Plasma and cerebrospinal fluid tau and neurofilament concentrations in rapidly progressive neurological syndromes: a neuropathology-based cohort

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Word count title: 12
Word count abstract: 248
Word count text: 3281
Number of tables: 3
Number of figures: 3

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Abstract

BACKGROUND AND PURPOSE:

Cerebrospinal fluid (CSF) tau and neurofilament light chain (NF-L) proteins have proved to be reliable biomarkers for neuronal damage; however, there is a strong need for blood-based tests.

METHODS:

The present study included 132 autopsy cases with rapidly progressive neurological syndromes, including Alzheimer disease (AD) (21), sporadic (65) and genetic (21) Creutzfeldt-Jakob disease (CJD), 25 cases with vascular, neoplastic and inflammatory alterations, and additionally 18 healthy control individuals. CSF tau and NF-L concentrations were measured by enzyme-linked immunosorbent assay. Plasma tau and NF-L concentrations were measured using ultra-sensitive single molecule array technology.

RESULTS:

Plasma and CSF tau ($R = 0.59$, $P < 0.001$) and NF-L ($R = 0.69$, $P < 0.001$) levels correlated significantly (Spearman test). Plasma tau and NF-L levels were significantly higher in all disease groups compared to healthy controls ($P < 0.001$). Receiver operating characteristic curves were used and area under the curve values for comparisons with controls were 0.82 (AD), 0.94 (sporadic CJD), 0.92 (genetic CJD) and 0.83 (other neurological disorders) for plasma tau and 0.99, 0.99, 1.00 and 0.96 for plasma NF-L, respectively. Molecular subtyping of sporadic CJD showed a strong effect (linear logistic regression) on plasma tau ($P < 0.001$) but not NF-L levels ($P = 0.19$).

CONCLUSION:

Plasma tau and NF-L concentrations are strongly increased in CJD and show similar diagnostic performance to the corresponding CSF measure. Molecular subtypes of sporadic CJD show different levels of plasma tau. Although not disease-specific, these findings support the use of plasma tau and NF-L as tools to identify, or to rule out, neurodegeneration.

Introduction

Neurodegenerative diseases are characterized by progressive loss of neurons and deposition of pathological forms of proteins predominantly in the central nervous system. Current molecular pathological classification of neurodegenerative disease is therefore protein-based (14). The most important proteins, such as tau, α-synuclein, transactive response (TAR) DNA-binding protein 43 (TDP-43), amyloid-β (Aβ), prion protein (PrP) are targets for body-fluid based biomarker development (14). In the diseased brain these proteins show several biochemical modifications (14); therefore either the physiological or the modified forms are selected for *in vivo* tests. Although markers are emerging detecting total and pathological forms of α-synuclein (34) or disease-associated PrP (8, 22), currently, the most widely applied tests focusing on neurodegeneration-associated proteins are used for supporting the clinical diagnosis of Alzheimer disease (AD). These include the examination of total and phosphorylated (p181) tau and Aβ levels and have relevance in the context of defining preclinical AD (7). Currently, these markers are analysed using the cerebrospinal fluid (CSF). However, most of the patients are seen by primary care doctors, therefore there is a strong need for blood-based tests (31).

Biomarker modelling of AD emphasizes the importance of total tau as a marker of neurodegeneration (11). Indeed, neurodegeneration-related proteins are important for disease-classification even in the context of frequent concomitant pathologies (27), however, the relevance of biomarkers reflecting the dynamics of neuronal degeneration remains of high value. In particular, these may allow assessment of prognosis or response to potential therapeutic efforts. Measurement of CSF total tau has proved to be a reliable marker of neuronal damage. Indeed, the most widely used surrogate markers for the clinical diagnosis of the most frequent human prion disease sporadic Creutzfeldt-Jakob disease (CJD) includes protein 14-3-3 and total tau (18, 35). These show altered levels in genetic CJD as well (18). Furthermore, recent studies emphasize that there might be differences between molecular subtypes of

sporadic CJD (10). Molecular subtypes of sporadic CJD are distinguished based on the polymorphic codon 129 of the prion protein gene (*PRNP*) and types of protease-resistant PrP (26). Detection of total PrP (6) and α-synuclein (20) has been recently added to the list of biomarkers helpful in the differential diagnosis of rapidly progressive dementias.

