Universitat de Girona
Facultat de Medicina

Diagnostic accuracy of t-tau and tau ratio combined with neurofilaments for the early diagnosis of sporadic Creutzfeldt-Jakob disease

A multicentric analytical cross-sectional study

FINAL DEGREE PROJECT

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INDEX

1. ABSTRACT

TITLE: Diagnostic accuracy of t-tau and tau ratio combined with neurofilaments for the early diagnosis of sporadic Creutzfeldt-Jakob disease.

BACKGROUND: Sporadic Creutzfeldt-Jakob disease (sCJD) is a fatal progressive neurodegenerative prion disease that affects the central nervous system. Although it has an heterogeneous clinical presentation, the principal and most frequent clinical features are progressive dementia, myoclonus or cerebellar manifestations as ataxia or nystagmus. It has a challenging diagnostic as it requires clinical compatibility with a positive result of another diagnostic tests such as real-time quaking-induced conversion (RT-QuIC) test, 14-3-3 protein cerebrospinal fluid (CSF) determination, magnetic resonance imaging (MRI) or electroencephalogram (EEG) in order to achieve a probable sCJD diagnosis. From all these tests, only RT-QuIC test and MRI can be abnormal at the beginning of the disease. For a definitive sCJD diagnosis, a brain biopsy or a brain autopsy is needed for its anatomopathological study. Nowadays, there is no curative available treatment for this disease. However, some clinical assays and other studies have suggested promising results for possible future curative treatments.

OBJECTIVE: The aim of this study is to evaluate the diagnostic accuracy of total tau (t-tau) and phosphorylated-tau/total-tau (p-tau/t-tau) ratio combined with neurofilament light chain protein (NfL) CSF biomarkers for the early diagnosis of patients with sCJD.

DESIGN AND SETTING: It is a multicentric analytical cross-sectional study. It will be carried out in hospitals from fifteen autonomous communities of Spain. Each autonomous community will centralize all the patients involved in the study to a reference hospital.

PARTICIPANTS: The target population of this study are patients with rapidly progressive dementia defined as an “acute or subacute decline in cognitive functions with impairment of basic activities of daily living in no more than two years” who have sporadic CJD as a differential diagnosis at the time of hospital admission.

METHODS: Patients enrolled in this study will be asked to answer some personal and clinical relevant data for the study and to undergo a basic neurological exploration. Subsequently, a set of diagnostic tests will be performed; blood test and lumbar puncture with biomarkers (t-tau, tau ratio and neurofilaments) determination, genetic study, MRI and EEG. Finally, a brain autopsy will be done to realize the anatomopathological study.

KEYWORDS: sporadic Creutzfeldt-Jakob disease, early diagnosis, tau protein, neurofilaments, RT-QuIC, accuracy.

2. ABBREVIATIONS

AUC	Area under the curve
CSF	Cerebrospinal fluid
CEIC	Clinical Research Ethics Committee
CJD	Creutzfeldt-Jakob disease
DWI	Diffusion-weighted images
EEG	Electroencephalogram
ELISA	Enzyme-linked immunosorbent assay
ECDC	European Centre for Disease Prevention and Control
FN	False negatives
FP	False positives
FLAIR	Fluid-attenuated inversion recovery
gCJD	Genetic Creutzfeldt-Jakob disease
HIV	Human immunodeficiency virus
iCJD	Iatrogenic Creutzfeldt-Jakob disease
IQR	Interquartile range
LR	Likelihood ratio
MRI	Magnetic resonance imaging
M	Methionine
NPV	Negative predictive value
NfL	Neurofilament light chain protein
PrPc	Cellular prion protein
PrPSc	Scrapie prion protein
P-tau/t-tau	Phosphorylated tau protein / total tau protein ratio
PCR	Polymerase chain reaction technique
PPV	Positive predictive value
qPCR	Quantitative polymerase chain reaction technique

RT-QuIC	Real-time quaking-induced conversion
ROC	Receiver operating characteristic curve
rec PrP	Recombinant prion protein
Simoa	Single molecule array
sCJD	Sporadic Creutzfeldt-Jakob disease
SPSS	Statistical package for social sciences
ThT	Thioflavin T
TSH	Thyroid stimulating hormone
t-PrP	Total prion protein
t-tau	Total tau
TN	True negatives
TP	True positives
V	Valine
vCJD	Variant Creutzfeldt-Jakob disease

3. INTRODUCTION

Prion diseases are a type of neurodegenerative diseases which have a rapid progression to death once symptoms appear. Creutzfeldt-Jakob disease (CJD) is the most common prion disease in humans (1).

There are three categories of human prion disease recognized which are sporadic, genetic and acquired prion disease. Sporadic Creutzfeldt-Jakob disease (sCJD) represents more than 90 percent of the sporadic prion diseases (2).

The **majority of CJD are sporadic Creutzfeldt-Jakob disease (sCJD)** (85-90 percent). The second most prevalent group is genetic Creutzfeldt-Jakob disease (gCJD) (5-15 percent). Iatrogenic Creutzfeldt-Jakob disease (iCJD) and variant Creutzfeldt-Jakob disease (vCJD) generally represent less than 1 percent of CJD (3) (*Figure 1*).

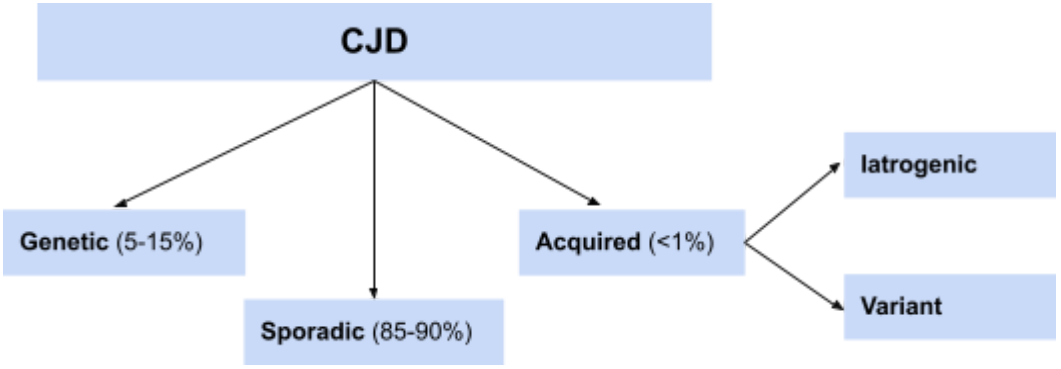


Figure 1: Scheme of Creutzfeldt-Jakob disease categories. CJD:Creutzfeldt-Jakob disease. *Made by the author.*

Even though CJD is a rare disease, it is universally known due to the variant CJD which is an acquired prion disease caused by the ingestion of contaminated beef meat. The first case was reported in 1996 in the United Kingdom at the same time that the epidemic of Bovine Spongiform Encephalopathy ("mad cow disease") appeared (4). Therefore, since 2001, CJD has been a **disease of obligatory notification** (5). Fortunately, after the application of protective measures, the vCJD has become almost non-existent. Meanwhile, sCJD cases continue to emerge, supposing a big challenge for health care professionals (5).

Sporadic Creutzfeldt-Jakob disease is a **rapidly progressive and fatal disease** without curative treatment. At the moment, it presents a challenging diagnostic which requires clinical compatibility plus other altered biomarkers such as 14-3-3 from cerebrospinal fluid (CSF), real-time quaking-induced conversion positive test (RT-QuIC), Magnetic Resonance Imaging (MRI) characteristic alterations or electroencephalogram (EEG) specific abnormalities in

order to diagnose a “probable sCJD”. To diagnose a “definitive sCJD”, an anatomopathological brain study with a biopsy or a necropsy is needed (6).

Most of the tests to diagnose CJD have normal results at the beginning of the disease, generating a gap between the suspicion of the illness and the confirmation. An **early diagnosis test** could benefit the patient and his family by providing early certainty on the diagnosis and could serve as a basis for future studies.

3.1. Epidemiology

3.1.1. Global Epidemiology

Sporadic CJD is a rare disease with an incidence of **1 to 2 cases per million people/year**. In general, there has been an increase in sCJD number of cases, which could be explained by aging population, improvements in diagnostic tests and improvements on clinical awareness of the disease and diagnostic criteria (7).

There is a globally geographical variation in sCJD incidence, and the continents with the highest incidence are Europe, America, East Asia and Oceania. The countries with an incidence superior to 1.2 per million people in Europe are Sweden, Finland, United Kingdom, Netherlands, Belgium, Luxemburg, Germany, Denmark, Switzerland, Italy, Austria, France and Spain. In the rest of the world, countries with more than 1.2 cases per million people are the United States of America, Japan and Australia. The country that presents the highest incidence in Europe and in the whole world is France *(Figure 2)*(7).

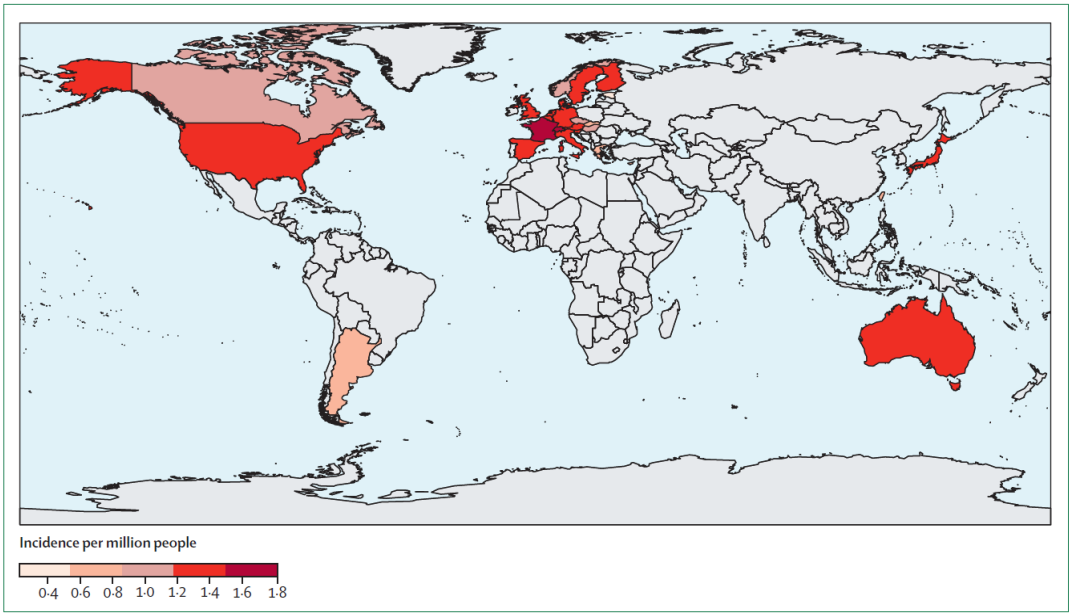


Figure 2: Global reporting of sporadic Creutzfeldt-Jakob disease incidence per million people (7).

3.1.2. Epidemiology in Spain

According to the Spanish National Registry, sCJD in Spain has an incidence of 1,14 cases per million people/year. This incidence is similar to other European countries. In Catalonia, between 10 and 20 cases are notified every year[1]. Between 1995 and 2021 there were 2318 notifications of human transmissible spongiform encephalopathies in Spain from which 1757 were sCJD cases (863 confirmed cases, 784 probable cases and 110 possible cases). In addition, 5 cases of vCJD, 8 cases of iCJD and 93 cases of gCJD were diagnosed. From the total of confirmed and probable CJD cases, 89% were sCJD and 10% were genetic forms (5).

Between 2005-2008 there was an increase in cases coinciding with the appearance of the five cases of vCJD, two of them were a family cluster who ingested offal products as bovine brain. However, in the last 8 years, no new cases of vCJD have been reported. A suspicion of clustering of 6 cases of sCJD was notified on the Canarian Islands in 2017-2018. A decrease in the number of notifications has been detected since 2016. It is worth highlighting that the coronavirus pandemic has promoted a decrease in the number of notifications that still persist nowadays and has had an impact on the surveillance system. The data from 2019 to 2021 is still not well defined (5).

In Spain, there is a significant geographic variation; whereas the Basque Country, Navarra, La Rioja, Catalonia, Castile and León and the Valencian Community are the autonomous communities that presented the highest rates of incidence, Asturias, Balearic Islands and Extremadura had the lowest rates of incidence *(Figure 3)* (5).

[1] Information provided in an interview with Carlos Nos, the neurologist responsible for CJD's epidemiological registry in Catalonia.

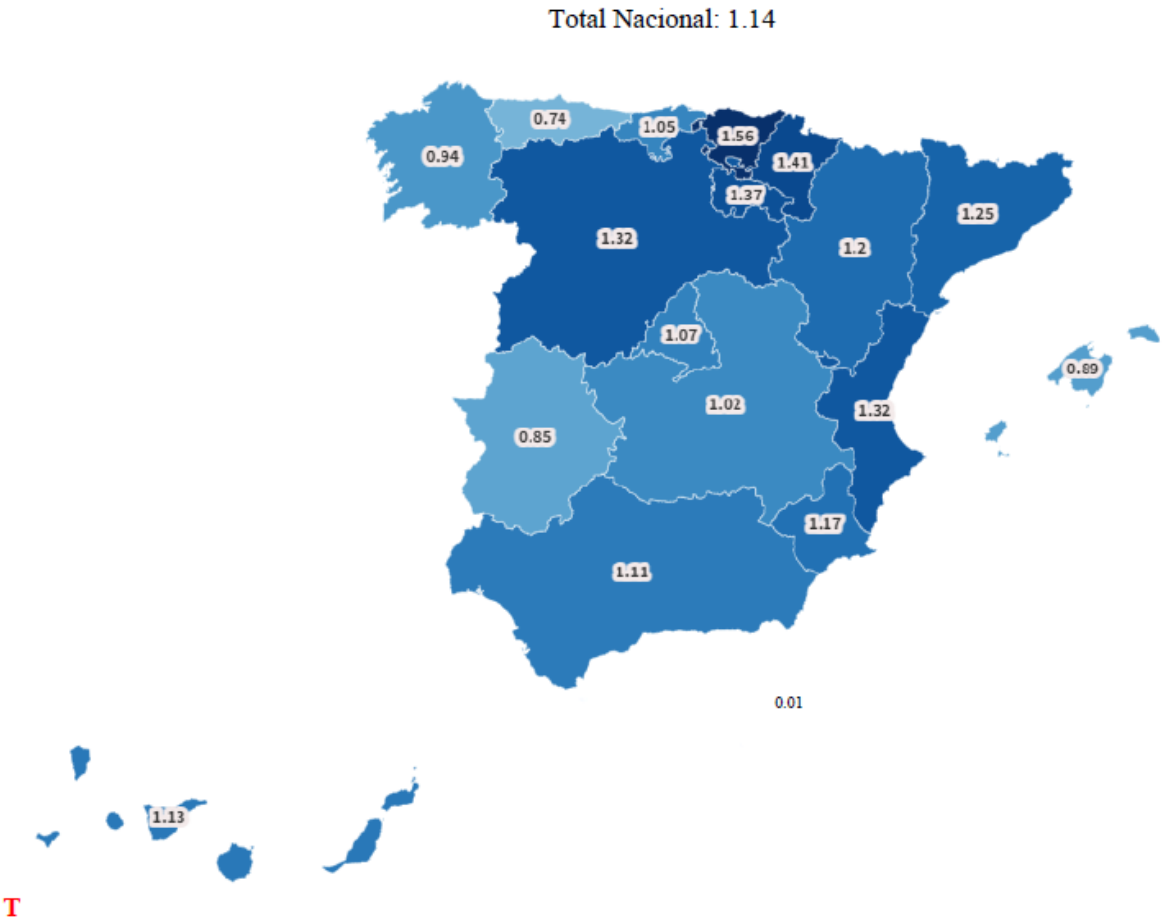


Figure 3: Average annual incidence of confirmed and probable sporadic Creutzfeldt-Jakob disease diagnosed between 1998-2021 in age-adjusted rates per million population (5).

Referring to distribution of sCJD by gender and age, women have slightly higher incidence than men in all age groups. Also, the majority (66%) of sCJD have been observed between the 60-79 years old (5).

