surveillance centers belong to the national CJD surveillance network which is integrated into the European surveillance network. Detection of 14-3-3 protein is usually performed by using western blot. The use of ELISA kits would probably save time compared to the western blot. However, in France, the ELISA kits for the detection of the 14-3-3 protein are reserved for research only. These kits do not have the CE marking which is mandatory for *in vitro* diagnostic devices used in medical practice. Moreover, a sufficiently large number of CSF samples is necessary to perform a series of analyses. CJD being a rare disease, the risk linked to ELISA is the freezing of the CSF for a long time and ELISA analysis on a small series is not financially attractive. For the determination of p-Tau/Tau, automatic analysers are present in hospitals (e.g. Lumipulse analysers from Fujirebio® or Cobas from Roche®). Thus, the measurement of these proteins takes place simultaneously which saves time. The cost of the p-Tau/Tau ratio

test and the 14-3-3 test is roughly equivalent in France (108 euros versus 94.5 euros, respectively).

Altogether, our data confirms the idea that CSF p-Tau/Tau tests are robust and probably early sCJD biomarkers, which opens new perspectives on diagnosis and rapid patient management. However, these results remain to be confirmed. Due to the focus on a specific hospital, the sample size is limited and lacks strength.

Taking into account the advanced elements, it would seem interesting that the WHO recommendations include in the future the dosage of the Tau ratio along with the detection of the 14-3-3 protein. Overall, we confirmed the relevant use of the p-Tau/Tau ratio in clinical practice to exclude probable cases of sCJD. This sporadic form is associated with a rapid evolution and an extraordinarily high neural lysis, which generates very high Tau protein concentrations in the CSF and consequently a low p-Tau/Tau ratio (ie, ≤ 0.0713) [33]. This threshold allows us to obtain excellent performance in our study. However, depending on the disease subtype whose progression is slower or different, it is possible that this ratio may be lower, potentially leading to false negatives. It is therefore still important to have several markers to validate the diagnosis [3,33]. One of the main objectives of our study was to examine the relevance of the p-Tau/Tau ratio to provide the first hint of a sCJD disease suspicion. The use of this ratio is not intended to establish with certainty the diagnosis of sCJD but it aims to warn health professionals that a case of sCJD cannot be excluded. Whatever the value of the Tau ratio, all paraclinical and biological examinations were performed (protein 14-3-3, MRI, EEG ...) to limit a false diagnosis. The interpretation of MRI in most European hospitals is not a coded or an encrypted data in the computerized patient record and therefore it cannot be taken into account by artificial intelligence, and more

specifically, by our alert system. In addition, given the waiting times in hospitals, the MRI examination generally takes place after the dosage of the Tau and p-Tau proteins.

Given the rapid evolution of the disease from the onset of the first symptoms, it's essential that the coordination of health professionals and social care be put in place as soon as probable diagnosis of sCJD is made. Hygiene and sterilization measures must be applied immediately to prevent transmission by contaminated human tissues (e.g., corneal graft, dura mater). Thus, we wanted to develop an alert system based on the combined Tau test to aid early diagnosis.

The risk of iatrogenic CJD according to recent direct evidence appears to be low. However, the long asymptomatic incubation periods (20 years and more in some cases), the difficulties of eradicating prions from surgical instruments and the high levels of infectivity of CJD in the brain mean that there is a margin of uncertainty around quantifying the risk of iatrogenic transmission of CJD [34]. Although no studies have identified a new case over the past few years, many case reports reported a relationship between CJD and a surgical intervention [34–37]. Hence, potential CJD instrument-contamination events may continue to occur, causing widespread hospital and patient concern. The current decision problem focuses on the risk of transmission of CJD via surgical instruments. Iatrogenic transmission can occur when surgical instruments, endoscopes or laryngoscopes are used during high-risk surgical procedures. Conventional cleaning and sterilization techniques applied in hospitals to surgical instruments are not sufficient to completely deactivate prions. The early and automatic alert described in this article allows these complementary techniques to be implemented without delay and only in cases where the risk is proven. Complementary techniques and sequestration of this equipment is necessary before re-use.

Any clinical suspicion of sCJD should be reported to the hospital hygiene team and the completion of any invasive procedure should be preceded by a benefit/risk assessment. According to French guidelines, in the event of positivity, single-use medical devices (or medical devices with single-use protection) should be favoured if they are compatible with safe and effective procedures for the patient. Failing this, reusable medical devices must be double-cleaned manually, inactivated and sequestered [21] (Canada, France, Germany, Switzerland) or incinerated (Australia, New-Zealand, UK, USA) [38]. Decontamination procedures may differ between European countries [39]. The prevention of prion transmission also involves the protection of medical staff in some specific circumstances (e.g., an accident involving exposure to tissues or biological fluids with a medical device that has been used and has not yet undergone the predisinfection and inactivation procedure). In addition, any probably sCJD case requires an approach whose purpose is to ensure that, previously, the patient has not undergone examination or intervention that may cause a risk for other patients. Finally, when dealing with a pathology of such gravity (poor prognosis in the short term after the first clinical symptoms), medico-social coordination is essential. It is necessary to take special care, including optimal organization of nursing and paramedical care, assistance with daily life, psychological support including that of relatives, pain management and palliative care [40]. To date, no similar alert system has been described in the literature. We have not been able to assess the benefits associated with it, especially because of the low incidence of sCJD.

Pending validation and availability of an easy-to-use etiologic biomarker for sCJD, the alert system developed can strongly and rapidly indicate to health professionals, with a high level of specificity and sensitivity, a probable diagnosis of sCJD, thus allowing them to set up infection control measures with adequate reprocessing of medical devices and thereby limit

iatrogenic transmission. The Tau and p-Tau protein concentrations in CSF present the desired criteria for use in current medical practice. The simplicity and speed of these dosages are valuable assets in fighting against healthcare-related infections.

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Declaration of interest

None

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