

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Cerebrospinal fluid biomarkers of neurodegeneration in narcolepsy type 1

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Availability:

This version is available at: <https://hdl.handle.net/11585/739035> since: 2020-02-27

Published:

DOI: <http://doi.org/10.1093/sleep/zsz215>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

(Article begins on next page)

This is the accepted manuscript of:

Baiardi S, Pizza F, Polischi B, Moresco M, Abu-Rumeileh S, Plazzi G, Parchi P.

Cerebrospinal fluid biomarkers of neurodegeneration in narcolepsy type 1.

Sleep. 2020 Feb 13;43(2):zsz215.

The final published version is available online at: <https://doi.org/10.1093/sleep/zsz215>

Rights / License:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

When citing, please refer to the published version.

Title

Cerebrospinal fluid biomarkers of neurodegeneration in narcolepsy type 1.

Authors

Simone Baiardi^{1,2}, Fabio Pizza^{1,2}, Barbara Polischi², Monica Moresco², Samir Abu-Rumeileh¹,
Giuseppe Plazzi^{1,2}, Piero Parchi^{2,3*}

Affiliations

¹ Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy

² IRCCS, Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy

³ Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy

***Corresponding Author:** Prof. Piero Parchi, MD, PhD, IRCCS Istituto delle Scienze Neurologiche di Bologna, Ospedale Bellaria, Via Altura 1/8, 40139 Bologna, Italy. Phone: +390514966740, Fax: +390514966208. E-mail: piero.parchi@unibo.it

ABSTRACT

Study objectives. To measure the levels of five neurodegenerative biomarkers in the cerebrospinal fluid (CSF) of patients with narcolepsy type 1 (NT1) with variable disease duration.

Methods. Following a standardized protocol of CSF collection and storage, we measured CSF total-and phosphorylated-tau, amyloid-beta 1-40 and 1-42, and neurofilament light chain proteins in 30 non-neurological controls and 36 subjects with NT1, including 14 patients with recent disease onset (i.e. ≤ 12 months, short disease duration group).

Results. CSF levels of all biomarkers were similar in NT1 subjects and controls. The comparison between NT1 with short and long disease duration only revealed slightly higher levels of CSF amyloid-beta 40 in the former group (median 9549.5, IQR 7064.2-11525.0 vs. 6870.0, IQR 5133.7-9951.2, $p=0.043$). CSF storage time did not influence the levels of the tested biomarkers.

Conclusions. The measurement of CSF total-tau, phosphorylated-tau, amyloid-beta 40 and 42, and neurofilament light chain proteins is not informative in NT1.

Keywords: amyloid, cerebrospinal fluid, narcolepsy, hypersomnia, sleepiness, neurodegenerative, tau, neurofilament protein.

Statement of Significance

In contrast with previous studies, here we demonstrated, in a sizeable cohort of patients with variable disease duration, that in narcolepsy type 1 the levels of several cerebrospinal fluid (CSF) neurodegenerative biomarkers are unremarkable. The findings argue against the utility of the current most common neurodegenerative CSF biomarkers in the diagnostic or prognostic evaluation of patients with narcolepsy type 1.

Accepted Manuscript

INTRODUCTION

Narcolepsy Type 1 (NT1) is a rare central sleep disorder clinically characterized by hypersomnolence and loss of boundaries between REM sleep and wake.¹ Several lines of evidence suggest that an autoimmune process mediated by T cells,^{2,3} which leads to a deficiency of hypothalamic hypocretin-1 (hcr-1) signaling pathway, drives the pathogenesis of NT1.^{4,5} A reduction of hcr-1 levels in cerebrospinal fluid (CSF) below the threshold of 110 pg/ml distinguishes NT1 from other hypersomnias of central origin with high specificity.⁶ Accordingly, the last update of the International Classification of Sleep Disorders (ICSD-3) introduced the detection of CSF hcr-1 ≤ 110 pg/ml by Radio Immune Assay a major diagnostic criterion for NT1.⁷ The availability of CSF prompted the search for biomarkers other than hcr-1 in NT1 cohorts to monitor disease pathogenesis and clinical outcome. However, initial studies, focusing on proteins involved in Alzheimer's disease and other neurodegenerative disorders, including tau and amyloid-beta (A β) peptides,⁸⁻¹³ yielded inconclusive and often divergent results. The small sample size of the cohorts, their clinical heterogeneity, including age, time lapse between the disease onset and lumbar puncture (LP), presence/absence of concomitant pharmacological treatment, combination of narcolepsy type 1 and 2, and eventually analytical and pre-analytical variables, which notoriously may interfere with biomarker dosages,^{14,15} likely contributed to these inconsistencies. In the present study, we systematically compared the CSF levels of five neurodegeneration biomarkers, namely total-tau, phosphorylated-tau, A β 1-40 and 1-42, and neurofilament light chain (NfL) proteins, between non-neurological controls and a large cohort of patients with NT1, stratified according to the time lapse from disease onset to LP.