In relation to genetic polymorphism association, from the confirmed and probable sCJD cases, 60% had methionine/methionine (M/M), 20% valine/valine (V/V) and 20% were heterozygous for the codon 129 polymorphism (5).

3.2. Etiology

Prion diseases are neurodegenerative diseases which present a rapidly progressive evolution towards death since the appearance of clinical symptomatology. Nowadays, still **there is not a clear explanation** for sCJD etiology and the origins of the pathological prion protein are not known. One study reported some sCJD cases following a disease-clustering mechanism which suggested that it could be related to the exposure to a common external

factor (8). However, another study suggested that the clusters were the result of a higher intensity surveillance in some specific areas (9).

Genetic CJD is caused by a range of autosomal dominant mutations on the prion protein gene PRNP located on chromosome 20 and the most common mutation is the E200K. Variant CJD is probably caused by the consumption of prion infected beef meat (1) and it has been proved that iCJD is the result of brain and meninges contamination due to instruments used in surgery in which routine sterilization has not been enough to disinfect from prions (1). Iatrogenic CJD can also be caused by dural and corneal transplants or cadaveric human pituitary hormones coming from patients who suffered from prion disease (10).

3.3. Pathogenesis

Non pathological neuronal and nonneuronal Brain's cells have anchored on their cell surface a normal prion protein designed as cellular prion protein (PrPc) in an alpha-helical conformation. This protein is encoded by the PRNP gene located on chromosome 20 (11). In physiological situations, Prion protein is involved in ionic homeostasis which plays a role in neurotransmission. It is synthesized in the rough endolpasmic reticulim, suffers some post-translational modifications and further changes in the Golgi apparatus. Finally, after different glycosylations, three isoforms of the protein are formed and all of them are soluble in detergent and sensitive to proteolytic digestion with proteinase K(11).

In contrast, prionic diseases present a pathological prion protein designed as scrapie prion protein (PrPSc) which has a higher amount of b-sheet structures, that results in some conformational alterations and a change in its properties and functions. In sporadic CJD the origin of this prion protein is yet unknown, but because of its conformational alteration, it is less soluble in detergent and partially resistant to proteinase K digestion (11). This pathological protein is able to convert normal PrPc from the brain to PrPSc and expand through the brain. Therefore, PrPSc accumulation acts as a neurotoxic substance and leads to synaptic degeneration and disorganization and neuronal loss (12).

3.4. Neuropathology

Neuropathological studies provide a definitive CJD diagnosis performing a brain biopsy or autopsy.

The common **histologic and immunohistochemical** features of prion diseases are (11,13):

- Neuronal loss and atrophy.
- Astroglial activation and proliferation: reactive gliosis as a response for severe neuronal loss.
- Absence of a classic inflammatory or immunitary response.

- Presence of small vacuoles (20 to 50 microns in diameter) which produce a spongiform appearance *(Figure 4)*. Although other neurodegenerative and cerebrovascular diseases can present this spongiform alterations in end stages, the micro-vacuoles are larger, more irregular and present in restricted zones of cerebral tissue.
- Accumulation of PrPSc in different patterns such as plaque-like, synaptic, perineuronal or perivacuolar form. Each sCJD subtype can present a specific prion protein distribution pattern.

Apart from histological and immunohistochemical characteristics, it is important to establish the **type of the pathological prion protein**. By Western Blot analysis we can establish the size and electrophoretic mobility of the protein and classify it as glycoform type 1 or type 2 (14,15).

Depending on the type of prion pathological protein and the polymorphisms at codon 129 of the PRNP gene (methionine M or valine V), we can classify sCJD in six molecular subtypes which present different tissue tropism, neuropathological changes, different incubation period and clinical manifestations. Depending on the sCJD subtype, it varies the prognosis and patients survival period (16). The most frequent subtypes are MM1 or MV1 (70 percent of the cases), secondary VV2 and MV2 subtypes (25 percent of the cases) and finally MM2 and VV1 (5 percent of the cases) (11).

Table 1: Summary of the most relevant characteristics of sporadic Creutzfeldt-Jakob disease molecular subtypes. sCJD: sporadic Creutzfeldt-Jakob disease, M: methionine, V: valine. (11,17–21).

sCJD molecular subtypes	% of cases	Survival time (months)	Main clinical features
MM1 or MV1	67 (MM1) 3 (MV1)	4	**Rapidly progressive dementia** with early and prominent myoclonus and ataxia.
VV2	15	7	**Ataxia** at onset, often as an isolated feature, and late dementia.
MV2	10	17	Progressive dementia with **prominent psychiatric features.**
MM2 thalamic	2	16	**Insomnia,** psychomotor hyperactivity, ataxia, dementia, dysarthria, quaking, myoclonus and spasticity.
MM2 cortical	2	16	Progressive dementia and **disturbances of higher cognitive functions, high-frequency aphasia and apraxia** and late myoclonus or epileptic seizures.
VV1	1	21	Progressive dementia with younger onset.

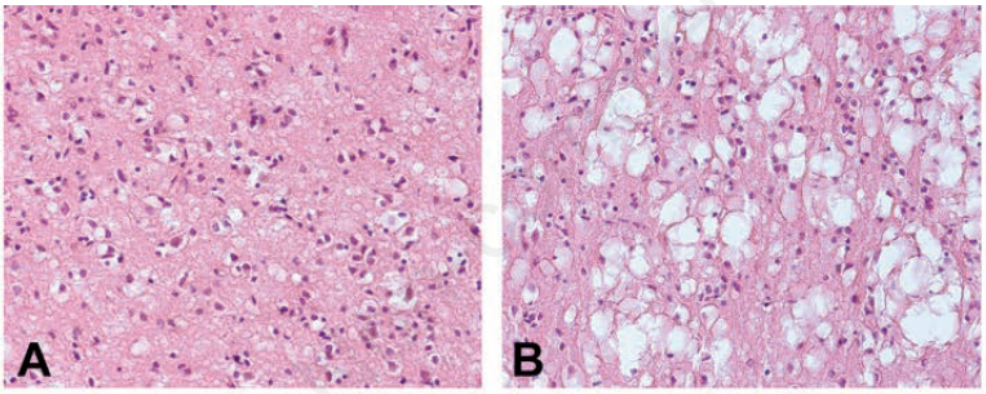


Figure 4: Small vacuolar lesionS (A) and confluent vacuolar lesions (B) in cortical gray matter (hematoxylin eosin stain) (11).

3.5. Clinical Features

Sporadic CJD is a rapidly progressive disease with a fatal prognosis and without curative treatment available. It presents a high degree of heterogeneity in its clinical presentation, which could complicate its diagnosis. Nevertheless, the vast majority course with a rapidly progressive dementia defined as an “acute or subacute decline in cognitive functions with impairment of basic activities of daily living in no more than two years” (22,23).

The most frequent features are:

- **Neuropsychiatric symptoms**: The most common symptoms are alterations in concentration, memory or judgment at early stages (24). As the disease progresses, patients suffer dementia that typically advances rapidly with behavioral alterations or cortical dysfunction (aphasia, apraxia, frontal lobe syndromes) (25). They can also present mood changes as depression or apathy (26) and sleep disturbances as hypersomnia. In some cases visual hallucinations or other psychotic features can be present (27).
- **Myoclonus:** rapid and involuntary muscular movements normally caused by startling that are present in a large part of the patients during the illness (28).
- **Cerebellar manifestations:** The most common cerebellar signs in patients affected by CJD are nystagmus and ataxia. Some of them develop ataxia at the onset of the disease symptomatology (24).
- Signs of corticospinal tract involvement such as hyperreflexia, extensor plantar responses and spasticity (29).
- Extrapyramidal signs such as hypokinesia, bradykinesia, dystonia, and rigidity can also appear in patients who suffer from sporadic CJD (29).

At the end-stage of the disease patients normally suffer from akinetic mutism and death normally within a median of six months except atypical or slow progression cases that die within one or two years (30).

3.6. Diagnostic tests

Sporadic CJD is a challenging diagnosis and at the moment, the diagnosis tests available for sCJD diagnosis are CSF biomarkers determination, MRI, EEG and RT-QuIC test.

The actual diagnostic criteria (since 2018) defines as a “probable sCJD” a neuropsychiatric disorder with a positive RT-QuIC test or a progressive dementia with either two clinical features or supportive findings in EEG / MRI / 14-3-3 protein CSF determination (6). The only way to definitively diagnose this disease is by brain biopsy or necropsy (31) (*see in Diagnostic criteria*).

In general, MRI and RT-QuIC test alterations may be observed in early stages of the disease (13) while EEG and 14-3-3 protein abnormalities will usually be pathological in more advanced stages (13,32).

3.6.1. Cerebrospinal fluid biomarkers

Although it is an invasive technique, lumbar puncture should be performed in all patients with suspicion of sCJD, as it is necessary to rule out other alternative treatable diagnoses similar to sCJD, as for example, an inflammatory etiology (33). When analyzing CSF, there are some biomarkers that have been studied for this disease that support the presence of prionic disease.

14-3-3 protein is an intraneuronal cytosolic protein that indicates cell lysis if there is recent neuronal damage (12,33) and is a validated biomarker included in the diagnostic criteria of sCJD (13)**.** Despite being **highly sensitive**, it is **not specific** for sCJD because its elevation is common in other neurodegenerative diseases or in diseases that cause acute neuronal injury such as central nervous system inflammation, stroke, status epilepticus or infiltrative neoplastic disease (12,13). Also, as 14-3-3 levels are higher when neuronal damage increases, sensitivity is lower in early stages of the disease and also in some disease subtypes (13).

In 2018, a new test, called the **RT-QuIC test**. was incorporated in diagnostic criteria of sCJD (12). It is a technique that directly detects prion protein in CSF by the misfolding of prion protein coupled to a fluorescent readout (13). For it, a buffered solution of recombinant PrP (recPrP) is prepared and aliquoted out into a 96-well plate. Then, each sample is seeded in four wells and if the added sample contains PrPsc seed, it will cause the recPrP misfolding and aggregation. Cyclic phases of heat and shaking promote this process. Thioflavin T (ThT) binds to the protein aggregation, after that, the fluorescent properties of the dye change and the fluorescent emission is recorded (34). During the aggregation process, fluorescence is plotted against time generating a kinetic curve which has three phases (11):

1. **Lag phase:** interaction of PrPsc with recPrP and induccion of its misfolding.
2. **Growth phase:** aggregation of misfolded proteins takes place and they bind with ThT which provokes an exponential increase of fluorescence.
3. **Plateau phase:** the majority of recPrP is incorporated into fibrils.

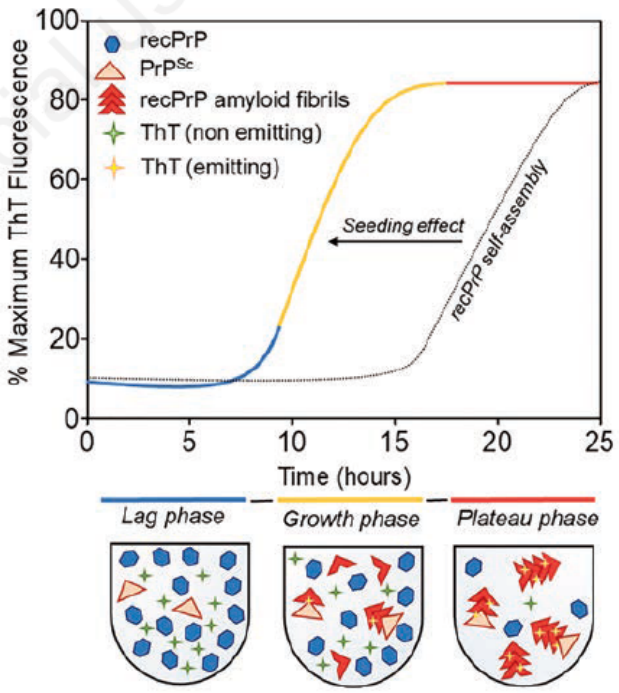


Figure 5. Schematic illustration of the Real-time quaking-induced conversion reaction. recPrP: Recombinant Prion Protein, PrPSc:Pathological prion protein, ThT: Thioflavin T (11).

It is worth mentioning that in normal conditions (samples with normal PrPc), recPrP will also spontaneously aggregate but later than the sample with pathological prion protein would do (11).

In *Figure 5* there is a schematic illustration of the RT-QuIC reaction and the three phases; the PrPsc *(pink triangle)* induces the conversion of recPrP *(blue hexagon)* into a misfolded form *(red arrow)* which starts to aggregate and form recPrP amyloid fibrils. The formation of the aggregates induces the emission of ThT fluorescence signal *(yellow star)*. In the absence of PrPsc, recPrP can aggregate following a well-defined kinetics *(dotted line)*, and in the presence of PrPsc, the kinetics of recPrP aggregation is significantly accelerated *(solid line)* (11).

A sample is considered positive when at least two out of four replicates show a seeding effect which means a large increase in relative fluorescence unit (higher than the cut-off value) during the RT-QuIC assay (11,13). If none of the replicates surpassed the chosen cut-off, the samples are considered negative and if only one replicate is over the cut-off value, normally it is considered unclear and the RT-QuIC assay is repeated (35).

Some studies and a meta-analysis support that RT-QuIC has **high sensitivity and the highest diagnostic specificity** (99-100 percent) (11,34,36). However, It has been shown to have low sensitivity for the most frequent sCJD subtype (MM1) (12) and also in subtypes with

pathological prion protein type 2 (35). In addition, it may be less sensitive to atypical or slowly progressive cases of sCJD (33) and it can not distinguish, on its own, sCJD from other human prion diseases such as Gerstmann-Sträussler-Scheinker and fatal insomnia (12).

Another limitation is that the RT-QuIC test cannot determine a quantitative value because it only detects the presence or absence of the pathological prion protein. Moreover, it **has not demonstrated the ability to differentiate between sCJD subtypes** because they generate very similar reaction products (37) and consequently it cannot determine the disease prognosis associated with every sCJD subtype (12).

Despite being included in new diagnostic criteria, it is **not universally available,** it is only accessible in a few hospitals and it has been established in only a few reference laboratories for molecular diagnosis due to the **complexity of the technique** and the **specific equipment required** (12)**.**

In Spain it has been used only in approximately 4 percent of diagnosis procedures since it was incorporated in 2018 (5). The low implementation of this test is probably explained by the fact that its procedure depends on the **external supply** of the recombinant prion protein substrate which is **very expensive** (38). Also, it has less interlaboratory standardization data in comparison with approved surrogate biomarkers which makes it difficult to be established (13).

3.6.2. Imaging studies

Neuroimaging studies are needed in the evaluation of a patient with rapidly progressive dementia and they can show abnormalities from early stages of the disease (13,39).

Protocol MRI recommendation includes T1-weighted axial images, T2-weighted axial images, fluid-attenuated inversion recovery (FLAIR) axial and sagittal images at 3 mm slice thickness, and diffusion-weighted images (DWI) sequences (40).

At the beginning, the first sequence that presents alterations is DWI with an increased signal in at least two regions of the cerebral cortex (ribboning) and/or corpus striatum (caudate head and putamen) *(Figure 6)* (41).

As the disease advances, we observe progression of the signal in DWI from unilateral to bilateral extension and from gray matter to secondary white matter affection. Also, FLAIR *(Figure 6)* and T2-weighted images might show high signal abnormalities and in late stages an evident and generalized ventricular dilation can be observed due to a generalized atrophy (40).