A further, potentially relevant biomarker of neuronal damage is neurofilament light chain (NF-L). This has been reported already in 1996 to be increased in several neurodegenerative and non-neurodegenerative conditions (30). Since NF-L is expressed in myelinated axons, studies focused on multiple sclerosis (37), furthermore, increased levels have been reported in traumatic brain injury (33). Importantly, recent studies suggest that NF-L can be reliably evaluated in the serum and can reflect disease intensity (3, 19, 29). Recent studies have shown that total tau levels can be also measured in the plasma (38), although on an individual level they are not helpful, *e.g.*, in the diagnostics of AD (21).

Based on the aforementioned studies, we hypothesized that the measurement of plasma tau and NF-L concentrations can be useful to support the diagnosis of human prion diseases. Therefore, we evaluated a cohort of neuropathologically classified CJD cases and compared with non-diseased controls and with a wide range of disorders defined by neuropathological examinations. Our study supports the concept that tau and NF-L concentrations can be reliably measured in plasma and that they probably reflect ongoing neuronal damage. They show similar diagnostic performance as their corresponding CSF measure but are not disease-specific.

Material and methods

Case selection and samples

The present study included 150 autopsy cases of neuropathologically confirmed AD (21 cases), sporadic CJD (65 cases), genetic CJD (21 cases with the E200K *PRNP* mutation) and cases with a wide range of neuropathological alterations, including cerebrovascular disease, inflammation (meningoencephalitis), PART, or Lewy body pathology (**Table 1**). Vascular and inflammatory lesions, tauopathies, Lewy body pathology (5) and neurofibrillary degeneration were (1, 4) documented and staged for all cases (**Table 1**). Tauopathy included argyrophilic grain disease or progressive supranuclear palsy type pathology (13). Molecular subtypes of sporadic CJD were defined following the classification by Parchi and colleagues (25, 26). Further neurodegenerative disorders and proteinopathies were excluded (16). In addition, 18

healthy control individuals were included. The controls had no subjective symptoms of cognitive dysfunction and were normal upon clinical neurological and psychiatric examination.

Of all the neuropathologically examined patients, CSF samples had previously been submitted for routine analysis of 14-3-3 protein (in the frame of surveillance for prion diseases). Together with plasma samples prepared from blood sent in EDTA tubes supernatants were stored at -80°C. This allowed us the investigation of CSF in combination with autopsy material from the same patient. The study was approved by the ethics committee of the Medical University of Vienna (Nr. 397/2011).

Analysis of tau and neurofilament in the plasma and CSF samples

CSF levels of total tau protein were measured using the INNOTEST ELISA (Fujirebio Europe, Ghent, Belgium), while NF-L levels were measured by the NF-light® ELISA kit (UmanDiagnostics AB, Umeå, Sweden). Plasma tau was measured using the Human Total Tau kit (Quanterix, Lexington, MA) on the Simoa HD-1 analyzer (CE marker). This assay differ from the previously published Simoa tau assay (28) in that it is based on different antibodies; one monoclonal antibody for capture that reacts with a linear epitope in the mid-region of tau, and one detection antibody that reacts with a linear epitope in the N-terminal region of T-tau. NF-L concentrations in blood were measured using the same monoclonal antibodies and calibrator as in the CSF assay, but the assay was transferred onto the Simoa platform using a homebrew kit (Quanterix, Lexington, MA, USA), as previously described (9). All CSF and plasma measurements were performed in one round of experiments using one batch of reagents by board-certified laboratory technicians who were blinded to clinical data.

Statistical analysis

Kruskal-Wallis (K-W) and Mann-Whitney (M-W) tests were used to compare different study groups. Linear logistic regression tests were performed to evaluate the effect of clinicopathological variables. Receiver-Operating-Characteristic (ROC) curves were used to evaluate the specificity and sensitivity of the tests used. For statistical analyses, we used SPSS Statistics (V23.0, SPSS Inc., Chicago, IL, USA).