METHODS

Patients and clinical evaluation

We analyzed CSF samples from 30 non-neurological controls and 36 patients with NT1, diagnosed at the Narcolepsy Center of the University of Bologna / Institute of the Neurological Sciences of

Bologna (ISNB). Each patient underwent a standardized assessment that included 48-hours polysomnography, multiple sleep latency test (MSLT), *HLA-DQB1*06:02* typing, and determination of CSF hypocretin-1. At the time of evaluation, all patients were drug-naïve. NT1 diagnosis was established by the current criteria, including pathological CSF hcr1 levels.⁷ The control group included age- and sex-matched subjects with a subjective complaint of excessive daytime sleepiness not confirmed at sleep studies (i.e. mean sleep latency at MSLT 14.6 ± 3.1 min, number of early onset REM sleep period ≤ 2 in each case) and lacking any neurophysiological or neuroradiological evidence of central nervous system diseases or respiratory/motor sleep disorders potentially responsible for the symptom(s). None had cataplectic episodes during a standardized video recording under emotional stimulation.¹⁶ The Local Ethics Committee approved the study protocol (CE-BI 17009), and all patients gave their written informed consent to participate in the study.

CSF biochemical analysis

CSF was taken at the L3-L4 or L4-L5 lumbar space during the diagnostic spinal tap for CSF hcr1 assay, following a standard procedure, centrifuged at 1000xg for 10 minutes, divided into aliquots, and stored in polypropylene tubes at -80°C until analysis. In each case, CSF was collected between 8:00 a.m. and 10:00 a.m.

The same senior technician handled and analyzed the samples at the Neuropathology Laboratory of the ISNB. CSF total-tau (t-tau), phosphorylated-tau (p-tau), A β 1-42 and A β 1-40 (hence abbreviated as A β 42 and A β 40, respectively) levels were analyzed using commercially available ELISA kits (INNOTEST htau-Ag, INNOTEST phosphorylated-Tau181, INNOTEST A β 1-42 and INNOTEST A β 1-40; Innogenetics/Fujirebio) according to the manufacturer's instructions. NfL protein levels were analyzed using a commercially available ELISA kit (IBL, Hamburg, Germany), as previously described.¹⁷ The ratio of A β 42 to A β 40 was calculated according to the formula $[(A\beta 42)/(A\beta 40) \times 10]$.

The mean intra-assay coefficients of variation (CVs) of the assays were 4.7% for t-tau, 1.1% for p-tau, 2.9% for A β 42, 4.8% for A β 40 and 4.4% for NfL, whereas the mean inter-assay CVs were 5.0% for t-tau, 2.9% for p-tau, 7.2% for A β 42, 5.8% for A β 40 and 6.5% for NfL.

Concerning pre-analytical variables, we collected for each case the CSF storage time (i.e., the interval between LP and each biomarker analysis) and the time interval between clinical onset and LP. We defined NT1 of “recent onset” when the elapsed time from the disease onset to LP was within a window of 12 months.¹⁸ CSF hcr1 levels were measured by radioimmune assay (¹²⁵I RIA kits from Phoenix Pharmaceuticals; Belmont, CA) according to the manufacturer’s instructions.

Statistical analysis

Statistical analysis was performed using the SigmaPlot 12.5 (Systat Software Inc) software. Based on the presence or not of a normal distribution of the values, data were expressed as mean \pm standard deviation (SD) or median and interquartile range (IQR), respectively. Depending on the data distribution the Mann-Whitney U test or the two-tailed t-test were used, as appropriate, to test differences between two groups of continuous variables, while the one-way ANOVA (followed by Tukey’s or Bonferroni’s post hoc test) or the Kruskal-Wallis test (followed by Dunn’s posthoc test) were applied for multiple group comparisons. The chi-square test was adopted for categorical variables. Spearman’s correlation was used to determine the relationship between two continuous or ordinal variables. We tested the effect of storage time on CSF biomarker results using univariate linear regression models. A *p* value of <0.05 was considered statistically significant.