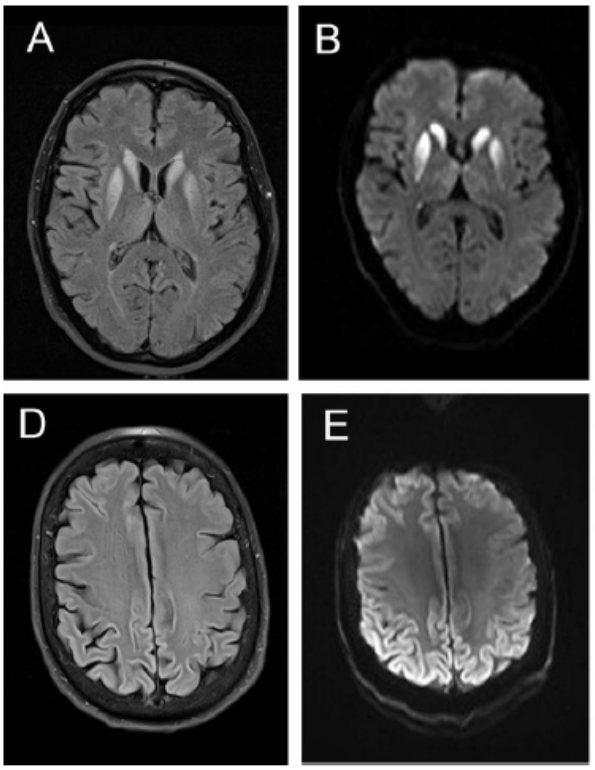


Figure 6. Different sequences of patients with definite sporadic Creutzfeldt-Jakob disease showing a typical increased signal in basal ganglia on Fluid-attenuated inversion recovery (FLAIR) (A), Diffusion-weighted images (DWI) (B) and typical features of cortical ribboning in cerebral cortex on FLAIR (D), DWI (E) sequences. (42).

MRI study helps us to rule out possible differential diagnosis of a patient with a rapidly progressive dementia in which we could see different structural alterations such as ischemia, encephalitis or neoplasia (13). A limitation of this test is that it depends on the personal experience of the image interpreters, the protocols and different scanners used (13).

3.6.3. Electroencephalogram

EEG is a functional non-invasive test which allows us to analyze brain activity and detect abnormalities. It has shown low sensitivity and a better specificity to diagnose a patient with CJD, although typical EEG abnormalities are not specific for this disease (12,13).

Typical abnormalities mainly appear in late symptomatic phases while in initial phases unspecific alterations or even normal EEG patterns appear (32). In addition, EEG alterations do not remain stable, but instead change during the disease progression (43).
At the *prodromal phase* of the disease there is diffuse slowing of the brain activity in theta or delta frequency range. As the disease progresses, frontal intermittent rhythmic delta activity

appears and subsequently, short periodic sharp-wave complexes appear with a symmetrical and synchronous distribution (*Figure 7*) (43). Those periodic activities of short duration and symmetrical and synchronous distribution can also be found on post anoxic encephalopathy, toxic and metabolic encephalopathies, other such as treatment with tricyclic antidepressant and hepatic or hypercalcemic encephalopathy. Therefore, these abnormalities are not specific to sCJD (44).

Afterwards, when degree of consciousness decreases, those biphasic or triphasic steep graph elements are replaced by slow theta or delta activity. In that moment, a cyclic altering pattern appears between these two phases. Finally, when the *terminal phase* arrives, the cyclic pattern disappears and background activity flattens (44).

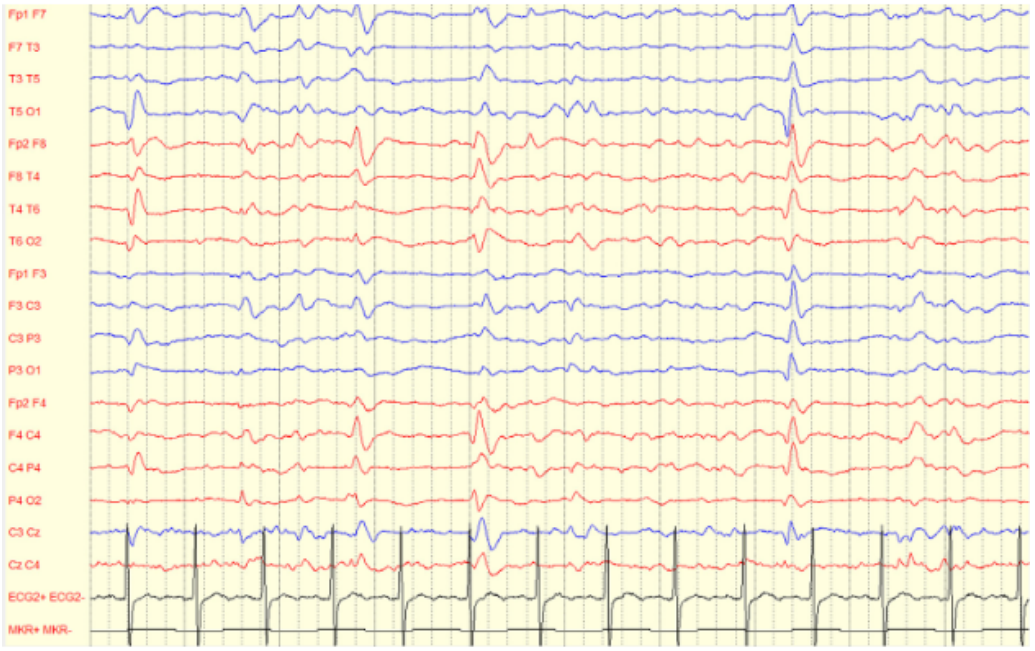


Figure 7 Periodic sharp-waves complexes can be observed in the electroencephalogram (43).

3.6.4. Genetic studies

Genetic studies should be considered in all cases of sCJD in order to determine de codon 129 polymorphism and subsequently identify the sCJD subtype (13).

On the other hand, sequencing the PRNP gene allows to identify any mutations indicative of genetic CJD and for this reason it should be considered for the differential diagnosis of sCJD, specially in atypical cases or when there is positive family history of this disease.(13)

3.7. Possible future diagnostic tests

Although not yet approved, several studies have shown promising results for the following diagnostic tests. Further studies are needed to confirm their benefit and diagnostic accuracy.

CSF biomarkers

Total tau protein (t-tau) is a microtubule-associated protein expressed in neuronal and glial cells (13) which indicates neuronal damage (12). It is used to diagnose some neurodegenerative diseases such as Alzheimer's disease and it can also be elevated in other neurodegenerative, inflammatory or neoplastic diseases (12). Some studies have demonstrated that total tau protein has high sensitivity and specificity to diagnose sCJD (45).
It has been observed that **diagnostic accuracy is superior to 14-3-3** for the diagnosis of sCJD (35,46), specially in terms of specificity and in those with "weak-positive 14-3-3" (13,47).
Other studies have shown that during the disease progression, levels of t-tau continue to increase (12) which indicates that it could be an accurate biomarker for **disease follow-up** and a prognostic marker for survival time. As a limitation, there is no general consensus on which is the best cut-off or enzyme-linked immunosorbent assay (ELISA) so more studies are needed (13).
In contrast to t-tau biomarker, phosphorylated tau levels decrease in sCJD and increase in Alzheimer's disease. Therefore, **phosphorylated tau / total tau ratio (p-tau / t-tau)** could be the CSF biomarker with better accuracy when differentiating neurodegenerative diseases such as Alzheimer (12,13,33,48).

Some studies have demonstrated that the **combination** of 14-3-3 protein with other surrogate biomarkers such as t-tau and tau ratio significantly increases specificity (12,12,38,49,50). One study supported that specificity increased when combining t-tau and p-tau/t-tau ratio (51) and another suggested that when combining 14-3-3, t-tau and p-tau/t-tau ratio the diagnostic precision increased (52).

Neurofilament light chain protein (NfL) are proteins from the cytoskeleton of neurological cells which are released into body fluids when there is neuroaxonal damage (13). It has an **outstanding sensitivity,** almost reaching the maximum value (4), and some studies support that low concentration of CSF NfL in a suspicious patient would exclude with high accuracy the diagnosis of prion disease (11). Opposed to the other biomarkers, NfL is **increased in all sCJD subtypes**, even in those in which 14-3-3 and t-tau show lower sensitivity values (12,13,33,35,48)). In addition, elevated NfL values have been demonstrated in atypical cases

of sCJD where other established CSF biomarkers had lower sensitivity, and for that reason, it is supported the use of NfL as a fast screening marker (33,48). However, increased NfL levels are not accurate enough to differentiate sCJD from other neurodegenerative dementia when they are used alone (11,13), with an inferior specificity than p-tal/t-tau and 14-3-3 combination (38).

Other surrogate CSF biomarkers have been studied but more studies are needed to determine their accuracy for sCJD diagnosis. **Alpha-synuclein** is a protein abundant in presynaptic regions (12) that in some studies have shown good diagnostic ability (53–56), but it is not approved in neurodegenerative diseases and it is still needed to confirm this benefit (12,33).

Total prion protein (t-PrP) levels are increased in plasma but decreased in CSF, probably explained by the aggregation of PrP in brain tissue (12,33). Despite being an accessible biomarker which increases at early stages of the disease (57), its accuracy to determine the disease is very low and it is not better than other biomarkers described (12,33).

Nasal mucosa RT-QuIC

RT-QuIC has been applied to less invasive and safer places as olfactory mucosa or skin biopsies which have demonstrated even better diagnostic accuracy than with CSF samples (11,13). The nasal brushing technique is used in order to collect olfactory epithelium and subsequently realize RT-QuIC test to determine pathological prion protein (1). This test has been shown to be a **less invasive** technique and to have **high sensitivity and maximum specificity**, outperforming the RT-QuIC test in CSF. Despite this, its use for diagnosis in clinical practice is unclear as only preliminary data are available and most patients will **still require a lumbar puncture** to rule out other neurodegenerative diseases using biomarkers that cannot currently be determined in the olfactory mucosa (12).

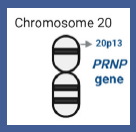
Blood biomarkers

Some studies suggest that blood NfL and t-tau levels are highly increased in sCJD (58,59) and reach **similar or even better sensitivity and specificity in serum than in CSF** (58–60). In addition, blood biomarkers have shown good results when discriminating prion diseases from control groups and even from other “CJD mimic” diseases (61) . **Sporadic CJD subtypes have shown different plasma t-tau levels** (58,59). Moreover, some studies expose that blood NfL levels distinguish sCJD from early stages of the disease with minimum symptomatology or even to predict onset in at-risk individuals (61).

Future work is needed in order to establish these biomarkers in the diagnostic process of the disease. The most studied blood biomarkers are:

- **14-3-3 protein:** it has shown high sensitivity and specificity although it has complex interpretation (13).
- **T-tau:** It presents incremented levels in sCJD compared to control groups and also to other neurological diseases as neurodegenerative dementias (12,13). It is particularly increased in homozygous for methionine sCJD and it has shown an association between survival and disease progression (59).
- **NfL:** it seems to be very useful when discriminating sCJD from control groups or neurodegenerative dementias (12,13,62) and it normally achieves high plasma levels at early stages of the disease, even before clinical symptoms appear and CSF levels increase (12,63). As with t-tau, NfL levels continue increasing during the disease progression, so it is a good predictor of high grade clinical impairment (63). Therefore, evidence suggests that blood NfL levels could be a good screening first-steep test in sCJD diagnostic (63).

Table 2. Summary of the main findings and clinical relevance for the most promising biomarkers (the approved and some of the under investigation biomarkers). CSF: Cerebrospinal fluid, CJD: Creutzfeldt-Jakob disease DWI: Diffusion-weighted images EEG: Electroencephalogram, MRI: Magnetic Resonance Imaging, NfL: Neurofilament light chain protein, FLAIR: Fluid-attenuated inversion recovery, RT-QuIC: Real-time quaking-induced conversion. *Adapted from* (12).

		Main findings	Clinical relevance for CJD diagnosis
Neurophysiological biomarkers 	EEG	● Periodic sharp-wave complexes. ● Theta and delta activity. ● Diffuse slowing of EEG background activity.	● Useful and non-invasive test. ● Abnormalities appear in late stages of sporadic CJD.
Neuroimaging biomarkers 	MRI	● Hyperintense signal in DWI at cortical regions and/or caudate nucleus, putamen or thalamus. ● Hyperintensities in FLAIR and T2 sequences, in cortex or basal ganglia.	● Useful diagnostic biomarker at an early stage. ● Non-invasive test. ● Helps to rule out possible differential diagnoses. ● Interobserver variability.
Genetics 		● It determines the polymorphism of codon 129. ● Determine E200K mutation	● Sporadic CJD subtype classification. ● Genetic CJD diagnosis.
CSF biomarkers 	14-3-3 protein	● Increased levels.	● Validated diagnostic biomarker.
	RT-QuIC test	● Positive for pathologic prion protein.	● It has high sensitivity and the highest specificity. ● Not universally available. ● Complex technique. ● Specific equipment required. ● Very expensive.
	Total tau*	● Increased levels.	● High sensitivity and specificity. ● Accurate for the disease follow-up. ● Different levels depending on the disease subtype.

	Tau ratio*	● Decreased ratio.	● High accuracy when differentiating neurodegenerative diseases such as Alzheimer.
	NfL*	● Increased levels.	● Very high sensitivity. ● Increased in all sporadic CJD subtypes. ● Allows quick disease screening. ● Low specificity.
	Alpha-synuclein*	● Increased levels.	● Not approved in neurodegenerative diseases.
	Total prion protein*	● Decreased levels.	● Not better than other biomarkers described.
Blood biomarkers 	14-3-3 protein*	● Increased levels.	● Similar sensitivity and specificity than in CSF. ● They could have potential utility in disease screening (NfL) and progression monitoring (total tau and NfL). ● Different total tau levels depending on the disease subtype.
	Total tau*		
	NfL*		

** Not currently approved or included in diagnostic criteria (2018).*

3.8. Diagnostic criteria

We can classify sCJD diagnosis in three categories: possible, probable and definitive sCJD. In order to diagnose possible and probable sCJD we need a combination of clinical features, specifically MRI and EEG changes or detection of biomarkers at CSF(31). However, to diagnose definitive sCJD, detection of pathological prion protein in the central nervous system tissue is needed with brain biopsies or autopsies (31).

Table 3. New diagnostic criteria released by the Centers of Disease Control and Prevention in 2018. sCJD: Sporadic Creutzfeldt-Jakob disease, CSF: Cerebrospinal fluid, EEG:Electroencephalogram, MRI: Magnetic Resonance Imaging (6).

Possible sCJD
Patients with progressive dementia syndrome and (*point 1, 2. 3 and 4 are required*) 1. At minimum two of the following clinical features: - Myoclonus - Visual or cerebellar signs - Pyramidal or extrapyramidal signs - Akinetic mutism 2. Absence of a positive result of any of the tests below that would classify the patient as a “probable sCJD” 3. Less than two years of illness duration 4. Without presenting other routine pathologic tests that indicate an alternative diagnosis
Probable sCJD
- Patients who present a neuropsychiatric disorder and also have a positive result in real-time quaking-induced conversion (RT-QuIC) test in CSF or other tissues. **or** - Rapidly progressive dementia, and (*point 1, 2 and 3 are required*) 1. At minimum two out of the following four clinical features: - Myoclonus - Visual or cerebellar signs - Pyramidal or extrapyramidal signs - Akinetic mutism 2. Positive tests on one or more of the following tests: - A typical EEG (periodic sharp wave complexes) during an illness of any duration - A positive 14-3-3 protein in CSF assay in patients with a disease duration of less than two years - MRI showing high signal in caudate nucleus/putamen and/or in at least two cortical regions (temporal, parietal, and occipital) on diffusion-weighted imaging (DWI) or fluid-attenuated inversion recovery (FLAIR). 3. Without presenting other routine pathologic tests that indicate an alternative diagnosis
Definitive sCJD
Patients with a neuropathological confirmation of the disease.

With biochemical, immunohistochemical and genetic analyses we can identify sCJD subtype (11):

- According to the pattern obtained on the Western Blot of the diseases-related PrP after partial protease digestion we define the prion strain type (33).
- Codon 129 genotype determined by genetic study.

Nowadays, we can only define sCJD and its subtype by brain tissue normally in postmortem analysis, therefore, the prognostic information that predicts the sCJD subtype generally loses utility (33). However, there are some references that support that CSF t-tau and NfL biomarkers are differentially altered depending on sCJD subtype, so they could be useful to determine the subtype during life without needing brain biopsy (48).