Results

Plasma tau and NF-L levels correlate well with CSF tau and NF-L levels

CSF tau levels were higher than plasma tau levels (CSF/plasma tau, mean: 779.1, range: 11.39-10076.9). For the total cohort (n=93) Spearman correlation test revealed a strong correlation

between plasma and CSF tau (R=0.59, p<0.001) and NF-L (R: 0.69, p<0.001). CSF tau levels correlated with CSF NF-L (R=0.42, p<0.001) and plasma tau levels with plasma NF-L (0.50, p<0.001). CSF tau correlated with plasma NF-L (R: 0.34, p=0.001) but CSF NF-L levels did not correlate with plasma tau levels (R: 0.16, p=0.12). When different disease groups were evaluated separately, similar results were seen for sporadic CJD, while for AD, genetic CJD and other neurological diseases not all of these were significant (**Table 2**).

We then evaluated whether age, gender and time interval from sampling to death (i.e., autopsy cohort) had any effect on these variables. We did not find any difference between men and women (M-W test, p>0.2 for all variables). Spearman correlation test revealed a negative correlation between age and CSF NF-L levels in the autopsy cohort (n= 93, R= - 0.22, p= 0.03). However, in a linear logistic regression model with diagnostic grouping and age added to the model this was not significant (p=0.1). Indeed, when evaluated in disease groups, age correlated inversely with CSF NF-L levels only in the sporadic CJD group (n=46, R= - 0.32, p=0.02). Importantly, the time interval from sampling to death (i.e., the whole autopsy cohort pooled) negatively correlated with CSF tau (R= - 0.26, p=0.01), plasma tau (R= -0.38, p<0.001) and plasma NF-L levels (R= - 0.18, p=0.03). Linear logistic regression test with diagnostic grouping and time interval to death added to the model, did not confirm an effect of time interval from sampling to death (p>0.07 for all). When diagnostic groups were evaluated separately, this time interval correlated negatively only in AD (plasma tau, R= - 0.46, p=0.03), sporadic CJD (plasma tau, R= -0.54, p<0.001; CSF tau, R= -0.3, p=0.04) and in genetic CJD (CSF tau, R= - 0.57, p=0.03). Braak NFT and Lewy-related pathology stages did not show any effect on these variables.

Elevated plasma and CSF tau levels characterize AD and CJD cases

When evaluating plasma tau levels, K-W test revealed significant differences between cases (p<0.0001) (**Fig. 1A**). High levels were observed in sporadic CJD, which was significantly (p<0.001) higher when compared to all other groups (M-W test) except to genetic CJD cases. Indeed, the mean plasma tau level in sporadic CJD cases showed more then ten-fold increase as compared to healthy controls and also prominently increased when compared to other neurological diseases (2.46-fold increase) or AD (2.28-fold increase). Plasma tau levels did not distinguish genetic CJD cases from other neurological diseases (p=0.08) but did from AD cases (p=0.03). Importantly, all disease groups showed significantly higher levels than the healthy control group (p>0.001). The mean plasma tau level in AD was 4.45-fold higher then in controls

(**Fig. 1A**). The highest levels of plasma tau (> 6 pg/ml) leading to an increased mean plasma tau value in the group of other neurological diseases were detected in single cases with i) cerebral lymphoma, ii) meningeal carcinomatosis, iii) severe ischemic/hypoxic panencephalopathy, iv) severe tick-borne encephalitis with ischemic/hypoxic brain damage, v) severe limbic encephalitis, and vi) multiple brain infarcts. On the other hand, cases with vascular encephalopathy including lacunar infarcts or strategic thalamus infarct, neurosyphilis, granulocytic meningoencephalitis, glioblastoma, or metabolic encephalopathy did not show high levels.

Next we evaluated the sensitivity and specificity of plasma tau levels using ROC curves. Area under the curve (AUC) for comparisons with AD revealed the following results: i) 0.821 (95% CI: 0.69-0.95) for comparison with healthy controls (**Fig. 2A**) and ii) 0.453 (95% COI: 0.28-0.62) for comparison with other neurological diseases. The AUC was i) 0.939 (95% COI: 0.89-0.98) when comparing sporadic CJD to healthy controls (**Fig. 2B**); ii) 0.756 (95% COI: 0.63-0.87) when comparing sporadic CJD to AD; iii) 0.722 (95% COI: 0.60-0.83) when comparing sporadic CJD to the group of other neurological diseases; and iv) 0.57 (95% COI: 0.43-0.71) when comparing sporadic to genetic CJD. AUCs for comparisons with genetic CJD revealed the following results: i) 0.918 (95% COI: 0.83-1.0) for comparison with healthy controls (**Fig. 2C**); ii) 0.694 (95% COI: 0.53-0.85) for comparison with AD; and iii) 0.651 (95% COI: 0.48-0.81) for comparison with the group of other neurological diseases. Finally comparing other neurological diseases and healthy controls (**Fig. 2D**) revealed an AUC of 0.829 (95% COI: 0.70-0.95).