RESULTS

The study cohort included middle-aged participants (mean age 40.7 \pm 7.3 years) with similar gender distribution (male: female ratio 1.3). Fourteen of the 36 NT1 patients (38.9%) had a recent disease onset, while the interval between clinical onset and LP in the whole group was 9.7 \pm 10.2 years (mean \pm SD). All NT1 patients had CSF hcr1 levels below the 110 pg/mL threshold, and all but one

carried the *HLA-DQB1*06:02* allele (Table 1). Sleep polysomnographic measures revealed more numerous awakenings during sleep, an increased percentage of N1 sleep stage and, conversely, a reduction of N3 sleep stage in NT1 patients with a recent onset than in controls and NT1 patients with a prolonged clinical course (Table 2).

All tested CSF biomarkers as well as the A β 42/A β 40, t-tau/A β 42 and p-tau/A β 42 ratios showed similar values in the NT1 and control groups (Table 3). Moreover, there was no correlation between CSF biomarkers levels and polysomnographic sleep parameters. Notably, CSF biomarker levels in both groups were within the range of our “in house” reference values, that are based on the analysis of 50 subjects who underwent LP due to tension-type headache, non-inflammatory polyneuropathies, or subjective complaints (IQR t-tau 152-256 pg/ml, IQR p-tau 32-45 pg/ml, IQR A β 42 698-1175 pg/ml, IQR A β 40 5831-10550 pg/ml, and IQR NfL 881-1680 pg/ml).

The comparison between NT1 patients with recent onset and those with a longer course revealed no significant differences in any biomarker results, except for higher median levels of A β 40 in the former group (9549.5 pg/ml, IQR 5133.7-9951.2 vs. 6870.0 pg/ml, IQR 5133.7-9951.2, $p=0.043$) (Figure 1). Moreover, the A β 42/A β 40 ratio, which allows the normalization of the A β peptide levels, did not significantly differ between the two groups.

Regarding pre-analytical variables, the overall median CSF storage time was slightly shorter in controls than in patients with NT1 (15.5 vs 23.0 months, $p=0.009$). However, on univariate linear regression models, including the storage time as the independent variable and the concentrations of each biomarker as the dependent variable, the latter were not influenced by the time lapse between LP and the biomarker analysis.

DISCUSSION

In this study, the assessment of a large set of neurodegenerative CSF biomarkers did not distinguish between patients with NT1 and non-neurological controls. Previous studies in this field reported discordant results. A single study showed increased levels of CSF A β 42, t-tau and p-tau in patients

with NT1 and NT2 and a long disease duration.⁹ To explain this unexpected finding some authors claimed a neurotoxicity from a chronic treatment with stimulants, i.e. amphetamines and methylphenidate.^{19,20} Most frequently, however, studies found reduced CSF A β 42 levels in patients NT1 in comparison to healthy subjects.¹¹⁻¹³ A marked reduction of CSF A β 42 was also reported in a young patient who developed NT1 after H1N1 flu-vaccination.⁸ However, the relatively young age of enrolled NT1 participants and the normal levels of CSF tau proteins (t-tau and p-tau)^{11,13} make it unlikely that the low CSF A β 42 reflects ongoing neurodegeneration in NT1. Given that reduced levels of CSF A β 42 are also found in patients affected by CNS inflammatory disorders of various etiologies,²¹⁻²³ some authors linked the finding to the inflammatory/autoimmune pathogenesis of NT1. Pursuing this hypothesis, Liguori and colleagues compared two subgroups of NT1 according to the duration of the disease and found a more pronounced CSF A β 42 decrease in the group with the shorter clinical course (≤ 5 years).¹² In contrast, however, we found no difference in CSF A β 42 levels between NT1 patients with recent onset and those with a longer disease course. Since the autoimmune pathogenic process in NT1 is thought to be more severe near clinical onset, our results strongly argue against the hypothesis of the reduction of CSF A β 42 levels due to neuroinflammation.

As the only possible difference related to the clinical course, we found higher levels of CSF A β 40 in NT1 group with recent disease onset. Recently, the disruption of nocturnal slow wave activity (SWA), which characterizes the sleep of NT1 patients,²⁴ has been correlated to increased levels of CSF A β 40 in a dose-dependent manner.²⁵ In our study, however, we failed to demonstrate a statistically significant correlation between the CSF levels of A β 40 and the polysomnographic sleep measures, including the absolute and percentage amount of N3 stage sleep. Nevertheless, the lower representation of N3 sleep suggests a more pronounced disruption of SWA in NT1 patients close to onset, a finding to be confirmed in larger studies.