3.9. Differential diagnosis

Some of the diseases and differential etiologies that can simulate sCJD are (63):

- Rapidly progressive neurodegenerative diseases
- Infectious or autoimmune encephalitis
- Cerebrovascular disease that causes cerebral ischaemia
- Toxico / metabolic encephalitis

It is necessary to rule out all these entities before diagnosing the disease. For this purpose, we can use imaging tests such as MRI or other tests such as lumbar puncture. The importance of detecting these pathologies that simulate sCJD is that most of them are treatable and the sooner they are treated, the better the patient's prognosis (13).

3.10. Treatment

We currently have no evidence of any curative treatment for sCJD, so it remains a universally fatal disease. Only some reports on specific drugs in CJD patients are available because there is a problem in assessing its effectiveness due to lack of appropriate preclinical tests, heterogeneous clinical presentation in humans, lack of information about molecular biology of prion diseases and the influence of sCJD subtype in the therapy response (16).

Nowadays, all we can offer at the moment is supportive and symptomatic treatment: myoclonus is treated with benzodiazepines (clonazepam), anxiolytic and antipsychotics (neuroleptics or benzodiazepines) for depression, anxiety, hallucinations and psychosis. When rigidity and akinesia appear we can use atypical neuroleptics (16) and morphine when patients suffer severe pain (64).

Some of the results from previous trials which evaluated curative or disease-modifying treatments are:

- Controversial results for pentosan polysulfate and quinacrine which were well tolerated but did not provide substantial benefit in CJD when improving survival months(16).
- Doxycycline administered in humans is controversial too because while a randomized, double-blind placebo controlled trial showed any significant difference between placebo and doxycycline (65), another study showed a life-prolonging effect of doxycycline (66). The difference was that the first study was applied on advanced stages of the disease and the second study on early disease stages. Therefore, the efficacy of a treatment is likely to change depending on the disease stage in which it is applied and it might be **greater at early stages.**

A human treatment programme evaluation has been recently published in which a new prion protein monoclonal antibody (PRN100) (anti-PrPc) therapy for CJD was studied. It was applied to six probable CJD who were **not in terminal disease stages**. Although all of them presented progressive neurological decline, this treatment seemed to be safe and achieved encouraging CSF and brain tissue concentrations (67).

In case of sCJD diagnosis, it is important to communicate the diagnosis to family members and to the social services so they can provide the necessary support, counsel families on end-of-life, provide care needs, arrange for hospice evaluation and give advice on financial matters (47).

It is interesting to note that the aspects that most worry the caregivers of patients with sCJD are the disease itself, the complexity of the diagnostic process, the difficulties in taking care of them in end-of-life stages and the increase in responsibilities and needs as the disease progresses. The aspects that help them the most are support with other caregivers, with expert people, resources and emotional support (68).

Finally, genetic therapies or engineering which modify or lower the expression of cellular prion protein (PrPc) could be effective, especially in sCJD and gCJD, but there is still no evidence and further studies need to be done (69,70).

4. JUSTIFICATION

Sporadic Creutzfeldt-Jakob disease is a rare disease that generates a very important negative impact on sCJD's patients and their families because of its neurological deterioration and its rapid progression to death. Unfortunately, nowadays there is not a curative treatment for this disease.

To diagnose a patient with sCJD supposes a big challenge for specialists due to the complexity of the diagnosis process. Different tests are needed to study these patients such as MRI, CSF biomarkers (14-3-3 protein), RT-QuIC and EEG to be diagnosed as a "probable sCJD". For definitive diagnosis it still requires demonstration of the pathological prion protein in the central nervous system requiring brain biopsy or brain autopsy.

Therefore, patients with sCJD often undergo a long diagnostic process in which they go through different specialists and many diagnostic tests before reaching a definitive diagnosis or even die without a definitive diagnosis due to the rapid progression of the disease. This process usually causes **uncertainty, anxiety and iatrogenesis** to our patients and to their families. An early diagnosis of this disease would reduce harm and suffering of the patient.
At the same time, an early stage diagnostic would **reduce healthcare resources and costs** in the diagnosis of sCJD as for example, working time of healthcare professionals, extra diagnostic tests, ineffective treatments, etc.
Furthermore, although there is currently no treatment for sCJD, it is important to diagnose it and to make an **early differential diagnosis** in order to identify other neurological etiologies with similar clinical features to sCJD because the majority of them have well-known treatment. Thus, they could start its treatment in the early stages of the disease and promote greater efficacy and better treatment outcomes.
Another important aspect to take into account is that an early diagnosis of the disease can provide us with possible **targets for future treatments**. In addition, if we define sensitive and specific biomarkers for sCJD disease in early stages, the evolution of these parameters will allow us to assess the efficacy and efficiency of future treatments in clinical trials and other research studies.
Recently, a study evaluating a possible treatment (PRN100, anti-PrPc therapy) in humans with possible CJD in non-terminal stages of the disease affirms that the treatment is safe and that it acquires good tissue and CSF concentrations (67). These promising data reaffirm that further investigations of this treatment and other possible future treatments are needed because they could improve disease prognosis and, although it is a minority disease, have an important clinical impact. Moreover, it has been proved that efficacy of treatments as

doxycycline may vary depending on the time when it is applied and that efficacy is greater in the early stages of the disease, which further justifies the importance of an early diagnosis (65).

Therefore, the early diagnosis of sCJD could generate a change and an impact in the management and clinical practice of this disease and it could serve as a basis for possible subsequent investigations, especially in sCJD's treatment.

Nowadays, since 2018, a new technique called RT-QuIC has been included in the diagnostic criteria for the sCJD (12). Although, to confirm diagnosis we still need postmortem anatomopathological study, RT-QuIC technique has shown the highest accuracy for the diagnosis of probable sCJD when neuropsychiatric disorders appear. It has been demonstrated that it can efficiently detect low amounts of sCJD prions in CSF (11); with a high sensitivity and a specificity of almost 100 percent (13,35). For that reason, in case of a RT-QuIC positive test with a previous clinical suspicion, we can diagnose a probable sCJD and rule out other neurological pathologies.

Nevertheless, this test presents some limitations that have led to a decrease in its use in clinical practice. Although the RT-QuIC test is starting to be established in some reference laboratories in Spain, since 2018, only 4 percent of all notifications of human transmissible spongiform encephalopathies have used RT-QuIC test for its diagnosis while 14-3-3 protein in CSF was determined in 89 percent of the notified cases (5). This data evidences the **poor accessibility** of this test and therefore, the difficulty of obtaining a probable diagnosis through the RT-QuIC test.

One of the reasons why it is difficult to establish this test is that it requires specific equipment and most of the laboratories depend on external supply of the recombinant prion protein substrate which is also very **expensive** (38). In addition, RT-QuIC test is more **complex** to realize than other CSF biomarkers and has **less inter-laboratory standardization** compared to other approved biomarkers (12,38).

Another relevant data is that RT-QuIC test detects the presence or absence of the pathological prion protein but does not have the ability to, without complementary tests, differentiate between the different forms of prion diseases (13) or to differentiate between different subtypes of sCJD which give us important prognostic information (12). Moreover, it has been reported that RT-QuIC sensitivity is lower in MM1, the most frequent subtype of sCJD (12).

Due to the lack of universal availability of the RT-QuIC test and the clinical relevance of an early and accurate diagnosis of sCJD, several studies have been conducted to demonstrate the sensitivity and specificity of other more accessible CSF biomarkers. The statistically

significant results have suggested that t-tau and p-tau/t-tau ratio have better accuracy in sCJD diagnosis than 14-3-3 (46) and that combining those CSF biomarkers with 14-3-3 protein can increase diagnostic accuracy; sensitivity and specificity (38,49,50,52). In addition, t-tau and tau ratio biomarkers have been validated for the diagnosis of other neurodegenerative diseases such as Alzheimer's disease, the most prevalent neurodegenerative disease in the world, allowing the availability of these tests in many centers. Moreover, some studies have demonstrated that p-tau/t-tau ratio has elevated specificity when differentiating sCJD from other rapidly progressive neurodegenerative diseases (48,51,71).

On the other hand, NfL studies are showing promising results for diagnosing neurodegenerative pathologies and seems to be the biomarker with the maximum sensitivity for sCJD (12,38). Therefore, although it has low specificity, low NfL levels would practically exclude prion disease diagnosis (38).

Recent studies suggest that determining t-tau, tau ratio and NfL biomarkers in blood based tests have similar or even better results than CSF biomarkers (58–61). Even more, they suggest that blood NfL levels are increased before clinical onset or with minimal symptomatology, so they could be useful in the initial screening of the disease (60,61). Being able to diagnose sCJD by blood biomarkers would have an important impact on patients suffering from this disease as it would reduce risks and damages of invasive tests. In addition, they could be used in the follow-up of the disease and to monitor the response to future treatments.

In conclusion, as a consequence of RT-QuIC test limitations for sCJD diagnosis and the importance of early diagnosis in patients who suffer from this disease, it would be useful to define and scientifically prove more accessible and economical CSF biomarkers which preserve high diagnostic accuracy for the early diagnosis of sCJD and that could be an alternative to RT-QuIC test.

T-tau and tau ratio are validated biomarkers that in some studies have demonstrated high diagnostic specificity for sCJD. Neurofilaments have been suggested to have high sensitivity for the diagnosis of sCJD, and as well as t-tau, indicate neuronal neurodegeneration. Therefore, we suggest that CSF t-tau and p-tau/t-tau ratio in combination with NfL levels have higher sensitivity and specificity than individual determination of those biomarkers and that they have similar diagnostic accuracy to RT-QuIC test. There is very little data available on the diagnostic accuracy of this biomarkers combination. For this reason, it would be very relevant to study the combination of these three biomarkers as an early diagnostic test.

It would be of relevance to study the same biomarkers (NfL, t-tau and and p-tau/t-tau ratio) in blood samples because establishing blood biomarkers for sCJD would cause a great impact on the patients who suffer from this disease as they could be diagnosed and followed-up in a less invasive and more accessible way.

Finally, some studies have observed variations in the biomarkers levels of some tests such as tau protein and NfL in CSF and in plasma determination, depending on the sCJD subtype. It would be relevant to determine the diagnostic accuracy of the biomarkers combination in each subtype and to evaluate their capability to differentiate between the CJD's subtypes.

5. HYPOTHESIS

Main hypothesis:

- T-tau and p-tau/t-tau ratio combined with NfL are accurate biomarkers when determined in **CSF** for the early diagnosis of patients with sporadic Creutzfeldt-Jakob disease.

Secondary hypothesis:

- T-tau and p-tau/t-tau ratio combined with NfL are accurate biomarkers when determined in **plasma** for the early diagnosis of patients with sporadic Creutzfeldt-Jakob disease.
- Accuracy of t-tau and p-tau/t-tau ratio combined with NfL biomarkers determined in CSF and in plasma for the early diagnosis of sporadic Creutzfeldt-Jakob disease varies depending on the **disease subtype**.

6. OBJECTIVES

Main objective:

- Evaluate the accuracy of t-tau and p-tau/t-tau ratio combined with NfL **CSF** biomarkers in the early diagnosis of patients with sporadic Creutzfeldt-Jakob disease.

Secondary objectives:

- Evaluate the accuracy of t-tau and p-tau/t-tau ratio combined with NfL **plasma** biomarkers in the early diagnosis of patients with sporadic Creutzfeldt-Jakob disease.
- Determine the accuracy of t-tau and p-tau/t-tau ratio combined with NfL CSF and plasma biomarkers in each **subtype of sCJD**.

7. SUBJECTS AND METHODS

7.1. Study design

The design of the study is a **multicentric analytical cross-sectional study** as the aim of this study is to evaluate the accuracy of a diagnostic test.
The duration of the study is estimated in three years (*see chronogram)*.

7.2. Study setting

In order to have more volume of patients, due to the low prevalence rate of sCJD, and to guarantee the time estimated of the study, this study will be a multicenter study in which hospitals from fifteen autonomous communities of Spain will participate. We assume that patients in these Spanish communities have similar baseline characteristics, for this reason we do not think that choosing these hospitals can induce a selection bias.
Each autonomous community will centralize all the patients involved in the study to a hospital of the same autonomous community that has been chosen as the reference hospital for the study (*Table 4*). In this reference center all the diagnostic tests will be performed. Afterwards, the samples and test results of all the patients will be sent to a receiving hospital (Hospital Universitari Clínic de Barcelona) where the necessary material will be available to analyze these tests by specialized professionals.

The principal research members from the hospitals of the fifteen autonomous communities will receive a previous explanation on how to identify and recognise the inclusion criteria of this study.
The reference hospital from each autonomous community will have the same required materials and diagnostic equipment with the standardized protocols to perform the data collection. In addition, team researchers from the reference centers will receive a previous training session to ensure that all the patients samples are acquired following the same and the standardized procedure.

Table 4: Selected reference hospitals for each autonomous community. *Made by the author.*

Autonomous Community	Hospital selected as the reference center
Andalucía	*Hospital Virgen del Rocío*
Aragón	*Hospital Universitario Miguel Servet*
Asturias	*Hospital General de Asturias*
Cantabria	*Hospital Universitario Marqués de Valdecilla*
Castilla y León	*Hospital Río Hortega*
Castilla y La Mancha	*Complejo Hospitalario Universitario de Albacete*
Catalunya	***Hospital Universitari Clínic****
Comunidad de Madrid	*Hospital Universitario Ramón y Cajal*
Comunidad Valenciana	*Hospital Universitario La Fe*
Extremadura	*Hospital de Mérida*
Galicia	*Hospital de A Coruña*
La Rioja	*Hospital General de La Rioja*
Navarra	*Hospital Universitario de Navarra*
País Vasco	*Hospital Universitario Cruces de Bilbao*
Región de Murcia	*Hospital Virgen de la Arrixaca*

*The **Hospital Universitari Clínic,** apart from being the reference center of Catalunya, will be the receiving hospital for the samples and test results of all the patients.

7.3. Study population

The target population of this study are patients from the fifteen autonomous communities with rapidly progressive dementia who have sporadic CJD as a differential diagnosis at the time of hospital admission.

7.3.1. Inclusion criteria

- Patients affected by rapidly progressive dementia, defined as an “acute or subacute decline in cognitive functions with impairment of basic activities of daily living in no more than two years” (22,23), who have sporadic CJD as a differential diagnosis at the time of hospital admission.

- Patients over 18 years old at the beginning of the study.
- Informed consent signed by the patient (if they are able to) or the legally authorized representative (a relative or legal guardian).

7.3.2. Exclusion criteria

- Patient without informed consent signed.
- Patients in whom there is suspicion of genetic CJD.
- Patients in whom there is suspicion of variant CJD or iatrogenic contamination of CJD.
- Patients with a previous neurodegenerative disease diagnosis.
- Patients with contraindications for lumbar puncture.

7.3.3. Withdrawal criteria

- Patients who die before we can realize the diagnostic tests.
- Patients with insufficient CSF sample volume to complete the assays (biomarkers detection and RT-QuIC test).
- Patients in whom lumbar puncture, blood tests or other complementary tests such as genetic study, complete neurological examination, magnetic resonance imaging or electroencephalogram cannot be performed.
- Patients who present a mutation in the PRNP gene on chromosome 20 compatible with genetic CJD.
- Patients in whom structural lesions such as vascular pathology or space-occupying lesions justifying neurological symptomatology are detected in their brain MRI.
- Patients with infectious, autoimmune or neoplastic diseases detected in the CSF analysis that are the cause of neurological symptomatology.
- Inability to perform the autopsy and the anatomopathological study of brain tissue.
- Annulation of informed consent.

7.4. Sample

7.4.1. Sample size

T-tau, tau ratio and NfL biomarkers have shown similar diagnostic accuracy (Area Under the Curve (AUC) = 0'95; AUC = 0'98 and AUC = 0'89, respectively) (13,60). It is assumed that the combination of the three of them will have a high diagnostic accuracy.

In a two-sided test with an alpha risk or significance level of 5% and statistical power (1 - beta) of 80% and assuming a high test accuracy (AUC >90%), we will need **87 subjects**. Assuming a drop-out rate of 15% we will finally need **100 subjects** to perform the study.

The 15% has been estimated taking into account that the long period of time between the performance of the initial diagnostic test (blood test, lumbar puncture, MRI and EEG) and the brain autopsy of the patient could increase the drop-out rate.