When evaluating CSF tau levels for the autopsy cohort, similar trends were observed (**Fig. 1B**) although the AUC was higher in comparisons with sporadic and genetic CJD (**Table 3**).

Elevated plasma and CSF NF-L levels in various diseases

When evaluating plasma NF-L levels, K-W test revealed significant differences between cases ($p<0.0001$) (**Fig. 1C**). All diseased groups showed higher levels than controls (other neurological disease 27-fold, AD 12-fold, sporadic CJD 8-fold and genetic CJD 7-fold increase of mean value). The highest levels were observed in a few cases of the group of other neurological diseases; these were the same as those showing the highest plasma tau levels. Indeed, these cases with severe brain damage showed 10-100 times higher values as the normal

controls. M-W test revealed significant differences only for the comparisons with healthy controls (i.e., versus diseased controls, AD, sporadic and genetic CJD; all $p<0.001$).

Next we evaluated the sensitivity and specificity of plasma NF-L levels using ROC curves (**Table 3**). The AUC was i) 0.992 (95% COI: 0.98-1.0) for the comparison of sporadic CJD and healthy controls; ii) 1.0 (95% COI: 1.0-1.0) for the comparison of genetic CJD and healthy controls; iii) 0.992 (95% COI: 0.97-1.0) for the comparison of AD and healthy controls, and iv) 0.96 (95% COI: 0.90-1.0) comparing diseased and healthy controls. The AUC was i) 0.497 (95% COI: 0.30-0.69) when comparing sporadic CJD to diseased controls; and ii) 0.657 (95% COI: 0.48-0.83) when comparing sporadic CJD to AD. AUCs for comparisons with genetic CJD revealed the following results: i) 0.705 (95% COI: 0.50-0.90) for comparison with AD; and ii) 0.520 (95% COI: 0.30-0.873) for comparison with diseased controls. Comparison of diseased controls to AD revealed an AUC of 0.576 (95% COI: 0.36-078).

When evaluating CSF NF-L levels for the autopsy cohort, similar trends were observed (**Fig. 1D** and **Table 3**). However, here further comparisons were significant as follows: i) sporadic versus genetic CJD ($p=0.041$); ii) sporadic CJD versus AD ($p=0.002$); iii) genetic CJD versus AD ($p<0.001$); and iv) diseased controls versus AD ($p=0.036$).

The effect of molecular subtyping on tau and NF-L levels in sporadic CJD cases

Linear logistic regression test indicated that molecular subtypes of sporadic CJD has a strong effect on the plasma ($p=0.001$) but not on CSF ($p=0.3$) tau levels, the first remaining significant ($p=0.002$) after the adjustment for Braak NFT stage and the time interval between sampling and death. Regarding plasma NF-L levels, molecular subtyping of sporadic CJD did not show a significant effect ($p=0.19$).

Plasma tau levels were highest in the MM/MV type 1 and MM type 1+2 subtypes. M-W test revealed significantly higher values of plasma and CSF tau levels for most of the molecular subtypes when compared to the MV type 2 kuru plaque type sporadic CJD cases (**Figs.1E, F**). NF-L plasma and CSF levels showed similar patterns, though less comparisons were significant (**Figs. 1G, H**). In ROC curve analysis for plasma tau MM/MV type cases compared to MV type 2K cases showed an AUC of 0.990 (95% COI 0.96-1.0) and 0.874 (95% COI 0.74-1.0) when compared to VV type 2 cases. Importantly, the lower tau and NF-L levels in MV type 2K cases were still higher when compared to non-diseased controls (M-W test: CSF tau, CSF and plasma

NF-L: $p<0.001$; plasma tau: $p = 0.1$), however, plasma tau and NF-L levels were not significantly different in AD and MV type K sporadic CJD cases.

