Supplementary Online Content

Staffaroni AM, Kramer AO, Casey M, et al. Association of blood and cerebrospinal fluid tau level and other biomarkers with survival time in sporadic Creutzfeldt-Jakob disease. *JAMA Neurol*. Published online May 6, 2019. doi:10.1001/jamaneurol.2019.1071

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods.

Sample Collection Protocol and Quantification of Fluid Biomarkers

CSF

CSF for research (up to 10-12 mL when possible) was collected with patients in either the sitting or lateral decubitus position in a polypropylene tube. CSF sent to the National Prion Disease Pathology Surveillance Center (NPDPSC), Athena, and Mayo Medical Laboratories was collected using a standard plastic lumbar puncture kit. Most samples were kept at room temperature and immediately delivered by courier to UCSF CRS Sample Processing Core Laboratories, where they were immediately aliquoted into 250 μL amounts, frozen on dry ice for twenty minutes and then transferred to -80°C for storage. If samples were collected at the end of the day and not able to be delivered to Core Labs before closing they were stored at 4°C overnight and delivered by courier for processing the next morning. CSF was not centrifuged or otherwise treated prior to aliquoting.

CSF neuron specific enolase (NSE) ELISA testing was performed by Mayo Medical Laboratories (Rochester, MN) and reported as a continuous variable (ng/mL) and as normal, intermediate or elevated (i.e. consistent with sJCD).[1] CSF 14-3-3 protein testing by Western blot was performed at the NPDPSC (n=142) and reported as positive, negative, or ambiguous. CSF 14-3-3 testing by ELISA was done at Mayo Clinic (n=13) but they only reported as positive (> 2.0 ng/ml) or negative. For analyses, negative and ambiguous (or intermediate) results were grouped as negative, a methodology employed by other studies[2] and supported by our own prior analysis.[1] T-tau testing was performed at the NPDPSC (sJCD cut-off ≥1150 pg/mL) or Athena Diagnostics (Worcester, MA; sJCD cut-off ≥ 1200 pg/mL); prior heterogeneity analysis by our group showed that the observed degree of variability between the two laboratories was not significant.[1] Samples stored at -80°C were shipped on dry ice to the University of Gothenburg, Sweden and analyzed for NfL using an in house ELISA as previously described,[3] and p-tau and the 42 amino acid form of amyloid-β ($A\beta_{42}$) using commercially available INNOTEST ELISAs (Fujirebio, Ghent, Belgium). RT-QuIC analysis of CSF samples was performed as previously described.[4] RT-QuIC was analyzed at the NIH Rocky Mountain Laboratories (n = 46) and NPDPSC (n = 34). For 16 patients, RT-QuIC was performed by both laboratories. Results were concordant for 13 cases. For 3 cases, the result was reported as inconclusive by the NPDPSC and negative by NIH RML.

Plasma

Whole blood tubes were collected from each participant by antecubital vein puncture in 4 mL EDTA tubes. For plasma, one tube was delivered immediately, at room temperature, to UCSF CRS Sample Processing Core Laboratories, centrifuged at 1300g for 10 minutes at 4°C. The resulting plasma was divided into 250 μL aliquots in cryovials and stored at -80°C. In rare circumstances, samples unable to be sent immediately for processing prior to lab closure were stored at 4°C overnight and delivered for processing the next morning. Plasma samples stored at -80°C were shipped on dry ice to the University of Gothenburg for analysis of NfL using an in house Single molecule array (Simoa) assay, as previously described,[5] and t-tau and GFAP using commercially available Simoa assays (Quanterix, Lexington, MA).

Of note, these plasma proteins, including tau, are not the primary pathogenic proteins in sJCD and increased concentrations would not be expected to increase the transmission of CJD. In a previous study, however, we have shown that hyperphosphorylated tau is not uncommon in sJCD and might be higher than expected given the relatively young age of sJCD.[6] Furthermore, in certain genetic forms of prion disease, such as stop codon mutations and some forms of Gertmann-Straussler-Scheinker, not only is there a prionopathy, but a prominent tauopathy can be present.[7,8] Although PrP prions likely are present in blood in sJCD, epidemiological evidence suggests that sJCD blood does not transmit prion disease. This could be for several reasons, including that the levels of PrP prions are too low. On the other hand, PrP prions from variant JCD (vJCD) blood has clearly been shown to transmit vJCD.[9] Misfolded tau has been shown in cell culture and in animal models to have prion-like spread and therefore could be transmissible.[7,10–13] There is also some less strong evidence that tau from cadaveric-derived human growth hormone pituitary extracts might be transmissible in humans.[10] It still is not definitively known if misfolded tau is truly transmissible in humans, nor whether tau found in blood could be transmissible.