Computations were carried out with the Prof. Dr. Marc Saez' software based on 'pwr' of the free environmental software R (version 4.2.2).

7.4.2. Estimated time of recruitment

The approximate time of the sample recruitment will be of two years. It has been estimated taking into account the global incidence of sCJD in Spain (1,14 cases per million people/year) (5), and also the total population of the fifteen autonomous communities (72).

With a total population of 43.956.152 people (*Table 5*) and an annual incidence of 1.14 per 1,000,000 people, we would collect a sample of **50 subjects** in one year. Therefore, within two years we would achieve the required sample for the study of **100 subjects**.

Table 5: Estimated population of each autonomous community (72).

Autonomous Community	Population (persons)
Andalucía	8.538.376
Aragón	1.315.523
Asturias	1.005.397
Cantabria	585.222
Castilla y León	2.375.970
Castilla y La Mancha	2.058.049
Catalunya	7.710.136
Comunidad de Madrid	6.825.005
Comunidad Valenciana	5.106.228

Extremadura	1.051.738
Galicia	2.693.451
La Rioja	316.806
Navarra	661.831
País Vasco	2.181.279
Región de Murcia	1.531.141
Total Population:	**43.956.152**

7.4.3. Sample selection

In order to collect our sample we will use a **non-probabilistic consecutive** method. We will apply this sample collection methodology because it is a low prevalent disease, so we need all the patients who meet requirements and sign their informed consent to participate.

7.5. Principal variables

7.5.1. Main objective variables

- **Gold Standard**

The gold standard for the definitive diagnosis of sCJD is the anatomopathological study of the brain autopsy/biopsy. This variable will be summarized as *"definitive sCJD"* or *"non-sCJD"*.

It is a dichotomous qualitative variable.

- **Test variables**

The diagnostic test variables will be t-tau, p-tau/t-tau and NfL levels in **CSF.**

These are continuous quantitative variables.

Subsequently, through the Receiver operating characteristic curve (ROC curve) we will establish the cut-off value of the biomarkers and transform the continuous quantitative variable into a dichotomous qualitative variable. It will be summarized as a "*positive"* or "*negative"* test.

7.5.2. Secondary objectives variables

- **Variables of plasma biomarkers objective**

In order to evaluate the diagnostic accuracy of plasma biomarkers, the gold standard test will be the same as for the main objective *(see Gold Standard).*

The diagnostic test variables will be t-tau, p-tau/t-tau and NfL levels in **plasma.** These are continuous quantitative variables.

Subsequently, through the ROC curve we will establish the cut-off value of the biomarkers and transform the continuous quantitative variable into a dichotomous qualitative variable. It will be summarized as a "*positive"* or "*negative"* test.

- **Stratification variables**

To evaluate the variation of the biomarkers diagnostic accuracy depending on the **sCJD subtype**, we will stratify the patients and determine the biomarkers diagnostic accuracy in CSF and in plasma for each stratum.

The sCJD subtype is defined by the polymorphism of the codon 129 and the type 1 or 2 of the pathological prion protein. The disease subtypes variable is a polychotomous nominal qualitative variable.The disease subtype variable will be summarized as:

  - MM1
  - MV1
  - VV2
  - MV2
  - MM2
  - VV1

7.5.3. Covariables

The main covariables that will be measured are:

- **Age:** brain tissue undergoes changes over time. Aging can promote physiological neurodegeneration and therefore, we must determine if age influences the diagnostic accuracy of the test. It is a polytomous ordinal qualitative variable. We are going to categorize this variable in five age ranges (years old):
    - From 18 to 38
    - From 39 to 58
    - From 59 to 78
    - From 79 to 98
    - More than 98

- **Gender:** Although there is no clear predominance of the disease in either males or females, it must be discarded that the diagnostic test accuracy varies depending on the gender. It is a dichotomous qualitative variable and it is summarized as "*male*" or "*female*".

- **Predominant clinical scenario**: As previously commented, sCJD presents a very heterogeneous clinical symptomatology as it manifests differently depending on each case. It should be determined how the diagnostic accuracy of the test variates depending on the predominant clinical symptom. It is a polytomous nominal qualitative variable and is summarized with six categories:
    - Rapidly progressive dementia: *acute or subacute cognitive decline*
    - Cerebellar manifestations: *nystagmus and ataxia*
    - Myoclonus: *rapid and involuntary muscular movements*
    - Extrapyramidal signs: *hypokinesia, bradykinesia, dystonia, and rigidity*
    - Pyramidal signs: *hyperreflexia, extensor plantar responses and spasticity*
    - Akinetic mutism: *inability to speak or move*

- **Disease duration when realizing the lumbar puncture:** depending on the time that has elapsed since the disease onset at the time of the first lumbar puncture, tests can have different diagnostic accuracy. The disease onset is defined as the starting of the neurological signs or typical sCJD symptoms. It is a polytomous ordinal qualitative variable and we are going to categorize the disease duration (months) in ten ranges:
    - Less than 1
    - From 1 to 3
    - From 4 to 6
    - From 7 to 9
    - From 10 to 12
    - From 13 to 15
    - From 16 to 18
    - From 19 to 21
    - From 22 to 24
    - More than 24

- **Place of residence:** some studies suggest that although the sCJD etiology is not clearly defined, some of the cases could be related with an exposure to a common external factor and follow a disease-clustering mechanism (*see in Etiology*). Therefore, it is relevant to determine whether geographical variations can vary the diagnostic accuracy of the tests evaluated. It is a polychotomous nominal qualitative variable. The variable is summarized as:
    - Andalucía
    - Aragón
    - Asturias
    - Cantabria
    - Castilla y la mancha
    - Castilla y león
    - Catalunya
    - Extremadura
    - Galicia
    - Comunidad de Madrid
    - Región de Murcia
    - Navarra
    - País Vasco
    - Comunidad Valenciana
    - La Rioja

Table 6: Summary of the study variables and covariables. CSF:Cerebrospinal fluid, M: Methionine, NfL: Neurofilament light chain protein, sCJD: Sporadic Creutzfeldt-Jakob disease, V: Valine. *Designed by the author.*

VARIABLE	TYPE	CATEGORY OF VALUES
GOLD STANDARD TEST		
Brain autopsy	Dichotomous qualitative	Definitive sCJD / Non-sCJD
MAIN OBJECTIVE VARIABLE		
Total tau and tau ratio combined with NfL CSF levels	Continuous quantitative	
SECONDARY OBJECTIVES VARIABLES		
Total tau and tau ratio combined with NfL blood levels	Continuous quantitative	
sCJD subtype stratification	Polychotomous nominal qualitative	MM1 / MV1 / VV2 / MV2 / MM2 / VV1
COVARIATES		
Age (years old)	Polytomous ordinal qualitative	From 18 to 38 / From 39 to 58 / From 59 to 78 / From 79 to 98 / More than 98
Gender	Dichotomous qualitative	Male / Female
Predominant clinical scenario	Polytomous nominal qualitative	Cognitive decline / Cerebellar manifestations / Myoclonus / Extrapyramidal signs / Pyramidal signs / Akinetic mutism
Disease duration (months)	Polytomous ordinal qualitative	Less than 1 / From 1 to 3 / From 4 to 6 / From 7 to 9 / From 10 to 12 / From 13 to 15 / From 16 to 18 / From 19 to 21 / From 22 to 24 / More than 24

Place of residence	Polytomous nominal qualitative	Andalucía / Aragón / Asturias / Cantabria/ Castilla y la mancha / Castilla y león / Catalunya / Extremadura / Galicia / Comunidad de Madrid / Región de Murcia / La Rioja / Navarra / País Vasco / Comunidad Valenciana

7.6. Data collection

- **Patients selection** will be performed in **all the hospitals of the fifteen autonomous communities.**The patients that meet inclusion criteria and do not have any exclusion criteria will be offered to participate in the study. They will be informed in detail about the aims of the study, the risks and benefits associated and will be given an information sheet (Annex 2). Afterwards, they will be able to voluntarily decide whether they want to participate in the study or not, and sign the informed consent (Annex 3).

The patients involved in the study will be referred to the reference hospital of its autonomous community to perform all the required tests.

- In the **reference hospital,** the neurologist will ask and fill out a questionnaire to patients enrolled in the study (Annex 1). All the data collected will be entered by the neurologist to a database.

  In the data collection questionnaire, the information required will be:

  - *General information:* gender, age, contact person and mobile number.
  - *Clinical history:* patient diagnosed pathologies or comorbidities, actual medications, family history of diagnosed rapidly progressive neurodegenerative disease, history of potential iatrogenic exposure (dura mater implants, corneal grafts or human cadaveric pituitary hormones), a trip to the United Kingdom more than ten years ago, and frequent beef meals.
  - *Relevant information for the study (some of the covariables):* predominant clinical scenario, disease duration since symptomatology onset and place of residence.

  Afterwards, a basic neurological examination will be performed by the same person.

- Subsequently, the patient will undergo:
    - **Blood test.** In the blood sample we will analyze:
        - General analytical parameters: Hemoglobin and leukocyte form, general biochemical parameters (glucose and lipid profile), renal and hepatic function, Human Immunodeficiency virus (HIV) and luetic serology, thyroid hormones (thyroid stimulating hormone (TSH)) and vitamins B12 and B9.

          If some of these parameters is altered, it may indicate an alternative etiology of the associated symptomatology.
        - T-tau, p-tau/t-tau ratio and NfL biomarkers determination.
        - DNA extraction to perform a genetic study in which we will determine de codon 129 polymorphism and the PRNP gene mutation.
    - **Lumbar puncture.** In the CSF sample we will perform:
        - General parameters such as biochemical parameters (glucose, cells and proteins), cytology and CSF culture in order to identify differential etiologies which could explain the patient's symptomatology.
        - T-tau, p-tau/t-tau ratio and NfL biomarkers determination.
        - RT-QuIC test
    - **MRI**. Following the protocol recommended sequences will be performed which are T1-weighted axial images, T2-weighted axial images, FLAIR axial and sagittal images at 3 mm slice thickness, and DWI (40).
    - **EEG**.
- Finally the **autopsy** will be performed when the patient dies in order to realize the anatomopathological study.

Afterwards, CSF samples, blood samples, MRI and EEG results and later the brain autopsy will be sent to the **receiving center (H. Clínic)** where the necessary equipment will be available to carry out the RT-QuIC test and the study of the brain tissue by two expert neuropathologists. The general analysis and the determination of the studied biomarkers in CSF and blood samples will also be performed at the receiving center, which will have the required ELISA and Single molecule array (Simoa) Kits, respectively. Also, two specialized neuroradiologists will analyze and report the patients results of the MRI and two specialized neurophysiologists will analyze and report the patients EEG results.

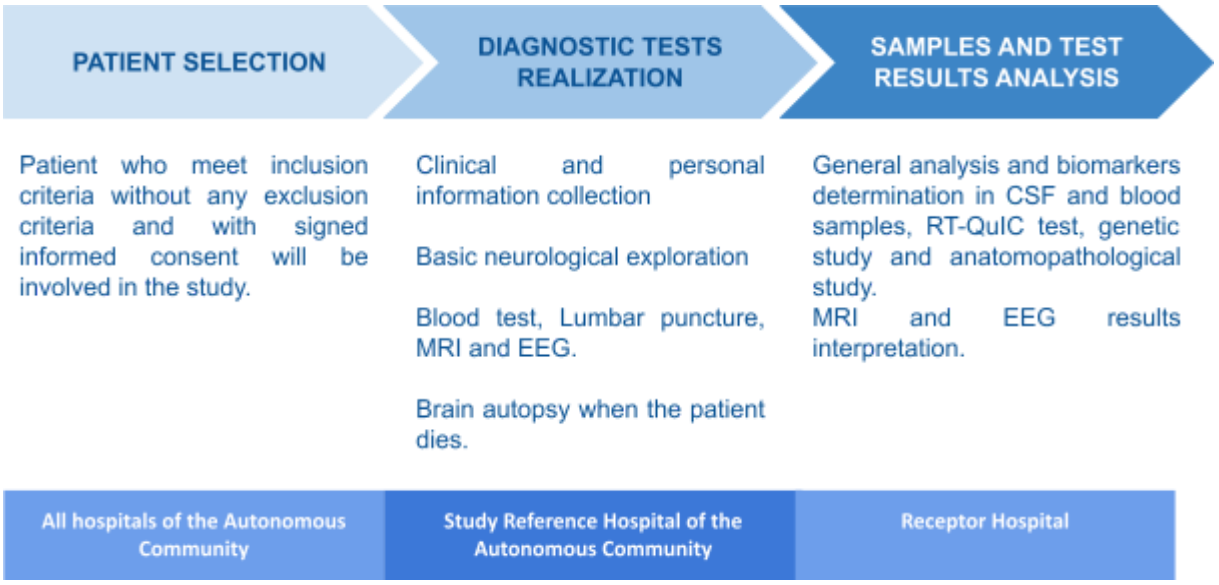


Figure 8: Scheme of the data collection procedure. CSF: Cerebrospinal fluid, EEG: Electroencephalogram, MRI: Magnetic Resonance Imaging, RT-QuIC: Real-time quaking-induced conversion. *Made by the author.*

In order to prevent possible loss of information, we will create a **computer-based database** using a statistical program in which data collected will be introduced. Each patient will be recorded with an identification number to preserve anonymity. All the collected data should be introduced in the study database and the date of collection and the professional who collected the data must be registered. There will be independence in data collection and analysation as the professionals who analyze the biomarkers test will be different from the ones who analyze the gold standard test.

Throughout the data collection and analysis process, **regular online meetings** will be done to evaluate the procedure and quality of the database, detect errors and solve possible problems that may have appeared.

7.7. Measuring instruments

All methodologies and procedures are going to be performed in accordance with the standardized guidelines and protocols.

7.7.1. CSF and blood samples

Following the standard procedure, from the lumbar puncture, CSF samples are acquired, centrifuged, aliquoted and stored at -80ºC until analysis. Blood samples will be processed the same way (35,60,73). CSF t-tau, p-tau/t-tau ratio and NfL levels are going to be measured with ELISA (35,60,73). Moreover, plasma t-tau, p-tau/t-tau ratio and NfL are going to be

determined using ultra-sensitive Simoa immuno-assay, which has shown greater sensitivity than standard sandwich based immunoassay techniques (33,58–60).

7.7.2. Genetic study

For the genetic study, DNA is extracted from the blood specimens in order to determine whether there is a mutation in the PRNP gene and the M/V polymorphism at codon 129 (73).
The PRNP gene mutation will be determined with the amplification of this region using the polymerase chain reaction (PCR) with specific primers and the gene sequencing (74).
The codon 129 polymorphism is going to be determined by performing the quantitative polymerase chain reaction technique (qPCR) using an allelic discrimination assay (75) .

7.7.3. MRI

Brain images will be acquired using a 1.5 Tesla scanner. The recommended protocol includes T1-weighted axial images, T2-weighted axial images, FLAIR axial and sagittal images at 3 mm slice thickness, and DWI (40).

7.7.4. EEG

It is recorded via surface electrodes placed according to the international and standardized 10-20 system (43). Devices needed are computer, electrode connection box and light stimulation lamp. In addition, the materials required are a cap of electrodes, harness, electrodes of clamp for ECG, syringe and needle without tip, pasta and conductive gel, alcohol and gauze, sensor and abdominal band, spoon electrodes and adhesive electrodes.

7.7.5. RT-QuIC

RT-QuIC test will be performed following the "*Laboratory standard operating procedure for detecting sCJD using RT-QuIC"* (34). It is a technical report developed by the European Centre for Disease Prevention and Control (ECDC), version 1.0 from February 2021.

7.7.6. Anatomopathological study of the brain

The anatomopathological study of the brain tissue will be carried out with the patient autopsy. According to the standardized protocol, histological and immunohistochemical analysis must be done in the left half of the brain that will be fixed in formalin. On the other hand, samples from frontal/temporal, occipital cortex and cerebellum will be taken from the right half of the brain and will be immediately frozen and stored at -80°C in order to realize the Western Blotting of PrPsc after partial digestion and determine the type of pathological prion protein. (33,35,74).