Discussion

The identification of proteins related to specific neurodegenerative diseases has opened new avenues for disease classification and biomarker development (14, 15). While there are efforts to detect disease-associated protein modifications in the blood (12, 34, 36), markers that reflect neuronal degeneration may be more tangible. In particular, those might be used to predict prognosis, clinical outcome (32), and eventually response to neuroprotective therapies. However, these indicators show lower levels in blood samples when compared to CSF, and highly sensitive immunochemical methods for their detection are needed. Importantly, recent studies have reported that tau and NF-L can be detected in blood (3, 9, 19, 21, 28, 29, 32, 38). To be able to define the reliability of these tests, different diseases and their combinations need to be examined. This was precisely our aim in this study and therefore we included cases with detailed neuropathological analysis.

First we show that both tau and NF-L levels measured in the plasma reflect the results of the CSF examination, even if at a much lower concentrations. This is important since both are widely accepted markers of neuronal damage due to various aetiologies. Importantly, we demonstrate significant correlation of tau with NF-L levels both in plasma and CSF. Combined evaluation of tau and NF-L can be used as complementary examinations. The sensitivity and specificity of the blood tests are comparable to those of the CSF. In particular, high AUCs were detected when compared to controls, while lower when different disease groups were compared to each other. However, in latter comparisons disease groups contained a pool of individual cases with various molecular subtypes (*i.e.*, sporadic CJD) or combined pathologies (*i.e.*, AD with or without vascular lesions). Therefore, on an individual level both tau and NF-L may be suitable to support the diagnosis of a disorder with neuronal damage; however, further neuroimaging or CSF tests are still needed to be able to classify a disease. We show that the UmanDiagnostics assay is suitable for the quantification of NF-L in human plasma also, however, recent studies on CSF samples (23) indicate that it should be validated for each laboratory prior to application for routine diagnostics.

One major focus of our analyses was CJD. Human prion diseases belong to the most dramatic neurodegenerative disorders with rapid and prominent neuronal loss. However, these show different disease course based on molecular characteristics, which can be defined with the examination of *PRNP* gene together with brain tissue usually *post mortem* (26). Our observations in plasma support the importance of total tau levels (35) to distinguish CJD from controls, but also from AD or other disorders with neuronal damage. In concordance with other reports (8, 10), we show, however, that the molecular subtypes of sporadic CJD might be associated with different levels of total tau and NF-L. This is compatible with the observations of longer duration of illness for example in MV type 2 cases (26). Indeed, sporadic CJD subtypes are both qualitatively and regionally distinct in the predominance of neuronal death markers, confirming regional vulnerabilities of sporadic CJD subtypes that potentially reflect distinct responses to pathogenic events (17). Interestingly, the lowest tau and NF-L levels have been detected in a patient with variably protease-sensitive prionopathy (VPSPr) treated with doxycycline and showing nearly five years duration of illness (2). VPSP is a neurodegenerative disorder with spongiform encephalopathy and an unusual immunostaining and immunoblotting pattern for the disease-associated PrP with long duration of disease (39). We show that the time interval between sampling and death correlates inversely with plasma tau levels and less with NF-L. Therefore, plasma tau level can be discussed also as a potential prognostic factor both in AD and CJD. This will be appreciated when therapies are available and rapid and easy blood tests are at disposal, which can be used for follow-up as well. It must be noted, that, our cohort included various disorders, including molecular subtypes, while for homogenous disease groups NF-L levels can be also used to predict clinical outcome (32).

The Braak stage of neurofibrillary degeneration or Lewy body pathology did not show a strong effect on the tau levels. This is not contrary to findings in AD that cognitive impairment seems to correlate best with the burden of neocortical neurofibrillary tangles (24), but rather suggests that tau and NF-L levels (i.e. plasma and CSF) reflect a relatively constant progression level of neuronal damage in AD. Higher values can be related to additional neuronal damage such as vascular lesions. Our study indicates, that inflammation, especially associated with prominent neuronal damage or hypoxic/ischemic brain damage, also associates with higher NF-L and tau levels. These disorders usually do not pose differential diagnostic issues for CJD or AD (i.e. increase cell counts), however, our observations further support the prognostic value of tau and NF-L in various disorders.