It is well-established that a correct interpretation of the results of CSF A β 42 assay relies on several factors. Firstly, among the commonly exploited neurodegenerative biomarkers, A β 42 is the most susceptible to variation in the pre-analytical process.^{15,26} To reduce this source of variability, we applied a standardized protocol of CSF collection and storage, had a single, experienced technician performing all the analyses, and excluded the possible influence of CSF storage time on biomarker results, using univariate linear regression analyses in both groups. Secondly, CSF levels of A β 42 may also vary among individuals depending on the constitutional production of A β peptides.²⁷ For the correct interpretation of CSF A β 42 results, increasing evidence recommends the evaluation of the CSF A β 42/A β 40 ratio,^{15,28-30} which allows the normalization of CSF A β 42 value concerning both the overall individual production of A β peptides and the confounding interference of preanalytical variables. In our NT1 cohort, the CSF A β 42/A β 40 ratio showed similar mean values in NT1 patients and controls. Finally, the concentration of A β shows physiological fluctuations over time as tested in healthy subjects.³¹ In human APP transgenic mice, this variation has been related to diurnal fluctuations, given that the A β increases in the interstitial fluid during wakefulness and decreases during sleep.³² In this study, we addressed the potentially confounding influence of the ultradian oscillation of CSF A β peptides by defining a standardized time window for CSF collection for both NT1 patients and controls.

The finding that NT1 patients have normal CSF levels of NfL, a biomarker found altered in a large variety of neurodegenerative disorders, is also consistent with the conclusion that the current neurodegenerative biomarkers lack sufficient sensitivity for the pathological process occurring in NT1. The latter result is also in line with that of the only previous study on this marker in NT1 patients.¹³

In conclusion, NT1 patients had normal CSF t-tau, p-tau, A β 42, and NfL irrespective of disease duration. Similar results were obtained by evaluation of CSF A β 42/A β 40, t-tau/A β 42, p-tau/A β 42

ratios. Hence, our data do not support the use of these CSF biomarkers for the clinical assessment of NT1 patients.

Accepted Manuscript

DISCLOSURE STATEMENT

Financial disclosure: none

Non-financial disclosure: G. Plazzi participates to advisory board of UCB Europe, Jazz pharmaceuticals, Bioprojet, IDORSIA. He is president of the Italian Sleep Medicine Association (AIMS) and vice president elected of the European Narcolepsy Network (EU-NN). Nothing to declare for the other co-authors.

REFERENCES

1. Kornum BR, Knudsen S, Ollila HM, et al. Narcolepsy. *Nat Rev Dis Primers*. 2017; 3:16100.
2. Mahoney CE, Cogswell A, Koralnik IJ, Scammell TE. The neurobiological basis of narcolepsy. *Nat Rev Neurosci*. 2019;20(2):83-93.
3. Latorre D, Kallweit U, Armentani E, et al. T cells in patients with narcolepsy target self-antigens of hypocretin neurons. *Nature*. 2018;562(7725):63-68.
4. de Lecea L, Kilduff TS, Peyron C, et al. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A*. 1998;95(1):322-327.
5. Sakurai T, Amemiya A, Ishii M, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*. 1998;92(4):573-585.
6. Mignot E, Lammers GJ, Ripley B, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. *Arch Neurol*. 2002;59(10):1553-1562.
7. American Academy of Sleep Medicine. *International Classification of Sleep Disorders*. 3rd ed. Darien, IL: American Academy of Sleep Medicine; 2014.
8. Kallweit U, Hidalgo H, Engel A, Baumann CR, Bassetti CL, Dahmen N. Post H1N1 vaccination narcolepsy-cataplexy with decreased CSF beta-amyloid. *Sleep Med*. 2012;13(3):323.
9. Heier MS, Skinningsrud A, Paus E, Gautvik KM. Increased cerebrospinal fluid levels of nerve cell biomarkers in narcolepsy with cataplexy. *Sleep Med*. 2014;15(6):614-618.
10. Dauvilliers YA, Lehmann S, Jaussent I, Gabelle A. Hypocretin and brain β -amyloid peptide interactions in cognitive disorders and narcolepsy. *Front Aging Neurosci*. 2014;6:119.
11. Liguori C, Placidi F, Albanese M, et al. CSF beta-amyloid levels are altered in narcolepsy: a link with the inflammatory hypothesis? *J Sleep Res*. 2014;23(4):420-424.
12. Liguori C, Placidi F, Izzi F, et al. Beta-amyloid and phosphorylated tau metabolism changes in narcolepsy over time. *Sleep Breath*. 2016;20(1):277-283.