8. STATISTICAL ANALYSIS

The statistical analysis will be carried out by the statistical analyst and it will be done using the Statistical Package for Social Sciences (SPSS) software version 28.1.

We will establish a $p<0.05$ value as statistically significant, defining a 95% confidence interval for all analyses.

8.1. Descriptive analysis

The gold standard variable is a qualitative dichotomous variable and will be summarized by proportions.

Plasma and CSF levels will be summarized by means, standard deviation, medians and interquartile range (IQR).

All these analyses will be stratified by the subtypes and additional stratification will be done by the covariates. To know if the differences between sCJD subtypes in the diagnostic accuracy of the biomarkers are statistically significant, the chi-square test will be used as proportions are being analyzed.

8.2. Accuracy parameters

We will analyze all accuracy parameters described below for each biomarker individually (t-tau, tau ratio and NfL). Subsequently, we will evaluate the same parameters for two biomarkers combinated (t-tau and tau ratio) and finally, we will calculate the same parameters for the combination of the three biomarkers (t-tau, tau ratio and NfL).

To estimate the cut-off value of each biomarker we will use the software SPSS. But, to estimate the cut-off value of each biomarker when combining them, instead of SPSS, we will use the package 'Epi' of R (version 4.2.2).

This procedure will be performed on CSF-determined biomarkers, plasma-determined biomarkers and it will be stratified by sCJD subtypes.

8.2.1. Sensitivity and specificity determination

Sensitivity and specificity are statistical measures used to evaluate the diagnostic accuracy of a test.

- **Sensitivity:** is the test probability to identify as "*positive*" those who actually have the disease (76).
- **Specificity:** is the test probability to identify as *"negative"* those who actually do not have the disease (76).

Therefore, in order to estimate these two measures, we must create a 2x2 table (*Table 7*) with the values obtained in the gold standard test and in the biomarkers test.

Then we will classify patients into (76):

- True positives (TP): patients with the disease and with a "positive" result in the test.
- True Negatives (TN): patients without the disease and with a "negative" result in the test.
- False Positives (FP): patients without the disease and with a "positive" result in the test.
- False Negatives (FN): patients with the disease and with a "negative" result in the test.

Afterwards, we will calculate the sensitivity and specificity following the corresponding operations (76):

- Sensitivity: TP / TP+FN
- Specificity: TN / FP+TN

Table 7: Basic table 2x2 for the sensitivity and specificity estimation. NfL: Neurofilament light chain proteins, CJD:Sporadic Creutzfeldt-Jakob disease, t-tau: Total tau. *Made by the author*.

	Positive for sCJD	**Negative for sCJD**	
Positive t-tau and NfL test	True positives (TP)	False positive (FP)	Total of patients with positive tests
Negative t-tau and NfL test	False negative (FN)	True negative (TN)	Total of patients with a negative test
	Total of definitive sCJD	Total of Non-sCJD	

As they are sample estimators of population parameters, they must be accompanied by an error estimation; a 95% confidence interval (76).

8.2.2. Predictive values

In order to know how much the result of a test can change the previous knowledge about the patient's condition, we use the predictive values. The result of these predictive values change according to the disease prevalence understood as the pre-test probability of suffering from the disease (76).

The Positive Predictive Value (PPV) is defined as "the conditional probability that individuals with a positive test actually have the disease" (76):
PPV = P (Disease / TP)

The Negative Predictive Value (NPV) is defined as "the conditional probability that individuals with a negative test really do not have the disease"(76):
NPV = P (No Disease / TN)

In the case of this study, since the sample is not random, we cannot estimate predictive values from the 2x2 table discussed above. Thus, in order to calculate the predictive values, we will use mathematical formulae derived from the application of Bayes' Theorem. We need to know:

- Sensitivity of the test = SE
- Specificity of the test = SP
- Disease Prevalence = P (Dis)
- Positive Predictive Value = PPV
- Negative Predictive Value = NPV

Applying Bayes' Theorem, the formulas are:

$$PPV = \frac{SE \times P(Dis)}{SE \times P(Dis) + (1-SP) \times \{1 - P(Dis)\}}$$

$$NPV = \frac{SP \times \{1 - P(Dis)\}}{SP \times \{1 - P(Dis)\} + (1-SE) \times P(Dis)}$$

8.2.3. Likelihood ratio

The Likelihood Ratio (LR) is defined as " the ratio between the probability of a test result in sick subjects and the probability of the same result in subjects who are not sick" (76). It combines the sensitivity and specificity information for its calculation.

The **Likelihood ratio for a positive test result** is an indicator that tells us how many times the test is more likely to be positive in sick people than in those who are not sick, while the **Likelihood ratio for a negative test result** is an indicator that tells us how many times the test is more likely to be negative in sick people than in those who are not sick. The formulas to calculate them are (76):

Positive LR = S / (1-E)

Negative LR = (1-S) / E

8.2.4. AUC by ROC curve analysis

The ROC curve analysis is a statistical method used to evaluate the diagnostic accuracy of a quantitative test by the determination of a cut-off value. The Y-axis corresponds to sensitivity and the X-axis to 1-specificity. The axis include values from 0 to 1 (0 to 100 percent) (77).

For the different values of the test, sensitivity and 1-specificity are calculated and represented on a graphic. With all the values together, we will obtain the ROC curve of the test. The value with a higher sensitivity and specificity will be defined as the **cut-off value** of the test which corresponds to the value that presents the major **Youden Index** = (sensitivity + specificity -1) (77). Visually, this cut-off value is the point that gets closer to the left-superior corner of the graphic. *Figure 9* shows an example of the ROC curve for two tests (A and B) (77). The values indicated with an arrow are the cut-off values in these cases.

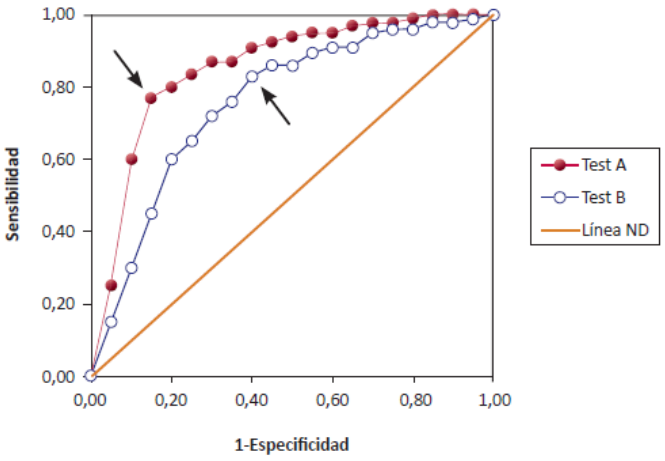


Figure 9: Example of a Receiver operating characteristic curve. (77).

The area under the curve (AUC) informs us about the ability of the test to discriminate the patients who are sick from the patients who are not sick (77).The diagonal line that goes from (0,0) to (1,1) is called non-discrimination line (orange line in Figure 9) and represents a diagnostic test with an AUC of 50% (0.5). If the ROC curve of a test follows this line, the test is not able to discriminate between patients with the disease from healthy patients. As the ROC curve moves away from this non-discriminative line and approaches the left-superior corner of the graphic, its ability to differentiate between sick and non sick patients increases and the AUC value will be closer to 1.To analyze whether the difference between the AUC of two different tests or combinations of biomarkers is statistically significant, the chi-square measure will be used (77).

As it is a sample estimator of a population parameter, it must be accompanied by an error estimation; a 95% confidence interval. Is important to notice that if the 95% confidence interval contains the value 0.5, then it cannot be affirmed that the cut-off value has a discriminative capacity (77).

9. WORK PLAN

Research team members

It is a multidisciplinary study, so the research team will be composed of:

- Neurologists from each reference hospital will perform the clinical data collection, neurological exploration and also the lumbar puncture to collect CSF samples. In addition, they will supervise and coordinate all the activity of their center. They will be the principal investigators of the study.
- Nursing staff from each reference hospital will participate in the realization of diagnostic tests and sample collection.
- Laboratory technicians from the Hospital Clinic who will perform:
    - CSF samples analysis, T-tau, p-tau/t-tau ratio and NfL determination and RT-QuIC test.
    - Blood samples analysis with t-tau, p-tau/t-tau ratio and NfL determination.
    - Genetic study from all the samples collected.
- Imaging technician from each reference hospital will perform MRI on the patients from the autonomous community.
- Neuroradiologist from the Hospital Clinic will report all the images collected.
- Neurophysiologist from the Hospital Clinic will report all the electroencephalograms performed.
- Neuropathologists from the Hospital Clinic will perform the histological and immunohistochemical analysis of the brain tissue.
- Statistician expert will be the person to carry out the statistical analysis of the study.
- Scientific researcher: will collaborate to interpret the results, write the paper and present results.
- Data manager: will design and create the database of the study and will periodically check its functioning.

The principal investigators will coordinate the project, organize the periodical meetings and keep in contact with all the professionals from the research team.

This project will be developed during an estimated period of three years but it can vary depending on the time required to obtain the sample and autopsy of all the patients. If needed, an extension will be requested. It will be organized into six stages.

STAGE 1: Protocol design and ethical evaluation

Study design: a bibliographic research and literature review is needed in order to learn more about the subject of study, define the areas where further research is needed and justify the study protocol. Once the topic is defined, the hypothesis, objectives, variables and type of study need to be designed as well as population (inclusion and exclusion criteria), sample size and sample collection. Finally, data collection and work plan have to be described as well as the chronogram, the budget and feasibility of the study.
This phase is being developed by principal investigators in a period of time of two months (December 2022 - January 2023).

Ethical evaluation: the protocol will be presented to the Clinical Research Ethics Committee (CEIC) to verify that it meets the necessary ethical requirements.
This phase will be developed in three months (February 2023 - April 2023).

STAGE 2: Coordination and health professionals training

Centers coordination: Once the protocol is approved by the CEIC, an informative meeting will be organized for the hospitals of each autonomous community to start the inclusion of the patients in the study.
In addition, another online meeting will be performed with all the principal investigators from the reference hospitals to remind the study protocol and its timeline. It will be a good opportunity to solve possible doubts or problems.
This phase will be developed by principal investigators and the estimated duration of this phase is one month (May 2023).

Research Team Formation:
All the neurologists, nursing staff and imaging technician from the reference hospitals will receive a **training session** in a telematic meeting. It will be explained the correct performance and the standardized protocols of all the procedures and techniques used in the sample collection process as well as how to transfer the personal and clinical data to the database. The objective is to unify the procedure and reduce the differences that may exist between the different professionals and services of the hospitals. Also, it will be explained the conditions under which the samples must be preserved.

An online meeting with specialized laboratory technicians, neuroradiologists, neurophysiologist and neuropathologists from the Hospital Clinic will be organized in order to explain how to transfer all the data collected to the database. In this case, there is no need to

unify any procedure as all the samples will be analyzed in the same center by highly specialized professionals with years of experience.

Taking into account all these measures, we ensure homogeneity in data collection procedure so that there can be obtained representative conclusions from this study.

Finally, study material such as personal and clinical questionnaires, informed consent sheets and informative papers will be prepared and distributed.

This phase will be developed by principal investigators and the estimated duration of this phase is one month (June 2023).

STAGE 3: Sample collection and data collection

- The patients visited in the neurology services of any of the hospitals from the areas included in this study, who meet inclusion criteria without any exclusion criteria will be asked to participate in the study. Neurologists from each center will explain the study protocol to the patients and will provide them with the **information sheet** (Annex 2).
- After being sure that they have understood the information given and solved any doubts or questions, the **informed consent** will be given to sign (Annex 3).
- **Sample collection and diagnostic tests** will be performed by the professionals from each reference hospital (clinical data collection, neurological exploration, blood test, lumbar puncture, RMI and EEG). All the samples collected will be sent to the receiving hospital (H. Clínic).
- Laboratory technicians, neuroradiologists and neurophysiologists from The Hospital Clínic will analyze and inform all the samples (CSF and blood samples) and the diagnostic test results (MRI and EEG). Subsequently, they will introduce all the information into the database which will be checked periodically to ensure that it is being used correctly.
- **Brain autopsy** (gold standard test) will be performed when the patient dies. As the rest of the samples, it will be sent to the receiving hospital (H. Clínic) where the anatomopathological study will be performed by specialized neuropathologists.
- Every three months, all the **research team will meet** in order to evaluate the study progression, review the database, assess that it is being well implemented and detect possible errors that can be corrected.

A recruitment of patients by neurologists will be done until the 100 patients required are included. The time estimated for data collection is two years (July 2023 - July 2025) although it can vary depending on the time needed to achieve the required sample size and the time at

which the data collection can be completed for all the patients of the study, in particular, the brain autopsy. The time of death of patients, especially of those who do not suffer from sCJD, can vary from the estimated time and is difficult to calculate accurately.

STAGE 4: Statistical analysis and interpretation of results

When data collection is being completed, data will be compiled and **analyzed by the statistical expert** using the necessary statistical tests depending on the study variables.

Subsequently, the **results** will be analyzed, described, **discussed and interpreted** by the principal investigators and the scientific researcher, and finally, from all this information collected, the **study conclusions** will be obtained.

This stage will take place in two months (August 2025 - September 2025) by the statistical expert, principal investigators and the scientific researcher.

STAGE 5: Redaction of the final report

An article with the study explanation, results and conclusions obtained will be **redacted** by the principal investigators of the team research and the collaboration of the scientific researcher.

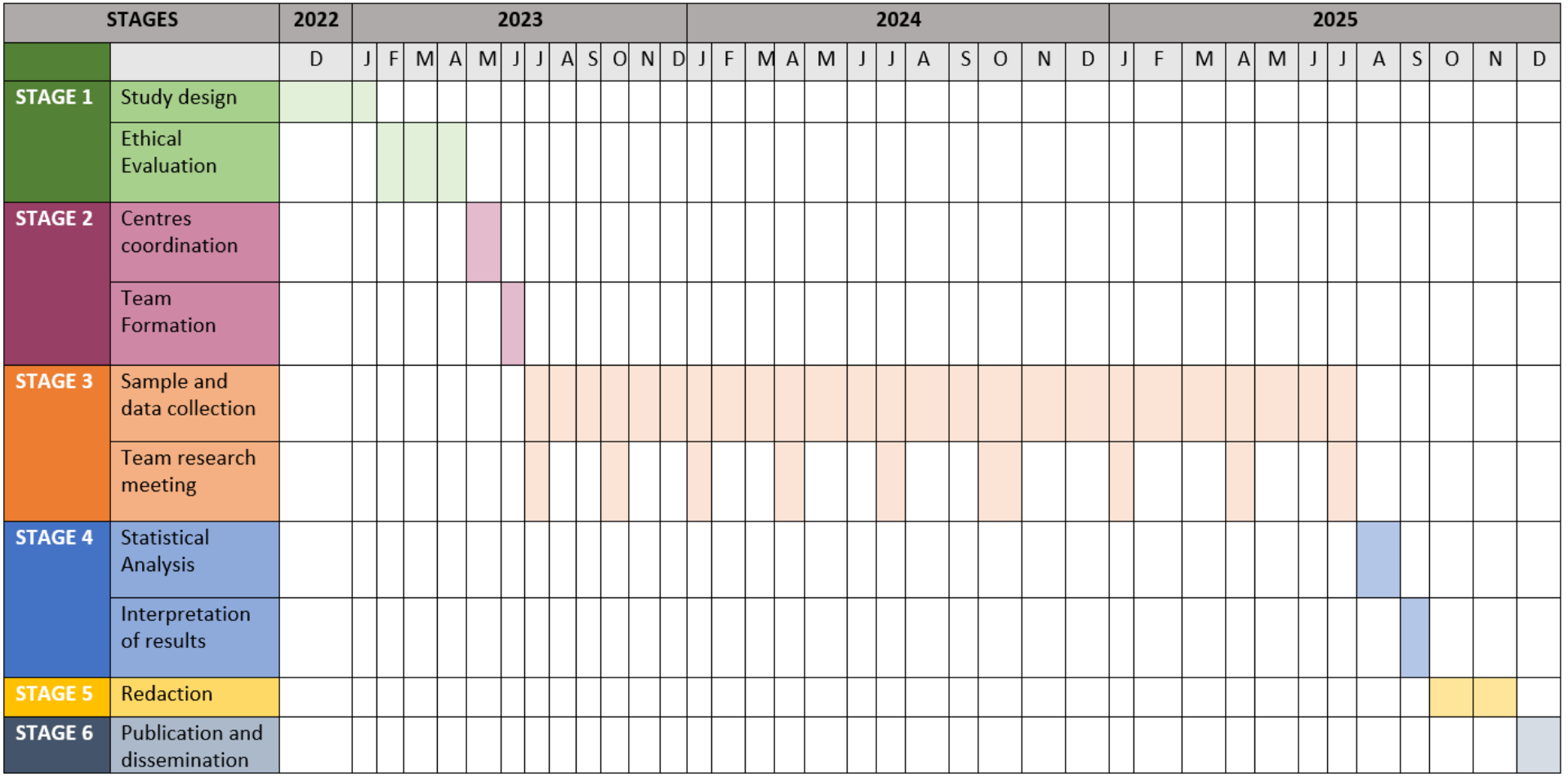
This procedure will be realized in two months (October 2025 - November 2025).

