SLEEPJ, 2021, 1–8

doi: 10.1093/sleep/zsaa203
Advance Access Publication Date: 9 October 2020
Original Article

ORIGINAL ARTICLE

Combining information on nocturnal rapid eye movement sleep latency and atonia to facilitate diagnosis of pediatric narcolepsy type 1

Alessandro Silvani[1,*,†], Stefano Vandi[1,2,†], Fabio Pizza[1,2], Elena Antelmi[3], Raffaele Ferri[4] and Giuseppe Plazzi[1,2]

[1]Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy, [2]IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy, [3]Neurology Unit, Movement Disorders Division, Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy and [4]Sleep Research Centre, Department of Neurology I.C., Oasi Research Institute-IRCCS, Troina, Italy

[†]Alessandro Silvani and Stefano Vandi contributed equally to this work.

*Corresponding author. Alessandro Silvani, Department of Biomedical and Neuromotor Sciences, University of Bologna, Piazza di Porta San Donato 2, 40126 Bologna, Italy. Email: alessandro.silvani3@unibo.it.

Abstract

Study Objectives: The diagnosis of narcolepsy type 1 (NT1) at its onset in children and adolescents is often difficult, with substantial diagnostic delay. We aimed to test and validate the effectiveness of rapid eye movement (REM) sleep latency (REML), the REM sleep atonia index (RAI), and their combination for the automatic identification of pediatric patients with NT1 based on the standard scoring of nocturnal polysomnograms.

Methods: A retrospective cohort of 71 pediatric patients with NT1 and 42 controls was subdivided in test and validation cohorts. A novel index (COM) was developed as a nonlinear function of REML and RAI. The effectiveness of REML, RAI, and COM in identifying patients with NT1 was assessed with receiver operating characteristic (ROC) curves.

Results: REML, RAI, and COM significantly identified patients with NT1 both in the test and validation cohorts. Optimal thresholds that maximized identification accuracy were estimated in the test cohort (REML, 49.5 min; RAI, 0.91; COM, 4.57 AU) and validated in the other cohort. COM performed significantly better in identifying patients with NT1 than either REML or RAI, with ROC area under the curve of 94%–100%, sensitivity 85%–96%, and specificity 92%–100%, and with good night-to-night agreement (Cohen's $k = 0.69$).

Conclusions: The analysis of REML, RAI, and particularly their combination in the COM index may help shorten diagnostic delay of NT1 in children and adolescents based on the standard scoring of nocturnal polysomnography.

Statement of Significance

Narcolepsy type 1 (NT1) is a rare neurological disorder often starting in childhood or adolescence. The diagnosis of pediatric NT1 is difficult, with substantial diagnostic delay. We validated a novel index (COM) for identification of pediatric patients with NT1 based on short nocturnal rapid eye movement (REM) sleep latency and lack of muscle atonia during REM sleep. The COM index identified patients with NT1 with approximately 90% sensitivity and specificity and with good night-to-night agreement. Computation of the COM index can be based on a single night's polysomnography that is currently performed in several pediatric sleep centers and does not require additional specialized expertise or time-consuming procedures. The COM index may help shorten diagnostic delay of NT1 in the pediatric population.

Key words: narcolepsy; pediatrics; sleep; electromyography; ROC curve; diagnosis

Submitted: 29 April, 2020; **Revised:** 30 August, 2020

Introduction

Narcolepsy type 1 (NT1) results from the functional loss of the hypothalamic neurons that release the orexin (hypocretin) neuropeptides [1], possibly due to an autoimmune reaction [2]. This loss entails a severe life-long disease characterized by excessive daytime sleepiness, cataplexy, hallucinations, and sleep paralysis, as well as by reduced rapid eye movement (REM) sleep latency (REML) and by the occurrence of REM sleep without atonia [3]. The prevalence of NT1 is estimated to be 14/100,000 in the United States [4], which indicates a rare disease. The age at onset of NT1 is bimodal, with one peak in adolescence [5]. The incidence of pediatric NT1 has increased markedly after 2010 at least in Taiwan, Sweden, and the United States [6–7].

Due to the rarity of NT1, expertise for NT1 diagnosis is often limited to specialized referral centers. The diagnosis of pediatric NT1 is particularly challenging because of its specific features, which include marked hypersomnolence and a complex and peculiar cataplexy pattern [8–9]. Lumbar puncture for the measurement of cerebrospinal fluid orexin levels, which is the disease marker of NT1, is not widely carried out in children because it may cause anxiety, pain, and anesthesiologic risks, although these may be limited with appropriate procedures [10]. As a result, the delay of NT1 diagnosis, which is dramatically long in adults (mean 8.9 years) [11], is even more common for children and adolescents [12].

One strategy to decrease NT1 diagnostic delay is to assist diagnosis with quantitative indexes that can be widely available without the need of invasive interventions. Pediatric patients with NT1 can be identified with high sensitivity and specificity based on sleep latency or on the occurrence of sleep-onset REM sleep periods (SOREMPs) during a multiple sleep latency test (MSLT) [13]. However, MSLT is a demanding and time-consuming test and must be preceded by a nocturnal polysomnography [14–15]. Unfortunately, a nocturnal SOREMP is highly specific but not sensitive for pediatric NT1 identification [16]. Nocturnal REML correlates strongly with the number of SOREMPs at MSLT in pediatric patients with NT1 [16], but its effectiveness in identifying these patients is still not known. Nocturnal REML identifies adult patients with NT1 with almost perfect specificity but low to moderate sensitivity [17]. Reduced REML also characterizes orexin knock-out mice with congenital deficiency of orexin peptides [18–19], demonstrating its close link with the lack of orexin transmission in NT1.