13. Jennum PJ, Østergaard Pedersen L, Czarna Bahl JM, et al. Cerebrospinal Fluid Biomarkers of Neurodegeneration Are Decreased or Normal in Narcolepsy. *Sleep*. 2017;40(1).
14. Toombs J, Foiani MS, Wellington H, et al. Amyloid β peptides are differentially vulnerable to preanalytical surface exposure, an effect incompletely mitigated by the use of ratios. *Alzheimers Dement*. 2018;10:311-321.
15. Hansson O, Mikulskis A, Fagan AM, et al. The impact of preanalytical variables on measuring cerebrospinal fluid biomarkers for Alzheimer's disease diagnosis: A review. *Alzheimers Dement*. 2018;14(10):1313-1333.
16. Vandi S, Pizza F, Antelmi E, et al. A standardized test to document cataplexy. *Sleep Med*. 2019;53:197-204.
17. Abu-Rumeileh S, Capellari S, Stanzani-Maserati M, et al. The CSF neurofilament light signature in rapidly progressive neurodegenerative dementias. *Alzheimers Res Ther*. 2018;10(1):3.
18. Plazzi G, Poli F, Franceschini C, et al. Intravenous high-dose immunoglobulin treatment in recent onset childhood narcolepsy with cataplexy. *J Neurol*. 2008;255(10):1549-1554.
19. Liguori C, Placidi F, Izzi F, et al. May CSF beta-amyloid and tau proteins levels be influenced by long treatment duration and stable medication in narcolepsy? *Sleep Med*. 2014;15(11):1424.
20. Heier MS, Gautvik KM. What happens in the brain in narcolepsy over time? *Sleep Med*. 2014;15(11):1427.
21. Sjögren M, Gisslén M, Vanmechelen E, Blennow K. Low cerebrospinal fluid beta-amyloid 42 in patients with acute bacterial meningitis and normalization after treatment. *Neurosci Lett*. 2001;314(1-2):33-36.
22. Krut JJ, Zetterberg H, Blennow K, et al. Cerebrospinal fluid Alzheimer's biomarker profiles in CNS infections. *J Neurol*. 2013;260(2):620-626.
23. Augutis K, Axelsson M, Portelius E, et al. Cerebrospinal fluid biomarkers of β -amyloid metabolism in multiple sclerosis. *Mult Scler*. 2013;19(5):543-552.
24. Khatami R, Landolt HP, Achermann P, et al. Insufficient non-REM sleep intensity in narcolepsy-cataplexy. *Sleep*. 2007;30(8):980-989.

25. Ju YS, Ooms SJ, Sutphen C, et al. Slow-wave sleep disruption increases cerebrospinal fluid amyloid- β levels. *Brain*. 2017;140(8):2104-2111.
26. Lewczuk P, Beck G, Esselmann H, et al. Effect of sample collection tubes on cerebrospinal fluid concentrations of tau proteins and amyloid beta peptides. *Clin Chem*. 2006;52(2):332-334.
27. Wiltfang J, Esselmann H, Bibl M, et al. Amyloid beta peptide ratio 42/40 but not A beta 42 correlates with phospho-Tau in patients with low- and high-CSF A beta 40 load. *J Neurochem*. 2007;101(4):1053-1059.
28. Lewczuk P, Lelental N, Spitzer P, Maler JM, Kornhuber J. Amyloid- β 42/40 cerebrospinal fluid concentration ratio in the diagnostics of Alzheimer's disease: validation of two novel assays. *J Alzheimers Dis*. 2015;43(1):183-191.
29. Janelidze S, Zetterberg H, Mattsson N, et al. CSF A β 42/A β 40 and A β 42/A β 38 ratios: better diagnostic markers of Alzheimer disease. *Ann Clin Transl Neurol*. 2016;3(3):154-165.
30. Baiardi S, Abu-Rumeileh S, Rossi M, et al. Antemortem CSF A β 42/A β 40 ratio predicts Alzheimer's disease pathology better than A β 42 in rapidly progressive dementias. *Ann Clin Transl Neurol* 2019. doi:10.1002/acn3.697
31. Bateman RJ, Wen G, Morris JC, Holtzman DM. Fluctuations of CSF amyloid-beta levels: implications for a diagnostic and therapeutic biomarker. *Neurology*. 2007;68(9):666-669.
32. Kang JE, Lim MM, Bateman RJ, et al. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. *Science*. 2009;326(5955):1005-1007

Figure legend

Figure 1. Comparison of CSF biomarker levels between NT1 patients with recent onset (green) and those with a longer course (orange). * $p < 0.05$.