STAGE 6: Publication and dissemination of the results

The paper will be sent to open-access and international neurologic journals and to centers of investigation. In addition it will be exposed in national and international neurological congresses in order to inform about this disease and about the conclusions obtained in the study.

The principal investigators with the cooperation of the scientific researcher will carry out this stage in one month (December 2025).

10. CHRONOGRAM



11. ETHICAL CONSIDERATIONS

This study will be realized according to the ethical principles defined in the Declaration of Helsinki (1964) of Ethical Principles for Medical Research Involving Human Subjects revised in October of 2013 by the World Medical Association.

It is mandatory that the study complies with the *"Ley 14/2007, de 3 de julio, de investigación biomédica y el Real Decreto 1716 /2011"*. The protocol will be presented to the CEIC and will be modified according to the committee evaluation.

All patients will be well informed about the study, its objectives, risks and benefits and will be given an information sheet (Annex 2). Respecting patient's autonomy and according to *"Ley 41/2002, de 14 de noviembre, básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica",* patients (if they are able to) or legally authorized representatives (a relative or legal guardian), will be able to decide and sign voluntarily the informed consent and to leave the study at any stage without being judged, injured or having any health care consequences (Annex 3). If a participant ends to be part of the study, all the data collected will be eliminated.

When collecting data, only those data that are necessary for the study will be collected (principle of minimization) and only the members of the research team will have available access to them. Professionals must be transparent throughout the entire process of data collection and patients will be constantly informed of the diagnostic tests that are being performed. All data and information will be used for the sole purpose of realizing the study.
Professionals collecting and analyzing the data must preserve the anonymity and privacy of patients according to *"Ley Orgánica 3/2018, de 5 de Diciembre, de Protección de Datos Personales y Garantía de los derechos digitales*." All the professionals with access to the diagnostic tests, results or database will have to sign a confidentiality agreement (Annex 4).

This study respects The Four Principles of Beauchamp and Childress: the principle of autonomy, beneficence, non-maleficence and justice.

- It respects the principle of autonomy because the patient will be properly informed about the study and will be able to decide voluntarily and during the whole study whether or not he/she wants to take part in it without any consequences. Therefore, once having all the information they are free to make their own decision.

- The study complies with the principle of beneficence as it could define a more accessible way to reach an early diagnosis of the disease, in a way that would reduce iatrogenesis and anxiety to patients and family members. In addition, it could serve as a new therapeutic target for future treatments.
- The study is carried out in accordance with the principle of non-maleficence, since it will be performed on people who are already ill and present symptomatology. In addition, most of the diagnostic tests are already routinely performed by protocol in patients with rapidly progressive dementia.
- It respects the principle of justice because all the patients meeting the inclusion and exclusion criteria will have equal opportunity to participate in the study and equal accessibility. There will be no discrimination and diagnostic tests and resources will be carried out in the same way to all the patients of the study.

12. STRENGTHS AND LIMITATIONS

The main limitations of this study are:

- Sporadic CJD is a **low prevalent disease** which indicates that it would take a long time to achieve the sample size. To reduce this limitation, we will carry out a multicentre study in fifteen autonomous communities in Spain in order to enlarge our study population.
- As it will be a multicentre study, an **information and measurement bias** could occur. In order to avoid this bias, a reference hospital has been selected in each autonomous community where all the diagnostic tests of the patients from the same community will be performed. These hospitals will have the same devices and the appropriate materials to carry out all the analyses. In addition, all the biologic samples (CSF sample, blood sample and brain autopsy) will be sent to a receiving hospital to ensure that they are analyzed using the same methodologies and standardized procedures. In the same way, the MRI and EEG results will be also sent to the receiving hospital where they will be reviewed by two specialized neuroradiologists and two neurophysiologists, respectively.
- A randomized sample is always better for controlling unknown confounding variables. However, in this study, due to the very low prevalence of the disease, the type of sampling cannot be randomized, so it will follow a **non-probabilistic consecutive** method.
- Sporadic CJD is a minority and **uncommon disease** in clinical practice. Therefore, some specialists may not easily suspect this disease and some patients who meet the inclusion criteria may not be included in the study. To try to make specialists more aware of the study, at the beginning of the study, an informative meeting will be organized for the hospitals participating in the study.
- **Study length**: to avoid performing a biopsy, which would involve an invasive test and associated iatrogenic complications for the patient, we will wait for the patient's autopsy to perform the anatomopathological study. This is why it is difficult to estimate the duration of the study until the data collection can be completed.
- The time period between the realization of the initial diagnostic tests and the autopsy may cause **case losses** in the study. However, it is not expected to cause any problem because it was taken into account during the estimation of the sample size.
- When the **interpretation of the results** is carried out, if characteristic alterations of a disease (sCJD or any other disease of the differential diagnosis) are detected, it could condition the interpretation of the rest of the tests. In order to avoid it, each diagnostic test will be analyzed by a different professional and they will not be able to know or to

have access to the results of the other tests. Thus, there will be independence between the professionals performing and interpreting each test without any possible exchange of information (blind analysis).

- The studied biomarkers (t-tau, tau ratio and NfL) could be altered by pathologies that cause neuronal degeneration and therefore, the test **result could be also altered.** To minimize this limitation, all patients who have been previously diagnosed with any neurodegenerative pathology that could modify the outcome will be excluded.
- This study has a **high cost** because some of the diagnostic tests required are very specific and expensive. The most expensive tests are the autopsy which is required for the definitive sCJD diagnosis and the RT-QuIC test. To be able to assume these costs, we will submit the protocol to multiple public and private funding calls.

The strengths of this study are:

- The early diagnosis of sCJD as well as the diagnostic accuracy of t-tau, tau ratio and NfL biomarkers evaluated in this study is a topic little studied in medicine. Therefore, a strength of this study is that it is a **novel topic** with little evidence in the literature.
- The inclusion in the study of several diseases that mimic sCJD clinical symptomatology as well as diagnostic test abnormalities, reflects that if biomarkers assessed show high diagnostic accuracy, they are not only good at distinguishing the disease from healthy people, but can also **differentiate sCJD from those with alternative diagnoses**. This greatly increases the value of the diagnostic test evaluated because these tests will be used to differentiate sCJD from the other possible differential diagnoses.
- Finally, it should be noted that achieving an accessible and more economical alternative for the early diagnosis of sCJD, apart from having a great impact on patients and their families, can serve as a **basis for future studies** (*see Further Studies*).

13. STUDY BUDGET

1. Protocol design

No compensation has been given for the bibliography search and the protocol design.

2. Staff costs

All the members of the research team (neurologists, nursing staff, laboratory technicians, imaging technicians, neuroradiologists, neurophysiologist, neuropathologists) are part of the National Health System and work in one of the fifteen reference hospitals of the autonomous communities included in the study. The required tasks for the study will be realized during their working hours, so there will not be any additional cost.

3. Subcontracted professional services

A **data manager** will help us to create the database (approx 10 hours) and he will connect to each regular meeting of the research team to check the correct use of the database and to solve possible doubts. It has been estimated to be 9 meetings of 2 hours duration. These meetings will be online so there will not be transport costs. The data manager will work a total of 30 hours at 30€/hour.

A **statistician** will be hired to perform the data analysis. The estimation is that 25 hours/week during one month will be required, resulting in a total of 100 hours, with a cost of 30€/hour.

A **scientific researcher** will be hired during the last four months after the statistical analysis is done. He will help to interpret all the results as well as to write them in the paper. In addition, he will also help in the publication and dissemination procedure; contacting investigation centers and organizing national meetings and congresses. It has been estimated to be 5 hours/week of work, with a total of 80 hours and a cost of 30€/hour.

4. Coordination and Research team formation

The meetings that will take place in order to inform and briefly explain the project to all the personnel who will participate in this study do not need extra funding as they will be done online.

The training session will have an estimated duration of 2h. The research members of the reference hospitals will be divided into three reduced groups to receive the training session. The expert trainer has a cost of 30€/hour. Any additional transport costs are needed as the training session will be online.

5. Implementation costs

The questionnaire to collect clinical and personal information and a basic neurological examination performed in the first visit will not have any cost.
As blood tests, lumbar puncture, MRI and EEG that we need for our study are tests usually performed in patients with rapidly progressive dementia, they are not included in the study budget.

However, apart from the basic analysis of CSF and blood samples, this study requires the determination of t-tau, tau ratio and NfL biomarkers in CSF and plasma. For it, CSF biomarkers will be determined using ELISA kit. 40 samples will be analyzed per kit. Therefore, we need 3 ELISA kits for each biomarker determination. In total, 9 ELISA kits are needed and each one of them costs 992€.
The RT-QuIC test will also be performed for each CSF sample and has a cost of 258€/patient.

In plasma, biomarkers determination will be carried out with Simoa Assay Kits. As with ELISA, 40 samples will be analyzed per kit, so we need 3 Simoa Assay Kits for each biomarker determination. Each Simoa Assay Kit has a price of 1200€, and in total 9 kits are required.

At the same time, a genetic study will be performed with the extracted DNA of plasma samples. For each genetic study we need to take into account the DNA extraction kit which has a cost of 330€ and is able to perform 50 reactions. Thus, we will need 2 units.
Afterwards, in order to determine the codon 129 polymorphism of the prion protein gene, we will perform the qPCR using an allelic discrimination assay with a total cost of 640€ (including all the samples).
Finally, for the determination of the E200K mutation of the PRNP gene, the gene will be amplified with the PCR using specific primers and subjected to sequencing analysis. This process has a cost of 95€ each sample.

To carry out all these procedures it is necessary to have general laboratory equipment (tips, plates, microtubes,etc) which have an approximated cost of 900€.

Some of the patient's will need a transport to the reference hospital of its autonomous community. Therefore, it will be an additional cost of approximately 50€/patient. When calculating the budget, we assume the maximum number of transports required.

Finally, when the patient dies, the anatomopathological study of the brain autopsy will be carried out for each patient. It has an approximate cost of 2.500€ each.

The transport of the biological samples has an approximate cost of €15 each CSF sample, 15€ each blood sample, and €110 each brain tissue. When estimating this price, the specific conditions required to transport the biological samples of this study have been taken into account (dry ice at -80ºC).

The regular meetings performed during the data collection procedures, will not have additional cost as they will be done online.

6. Material

We need to take into account that some informative sheets need to be printed such as information sheets (4 pages), informed consent (2 pages) and professional confidentiality agreement (1 page). The printing has a cost of 0'03€/page (black and white).
In addition, the creation of the software and database has a cost of 1.000€.

7. Publication and dissemination

For publication fees, 2.000€ have been estimated. In addition, study results will be exposed in national and international congresses with an estimated cost of 1.000€/attendant and 2.000€/attendant, respectively. This price includes inscription, transport, accommodation and diets. Two attendants will be invited to each congress.

Table 8: Budget of the study. CSF: Cerebrospinal fluid, ELISA: Enzyme-linked immunosorbent assay, PCR: Polymerase chain reaction technique, RT-QuIC: Real-time quaking-induced conversion, qPCR; Quantitative polymerase chain reaction technique. *Made by the author.*

STUDY BUDGET	QUANTITY	COST	SUBTOTAL
1. Protocol design			
Bibliography search and protocol design		0€ /hour	0€
2. Staff cost			
Research team		0€ /hour	0€
3. Subcontracted professionals services			
Data manager	30 hours	30€ /hour	900€
Statistician expert	100 hours	30€ /hour	3.000€
Scientific researcher	80 hours	30€ /hour	2.400€
4. Coordination and Research team formation			
Meetings for centers coordination		0€ /hour	0€
Training session	6 hours	30€ /hour	180€
5. Implementation costs			
First visit: - Questionnaires - Basic neuronal exploration	100	0€ /hour	0€
ELISA test for CSF biomarkers determination	100 (9 kits)	992€/kit	8.928€
RT-QuIC test	100	258€/test	25.800€
Simoa test plasma biomarkers determination	100 (9 kits)	1200€/kit	10.800€

Genetic study: - DNA extraction - qPCR kit for the polymorphism codon 129 - PCR and PRNP gene sequencing	 - 2 - 1 - 100	 - 330€/kit - 640€/kit - 95€/patient	 660€ 640€ 9.500€
Laboratory materials (tips, plates, microtubes, etc)			900€
Autopsy	100	2.500€/patient	250.000€
Patients transport	100	50€/patient	5.000€
Sample Transport - CSF sample - Blood sample - Brain autopsy	 - 100 - 100 - 100	 - 15€/patient - 15€/patient - 110€/patient	14.000€
Regular meetings		0€ /hour	0€
6. General material			
Printing expenses: - Information sheet (4 pages) - Informed consent (2 pages) - Professionals confidentiality agreement (1 page)	4 x 100 = 400 2 x 100 = 200 1 x 150 = 150	0’03€/page (black and white)	22’50€
Software and database creation	1	1.000€	1.000€
7. Publication and dissemination			
Article publication fees			2.000€
Inscription to national congresses	2 attendants	1.000€/ attendant	2.000€
Inscription to international congresses	2 attendants	2.000€/attendant	4.000€
		TOTAL COSTS:	**341.730,5€**

14. FEASIBILITY

This cross-sectional multicenter study will be carried out in hospitals of fifteen autonomous communities in Spain. We assume that they have similar baseline characteristics. With the participation of all these centers in the study, it is feasible to achieve the required sample size (100 patients) in two years and the expected duration of the whole study is three years.

The study will be carried out by a multidisciplinary team composed of different professionals and specialists; neurologists, nursing staff, laboratory technicians, imaging technicians, neuroradiologist, neurophysiologist and neuropathologists. All of them have the knowledge and the experience to perform and interpret the diagnostic tests realized. Thus, they are all fully capable of developing each procedure of the study.
To avoid possible differences between specialists, a training session will be held in which the standardized protocols for performing and interpreting diagnostic tests will be explained in detail.
In addition, for the study it will be necessary to hire a data manager to periodically revise the data collection, a statistician to perform the statistical analyses and also a scientific researcher to collaborate with the results interpretation and the paper redaction.

We are aware that this study will have a high cost because the diagnostic tests used (biomarker detection, RT-QuIC test, anatomopathological study, etc.) are specific for this disease and require very expensive equipment. In order to carry out this study, we will apply for the Convocatoria de Acción Estratégica de Salud 2024 of the Instituto de Salud Carlos III (Gobierno de España) and we will submit the protocol to multiple public and private funding calls.

In summary, with all this information, we conclude that this study has a feasible realization as it meets the main requirements.

15. IMPACT ON THE NATIONAL AND INTERNATIONAL HEALTH SYSTEM

Sporadic CJD is a minority and neurodegenerative disease which has an important impact on the people who suffer it and on their families because it has a challenging diagnostic, fatal prognosis and no treatment available. As few tests with high diagnostic accuracy are available, patients undergo a long and hard diagnostic period causing uncertainty, anguish, and iatrogenesis to patients and family members and they are normally diagnosed at advanced stages or even die without a definitive diagnosis. Despite RT-QuIC test, with high sensitivity and maximum specificity, has recently been incorporated into diagnostic criteria of the disease, in some sCJD subtypes has low sensitivity and it is not universally accessible and available due to the necessity of external material and its elevated cost.