Conclusion

Our neuropathology-based study shows that plasma tau and NF-L levels reflect those in CSF and may be used to substitute them for example in studies where CSF sampling is not feasible. We show that the highest levels of tau can be detected in CJD, however, rare molecular subtypes may show lower levels. Thus, combined evaluation of the polymorphic codon 129 of the *PRNP* can be helpful to interpret these results. We demonstrate increased levels of tau and NF-L in AD as compared to controls. Unexpected higher levels can be observed in additional vascular lesions or inflammatory changes associated with neuronal damage. We are aware that the evaluation of plasma tau and NF-L levels alone will be not suitable to specifically diagnose a disease; however, due the practicability of the blood tests and their strong association with tissue lesioning they can reliably support the clinical practice for evaluating the degree and dynamics of neuronal damage. Finally, the extreme elevation of plasma tau, together with the constellation of clinical symptoms, might support the suspicion of human prion disease.

Acknowledgements

The study was supported by grants from VINNOVA, the Swedish Research Council, the Torsten Söderberg Foundation, the Knut and Alice Wallenberg Foundation, the European Research Council, Alzheimerfonden and Frimurarestiftelsen.

Conflict of interest statements

HZ and KB are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg.

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Table 1. Demographic and neuropathological data of the examined cases.

AD: Alzheimer disease; sCJD and gCJD: sporadic and genetic Creutzfeldt-Jakob disease; OND: other neurological disorders; Co: clinically healthy controls; NFT: neurofibrillary tangle; LRP: Lewy-related pathology; Molpath: molecular pathological subtype; - indicates not applicable.

Variable	n/%/type	OND	AD	sCJD	gCJD	Co	Total
Men	n	16	5	28	9	9	67
	%	64	23.8	43.1	42.9	50	44.7
Women	n	9	16	37	12	9	83
	%	36	76.2	56.9	57.1	50	55.3
Sum	n	25	21	65	21	18	168
Age	Mean	67.6	77	69	60.8	73.7	69.3
	Min	48	51	55	42	65	42
	Max	89	89	85	79	81	89
Vascular lesion	n	8	11	1	0	-	20
	%	32	52.4	1.5	0	-	15.2
Inflammation	n	7	1	0	0	-	8
	%	28	4.8	0	0	-	6.1
Tauopathy	n	0	3	3	1	-	7
	%	0	14.3	4.6	4.8	-	5.3
Neoplasm	n	5	1	0	0	-	6
	%	20	4.8	0	0	-	4.5
Braak NFT (n)	0	14	0	20	17	-	51
	I	2	0	20	2	-	24
	II	5	0	15	1	-	21
	III	4	1	6	1	-	12
	IV	0	3	3	0	-	6
	V	0	7	0	0	-	7
	VI	0	10	0	0	-	10
Brak LRP (n)	0	22	16	62	20	-	120
	1	0	0	0	0	-	0
	2	1	0	2	1	-	4
	3	0	0	1	0	-	1
	4	0	3	0	0	-	3

	5	1	1	0	0	-	2
	6	1	1	0	0	-	2
Prion Molpath	MM1	-	-	34	-	-	34
	MM1+2	-	-	7	-	-	7
	MM2	-	-	3	-	-	3
	MV2K	-	-	6	-	-	6
	VV2	-	-	10	-	-	10
	VV1	-	-	4	-	-	4
	VPSPVV	-	-	1	-	-	1

Table 2. Spearman correlation test comparisons of cerebrospinal fluid and plasma tau and neurofilament levels. For the disease groups only the significant results are shown. R: correlation coefficient; AD: Alzheimer disease; CJD: Creutzfeldt-Jakob disease.

Total cohort					
		CSF NF-L	**CSF Tau**	**Plasma Tau**	**NF-L Plasma**
CSF NF-L	R	-	0.42	0.161	0.69
	p	-	<0.001	0.123	<0.001
	N	-	91	93	93
CSF Tau	R	0.42	-	0.59	0.34
	p	<0.001	-	<0.001	0.001
	N	91	-	91	91
Plasma Tau	R	0.161	0.59	-	0.50
	p	0.123	<0.001	-	<0.001
	N	93	91	-	150
NF-L Plasma	R	0.69	0.34	0.50	-
	p	<0.001	0.001	<0.001	-
	N	93	91	150	-
Other neurological diseases					
		CSF NF-L	**CSF Tau**	**Plasma Tau**	**NF-L Plasma**
CSF NF-L	R	-	0.474	0.373	0.83
	p	-	0.064	0.141	<0.001
	N	-	16	17	17
AD					
		CSF NF-L	**CSF Tau**	**Plasma Tau**	**NF-L Plasma**
CSF NF-L	R	-	0.71	0.06	0.54
	p	-	0.004	0.829	0.045
	N	-	14	14	14
Sporadic CJD					
		CSF NF-L	**CSF Tau**	**Plasma Tau**	**NF-L Plasma**
CSF NF-L	R	-	0.34	-0.004	0.57
	p	-	0.02	0.981	0
	N	-	45	46	46
CSF Tau	R	0.34	-	0.51	0.33
	p	0.02	-	<0.001	0.025
	N	45	-	45	45
Plasma Tau	R	-0.004	0.51	-	0.33
	p	0.981	<0.001	-	0.007
	N	46	45	-	65
NF-L Plasma	R	0.57	0.33	0.33	-
	p	<0.001	0.025	0.007	-

	N	46	45	65	-
Genetic CJD					
		CSF NF-L	**CSF Tau**	**Plasma Tau**	**NF-L Plasma**
CSF NF-L	R	-	0.028	-0.152	0.50
	p	-	0.918	0.575	0.044
	N	-	16	16	16
CSF Tau	R	0.028	-	0.52	0.224
	p	0.918	-	0.039	0.405
	N	16	-	16	16

Table 3. Area under the curve (AUC) values and 95% confidence intervals (CI) for Receiver-Operating-Characteristic curves for the evaluation of plasma and cerebrospinal fluid (CSF) tau and neurofilament (NF-L) levels. AD: Alzheimer disease; CJD: Creutzfeldt-Jakob disease (s: sporadic, g: genetic); OND: other neurological disorder.

	Plasma tau		**Plasma NF-L**		**CSF tau**		**CSF NF-L**	
Comparison	**AUC**	**95% CI**	**AUC**	**95% CI**	**AUC**	**95% CI**	**AUC**	**95% CI**
sCJD-AD	0.756	0.63-0.87	0.657	0.48-0.83	0.894	0.78-1.0	0.768	0.60-0.93
sCJD-OND	0.722	0.60-0.83	0.497	0.30-0.69	0.883	0.78-0.98	0.451	0.27-0.63
sCJD-gCJD	0.57	0.43-0.71	0.467	0.33-0.60	0.515	0.36-0.66	0.676	0.53-0.81
gCJD-AD	0.694	0.53-0.85	0.705	0.50-0.90	0.902	0.77-1.0	0.862	0.71-1.0
gCJD-OND	0.651	0.48-0.81	0.520	0.30-0.87	0.901	0.79-1.0	0.570	0.36-0.77
AD-OND	0.453	0.28-0.62	0.441	0.27-0.61	0.429	0.21-0.63	0.759	0.58-0.93
sCJD-CO	0.939	0.89-0.98	0.992	0.98-1.0	0.979	0.93-1.0	0.979	0.95-1.0
gCJD-CO	0.918	0.83-1.0	1.0	1.0-1.0	1.0	1.0-1.0	0.997	0.98-1.0
AD-CO	0.821	0.69-0.95	0.992	0.97-1.0	0.720	0.52-0.92	0.817	0.66-0.97
OND-CO	0.829	0.70-0.95	0.96	0.90-1.0	0.799	0.64-0.95	0.902	0.78-1.0

Legend to figures

Figure 1.Scatter plots of plasma tau (A, E), NF-L (C, G) and CSF tau (B, F) and NF-L (D, H) in all disease groups examined (A-D) and in molecular subtypes of sporadic CJD (E-H). Horizontal lines in each plot for each disease group indicate the mean value observed for that group. The horizontal line spanning the whole graph area in E-H represents the mean values for the total cohort of sporadic CJD. * indicates p< 0.05; **: p< 0.01; ***: p<0.001; OND: other neurological disorder; AD: Alzheimer disease; sCJD: sporadic Creutzfeldt-Jakob disease; gCJD: genetic CJD, CO: healthy controls.

Figure 2. Receiver operator characteristic curves and area under the curve (AUC) values for plasma tau examination for A) Alzheimer disease, B) sporadic CJD, C) genetic CJD, and D) other neurological disease versus healthy controls.