eTable 1. Participant demographic characteristics for patients with CSF t-tau measurements

sJCD Patients with CSF T-TAU	
Age at Study visit (mean ± SD, median [IQR], range)	64.0 ± 9.2, 65 [57, 70.5],38-84
% Female	48
	N (%)
Total	125
Pathologically-Confirmed	90(72)
Probable	35(28)
PRNP codon 129 and molecular classification	116 (n = 82 with typing, 71%)
MM	49(42)
MM 1	13(16)
MM 2	12(15)
MM 1 + 2	10(12)
MV	48(41)
MV 1	13(16)
MV 2	12(15)
MV 1 + 2	9(11)
VV	19(16)
VV 1	1(1)
VV 2	8(10)
VV 1 + 2	4(5)
Barthel score at first visit (n=88; mean ± SD, median [IQR], range)	61.9 ± 34.7, 70 [30, 93.8], 0-100
MRC Scale score at first visit (n=14; mean ± SD, median [IQR], range)	15.5 ± 3.9, 16.5 [12, 18.3], 6-20
Average months from first symptom to study visit (n=121; mean ± SD, median [IQR], range)	10.0 ± 8.6, 7.7 [3.9, 13.2], 0.4-57.5
Average months from first study visit to death (n=125; mean ± SD, median [IQR], range)	6.4 ± 7.2, 3.7 [1.2, 9.0], 0.1-38.3
Average months from first symptom to death (n=121; ± SD, median [IQR], range) MM (n=49; mean ± SD) MV (n=48; mean ± SD) VV (n=17; mean ± SD)	16.6 ± 12.3, 15.0 [7.1, 23.3], 1.2-64.4 14.4 ± 10.6 21.4 ± 14.3 11.2 ± 7.4

eTable 2. Participant demographic characteristics for patients with CSF NSE measurements

sJCD Patients with NSE	
Age at Study visit (mean ± SD, median [IQR], range)	63.7 ± 9.5, 64.5 [57-70],38-84
% Female	49
	N(%)
Total	123
Pathologically-Confirmed	89 (72)
Probable	34(28)
PRNP codon 129 and molecular classification	113 (n = 81 with typing, 72%)
MM	49(43)
MM 1	14(17)
MM 2	14(17)
MM 1 + 2	9(11)
MV	44(39)
MV 1	10(12)
MV 2	10(12)
MV 1 + 2	11(14)
VV	20(18)
VV 1	2(3)
VV 2	8(10)
VV 1 + 2	3(4)
Barthel score at first visit (n=90; mean ± SD, median [IQR], range)	63.5 ± 33.1, 75 [35, 95], 0-100
MRC Scale score at first visit (n=15; mean ± SD, median [IQR], range)	14.9 ± 4.0, 16 [12, 18], 6-20
Average months from first symptom to study visit (n=116; mean ± SD, median [IQR], range)	9.9 ± 7.6, 7.6 [4.3, 12.6], 0.5-35.2
Average months from first study visit to death (n=123; mean ± SD, median [IQR], range)	6.6 ± 7.3, 3.6 [1.3, 9.8], 0-33
Average months from first symptom to death (n=117; mean ± SD, median [IQR], range) MM (n=48; mean ± SD) MV (n=44; mean ± SD) VV (n=19; mean ± SD)	16.6 ± 11.8, 14.8 [7.9, 23.6], 1.1- 60.1 15.0 ± 10.5 20.5 ± 13.7 12.5 ± 8.2

eTable 3. Participant demographic characteristics for patients with RT-QuIC measurements