Having an accurate and accessible test for the early diagnosis of sporadic CJD would reduce patients' harm and anguish as well as healthcare resources employed in this process (working time of healthcare professionals, extra diagnostic tests or treatments). At the same time, a differential diagnosis of other similar pathologies would allow patients with alternative diagnoses to be treated appropriately and in an early stage of the disease which will lead to better results.

On the other hand, although nowadays sCJD has not a curative and available treatment, some studies are being done and they have shown safeness and encouraging central nervous system treatment concentrations (67). Therefore, further treatment investigation must be done and an early sCJD diagnosis could provide therapeutic targets for future treatments. In addition, future treatments will be applied at an early stage of the disease and consequently, improve its efficacy. Early diagnostic biomarkers will allow disease monitorization and the evaluation of future treatments in clinical trials.

In conclusion, an early diagnosis of sCJD may involve an enormous change in national and international disease management and clinical practice and form the basis for future treatment and disease research.

16. FURTHER STUDIES

Due to the poor accessibility of RT-QuIC test and the clinical relevance of an early and accurate diagnosis of sCJD, this study proposes the evaluation of the diagnostic accuracy of the combination of t-tau, tau ratio and NfL biomarkers both in CSF and plasma for the early diagnosis of sCJD. Furthermore, it proposes to stratify the sample into the disease subtypes in order to determine if significant differences appear in the diagnostic accuracy of the test depending on the subtype.

For future studies, it would be relevant to compare the diagnostic accuracy (sensitivity, specificity, predictive values, AUC, likelihood ratio, etc.) of the biomarkers combination in both CSF and plasma with those of the RT-QuIC test in the early diagnosis of sCJD. Therefore, if they have similar diagnostic accuracy, as is believed in this study, the combination of t-tau, tau ratio and NfL biomarkers could be a powerful alternative to RT-QuIC, being a much more accessible and cost-effective test.

On the other hand, if promising results are obtained from this study, it would be relevant to monitor and study how these biomarkers levels in both CSF and plasma change throughout disease progression and whether they are related to longer or shorter survival. Stratification for sCJD subtypes could also be done as they can also have an effect on the disease prognosis.

The information obtained from this research and complemented by information from possible subsequent studies, could serve as a therapeutic target for potential new treatments for the disease. In addition, it could serve as a basis for monitoring future clinical trials in treatment investigation. These advances could improve patients' management, the prognosis of the disease, and have an important clinical impact.

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18. ANNEXES

ANNEX 1: DATA COLLECTION QUESTIONNAIRE

DATA COLLECTION QUESTIONNAIRE

“Diagnostic accuracy of t-tau and tau ratio combined with neurofilament biomarker in the early diagnosis of sporadic Creutzfeldt-Jakob disease”.

Date of the collection:

Name of the investigator:

Information required in the screening visit:

General information:

Hospital identification	
Patient identification	
Gender (male / female)	
Date of birth (age)	
Contact person	
Telephone or mobile number	

Clinical history:

Patient diagnosed pathologies or comorbidities	
Actual medications	
Family history of diagnosed rapidly progressive neurodegenerative diseases.	
History of potential iatrogenic exposure (dura mater implants, corneal grafts or human cadaveric pituitary hormones)	
A trip to the United Kingdom more than ten years ago	
Frequently beef meals	

Relevant study information:

- **Predominant clinical scenario**

Rapidly progressive dementia	
Cerebellar manifestations	
Myoclonus	
Extrapyramidal Signs	
Pyramidal signs	
Akinetic mutism	

- **Disease duration since symptomatology onset (months)**

Less than 1	
From 1 to 3	
From 4 to 6	
From 7 to 9	
From 10 to 12	
From 13 to 15	
From 16 to 18	
From 19 to 21	
From 22 to 24	
More than 24	

- **Place of residence**

Andalucía	
Aragón	
Asturias	
Cantabria	
Castilla y la mancha	
Castilla y león	
Catalunya	
Extremadura	
Galicia	
Comunidad de Madrid	
Región de Murcia	
La Rioja	
Navarra	
País Vasco	
Valencian community	

ANNEX 2: STUDY INFORMATION SHEET

HOJA INFORMATIVA

Título del estudio: “Precisión diagnóstica de los biomarcadores t-tau y tau ratio combinados con los neurofilamentos en el diagnóstico precoz de la enfermedad de Creutzfeldt-Jakob esporádica.”

Investigador principal:

Hospital:

Estimado paciente, nos dirigimos a usted porque nos gustaría informarle sobre un estudio que estamos realizando y ofrecerle la oportunidad, totalmente voluntaria, de participar. El estudio ha sido aprobado por el Comité Ético de Investigación Clínica (CEIC) y todas las direcciones de los hospitales donde se está realizando. Este estudio también cumple con la legislación vigente y con los principios de la declaración de Helsinki.

Le pedimos por favor que lea con atención este documento para estar bien informado sobre los objetivos del estudio, beneficios y riesgos asociados. Posteriormente, podrá plantear todas las preguntas y dudas que tenga para que el investigador pueda resolverlas.

Participación voluntaria

Queremos recordarle que la participación en este estudio es totalmente voluntaria y en todo momento tiene derecho a retirarse del mismo sin necesidad de justificación y sin ninguna consecuencia o perjuicio para su asistencia o tratamiento.

¿Qué es la enfermedad de Creutzfeldt-Jakob esporádica?

La enfermedad de Creutzfeldt-Jakob esporádica es una enfermedad priónica neurodegenerativa progresiva que afecta al sistema nervioso central. Es una enfermedad minoritaria con una incidencia de 1 a 2 casos por millón de personas/año y que suele afectar entre los 60-80 años de edad.

Aunque tiene una presentación clínica heterogénea, las características clínicas principales y más frecuentes son demencia progresiva, así como movimientos musculares rápidos e involuntarios o falta de coordinación en el movimiento o la marcha.

Presenta seis subtipos diferentes de la enfermedad con características clínicas y pronósticas diferentes que actualmente solo se pueden determinar a partir del estudio genético y del estudio anatomopatológico del tejido cerebral.

Tiene un diagnóstico difícil que requiere la compatibilidad clínica y la realización de otras pruebas diagnósticas como la punción lumbar, la resonancia magnética (RM) o el electroencefalograma (EEG) para alcanzar un diagnóstico probable de la enfermedad. Para alcanzar el diagnóstico definitivo es necesario un estudio anatomopatológico del tejido cerebral.

En la actualidad, no existe ningún tratamiento curativo disponible, pero diversos estudios de investigación están siendo realizados.

¿Cuál es el objetivo de este estudio?

El objetivo principal de este estudio es evaluar la precisión diagnóstica de una combinación de biomarcadores (t-tau, tau ratio y neurofilamentos) determinados en líquido cefalorraquídeo en el diagnóstico precoz de pacientes con enfermedad de Creutzfeldt-Jakob esporádica.

Otros objetivos secundarios son valorar la precisión diagnóstica de estos mismos biomarcadores determinados en sangre en el diagnóstico precoz de la enfermedad y valorar si cambia la capacidad diagnóstica de estos biomarcadores dependiendo del subtipo de enfermedad que presente el paciente.

¿Por qué es necesario realizar este estudio?

Disponer de un diagnóstico precoz universalmente accesible de la enfermedad de Creutzfeldt-Jakob esporádica reduciría la angustia de los pacientes durante el procedimiento de diagnóstico, así como los recursos sanitarios empleados en este proceso (tiempo de trabajo de los profesionales sanitarios, pruebas diagnósticas o tratamientos adicionales). Además, el diagnóstico precoz de la enfermedad de Creutzfeldt-Jakob esporádica puede proporcionar dianas terapéuticas para futuros tratamientos curativos y sentar las bases para la investigación futura de la enfermedad, y puede suponer un cambio en la gestión nacional e internacional de la enfermedad y en la práctica clínica.

¿Cuántos centros participan y cuánto tiempo dura?

Es un estudio multicéntrico en el que participan los hospitales de 15 comunidades autónomas de España. Cada comunidad autónoma dispone de un hospital de referencia en el que se realizan las pruebas diagnósticas. Se estima una duración total del estudio de 3 años, aunque se puede modificar dependiendo del tiempo requerido para conseguir el tamaño de la muestra necesario o para completar la recolecta de datos.

¿Qué características deben tener los/las pacientes para participar en el estudio?

Los/las pacientes, mayores de 18 años, afectados por demencia rápidamente progresiva en el momento del ingreso hospitalario. Además, se precisa de un consentimiento informado para poder participar en el estudio.

¿En qué consiste mi participación en el estudio?

Su participación en el estudio consistirá en inicialmente, asistir a una visita con un neurólogo donde se le preguntará información personal y clínica relevante y necesaria para la realización del estudio y se rellenará un cuestionario. También se le realizará una exploración neurológica básica.

Posteriormente se le realizarán **cuatro pruebas** diferentes (análisis de sangre, punción lumbar, resonancia magnética y un electroencefalograma) en el hospital de referencia de su comunidad autónoma. Estas cuatro pruebas se realizan en la práctica clínica habitual en el diagnóstico de pacientes con una demencia rápidamente progresiva. Las muestras biológicas recogidas y los resultados de las pruebas complementarias serán enviados al Hospital Clínic de Barcelona que dispone del material y de los equipos necesarios para realizar el análisis correspondiente. Durante el análisis de estas pruebas, además de analizar los parámetros habituales, se determinarán los biomarcadores de estudio (total tau, tau ratio y la proteína de cadena ligera de neurofilamentos) en líquido cefalorraquídeo y en sangre. También se realizará la determinación del RT-QuIC (real-time quaking-induced conversion) en el líquido cefalorraquídeo.

A partir de la muestra de sangre, se realizará un **estudio genético** para determinar el polimorfismo concreto del codón 129 y la presencia o ausencia de una mutación del gen PRNP que puede causar la enfermedad de Creutzfeldt-Jakob.

Finalmente, a partir de la autopsia cerebral se realizará un **estudio anatomopatológico** específico para la detección de esta enfermedad.

¿Puedo consultarlo con otros profesionales?

En todo momento puede pedir segundas opiniones a diferentes profesionales antes de aceptar participar en el estudio.

¿Qué riesgos y beneficios obtendré de mi participación en el estudio?

Las pruebas realizadas son pruebas seguras y habituales en la práctica clínica habitual. Su participación en el estudio ayudará a obtener un conocimiento mayor sobre esta enfermedad que aportará beneficios a las personas que la padezcan y servirá de base para futuros estudio de posibles tratamientos.

¿Hay alguna recompensa económica?

Ni usted ni los miembros del equipo de investigación recibirán recompensa económica por participar en este estudio, pero tampoco tendrán ningún gasto económico adicional.

¿Cómo se garantiza la confidencialidad y la protección de datos?

Se recogerán los datos necesarios para el estudio y sólo los miembros del equipo de investigación tendrán acceso a ellos. Los profesionales le informarán de la prueba diagnóstica que se le realizará y todos los datos e información se utilizarán con el único fin de realizar el estudio.

Además, los profesionales que recojan y analicen los datos preservarán el anonimato y la privacidad de los pacientes y deberán firmar un acuerdo de confidencialidad.

En la redacción y difusión de los resultados no aparecerán datos identificativos en las publicaciones.

En caso de duda o problema, ¿con quién debo contactar?

Si necesita contactar con profesionales del equipo de investigación de su hospital, puede escribir un correo electrónico a esta dirección (*...rellenar los datos del hospital correspondiente...)* o llamar por teléfono al *(...rellenar los datos del hospital correspondiente....*) y le atenderemos lo antes posible.

Le pediremos su autorización y podrá quedarse con una copia del presente documento.

Muchas gracias por su tiempo y su dedicación.

Signatura del/a paciente

Signatura del/a investigador/a

Nombre:

Nombre:

Fecha:

Fecha:

ANNEX 3: INFORMED CONSENT AND WITHDRAWAL OF CONSENT

CONSENTIMIENTO INFORMADO:

Yo o los representantes legalmente autorizados (en caso de que el paciente no pueda firmar) ..... con número de identificación ....., acepto participar en el estudio de cohortes sobre la "Precisión diagnóstica de los biomarcadores t-tau y tau ratio combinados con los neurofilamentos en el diagnóstico precoz de la enfermedad de Creutzfeldt-Jakob esporádica", y declaro que:

- He leído y comprendido la **hoja informativa** del estudio que se me ha entregado.

- He podido formular las **preguntas** necesarias sobre el estudio.

- He obtenido **respuestas satisfactorias** a mis preguntas.

- He sido informado de las implicaciones y **objetivos** del estudio, así como de los **beneficios y riesgos asociados y de su relevancia clínica**.

- Entiendo que mi participación es **voluntaria y no remunerada.**

- Doy permiso y consentimiento para que se me realicen las pruebas necesarias para el estudio: análisis de sangre, punción lumbar, resonancia magnética y un electroencefalograma.

- Doy permiso para que se analicen los resultados de las pruebas con la determinación de biomarcadores en sangre y en líquido cefalorraquídeo necesarios para la realización del estudio.

- Doy permiso para que se realice un estudio genético para determinar el polimorfismo concreto del codón 129 y la presencia o ausencia de una mutación del gen PRNP a partir de la muestra de sangre recogida.

- Doy permiso para que se realice el estudio anatomopatológico correspondiente a partir de mi autopsia cerebral.

- Doy permiso para que la información de mi historial personal y clínico, los resultados de las pruebas diagnósticas y la base de datos sean utilizados por el equipo de investigación para fines relacionados con este estudio.

- Entiendo que se respetará en todo momento la **confidencialidad y privacidad** de mis datos y que se utilizarán **exclusivamente para la investigación clínica.**

- Entiendo que puedo **revocar mi consentimiento** sin justificación y sin que ello suponga ningún cambio en mi asistencia sanitaria y/o escolar.

❑ Deseo recibir información por teléfono o correo electrónico sobre futuros resultados del estudio:

Correo electrónico:..... o teléfono de contacto:.....

Nombre y apellidos del investigador que me facilitó la información para este estudio:

Firma del paciente Firma del investigador

Lugar y fecha: Lugar y fecha:

REVOCACIÓN DEL CONSENTIMIENTO

Yo ....., con número de identificación ....., revoco el consentimiento informado previamente firmado para participar en el estudio "Precisión diagnóstica de los biomarcadores t-tau y tau ratio combinados con los neurofilamentos en el diagnóstico precoz de la enfermedad de Creutzfeldt-Jakob esporádica".

Firma del paciente Firma del investigador

Lugar y fecha: Lugar y fecha:

ANNEX 4: PROFESSIONALS CONFIDENTIALITY AGREEMENT

ACUERDO DE CONFIDENCIALIDAD DE LOS PROFESIONALES

Yo, ..., con DNI ....., y con la profesión de ....., en el hospital ..... de ..... para el estudio "Precisión diagnóstica de los biomarcadores t-tau y tau ratio combinados con los neurofilamentos en el diagnóstico precoz de la enfermedad de Creutzfeldt-Jakob esporádica":

ESTOY DE ACUERDO CON:

1. Recoger sólo los datos necesarios para el estudio y siempre después de haber informado correctamente a los pacientes del procedimiento. Es obligatorio que todos los pacientes (si son capaces) o representantes legalmente autorizados (un familiar o tutor legal) hayan firmado el consentimiento informado.

2. Presentar conformidad respecto a tener acceso y utilizar la información médica, datos personales, resultados de pruebas diagnósticas y base de datos de los pacientes con la única finalidad de los servicios profesionales de este estudio y de acuerdo con la normativa vigente sobre protección de datos de carácter personal definida en la "Ley Orgánica 3/2018, de 5 de Diciembre, de Protección de Datos Personales y garantía de los derechos digitales (LOPD-GDD)".

3. Mantener la confidencialidad y privacidad de la información y datos personales y médicos del paciente de acuerdo con el secreto profesional y el deber de protección de los mismos. Este compromiso será durante la realización del estudio y persistirá aunque finalice el proyecto.

Firma de conformidad con los puntos anteriores sobre confidencialidad y protección de datos de los pacientes.

Firma

Lugar y fecha: