Original Article

Intermediate hypocretin-1 cerebrospinal fluid levels and typical cataplexy: their significance in the diagnosis of narcolepsy type 1

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Abstract

Study Objectives: The diagnosis of narcolepsy type 1 (NT1) is based upon the presence of cataplexy and/or a cerebrospinal fluid (CSF) hypocretin-1/orexin-A level ≤ 110 pg/mL. We determined the clinical and diagnostic characteristics of patients with intermediate hypocretin-1 levels (111–200 pg/mL) and the diagnostic value of cataplexy characteristics in individuals with central disorders of hypersomnolence.

Methods: Retrospective cross-sectional study of 355 people with known CSF hypocretin-1 levels who visited specialized Sleep-Wake Centers in the Netherlands. For n = 271, we had full data on cataplexy type (“typical” or “atypical” cataplexy).

Results: Compared to those with normal hypocretin-1 levels (>200 pg/mL), a higher percentage of individuals with intermediate hypocretin-1 levels had typical cataplexy (75% or 12/16 vs 9% or 8/88, p < .05), and/or met the diagnostic polysomnographic (PSG) and Multiple Sleep Latency Test (MSLT) criteria for narcolepsy (50 vs 6%, p < .001). Of those with typical cataplexy, 88% had low, 7% intermediate, and 5% normal hypocretin-1 levels (< .001). Atypical cataplexy was also associated with hypocretin deficiency but to a lesser extent. A hypocretin-1 cutoff of 150 pg/mL best predicted the presence of typical cataplexy and/or positive PSG and MSLT findings.

Conclusion: Individuals with intermediate hypocretin-1 levels or typical cataplexy more often have outcomes fitting the PSG and MSLT criteria for narcolepsy than those with normal levels or atypical cataplexy. In addition, typical cataplexy has a much stronger association with hypocretin-1 deficiency than atypical cataplexy. We suggest increasing the NT1 diagnostic hypocretin-1 cutoff and adding the presence of clearly defined typical cataplexy to the diagnostic criteria of NT1.

Clinical trial information: This study is not registered in a clinical trial register, as it has a retrospective database design.

Statement of Significance

The diagnostic value of intermediate cerebrospinal fluid (CSF) hypocretin-1 levels, 111–200 pg/mL, has not been established. Neither has the relevance of distinguishing typical and atypical cataplexy been evaluated. Additionally, the currently used cutoff value to diagnose narcolepsy was determined when the current classification (narcolepsy type 1 and 2) was not in place. A (re-)examination of this cutoff value and more information regarding the diagnostic implications of typical cataplexy and clinical characteristics of individuals with intermediate CSF hypocretin-1 levels will improve the diagnostic process of people with suspected narcolepsy.

Key words: orexin-A; cataplexy; sleep disorder; central disorders of hypersomnolence

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Introduction
Narcolepsy type 1 (NT1) is a debilitating sleep-wake disorder with a prevalence of 0.02%–0.06% [1, 2]. The clinical presentation involves excessive daytime sleepiness (EDS), disrupted nighttime sleep, and cataplexy. Cataplexy is currently defined in the International Classification of Sleep Disorders (third edition, ICSD3) as more than one episode of generally brief (<2 min), usually bilateral symmetric, sudden loss of muscle tone with retained consciousness. The episode is provoked by strong emotion (mainly of a positive nature) and ends with an abrupt return of muscle activity [3]. The clinical symptoms of narcolepsy are presumed to be caused by insufficient cerebral hypocretin (also named orexin) transmission. In idiopathic narcolepsy (about 95% of cases in humans) [4], the probable cause is an autoimmune-induced loss of hypocretin-producing neurons in the lateral hypothalamus due to a combination of genetic and environmental factors. The loss of these cells leads to low or undetectable hypocretin-1 levels in the cerebrospinal fluid (CSF) [5–7]. A CSF hypocretin-1 deficiency (<110 pg/mL) combined with EDS is sufficient for the diagnosis of NT1. As an alternative to the assessment of CSF hypocretin-1 concentration, narcolepsy can be diagnosed by polysomnography (PSG) during the night, followed by a Multiple Sleep Latency Test (MSLT) during the day [3]. According to the ICSD3, NT1 is diagnosed when EDS is present for ≥3 months, and when the MSLT yields a mean sleep latency (SL) of ≥8 min and ≥2 sleep-onset rapid eye movement periods (SOREMPs). A nocturnal SOREMP during the PSG may count as one of the required SOREMPs. When applying these diagnostic criteria, cataplexy must be present.

Mignot et al. [8] established the currently used CSF hypocretin-1 cutoff value of ≤110 pg/mL within a subject group of 274 people with sleep disorders, including 157 with narcolepsy, and 296 controls (healthy controls and individuals with other neurological disorders). This threshold had a sensitivity of 60% and specificity of 98% for the diagnosis of ICSD2-defined narcolepsy, while a threshold of >200 pg/mL had the best sensitivity/specificity ratio for healthy controls versus all other subject samples [8]. Subsequent studies have implemented these cutoffs [9, 10], as has the ICSD3 [3, 11]. Consequently, an intermediate CSF hypocretin-1 range of 111–200 pg/mL was created, resulting in a patient subgroup that is more difficult to diagnose unless there are clear features of typical cataplexy and positive MSLT and PSG findings [12]. Moreover, at the time these hypocretin-1 cutoff values were determined, the criteria for the differential diagnosis of NT1 and narcolepsy type 2 (NT2) were not yet established, as narcolepsy was then classified as narcolepsy with or without cataplexy [8] We need to characterize individuals with intermediate CSF hypocretin-1 levels, by re-examining the current hypocretin-1 cutoff value.

The presence of cataplexy is considered pathognomonic for NT1, and early recognition can help to avoid misdiagnoses and reduce diagnostic delay [13, 14]. The determination of cataplexy is usually solely based on patient reports, which can be quite difficult to interpret [15, 16]. For example, some symptoms seen in syncope, epileptic, and psychogenic attacks, may resemble cataplexy [16]. Generally, loss of consciousness from the start of the attack indicates that the attacks are not cataplectic. Also, cataplexy attacks sometimes change in frequency and intensity over time [17] and can develop years after EDS onset [18]. There is also diversity in the expression of cataplexy, which led to the introduction of the terms “typical” and “atypical” cataplexy, which are however not clearly defined [19]. Typical cataplexy has been reported to be associated with CSF hypocretin-1 levels ≤110 pg/mL in 90%–95% of people. Thus, typical cataplexy is hypothesized to be more specific for NT1 than its atypical form [20].

The diagnostic significance of intermediate hypocretin-1 levels and of clearly defined typical and atypical cataplexy in the diagnosis of NT1 is yet unresolved. We aimed to: (1) assess the prevalence of intermediate hypocretin-1 levels in people with hypersomnolence complaints; (2) evaluate how clinical aspects (the results of auxiliary investigations as well as cataplexy presence and characteristics) relate to low, intermediate, and normal hypocretin-1 levels; (3) examine the diagnostic value of defined typical versus atypical cataplexy; and (4) evaluate the current CSF hypocretin-1 cutoff point for NT1. The results of this study may improve the diagnostic accuracy of NT1.

Methods
In this retrospective cross-sectional study, we analyzed data of individuals who were referred with complaints of hypersomnolence to any of the following Dutch sleep-wake clinics, between October 2001 and December 2019: Leiden University Medical Center (LUMC, n = 116), Stichting Epilepsie Instellingen Nederland (SEIN, n = 158), and/or Kempenhaeghe (n = 81). Individuals were included if their hypocretin-1 CSF level was assessed and if their electronic health record was available. Hypocretin-1 CSF levels were determined at the LUMC Department of Clinical Chemistry and Laboratory Medicine using the radioimmunoassay (RIA) kit of Phoenix Pharmaceuticals (Phoenix Pharmaceuticals Inc, Burlingame, CA) and harmonized using a Stanford reference sample with a known concentration (used to correct for inter-assay variation between RIAs). Hypocretin-1 levels below 75 pg/mL are deemed “undetectable” in this article, as there is currently local consensus at the LUMC that levels below this value cannot be measured reliably.

Individual characteristics (gender and age at time of CSF assessment) and diagnoses and results of auxiliary investigations (human leukocyte antigen [HLA] DQ beta 1 [DQB1]*06:02 positivity, and PSG and MSLT results at time of diagnostic evaluation) were extracted from the electronic health records. In 77% of patients, PSG reports were available, and the parameters total sleep time, time in bed, sleep efficiency, and the presence of a nighttime SOREMP were extracted. When reports were not available, data could sometimes be extracted from physician notes, referral letters, and letters to the family doctor, resulting in nighttime SOREMP data being available in 86% of cases. From the MSLT reports, the presence of SOREMPs and the SL were extracted. Data on cataplexy presence and type were available for people from the LUMC and SEIN clinics (n = 271, for three individuals from these centers cataplexy data were not available). At SEIN and the LUMC, the MSLT was performed according to Littner et al. [21] with five nap opportunities starting at 09:00 am, at least an hour and a half after the termination of nocturnal sleep. The MSLT was preceded by a PSG, usually in an ambulant setting. Individuals were instructed to try to sleep for at least 6 hours. However, this procedure was not followed in all instances, as a substantial subgroup of individuals was referred after the MSLT and PSG were already performed at the referring sleep centers. These registrations were not always repeated, particularly not if pharmacotherapy was started and the earlier registrations were performed in certified centers (according to European Sleep Research Society
Typical and atypical cataplexy

The definitions of typical and atypical attacks are shown in Table 1. "Typical" cataplexy was defined as the presence of all phenomena in the current ICSD3 definition as noted in the left column of Table 1 and in addition the absence of atypical characteristics [23] as noted in the right column of Table 1. If only one of these atypical characteristics was present, cataplexy was defined as “atypical”, as defined by Lammers et al. [23] mainly based on expert opinion and the results from Overeem et al. [19]. If more than two atypical characteristics we defined the attacks as non-cataplexic.