sJCD Patients with RT-QuIC	
Age at Study visit (mean ± SD, median [IQR], range)	64.3 ± 9.0, 65 [58, 70.3], 43-83
% Female	51.6
	N(%)
Total	64
Pathologically-Confirmed	41(64)
Probable	23(36)
PRNP codon 129 and molecular classification	59 (n=38 with typing, 64%)
MM	24(41)
MM 1	7(18)
MM 2	5(13)
MM 1 + 2	5(13)
MV	29(49)
MV 1	4(11)
MV 2	5(13)
MV 1 + 2	7(18)
VV	6(10)
VV 1	0(0)
VV 2	4(11)
VV 1 + 2	1(3)
Barthel score at first visit (n=43; mean ± SD, median [IQR], range)	65.6 ± 37.0, 80 [25, 100], 0-100
MRC Scale score at first visit (n=15; mean ± SD, median [IQR], range)	15.7 ± 3.8, 17 [12, 18], 6-20
Average months from first symptom to study visit (n=60; mean ± SD, median [IQR], range)	10.4 ± 9.5, 8.2 [4.3, 12.3], 0.5-57.5
Average months from first study visit to death (n=64; mean ± SD, median [IQR], range)	6.2 ± 6.5, 3.6 [1.3, 7.7], 0.3-26.6
Average months from first symptom to death (n=60; mean ± SD, median [IQR], range)	16.8 ± 12.9, 14.9 [8, 23.1], 1.2-64.4
MM (n=24; mean ± SD)	14.1 ± 10.6
MV (n=29; mean ± SD)	20.4 ± 14.6
VV (n= 4; mean ± SD)	10.1 ± 9.9

eTable 4. Participant demographic characteristics for patients with plasma measurements

sJCD Patients with Plasma Markers	
Age at Study visit (mean ± SD, median [IQR], range)	64.7 ± 7.4, 66 [62.5, 69], 49-80
% Female	58.3
	N(%)
Total	24
Pathologically-Confirmed	18(75)
Probable	6(25)
PRNP codon 129 and molecular classification	7 (n=5 with typing, 71%)
MM	2(29)
MM 1	0(0)
MM 2	1(20)
MM 1 + 2	0(0)
MV	4(57)
MV 1	1(20)
MV 2	1(20)
MV 1 + 2	1(20)
VV	1(14)
VV 1	0(0)
VV 2	1(20)
VV 1 + 2	0(0)
Barthel score at first visit (n=21; mean ± SD, median [IQR], range)	68.8 ± 30.8, 80 [50, 100], 0-100
MRC Scale score at first visit (n=11; mean ± SD, median [IQR], range)	14.7 ± 4.3, 17 [12, 18], 6-20
Average months from first symptom to study visit (n=22; mean ± SD, median [IQR], range)	8.9 ± 5.5, 7.2 [5.1, 12.7], 1.1-24.6
Average months from first study visit to death (n=24; mean ± SD, median [IQR], range)	6.1 ± 7.3, 3.0 [1.4, 7.6], 0.5-26.3
Average months from first symptom to death (n=22; mean ± SD, median [IQR], range) MM (n=2; mean ± SD) MV (n=4; mean ± SD) VV (n=1)	15.5 ± 10.3, 13.0 [8.5, 20.3], 2.4-39.3 18.0 ± 1.8 17.0 ± 14.7 8.0

eTable 5. Participant demographic characteristics for patients with CSF measurements (analyzed in Sweden)

sJCD Patients with CSF P-TAU, NfL, & Aß$_{42}$ (Sweden)	
Age at Study visit (mean ± SD, median [IQR], range)	63.8 ± 8.4, 65 [58.5, 69], 43-82
% Female	53.1
	N(%)
Total	49
Pathologically-Confirmed	36(73)
Probable	13(27)
PRNP codon 129 and molecular classification	47 (n=33 with typing, 70%)
MM	19(40)
MM 1	4(12)
MM 2	7(21)
MM 1 + 2	3(9)
MV	23(49)
MV 1	3(9)
MV 2	5(15)
MV 1 + 2	7(21)
VV	5(11)
VV 1	0(0)
VV 2	3(9)
VV 1 + 2	1(3)
Barthel score at first visit (n=40; mean ± SD, median [IQR], range)	62.0 ± 37.6, 80 [25, 98.8], 0-100
MRC Scale score at first visit (n=12; mean ± SD, median [IQR], range)	14.8 ± 4.2, 16.5 [12, 18], 6-20
Average months from first symptom to study visit (n=47; mean ± SD, median [IQR], range)	10.1 ± 7.6, 8.1 [5, 13], 0.8-35.2
Average months from first study visit to death (n=49; mean ± SD, median [IQR], range)	6.2 ± 6.9, 3.3 [1.3, 9.1], 0.3-26.3
Average months from first symptom to death (n=47; mean ± SD, median [IQR], range) MM (n=19; mean ± SD) MV (n=23; mean ± SD) VV (n=4; mean ± SD)	16.4 ± 11.8, 14.7 [8.1, 25], 1.2-60.1 15.3 ± 11.0 19.1 ± 12.5 10.1 ± 9.9

eReferences.

1. Forner SA, Takada LT, Bettcher BM, et al. Comparing CSF biomarkers and brain MRI in the diagnosis of sporadic Creutzfeldt-Jakob disease. *Neurol Clin Pract*. 2015;5(2):116-125. doi:10.1212/CPJ.0000000000000111.
2. Chohan G, Pennington C, Mackenzie JM, et al. The role of cerebrospinal fluid 14-3-3 and other proteins in the diagnosis of sporadic Creutzfeldt-Jakob disease in the UK: a 10-year review. *J Neurol Neurosurg Psychiatry*. 2010;81(11):1243-1248. doi:10.1136/jnnp.2009.197962.
3. Gaetani L, Höglund K, Parnetti L, et al. A new enzyme-linked immunosorbent assay for neurofilament light in cerebrospinal fluid: analytical validation and clinical evaluation. *Alzheimers Res Ther*. 2018;10(1):8. doi:10.1186/s13195-018-0339-1.
4. Orrú CD, Groveman BR, Hughson AG, Zanusso G, Coulthart MB, Caughey B. Rapid and Sensitive RT-QuIC Detection of Human Creutzfeldt-Jakob Disease Using Cerebrospinal Fluid. *MBio*. 2015;6(1):e02451-14. doi:10.1128/mBio.02451-14.
5. Gisslén M, Price RW, Andreasson U, et al. Plasma Concentration of the Neurofilament Light Protein (NFL) is a Biomarker of CNS Injury in HIV Infection: A Cross-Sectional Study. *EBioMedicine*. 2016;3:135-140. doi:10.1016/j.ebiom.2015.11.036.
6. Tousseyn T, Bajsarowicz K, Sánchez H, et al. Prion Disease Induces Alzheimer Disease-Like Neuropathologic Changes. *J Neuropathol Exp Neurol*. 2015;74(9):873-888. doi:10.1097/NEN.0000000000000228.
7. Jayadev S, Nochlin D, Poorkaj P, et al. Familial prion disease with Alzheimer disease-like tau pathology and clinical phenotype. *Ann Neurol*. 2011;69(4):712-720. doi:10.1002/ana.22264.
8. Fong JC, Rojas JC, Bang J, et al. Genetic Prion Disease Caused by PRNP Q160X Mutation Presenting with an Orbitofrontal Syndrome, Cyclic Diarrhea, and Peripheral Neuropathy. *J Alzheimer's Dis*. 2016;55(1):249-258. doi:10.3233/JAD-160300.
9. Knight R. The risk of transmitting prion disease by blood or plasma products. *Transfus Apher Sci*. 2010;43(3):387-391. doi:10.1016/j.transci.2010.09.003.
10. Duyckaerts C, Sazdovitch V, Ando K, et al. Neuropathology of iatrogenic Creutzfeldt-Jakob disease and immunoassay of French cadaver-sourced growth hormone batches suggest possible transmission of tauopathy and long incubation periods for the transmission of Abeta pathology. *Acta Neuropathol*. 2018;135(2):201-212. doi:10.1007/s00401-017-1791-x.
11. Woerman AL, Aoyagi A, Patel S, et al. Tau prions from Alzheimer's disease and chronic traumatic encephalopathy patients propagate in cultured cells. *Proc Natl Acad Sci U S A*. 2016;113(50):E8187-E8196. doi:10.1073/pnas.1616344113.
12. Sanders DW, Kaufman SK, DeVos SL, et al. Distinct tau prion strains propagate in cells and mice and define different tauopathies. *Neuron*. 2014;82(6):1271-1288. doi:10.1016/j.neuron.2014.04.047.
13. Frost B, Jacks RL, Diamond MI. Propagation of tau misfolding from the outside to the inside of a cell. *J Biol Chem*. 2009;284(19):12845-12852. doi:10.1074/jbc.M808759200; 10.1074/jbc.M808759200.