Accepted Manuscript

Table 1. Demographic features, results of HLA DQB1*06:02 typing and CSF hypocretin-1 values.

	NT1 n=36 [%]	Controls n=30 [%]	<i>p</i> value
Mean age at LP – years ± SD	41.1±8.6	40.2±5.5	0.969
Female	16 [44.4]	12 [40.0]	0.909
HLA-DQB1*06:02	35 [97.2]	3 [10.0]	<0.001
CSF hypocretin-1 pg/ml (IQR)	19.9 (0.7-47.9)	358.5 (322.5-385.2)	<0.001
NT1 of recent onset (<12 months)	14 [38.9]	-	-
Interval between clinical onset and LP – years ± SD	9.7±10.2	-	-

Table 2. Sleep polysomnographic results in NT1 with long duration and recent onset, and in controls.

	Controls	NT1	<i>p</i> value	NT1 with long duration	NT1 with recent onset	<i>p</i> value[¥]
Total sleep time (min)	401.0 (325.0-431.6)	369.5 (342.2-424.2)	0.822	385.0 (349.7-430.5)	350.2 (329.4-416.1)	0.490
Sleep efficiency (%)	86.0 (77.0-90.4)	83.4 (76.4-88.9)	0.694	87.4 (75.9-90.8)	80.0 (75.8-87.6)	0.392
Awakenings (n)	27.0 (18.0-35.0)	27.0 (24.0-44.0)	0.147	26.0 (23.0-35.7)	44.0 (25.0-54.5)	0.101
N1 stage (%)	5.4 (3.7-6.9)	10.1 (5.8-14.2)	<0.001	9.9 (5.6-11.9)	13.0 (6.2-21.5)	<0.001 [§] #
N2 stage (%)	42.5 (34.9-50.3)	41.0 (35.3-49.9)	0.662	39.4 (35.1-50.3)	41.1 (36.2-46.9)	0.853
N3 stage (%)	29.9 (22.3-33.3)	19.2 (13.9-30.9)	0.004	24.7 (18.9-31.9)	14.5 (12.2-20.5)	<0.001 [§] *
REM stage (%)	21.9 (17.4-25.9)	24.9 (20.5-28.5)	0.071	23.6 (19.9-26.4)	27.5 (21.0-33.8)	0.120

Data are expressed as median and interquartile range (IQR). [¥] ANOVA or the Kruskal-Wallis test compared controls vs NT1 with long duration vs NT1 with recent onset. [§]Controls vs NT recent onset *p* value <0.05, [#]Controls vs NT1 long duration *p* value <0.05, *NT1 long duration vs NT1 recent onset *p* value <0.05.

Table 3. CSF biomarker levels in the NT1 and control groups.

	NT1 n=36	Controls n=30	<i>p</i> value
t-tau pg/ml	148.0 (117.2-235.7)	164.5 (130.7-184.0)	0.979
p-tau pg/ml	34.0 (26.0-48.2)	35.5 (29.0-40.7)	0.986
Aβ42 pg/ml	940.5 (776.0-1191.0)	959.0 (835.5-1103.2)	0.953
Aβ40 pg/ml	7421.0 (5820.2-10700.0)	8359.0 (6699.7-10050.0)	0.562
Nfl pg/ml	619.0 (478.7-826.2)	658.5 (558.2-830.2)	0.260
Aβ42/Aβ40	1.207 (1.097-1.360)	1.140 (1.053-1.239)	0.382
t-tau/Aβ42	0.160 (0.133-0.200)	0.170 (0.142-0.189)	0.718
p-tau/Aβ42	0.037 (0.031-0.042)	0.036 (0.032-0.042)	0.730
CSF storage time - months	23.0 (13.2-30.7)	15.5 (9.5-22.2)	0.009

Data are expressed as median and interquartile range (IQR)

Figure 1