Ethics statement

The study was conducted per the Helsinki Declaration as revised in 2013. Due to the retrospective design of this study, a waiver of the requirement for informed consent was obtained from the Medical Ethical Committee of Leiden-Den Haag-Delft (registration number G20.139).

Data availability statement

The datasets generated during and/or analyzed during the current study are not publicly available to protect participant confidentiality but are available from the corresponding author on reasonable request.

Statistical analysis

Frequencies (in numbers or percentages) were used to describe categorical variables, and continuous data were presented using means and SDs, or medians and interquartile range (IQR) depending on the distribution of the data.

Differences in continuous variables between two groups were analyzed using t-tests when normally distributed and the Mann–Whitney U-test when non-normally distributed. Differences between more than two groups were computed using a one-way analysis of variance or the Kruskal Wallis test, depending on the distribution of the data. To analyze differences between categorical variables, a chi-square test was used: if one or more cells had an expected frequency < 5, Fisher’s exact test was used instead.

To establish the optimal CSF hypocretin-1 threshold for diagnosis of NT1, we performed multiple receiver operating characteristic (ROC) analyses, using various outcome parameters for NT1. The following four sets of parameters were used: (1) positive MSLT and PSG findings according to the ICSD3 criteria for narcolepsy, (2) typical cataplexy, (3) any cataplexy (typical or atypical), (4) typical cataplexy and/or positive PSG and MSLT findings, and (5) typical cataplexy and positive PSG and MSLT findings. To determine the optimal cutoff for hypocretin-1, we chose the point of the ROC curve as defined by the Index of Union method, where (1) the sensitivity and specificity are simultaneously close to the area under the curve (AUC) value and (2) the difference between sensitivity and specificity is minimal [24].

The data were analyzed using SPSS version 25.0. Pairwise deletion was applied in case of missing data, as automatically performed by SPSS. Graphs were made using GraphPad Prism 8.4.2. A statistical significance level of $\alpha = 0.05$ (two-tailed) was used.

Results

Patients

CSF hypocretin-1 levels and electronic health records were available for 355 individuals. Cataplexy characteristics were available for 271 of them. As shown in Figure 1, 342 out of 355 individuals
(96.3%) were categorized with the clinical diagnosis NT1, NT2, familial narcolepsy, idiopathic hypersomnia, or “other.” The remaining 13 individuals had no final clinical diagnosis. Two thirds \( (n = 235) \) were clinically diagnosed with NT1, 4.4\% \( (n = 15) \) with NT2, 1.5\% \( (n = 5) \) with familial narcolepsy, 6.1\% \( (n = 21) \) with idiopathic hypersomnia, and 19.3\% \( (n = 66) \) received a different (or no) sleep diagnosis. Low \( \leq 110 \text{ pg/mL} \) hypocretin-1 level was present in 58.9\% of people, intermediate level \( 111–200 \text{ pg/mL} \) in 5.3\%, and normal hypocretin-1 level \( > 200 \text{ pg/mL} \) in 35.8\%. The medians and IQRs of the CSF hypocretin-1 levels of the whole group and the diagnostic groups (based on clinical diagnoses) are displayed in Figure 1.

In the group with information on the presence of typical cataplexy a categorization according to the ICSD3 criteria was also made. Out of 271, 175 were classified with NT1 and 5 as with NT2 according to official criteria. Respectively 19 and 20 cases could not be classified due to missing data.

As shown in Table 2, 53\% were male and the median age was 28 years.

### Hypocretin-1 level groups

An overview of the diagnostic outcomes of patients with low, intermediate, and normal hypocretin-1 levels is shown in Table 2.

In the group with an intermediate hypocretin-1 level, there was a significantly higher percentage of HLA positivity for DQB1*0602 compared to the normal hypocretin-1 group \( (94.7 \text{ vs } 36.8\%, \ p = .001) \). The MLST results of the intermediate hypocretin-1 group showed that their sleep latency was longer than the low hypocretin-1 group \( (6.2 \text{ vs } 3.3 \text{ min}; \ p < .005) \), and the frequency of SOREMPs was higher as compared to the normal hypocretin-1 group. The PSG results showed that nighttime SOREMPs occurred significantly more often in the intermediate group as compared to the normal hypocretin-1 group \( (46.7 \text{ vs } 3.7\%, \ p < .001) \).

In the group with intermediate hypocretin-1 levels, there were significantly more NT1 diagnoses according to the ICSD3 criteria than in those with normal hypocretin-1 levels \( (41.7 \text{ vs } 0\%, \ p < .001) \), while the percentage was not significantly different from those with low hypocretin-1 levels \( (64.8\%) \). This means that only 83 out of 128 individuals with low hypocretin-1 levels and available MSTL and PSG findings received the diagnosis of NT1 based solely on their MSTL and PSG results and cataplexy presence. Non-NT1 diagnoses based on ICSD3 criteria were adjusted to NT1 as a result of an established CSF hypocretin-1 deficiency in 5 out of 10 individuals thought to have NT2, and 45 out of 121 (35.2\%) individuals not meeting the ICSD3 criteria for NT1, 36 (80\%) of whom did have typical cataplexy.

In addition to the analyses using diagnoses according to the ICSD-3 criteria that can be seen in Table 2, an analysis of the clinical diagnoses of individuals with intermediate hypocretin-1 levels was performed. More clinical diagnoses than ICSD3 diagnoses were available as some of the MSTL and PSG results, needed to diagnose according to the ICSD3 criteria, were missing. Of the 19 patients with intermediate hypocretin-1 levels, 13 patients received the clinical (ie, not necessarily ICSD3 compliant) diagnosis NT1 (of whom one had a secondary

![Figure 1.](https://example.com/figure1.png) Cerebrospinal fluid hypocretin-1 levels of the total group and the clinical diagnostic groups, \( N = 355 \). The bars represent median and interquartile ranges. The dashed lines represent the lower reliable hypocretin-1 detection limit \( (75 \text{ pg/mL}) \), and the “intermediate” hypocretin-1 range \( (111–200 \text{ pg/mL}) \). NT1, narcolepsy type 1; NT2, narcolepsy type 2; IH, idiopathic hypersomnia.
The presence and type of cataplexy per hypocretin-1 level group is shown in Table 4. Most (88.1%) of the people with typical cataplexy had low hypocretin-1 levels compared to 56.3% of those with atypical cataplexy and 11.5% of those without cataplexy (p < .01). Figure 2A also shows the distribution of hypocretin-1 levels per cataplexy type, clearly showing a relationship between typical cataplexy and lower hypocretin-1 levels. The distributions of hypocretin-1 levels per cataplexy type of only HLA DQB1*0602 negative patients are displayed in Figure 2B. None of the HLA-negative individuals without cataplexy (n = 21) had a hypocretin-1 level below 200 pg/mL.

The presence of HLA DQB1*0602 positivity was significantly higher in those with typical cataplexy (94%) as compared to those without cataplexy (61%) and those with atypical cataplexy (73%; p < .05). Those with typical cataplexy had a significantly shorter sleep latency and higher number of SOREMPs during the MSLT than those without cataplexy (p < .001). The prevalence of nighttime SOREMPs during the PSG was significantly higher in those with typical cataplexy (47%) than in those without cataplexy (9.6%, p < .001). Other PSG parameters did not differ between groups.

The percentage of people with an NT1 diagnosis according to the ICSD3 criteria differed significantly between all groups, with 93.9% of people with typical cataplexy meeting the criteria for narcolepsy as it was likely caused by angiitis, 4 had the clinical diagnosis NT2, and 2 were not diagnosed with a disorder of hypersomnolence (EDS e.g., i.e., EDS complaints without explanatory diagnosis).

The five adults with familial narcolepsy (four females, one male) all had normal hypocretin-1 levels. As familial narcolepsy has an atypical presentation, these individuals did not meet all formal PSG and MSLT criteria for narcolepsy type 1 or 2.

**Cataplexy presence**

The presence and type of cataplexy per hypocretin-1 level group is shown in Table 3. Of the five people with familial narcolepsy, four had cataplexy (two typical and two atypical). The prevalence of cataplexy (typical and atypical) differed significantly between all groups, with the highest prevalence in those with low, and lowest prevalence in those with normal hypocretin-1 levels (p < .001). The prevalence of typical cataplexy was significantly higher in those with low hypocretin-1 levels (88.6%) as well as those with intermediate hypocretin-1 levels (75%), compared with those with normal hypocretin-1 levels (9.1%, p < .001). When excluding familial narcolepsy cases the percentage of patients with normal hypocretin-1 levels and typical cataplexy dropped to 7.0%.

### Table 2. Demographic characteristics, HLA, MSLT, PSG, and diagnostic characteristics of the total group and of the groups with low, intermediate, and normal CSF hypocretin-1 levels (N = 355)

|                          | Total (N = 355) | Low: ≤ 110 pg/mL (n = 209) | Intermediate: 111–200 pg/mL (n = 19) | Normal: > 200 pg/mL (n = 127) | Test statistic | P     |
|--------------------------|----------------|----------------------------|--------------------------------------|-------------------------------|----------------|-------|
| Age at lumbar puncture, n | 28.0 (20–40), 312 | 27.3 (18–39), 184          | 34.0 (23–44), 18                     | 29.7 (21–41), 110              | H = 5.538       | .063  |
| Gender, count/n (% male) | 188/355 (53.0) | 114/209 (54.5)             | 13/19 (68.4)                         | 61/127 (48.0)                 | χ² = 3.272     | .204  |
| HLA positive, count/n (%)| 225/285 (78.9) | 175/179 (97.8)             | 18/19 (94.7)                         | 32/87 (36.8)                  | FET             | <.001 |
| MSLT results             | n = 302         | n = 180                    | n = 15                               | n = 107                       |                |       |
| Sleep latency in minutes, n | 5.0 (2.5–8.6), 280 | 3.3 (1.9–5.4), 161          | 6.2 (4.1–10.3), 15                   | 8.5 (5.1–13.9), 104            | H = 83.513     | <.001 |
| Number of SOREMPs, n     | 2 (0–3), 300    | 3 (1–4), 180               | 2 (0–3), 14                          | 0 (0–0), 106                   | H = 98.485     | <.001 |
| ≥2 SOREMPs, count/n (%)  | 185/302 (52.3) | 134/180 (74.4)             | 9/15 (60.0)                          | 15/107 (14.0)                 | χ² = 98.596    | <.001 |
| PSG results              | n = 302         | n = 179                    | n = 15                               | n = 108                       |                |       |
| TIB in minutes (mean ± SD), n | 504 ± 95, 274  | 505 ± 100, 155             | 501 ± 72, 15                         | 505 ± 90, 104                 | F = .009        | .991  |
| TST in minutes (mean ± SD), n | 437 ± 86, 280  | 437 ± 92, 159             | 441 ± 89, 15                         | 435 ± 78, 106                 | F = .028        | .973  |
| SE %, n                  | 90.1 (84–94), 284 | 88.9 (84–94), 162          | 88.7 (84–95), 15                     | 91.0 (83–95), 107             | H = .385       | .825  |
| SOREMP present, count/n (%) | 91/302 (30.1) | 80/179 (44.7)              | 7/15 (46.7)                          | 4/108 (3.7)                   | χ² = 98.596    | <.001 |
| Diagnosis                | n = 252         | n = 170                    | n = 12                               | n = 70                        |                |       |
| Narcolepsy diagnoses (ICSD3) per group, count/n (%) | 180/251 (71.7) | 170/170 (100.0)            | 6/12 (50.0)                         | 4/69 (5.8)                    | FET             | <.001 |

Of whom the diagnosis is based on

- NT1 (ICSD3 criteria excl. hypocretin-1 measurement), count/n (%) 88/128 (42.1) 83/128 (64.8) 5/12 (41.7) 0/69 (0.0) χ² = 77.332 <.001
- NT2 (ICSD-3 criteria excl. hypocretin-1 measurement), count/n (%) 175/252 (69.4) 170/170 (100.0) 5/12 (41.7) 0/70 (0.0) FET <.001
- NT2 (ICSD-3 criteria excl. hypocretin-1 measurement), count/n (%) 10/209 (4.8) 5/128 (3.9) 1/12 (8.3) 4/69 (5.8) FET 0.450
- NT2 (ICSD-3 criteria excl. hypocretin-1 measurement), count/n (%) 5/251 (2.0) 0/170 (0.0) 1/12 (8.3) 4/69 (5.8) FET .003

Median (IQR) is used unless specified otherwise.

FET, Fisher’s exact test; MSLT, Multiple Sleep Latency Test; SOREMP, sleep-onset rapid eye movement period; PSG, polysomnography; TIB, time in bed; TST, total sleep time; SE, sleep efficiency; NT1, narcolepsy type 1; NT2, narcolepsy type 2; IQR, interquartile range; ICSD3, International Classification of Sleep Disorders third edition, patients meet the following criteria: sleepiness > 3 months, ≥2 SOREMPs, sleep latency ≤ 8 minutes and/or cerebrospinal fluid hypocretin-1 ≤ 110 pg/mL and/or cataplexy (in the case of NT1).

*Significant differences between the normal hypocretin-1 group and the low and intermediate groups.

†The difference is significant between the low hypocretin-1 group and intermediate and normal groups.

‡The difference is significant between all groups.

§The difference is significant between the low and normal groups.
Diagnosis of typical cataplexy

The diagnostic value of typical cataplexy versus atypical and no cataplexy to determine a CSF hypocretin-1 level ≤ 110 pg/mL has a sensitivity of 88.6% with a specificity of 80.8% (n = 271). The presence of typical cataplexy results in a positive predictive value (PPV) of 88.1% and a negative predictive value (NPV) of 81.6% when predicting the presence of a low hypocretin-1 level (≤ 110 pg/mL).

In contrast, when positivity for the ICSD3 PSG and MSLT criteria is used (in the case of NT1), the sensitivity increases (94.0%) while the specificity decreases (74.0%) when using cataplexy in general (typical and atypical) to test for hypocretin-1 deficiency (PPV is 85.3% and NPV is 88.5%).

Significant differences between the normal hypocretin-1 group and low and intermediate groups exist.

The presence of typical cataplexy results in a positive predictive value (PPV) of 88.1% and a negative predictive value (NPV) of 81.6% when predicting the presence of a low hypocretin-1 level (≤ 110 pg/mL).

In contrast, when positivity for the ICSD3 PSG and MSLT criteria is used to predict hypocretin-1 deficiency, the sensitivity and specificity are 67.9% and 86.6% (n = 213), and the PPV is 89.0% and NPV is 62.8%.

## Table 3. Comparison of characteristics in people with no cataplexy, cataplexy, and atypical cataplexy (n = 271)

|                          | No cataplexy | Typical cataplexy | Atypical cataplexy | Test statistic | P   |
|--------------------------|--------------|------------------|--------------------|----------------|-----|
| Age at lumbar puncture, n | 29.0 (21–42), 75 | 27.8 (18–40), 142 | 26.6 (15–49), 14 | H = .995 | .608 |
| Gender, count/n (% male) | 46/87 (52.9)  | 88/168 (52.4)    | 8/16 (0.0)         |                |     |
| Hypocretin-1 level:      |              |                  |                    |                |     |
| Low, count/n (%)         | 10/87 (21.1) | 148/168 (88.1)   | 9/16 (56.3)        |                |     |
| Intermediate, count/n (%)| 4/87 (4.6)   | 12/168 (7.1)     | 0/16 (0.0)         |                |     |
| Normal, count/n (%)      | 7/87 (83.7)  | 8/168 (4.8)      | 7/16 (43.8)        |                |     |
| HLA+, count/n (%)        | 33/54 (61.1) | 134/142 (94.4)   | 11/15 (73.3)       |                |     |
| MSLT (n = 222)           | n = 71       | n = 138          | n = 13             |                |     |
| Sleep latency in minutes, n | 8.2 (4.9–13.2), 69 | 4.0 (2.0–6.4), 121 | 5.9 (4.0–8.7), 13 | H = 38.763 | <.001‡ |
| ≥2 SOREMPs, count/n (%)  | 15/71 (21.1) | 96/138 (69.6)    | 6/13 (46.2)        |                |     |
| PSG (n = 224)            | n = 73       | n = 137          | n = 14             |                |     |
| TIB in minutes (mean ± SD), n | 503 ± 109, 71 | 490 ± 94, 117    | 509 ± 78, 10       | F = .474 | .623 |
| TST in minutes (mean ± SD), n | 426 ± 82, 70 | 423 ± 92, 120    | 474 ± 111, 13      | F = 1.856 | .159 |
| SE, %, n                 | 87.0 (82–94), 73 | 89.7 (83–94), 124 | 89.9 (81–95), 11   | H = .475 | .789 |
| SOREMP, count/n (% present) | 7/73 (9.6)  | 65/137 (47.4)    | 4/14 (28.6)        |                |     |
| Diagnosis (n = 249)      | n = 72       | n = 163          | n = 14             |                |     |
| NT1 (ICSD3 criteria excl. hypocretin-1 measurement), count/n (%) | 0/68 (0.0) | 82/1278 (64.1) | 6/13 (46.2) | χ² = 74.858 | <.001‡ |
| NT1 (ICSD3), count (%)   | 10/72 (13.9) | 153/163 (93.9)   | 9/14 (64.3)        |                |     |
| NT2 (ICSD3 criteria excl. hypocretin-1 measurement), count/n (%) | 10/68 (14.7) | 0/128 (0.0) | 0/13 (0.0) | χ² = 149.698 | <.001‡ |
| NT2 (ICSD3), count (%)   | 5/71 (7.0)  | 0/163 (0.0)      | 0/14 (0.0)         |                |     |

### Table 4. Comparison of characteristics in people with no cataplexy, cataplexy, and atypical cataplexy (n = 271)

|                          | No cataplexy | Typical cataplexy | Atypical cataplexy | Test statistic | P   |
|--------------------------|--------------|------------------|--------------------|----------------|-----|
| Total group (n = 271)    |              |                  |                    |                |     |
| Low: ≤110 pg/mL (n = 167)| 184/271 (67.9)| 157/167 (94.0)   | 12/16 (75.0)       |                |     |
| Intermediate: 111–200 pg/mL (n = 16) | 271 | 15/88 (17.0) | 8/88 (9.1) | χ² = 157.020 | <.001† |
| Normal: >200 pg/mL (n = 88) | 157/167 (94.0) | 8/88 (9.1) | 2/88 (2.5) | FET | .634 |

**Median (IQR) is used unless specified otherwise.**

FET, Fisher’s exact test; MSLT, Multiple Sleep Latency Test; SOREMP, sleep-onset rapid eye movement period; PSG, polysomnography; TIB, time in bed; TST, total sleep time; SE, sleep efficiency; NT1, narcolepsy type 1; NT2, narcolepsy type 2; IQR, interquartile range; ICSD3, International Classification of Sleep Disorders third edition, patients meet the following criteria: sleepiness > 3 months, ≥2 SOREMPs, sleep latency ≤ 8 min and/or cerebrospinal fluid hypocretin-1 ≤ 110 pg/mL and/or cataplexy (in the case of NT1).

†Significant between the typical cataplexy and the no and atypical cataplexy groups.

‡Significant between the no cataplexy and the typical and atypical cataplexy groups.

§Significant between the normal hypocretin-1 group and low and intermediate groups exist.
Characteristics of atypical cataplexy

The criterion of atypical cataplexy most often seen was the lack of an identifiable trigger or only negative emotions being the trigger for a cataplexy attack. Seven out of the 16 individuals suspected of having atypical cataplexy displayed this criterion, with 5 of those having no identifiable trigger and 2 having only negative emotions as a trigger.

Atypical features did not significantly differ between those without eventual diagnosis of narcolepsy (n = 4) and those with NT1 or familial narcolepsy (n = 12, p = .608), nor did they significantly differ between hypocretin-1 range groups (p = .927).

Establishing the optimal hypocretin-1 level threshold

A CSF hypocretin-1 threshold of 55.0 pg/mL was optimal to determine whether patients had positive PSG and MLST findings (1), with a sensitivity of 78.0% and a specificity of 77.9%. The AUC was .837 (Figure 3A, p < .001). When using the presence of typical cataplexy versus atypical or no cataplexy as diagnostic for narcolepsy (2), a threshold of 101.5 pg/mL was found with a specificity of 83.5% and a sensitivity of 85.1%, with an AUC of .894 (Figure 3B, p < .001). A hypocretin-1 threshold of 119.5 pg/mL was optimal to determine cataplexy (typical and atypical) presence (3), with a sensitivity of 87.5% and specificity of 87.4% and an AUC of .915 (Figure 3C, n = 271, p < .001). Lastly, the outcome parameter perhaps closest to the clinical practice of diagnosing narcolepsy—typical cataplexy and/or positive PSG and MLST findings (4)—resulted in an optimal cutoff value of 149.5 pg/mL, with a sensitivity of 93.0% and a specificity of 92.3%. In this case, the AUC was .952 (Figure 3D, n = 250, p < .001). Notably, when applying the outcome parameter typical cataplexy and positive PSG and MSLT findings (5) the found threshold was far lower, namely 40.5 pg/mL with both a sensitivity and specificity of 78.0% and an AUC of .846.

Discussion

We examined the significance of intermediate CSF hypocretin-1 values and presence of typical cataplexy, using historic data of people with EDS from highly specialized sleep-wake centers. Our results show that: (1) only 5.3% had hypocretin-1 levels in the intermediate range; (2) in general, individuals with intermediate hypocretin-1 levels had more features (both of cataplexy presence and auxiliary findings) associated with NT1 than those with normal hypocretin-1 levels (> 200 pg/mL); (3) When categorizing groups based on cataplexy type, we found that those with typical cataplexy had more positive diagnostic findings for NT1 than those with atypical or no cataplexy. Compared to cataplexy in general (atypical and typical cataplexy), typical cataplexy has a higher specificity, but a lower sensitivity for NT1 (as determined by hypocretin-1 ≤ 110 pg/mL). (4) We found that a higher cutoff value for hypocretin-1 is needed to increase diagnostic accuracy for NT1. These results suggest a re-appraisal of both the diagnostic value of typical cataplexy and of the current hypocretin-1 threshold values in narcolepsy.

The diagnostic value of typical cataplexy

People with typical cataplexy more often fulfill the diagnostic criteria for NT1 and more often have low hypocretin-1 levels than those with atypical or no cataplexy. When using typical cataplexy as a predictor for hypocretin-1 deficiency, a sensitivity of 88% and a specificity of 81% were found. In short, typical cataplexy is a good predictor of NT1 and should therefore have more weight when diagnosing NT1.

While the term “typical cataplexy” (as opposed to cataplexy in general) has been used in scientific research, including in the article from Mignot et al. [8] on which the current hypocretin-1 cutoff is based, it generally has not been defined clearly. We clearly define both typical and atypical cataplexy and at the same time show the usefulness of typical cataplexy to diagnose NT1.
Individuals with intermediate CSF hypocretin-1 levels

There were only 19 people (5.3%) with an intermediate CSF hypocretin-1 level. Of these, 17 were diagnosed with either NT1 or NT2 based on clinical characteristics (i.e., they not necessarily met all ICSD3 criteria for NT1 or NT2, only 40% did). These individuals had a median MSLT sleep latency ≤ 8 min and significantly more SOREMPs (during PSG and MSLT) than those with normal hypocretin-1 levels. This suggests that the current diagnostic criteria, the MSLT and PSG findings and the hypocretin-1 cutoff of 110 pg/mL, are insufficient accurately to diagnose individuals who have hypocretin-1 levels in the intermediate range. A higher CSF hypocretin-1 cutoff may mitigate this problem. Visual inspection of the range of hypocretin-1 levels in our sample (see Figure 1) would lend support to this option. Two distinct clusters can be distinguished, with a lower cluster that ranges well beyond the current cutoff of 110 pg/mL. Other studies have also suggested a higher cutoff. Andlauer et al. [9] found an optimal cutoff CSF hypocretin-1 level for narcolepsy without cataplexy of 200 pg/mL rather than 110 pg/mL, with a high specificity of 99% but a low sensitivity of 33%. A similar conclusion, that a higher cutoff value may be feasible to determine hypocretin-1 deficiency, was drawn by Heier et al. [25].

Diagnosing patients with intermediate hypocretin-1 levels is further complicated by variability in the determination of hypocretin-1 concentrations using Phoenix Pharmaceuticals RIA kits. Inter-assay variability is quite high when not corrected using a reference sample. This could cause a patient to fall within a different hypocretin-1 range category (i.e., intermediate...
fluenced by inclusion bias given that the a priori probability of a lumbar puncture. Thus, it is possible that our analysis is in-
narcolepsy diagnosis and would normally not have undergone as part of scientific research. These individuals already had a
in some people, the CSF hypocretin-1 levels were determined to complaints of EDS and suspected narcolepsy. As such, the re-
data from people who visited a sleep-wake clinic because of
predicts this combination.

The optimal CSF hypocretin-1 cutoff
The results of our own ROC analyses varied depending on the chosen outcome parameter used as substitute or "gold standard" for the NT1 diagnosis. At the moment, the closest to a gold standard is a CSF hypocretin-1 level ≤ 110 pg/mL. As this outcome measure cannot be used to determine the optimal CSF hypocretin-1 threshold value, other outcome parameters were chosen based on the ICDSD3 criteria.

We found that using positive PSG and MSLT findings results in a cutoff value of 55 pg/mL (sensitivity and specificity 78%), far lower than the currently used cutoff value. This, however, does not mean that a 55 pg/mL threshold is suitable to diagnose narcolepsy. In our study 29% of individuals with a CSF hypocretin-1 level ≤ 110 pg/mL did not meet the PSG and MSLT criteria for narcolepsy. It seems that PSG and MSLT findings tend to be especially positive in people with a more severely decreased CSF hypocretin-1 level, resulting in a lower cutoff value, while we are also interested in those with intermediate levels. Thus, positive PSG and MSLT findings alone may not be the best measure of narcolepsy.

When we use cataplexy (typical or both typical and atypical) as outcome measure, the optimal cutoff value (101 pg/mL) comes closer to the one currently in use. However, just as is the case with using positive PSG and MSLT findings as outcome measure, typical cataplexy is not the gold standard for NT1. In spite of cataplexy being highly pathognomonic for NT1, not all people with NT1 have cataplexy (6% of people in our study with CSF hypocretin-1 ≤ 110 pg/mL had no cataplexy). It is not uncommon for people to develop cataplexy a couple of years after their diagnosis [18]. We had follow-up data of 10 out of 14 patients who had a low hypocretin-1 level despite the absence of cataplexy. Of these, six developed typical cataplexy at a later stage, emphasizing the strong correlation of typical cataplexy and hypocretin-1 deficiency. In addition, 12% of patients with typical cataplexy had a CSF hypocretin-1 level > 110 pg/mL. It should however be mentioned that the AUC is considerably higher when typical cataplexy or cataplexy, in general, is used compared to when PSG and MSLT findings are used as outcome measures (see Figure 3). This would suggest that measured hypocretin-1 level is a better predictor of the presence of (typical) cataplexy than of positive PSG and MSLT findings.

The diagnosis of NT1 is complicated and a perfect gold standard does not exist. Given that no single option is a perfect substitute that can be used to predict hypocretin-1 deficiency, we consider the combination of typical cataplexy and/or positive PSG and MSLT findings the best approximation of an NT1 diagnosis to be used for this purpose. We found that, with an AUC of 0.952, a hypocretin-1 cutoff value of < 150 pg/mL best predicts this combination.

Some limitations should be mentioned. First of all, we used data from people who visited a sleep-wake clinic because of complaints of EDS and suspected narcolepsy. As such, the results are not generalizable to the general population. Moreover, in some people, the CSF hypocretin-1 levels were determined as part of scientific research. These individuals already had a narcolepsy diagnosis and would normally not have undergone a lumbar puncture. Thus, it is possible that our analysis is influenced by inclusion bias given that the a priori probability of a narcolepsy diagnosis was higher than would be the case in clinical practice. Secondly, because the number of individuals with NT1 is far higher than those with NT2 in our population, this may impact the hypocretin-1 cutoff we found. However, this reflects the prevalence of these disorders in Europe. Thus, we believe the result to be relevant in clinical practice. Lastly, as this is a retrospective study, we were not able to use the newly defined hypocretin-1 cutoff value and use of typical cataplexy in a test sample to determine actual positive and NPVs of NT1. Future studies with a new sample should evaluate this prospectively.

Conclusion
We come to several conclusions that could improve the diagnostic process of NT1, especially where it concerns individ-
als with narcolepsy symptoms and intermediate hypocretin-1 levels. Firstly, given that all non-cataplectic HLA-negative pa-
tients in our sample had CSF hypocretin-1 levels above 200 pg/
ML, we conclude that determining hypocretin-1 levels in pa-
tients with these characteristics is largely redundant.

Secondly, the current diagnostic process is mainly focused on the hypocretin-1 CSF concentration combined with PSG and MSLT results. The presence of typical cataplexy has a higher diagnostic than PSG and MSLT findings. Correct identification of typical cataplexy thus improves diagnostic accuracy. Lastly, modification of the currently used hypocretin-1 cutoff should be considered. We suggest a new cutoff of < 150 pg/mL. In con-
clusion, adding typical cataplexy to the diagnostic criteria, preventing unnecessary lumbar punctures, and altering the hypocretin-1 cutoff value would enhance the diagnostic ac-
curacy and patient care in narcolepsy.

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