The occurrence of REM sleep without atonia is common in adult [20–21] and pediatric [22–23] patients with NT1, and occurs in orexin knock-out mice so robustly as to allow their discrimination from wild-type controls [24]. The lack of submentalis muscle atonia during nocturnal REM sleep, determined with a careful manual analysis of the tracings, is sensitive but not specific for the identification of pediatric patients with NT1 [23].

The aim of this study was to test the effectiveness of combined information on nocturnal REML and REM sleep atonia for the identification of pediatric patients with NT1. We hypothesized that pediatric patients with NT1 could be significantly identified based on relatively low values of nocturnal REML, similar to what has been reported for adult patients with NT1 [17]. Furthermore, we hypothesized that pediatric patients with NT1 could be significantly identified based on the occurrence and severity of nocturnal REM sleep without atonia, as already reported by others [23], even without the need for time-consuming manual analysis of the tracings. To this aim, we assessed REM sleep atonia based on an automatic analysis of the submentalis muscle electromyogram (EMG) with the REM sleep atonia index (RAI) developed by Ferri and coworkers [25]. This index is effective in the identification of adult patients with NT1 [20] or with REM sleep behavior disorder [24, 26]. Finally, we hypothesized that combination of information on low nocturnal REML and the occurrence and severity of nocturnal REM sleep without atonia would afford a greater effectiveness in identifying patients with NT1 than either of the two indexes taken in isolation. To combine information on REML and RAI, we developed a novel index (COM) based on their nonlinear transformation. Our study consisted of three phases: test, validation, and comparison of the effectiveness of REML, RAI, and COM in identifying pediatric patients with NT1.

Methods

Subjects

The study was based on two independent retrospective cohorts (a test cohort and a validation cohort) of children and adolescents with NT1 and of control subjects. Recruitment involved all subjects of pediatric age who had been consecutively referred to the Center for Narcolepsy of the Department of Biomedical and Neuromotor Sciences at the University of Bologna, Italy, for a clinical suspicion of hypersomnia and had undergone two consecutive 24-h video-polysomnographic recordings (the first for adaptation and the second for diagnostic purposes) in the sleep laboratory.

The inclusion criterion for patients with NT1 was a clinical and laboratory diagnosis of NT1 according to *The International Classification of Sleep Disorders 3rd ed.*: (1) unequivocal cataplexy documented during in-laboratory testing [27]; (2) persistent daytime sleepiness; (3) at least two SOREMPs and mean sleep latency <8 min during MSLT; and (4) when available, evidence of cerebrospinal fluid orexin A deficiency. The presence of human leukocyte antigen (HLA) DQB1*0602 was assessed in all patients. The inclusion criterion for clinical control subjects was a clinical suspicion of hypersomnia not confirmed after diagnostic evaluation.

Exclusion criteria for all subjects were comorbidity with other neurological disorders or drug use in the 3 weeks prior to polysomnography. No subjects thus received selective serotonin reuptake inhibitors, whose use can be associated with augmented REM sleep EMG activity, in the 3 weeks prior to polysomnography. Another exclusion criterion for all subjects was the occurrence of signal artifacts on the submentalis (chin) EMG during the second recording night, including signal loss and alternating current interference. Artifact detection was performed by a single trained investigator (S.V.) blind to the results of the data analysis. The analysis focused on the second recording night to avoid first-night effects [28]. Artifact-free data on the first recording night, when available, were analyzed to estimate night-to-night variability of the results.

The test cohort was the same as that characterized in a recent publication by our group [22] and consisted of 23 patients with NT1 and 18 clinical control subjects. The validation cohort consisted of 48 patients with NT1 and 24 clinical control subjects, without overlap with the first cohort. Artifact-free data on the first (adaptation) recording night were available of 20 patients with NT1 and 17 controls of the test cohort and of 42 patients with NT1 and 20 controls of the validation cohort.

All subjects and/or their parents/tutors gave written informed consent to the study protocol, in agreement with the Convention of Helsinki. The study was approved by our local Ethical Committee (Comitato Etico Interaziendale Bologna-Imola, CE-BI, code 17009).

Study design

The study design consisted of three phases: test, validation, and comparison of the effectiveness of REML, RAI, and their combination in identifying pediatric patients with NT1. Phase 1 (test) was performed by analyzing the second recording night of the test cohort. This phase included the estimation of the optimal thresholds of each index for maximal accuracy of identification of patients with NT1. Phase 2 (validation) was performed by analyzing the second recording night of the validation cohort and included the validation of the optimal index thresholds estimated in phase 1. Phase 3 (comparison) was performed on the whole dataset under study (test and validation cohorts together) to maximize sample size, and consisted of three steps: (1) comparison of the effectiveness of REML, RAI, and their combination (index COM) in identifying pediatric patients with NT1 on the second recording night; (2) comparison of the effectiveness of the index COM in the second recording night with that of the sleep latency and SOREMP number at MSLT, the neurophysiological gold standard for identification of patients with NT1; (3) evaluation of the variability in identification of patients with NT1 based on the different indexes computed on the first versus the second recording night.

Recordings and sleep scoring

The recorded signals during overnight polysomnography included: electroencephalogram (with frontal, central, and occipital leads referred to the contralateral mastoid); electrooculogram (electrodes placed 1 cm above the right outer cantus and 1 cm below the left outer cantus and referred to the left mastoid); and EMG of the submentalis muscle (bipolar derivations with electrode pairs placed 3 cm apart and impedance ≤10 kΩ). Sleep signals were recorded with a polysomnography ambulatory device, sampled at 256 Hz, and stored on hard disk in European data format for further analysis. The recordings were carried out in a single sleep laboratory room. Subjects could sleep until spontaneous morning awakening. Caffeinated beverages were prohibited from the afternoon preceding recording. Light-out time was based on individual habitual bedtime.

Sleep stages were scored following standard criteria on 30-s epochs [29]. The sleep architecture was characterized with standard indexes, including REML. REML was assessed with a double scoring, with the first scoring always supervised by the senior technician (S.V.).

Submentalis muscle atonia during REML sleep was quantified with the RAI, which reflects the fraction of REM sleep time with muscle atonia. To compute RAI, the submentalis EMG of all 30-s epochs of REM sleep was band-pass filtered at 10–100 Hz with a notch filter at 50 Hz and rectified, and its amplitude was averaged over 1-s mini-epochs. A noise correction was applied by subtracting from the rectified and averaged EMG amplitude (raEMGa) in each 1-s mini-epoch the minimum value of raEMGa in a moving window of 60 mini-epochs surrounding that mini-epoch. The RAI was computed as the ratio between the number of mini-epochs with raEMGa ≤ 1 μV and the total number of mini-epochs, excluding those with 1 μV < raEMGa ≤ 2 μV. The RAI is bounded between 0 (complete lack of muscle atonia during REM sleep) and 1 (occurrence of atonia in all REM sleep epochs) [25].

Computation of the COM index combining REML and RAI

In order to combine the information on REML and RAI, a novel index (COM) was computed as the product of the hyperbolic arctangent of RAI (expressed in fractional units) times the natural logarithm of REML (expressed in mins), according to the formula:

$$COM = \begin{cases} \frac{1}{2} \cdot ln\left(\frac{1+RAI}{1-RAI}\right) \cdot ln(REML) \text{ if } REML > 1 \\ 0 \text{ if } REML \leq 1 \end{cases}$$

where ½ ln ([1 + RAI]/[1 − RAI]) corresponds to the hyperbolic arctangent of RAI and ln indicates the natural logarithm. The rationale for this formula was as follows. Prior evidence indicates that compared with control subjects, patients with NT1 are characterized by shorter REML and lower RAI [22]. This suggested to compute COM based on the product of REML and RAI, so that the contributions of REML and RAI to identification of patients with NT1 would be mutually reinforced. However, the simple product of REML and RAI would be overly sensitive to the variability of REML, which may range from 0 to hundreds of minutes, whereas RAI is bounded from 0 to 1 [22]. In order to overcome this difficulty, we made COM depend on the hyperbolic arctangent (Fisher's z-transformation) of RAI, a standard transformation employed for the statistical analysis of Pearson's correlation coefficients. For values of RAI > 0.5, the hyperbolic arctangent of RAI increases nonlinearly and tends to infinite when RAI tends to 1, with the effect of enhancing the variance of RAI among subjects. In addition, we made COM depend on the natural logarithm of REML, which had the opposite effect of decreasing the variance of REML among subjects. The value of COM was set to 0 if REML was ≤ 1 min, thus avoiding negative values of COM. The effectiveness of the COM index in identifying patients with NT1 was compared with that of the simple product of REML times RAI in an ancillary analysis during phase 3.

Receiver operating characteristic (ROC) curve analysis

The effectiveness of REML, RAI, COM, and of the sleep latency and SOREMP number at MSLT in identifying NT1 subjects was estimated by the area under the curve (AUC) of ROC curves. Five individual ROC curves were computed as binary classifiers, one for each index considered in this study (i.e. REML, RAI, COM—cf. the defining equation above—sleep latency at MSLT, or SOREMP number at MSLT). For example, the ROC curve for the index REML was computed as a bidimensional plot of the true positive rate (sensitivity) versus the false positive rate (1—specificity) of NT1 subject identification for different threshold values of REML [30]. Effective subject classification is indicated by ROC AUC values >50%, which corresponds to the performance of a random classifier, or half the performance (100%) of a perfect

classifier. Optimal thresholds were computed based on ROC curves as the index values that yielded the maximal accuracy of classification (number of correctly classified cases divided by the total number of cases) [30]. The values of sensitivity (ratio of true positive cases to total positive cases) and specificity (ratio of true negative cases to total negative cases) corresponding to the optimal thresholds were computed for each index.

Statistical analysis

The analysis of ROC curves was performed with the StAR web-based application (http://melolab.org/star/roc_analysis.php) [30]. The ROC AUC results are reported as mean ± SEM. Other statistical tests were performed with SPSS V.18 (SPSS Inc.). Categorical differences in sex and in the occurrence of apnea–hypopnea index (AHI) values recorded as <1 were analyzed with chi-square tests. Differences in age and in sleep architecture including REML, RAI, and COM in the second recording night were compared with Mann–Whitney U-tests and Bonferroni correction for multiple comparisons. Normality of the distributions of these variables was assessed with Kolmogorov–Smirnov tests. The occurrence of significant monotonic relationships between the values of REML, RAI, and COM during the first and second recording nights in all subjects under study was quantified with Spearman's rank-order correlation coefficients. The night-to-night agreement in NT1 subject classification was quantified with Cohen's κ coefficient, reported as mean ± SEM. Values of κ of 0 and 1 are consistent with chance and perfect agreement, respectively. All other data were reported as median (interquartile range). Significance was set at $p < 0.05$. The sample size was determined based on subject recording availability, with no statistical power analysis performed a priori.

Results

Characteristics of the study cohorts

In the test cohort, patients with NT1 and control subjects were matched for sex (8 vs 5 females, $p = 0.742$, chi-square test) and age (12.7 [4.8] vs 13.3 [5.2] years, $p = 0.198$, Mann–Whitney U-test). In the validation cohort, patients with NT1 and control subjects were also matched for age (11.5 [5.1] vs 12.3 [8.8] years, $p = 0.424$, Mann–Whitney U-test) but not for sex (25 vs 4 females, $p = 0.005$, chi-square test). Neither the patients with NT1 nor the control subjects differed in age between cohorts ($p = 0.796$ and $p = 0.140$, Mann–Whitney U-tests). In the test cohort, eight patients with NT1 and four control subjects were 10 years of age or younger. The corresponding figures in the validation cohort were 14 patients with NT1 and 10 control subjects.

The orexin concentration in the cerebrospinal fluid was measured in 64 out of 71 patients with NT1, with values of 12 (43) pg/mL. The results of the MSLT are shown in Table 1 and were available for all subject except for 1 control subject of the test cohort. As expected, both sleep latency and the number of SOREMPs at MSLT differed significantly between patients with NT1 and control subjects of both cohorts, and did not differ significantly between cohorts ($p < 0.001$ and $p \geq 0.082$, respectively, Mann–Whitney U-test).

The values of indexes of nocturnal sleep architecture, REML, RAI, and COM for the test and validation cohorts are reported in Table 2. The patients with NT1 of either cohort had shorter sleep latency, shorter REML, more stage N1 sleep, lower RAI (i.e. more REM sleep without atonia), and lower COM than control subjects of the same cohort. In the validation cohort, patients with NT1 also had significantly higher WASO than control subjects. The only significant difference between cohorts was a slightly higher nocturnal sleep latency of patients with NT1 of the validation cohort compared with those of the test cohort (all differences: $p < 0.05$ with Bonferroni correction, Mann–Whitney U-tests).

Numerical values of the AHI were recorded for all subjects of the test cohort and did not differ significantly between patients with NT1 and control subjects (0.5 [0.7] h^{-1} vs 0.3 [0.5] h^{-1}, $p = 0.281$, Mann–Whitney U-test). In the validation cohort, AHI was recorded as <1 h^{-1} in 31/48 patients with NT1 and 11/24 control subjects, without significant difference between groups ($p = 0.128$, chi-square test). Numerical values of AHI were recorded in the remaining subjects and did not differ significantly between patients with NT1 and control subjects (0.2 [0.8] h^{-1} vs 0.2 [0.3] h^{-1}, $p = 0.563$, Mann–Whitney U-test).

Table 1. Results of the MSLT

	Test cohort		Validation cohort	
	NT1	Controls	NT1	Controls
SL (min)	3 (2)*	17 (3)	3 (3)*	17 (6)
SOREMP (*n*)	5 (1)*	0 (0)	4 (1)*	0 (0)

NT1, narcolepsy type 1; SL, sleep latency during the MSLT; SOREMP, sleep-onset REM sleep episodes during the MSLT. Data are shown as median (interquartile range). In the test cohort, N = 23/17 patients with NT1/control subjects. In the validation cohort, N = 48/24 patients with NT1/control subjects.
*$p < 0.001$, Mann–Whitney U-test versus control subjects.

Table 2. Nocturnal sleep characteristics

	Test cohort		Validation cohort	
	NT1	Controls	NT1	Controls
TST (min)	485 (57)	466 (104)	491 (106)	481 (85)
SL (min)	3 (4)*	12 (17)	5 (4)*,†	11 (17)
SE (%)	93 (7)	92 (3)	92 (8)	93 (5)
WASO (min)	35 (34)	22 (15)	36 (51)*	16 (15)
N1 (%)	11 (5)*	4 (7)	9 (6)*	4 (5)
N2 (%)	38 (13)	37 (14)	38 (10)	43 (16)
N3 (%)	24 (18)	30 (20)	26 (13)	30 (22)
R (%)	25 (8)	24 (3)	23 (6)	23 (7)
REML (min)	3 (10)*	70 (75)	5 (33)*	74 (60)
RAI	0.84 (0.15)*	0.92 (0.06)	0.77 (0.19)*	0.92 (0.07)
COM (AU)	1.16 (2.27)*	7.01 (2.48)	1.27 (2.17)*	6.38 (2.03)

NT1, narcolepsy type 1; TST, total sleep time; SL, sleep latency; SE, sleep efficiency; WASO, wakefulness after sleep onset; N1, N2, N3, and R, % of TST spent in stages N1, N2, and N3 of NREM sleep and in REM sleep, respectively. Data of the second recording night are shown as median (interquartile range). In the test cohort, N = 23/18 patients with NT1/control subjects. In the validation cohort, N = 48/24 patients with NT1/control subjects.
*,†, $p < 0.05$ with Bonferroni correction (corrected $p < 0.005$), Mann–Whitney U-test versus control subjects and versus test cohort, respectively.

Phase 1: test

The values of COM for each subject under study during the second recording night are shown as a function of those of REML and RAI in Figure 1 as scatterplots, emphasizing the nonlinear relationships between COM and the other indexes.

Data of patients with NT1 tended to form clusters distinct from those of control subjects, supporting the feasibility of automatic identification.

The ROC curves for the identification of patients with NT1 based on REML, RAI, and COM during the second recording night of the test cohort are shown in panel A of Figure 2. Each index performed significantly better than a random classifier (ROC AUC > 50%, $p < 0.001$). The ROC AUCs based on REML and RAI indicated excellent performance (approximately 90% in both cases), and the ROC AUC based on COM came close (99.8%) to the performance of the perfect classifier. The optimal thresholds of REML, RAI, and COM that maximized accuracy of identification of patients with NT1 were 49.5 min, 0.91, and 4.57 AU, respectively. The corresponding values of sensitivity and specificity are reported in Table 3. REML afforded excellent specificity, with lower but still very good sensitivity. To the contrary, RAI afforded excellent sensitivity but low specificity. COM seemed to combine the strengths of REML and RAI, affording excellent sensitivity and complete specificity for the identification of patients with NT1.

Phase 2: validation

The ROC curves for the identification of patients with NT1 in the validation cohort based on REML, RAI, and COM during the second recording night are shown in panel B of Figure 2. The values of sensitivity and specificity corresponding to the optimal thresholds of phase 1 are reported in Table 3. Taken together, the phase 2 results confirmed exactly and thereby validated those of phase 1, although with a somewhat lower performance. The COM index still afforded high values of sensitivity (85.4%) and specificity (91.7%) for the identification of patients with NT1 of the validation cohort.

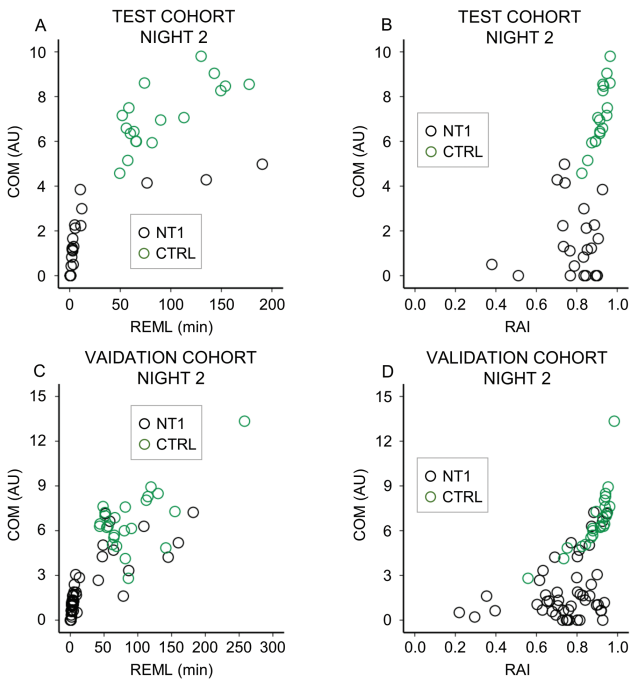

Figure 1. Relationship between the indexes REML, RAI, and COM in individual subjects. REML, REM sleep latency; RAI, REM sleep atonia index; COM, index based on the combination of REML and RAI (arbitrary units, AU). Each circle corresponds to one child or adolescent during the second recording night. NT1, narcolepsy type 1 (black); CTRL, clinical control subjects (green). See text for details on composition and size of the test and validation cohorts.

Phase 3: comparisons

On the second recording night of the test and validation cohorts, the index COM (ROC AUC 95.9 ± 1.7%) performed significantly better in identifying patients with NT1 than either REML (ROC AUC 88.9 ± 3.3%, $p = 0.015$) or RAI (ROC AUC 85.1 ± 3.8%, $p < 0.001$), whereas the performance of REML and RAI was not significantly different ($p = 0.411$). The effectiveness of COM was also significantly higher than that of the simple REML times RAI product (ROC AUC 91.1 ± 2.9%, $p = 0.038$).

As expected, sleep latency and SOREMP number at MSLT had almost perfect effectiveness in identifying patients with NT1 (ROC AUC 100.0 ± 0.0% and 100.0 ± 0.1%, both $p < 0.001$), and this effectiveness was, for both indexes, significantly higher than that of COM ($p \leq 0.016$).

The relationships between values of REML, RAI, and COM during the first and the second recording night are shown as scatterplots in Figure 3. The distributions of REML, RAI, and COM during either recording night differed significantly from normal ($p \leq 0.027$, Kolmogorov–Smirnov test). In the cohorts under study, the monotonic relationship between COM values of the two recording nights ($\rho = 0.712$) was stronger than that of REML ($\rho = 0.541$) but weaker than that of RAI ($\rho = 0.920$).

The results of ROC AUC analysis performed on the first recording night of both cohorts mirrored the results obtained on the second night. The ROC AUC based on COM (97.2 ± 1.4%) was significantly higher than that based on RAI (86.7 ± 3.8%, $p = 0.002$) and was also higher than that based on REML (93.8 ± 2.4%), although the latter difference was not statistically significant ($p = 0.066$). The night-to-night agreement of NT1 patient identification as estimated by Cohen's κ index was moderate for REML ($\kappa = 0.55 \pm 0.08$), whereas it was substantial and similar for RAI ($\kappa = 0.70 \pm 0.08$) and for COM ($\kappa = 0.69 \pm 0.07$).

Discussion

The novel findings of our study are that nocturnal REML, nocturnal RAI, and particularly their combination in the new index COM are highly effective in identifying pediatric patients with NT1 based on standard scoring and automatic chin EMG tone analysis of nocturnal polysomnography.

We found that nocturnal REML was effective (ROC AUC 87%–90%) in identifying pediatric patients with NT1, albeit significantly less than COM. We estimated an optimal REML threshold of 49.5 min, which afforded high sensitivity (82%–87%) and even higher specificity (87%–94%). To our knowledge, the effectiveness of REML in identifying pediatric patients with NT1 has not been previously tested. In a previous study, identification of adult patients with NT1 based on REML had ROC AUC values of 70%–80%, and a REML threshold of 15 min yielded almost perfect specificity (99%–100%) but low to moderate sensitivity (36%–51%) [17]. A study on pediatric patients with NT1 reported that the occurrence of a nocturnal SOREMP, which may be regarded as a REM sleep episode with REML < 15 min, identified these patients with almost complete specificity (97%) but with moderate sensitivity (55%) [16]. Thus, our estimates of the specificity of REML-based identification appear consistent with previous work, whereas our sensitivity estimates appear higher.

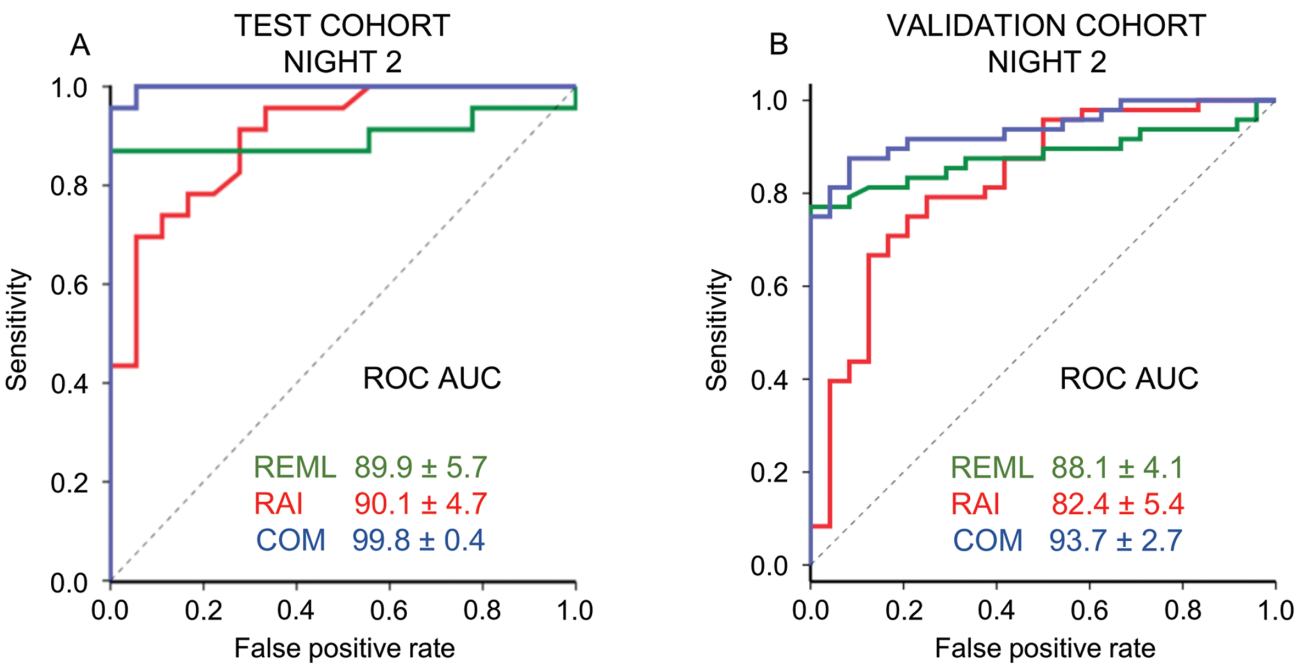

Figure 2. ROC curves for the identification of patients with NT1 based on REML, RAI, and COM. ROC, receiver operating characteristic; AUC, area under the curve; REML, REM sleep latency (green); RAI, REM sleep atonia index (red); COM, index based on the combination of REML and RAI (blue). Dashed diagonal lines indicate the performance of a random classifier with ROC AUC = 50%. See text for details on composition and size of the test and validation cohorts. Data are shown as mean ± SEM.

Table 3. Sensitivity and specificity of identification of patients with NT1 based on REML, RAI, and COM

		Test cohort	Validation cohort
Sensitivity	REML	87.0	81.3
	RAI	95.7	87.5
	COM	95.7	85.4
Specificity	REML	94.4	87.5
	RAI	66.7	58.3
	COM	100	91.7

NT1, narcolepsy type 1; REML, REM sleep latency; RAI, REM sleep atonia index; COM, novel index combining information on REML and RAI (arbitrary units, AU).

We found that nocturnal RAI was also effective (ROC AUC 84%–90%) in identifying pediatric patients with NT1, albeit, again, significantly less than COM. We estimated an optimal RAI threshold of 0.91, which afforded high sensitivity (88%–96%) but moderate specificity (62%–67%) for the identification of pediatric patients with NT1. To our knowledge, this was the first application of RAI to the identification of pediatric patients with NT1. In a previous study, Bin-Hasan and coworkers showed that lack of submentalis muscle atonia, evaluated with manual scoring of EMG tracings, identified pediatric patients with NT1 with ROC AUC of 87% [23]. A threshold of ≥1% of REM sleep epochs without atonia afforded 88% sensitivity and 61% specificity for the identification of pediatric patients with NT1, whereas a threshold of ≥8% of REM sleep epochs without atonia decreased sensitivity to 53% and increased specificity to 96% [23]. The atonia index computed by Bin-Hasan et al. had a time resolution of 30 s, scoring each 30-s epochs of REM sleep as atonic if atonia occured for ≥50% of the epoch [23]. RAI is computed with a time resolution of 1 s [25]. Thus, the values of these two indexes of atonia are not directly comparable. It is worth remarking that RAI is computed automatically on the submentalis EMG tracings based on standard sleep scoring [25]. Our validation of RAI for the identification of pediatric patients with NT1 thus overcomes the difficulty associated with the time and skill requirements of manual scoring of REM sleep atonia as well as its inherent inter-rater variability issues. While RAI also has limitations, as detailed below, this may open the way to a wider application.

The main finding of our study was that the novel COM index combining information on REML and RAI may provide superior effectiveness in identifying pediatric patients with NT1 compared with either REML or RAI alone. In support of this conclusion, the ROC AUC based on COM was higher than those based on REML and RAI both in the test cohort and in the validation cohort (Figure 2). Both differences were statistically significant on the second recording night of the whole database under study, whereas only the difference between COM and RAI retained significance on the first (habituation) night. Nevertheless, in our cohorts, the night-to-night agreement of NT1 identification based on COM appeared better than that based on REML, as estimated with the Cohen's κ index, and the monotonic relationship between COM values of the two recording nights was stronger than the corresponding relationship between REML values (Figure 3). Further work is needed to clarify whether a first-night effect [28] modulates the relative effectiveness of COM versus REML in identifying pediatric patients with NT1.

The effectiveness of COM in identifying pediatric patients with NT1 (ROC AUC 94%–100% on single cohorts, ROC AUC 96%–97% on the first and second recording night of both cohorts together) was similar to, but slightly lower than, that previously

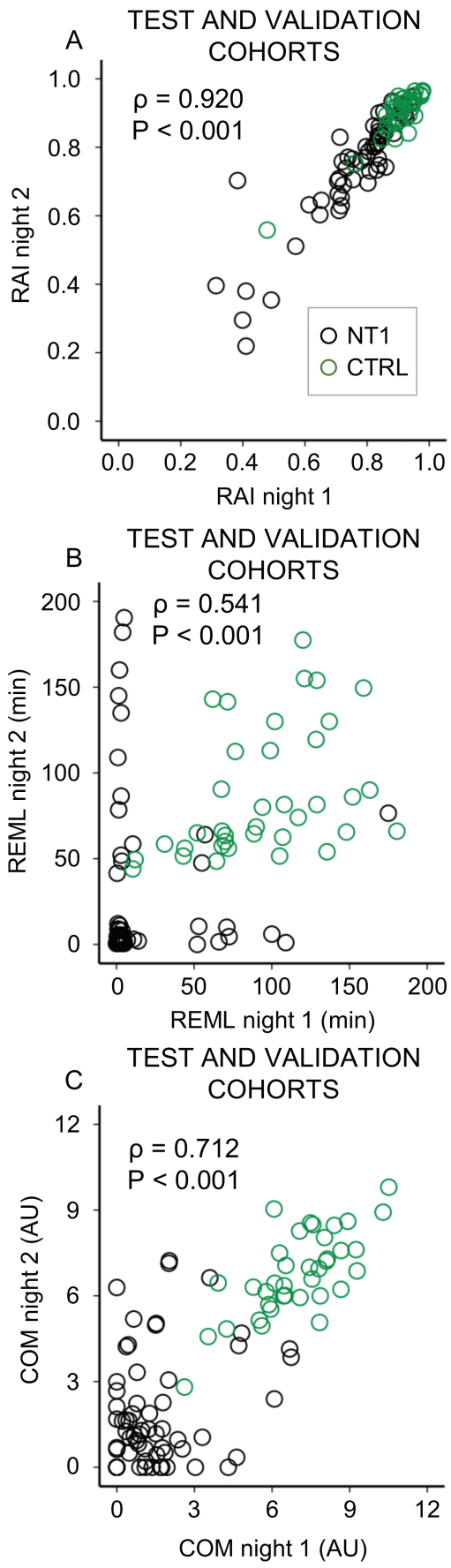

Figure 3. Relationships between night-to-night values of REML, RAI, and COM. REML, REM sleep latency; RAI, REM sleep atonia index; COM, index based on the combination of REML and RAI (arbitrary units: AU). Each circle corresponds to one child or adolescent. NT1, narcolepsy type 1 (black); CTRL, clinical controls (green). See text for details on composition and size of the test and validation samples. Insets indicate Spearman's ρ correlation coefficient, an index of monotonic relationship between two variables, with its statistical significance, *p*.

reported for a mean sleep latency ≤ 8.2 min at the MSLT (ROC AUC 99%) [13]. We also found that the effectiveness of MSLT in classifying pediatric patients with NT1 and control subjects was virtually perfect, and slightly but significantly higher than that of COM. However, the MSLT procedure is highly time consuming, must be preceded by nocturnal polysomnography, and requires a dedicated sleep laboratory [14–15]. Conversely, the COM index requires only standard scoring of a nocturnal polysomnography, that could also be recorded in an ambulatory setting. Computation of the COM index may thus represent a cost- and time-effective tool to prioritize subjects for MSLT, which remains the neurophysiological gold standard for identification of patients with NT1. Recently, the number of transitions from any sleep stage to wake or nonrapid eye movement (NREM) sleep stage 1 normalized by total sleep time during the first night of polysomnography (Wake/N1 index) combined with the occurrence of a nocturnal SOREMP was reported to identify pediatric patients with NT1 with ROC AUC of 91% [31]. The performance of the COM index in identifying pediatric patients with NT1 compares favorably with these figures.

It is worth remarking that we analyzed only recordings that had been judged as free of significant submentalis EMG artifacts by an experienced scorer blind to the results of the analysis. This quality check can be performed simultaneously with the sleep scoring procedure. Further work is needed to determine the sensitivity of RAI and COM to submentalis EMG artifacts and to test algorithms for automatic artifact detection and removal.

Our study has a few limitations. First, our control subjects were mostly children and adolescents in whom a clinical suspicion of hypersomnia was not supported by clinical evaluation. However, this reflects real-life situations in which a differentiation between these subjects and patients with NT1 is needed. Other limitations of our cohorts are that they were retrospective, and that female sex was more represented in patients with NT1 than in control subjects of the validation cohort. Moreover, although both cohorts included children in the age range 5–10 years, their number did not allow a meaningful sub-analysis. All these limitations may be addressed with a multicenter study to support the clinical application of the index COM in the pediatric population, in comparison or combination with other indexes based on the analysis of nocturnal sleep [31], and to evaluate its potential for extension to the adult population. Another limitation of our study was that RAI requires home-made software or scripts for the analysis of EMG amplitude, which limit its generalization. However, the algorithm for RAI computation is straightforward [25], making its implementation feasible [24, 26].

In conclusion, we developed, tested, and validated a novel index, COM, based on REML and RAI, which proved highly effective in identifying pediatric patients with NT1 based on standard scoring of a single-night polysomnography. The COM index may contribute useful information to the triage of children and adolescents with a diagnostic suspect of NT1, potentially helping to reduce diagnostic delay.

Acknowledgments

We are indebted to all the children, adolescents, and families who participated in this study, most notably to the Italian Association of Narcolepsy (AIN Onlus) patients. Without their contributions, this study would not have been possible. R.F. is partially funded by a research grant by the Italian Ministry of Health ("Ricerca Corrente", n.2757319).

Disclosure statement

Financial disclosures: G.P. participated to advisory board for Jazz, Bioprojet, and Idorsia; S.V., F.P., E.A., R.F., and A.S. have nothing to disclose.

Non-financial disclosures: G.P. participated to advisory board for Jazz, Bioprojet, and Idorsia; S.V., F.P., E.A., R.F., and A.S. have nothing to disclose.

References

1. Peyron C, *et al*. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med*. 2000;**6**(9):991–997.
2. Kornum BR. Narcolepsy type 1: what have we learned from immunology? *Sleep*. 2020. doi:10.1093/sleep/zsaa055
3. Scammell TE. Narcolepsy. *N Engl J Med*. 2015;**373**(27):2654–2662.
4. Scheer D, *et al*. Prevalence and incidence of narcolepsy in a US health care claims database, 2008–2010. *Sleep*. 2019;**42**(7). doi:10.1093/sleep/zsz091
5. Dauvilliers Y, *et al*. Age at onset of narcolepsy in two large populations of patients in France and Quebec. *Neurology*. 2001;**57**(11):2029–2033.
6. Dodd CN, *et al*. Incidence rates of narcolepsy diagnoses in Taiwan, Canada, and Europe: The use of statistical simulation to evaluate methods for the rapid assessment of potential safety issues on a population level in the SOMNIA study. *PLoS One*. 2018;**13**(10):e0204799.
7. Dye TJ, *et al*. Epidemiology and Pathophysiology of Childhood Narcolepsy. *Paediatr Respir Rev*. 2018;**25**:14–18.
8. Postiglione E, *et al*. The clinical spectrum of childhood narcolepsy. *Sleep Med Rev*. 2018;**38**:70–85.
9. Plazzi G, *et al*. Complex movement disorders at disease onset in childhood narcolepsy with cataplexy. *Brain*. 2011;**134**(Pt 12):3477–3489.
10. Testoni C, *et al*. Use and safety of nitrous oxide during lumbar puncture for the diagnosis of childhood narcolepsy. *Sleep Med*. 2019;**59**:120–122.
11. Taddei RN, *et al*. Diagnostic delay in narcolepsy type 1: combining the patients' and the doctors' perspectives. *J Sleep Res*. 2016;**25**(6):709–715.
12. Maski K, *et al*. Listening to the patient voice in narcolepsy: diagnostic delay, disease burden, and treatment efficacy. *J Clin Sleep Med*. 2017;**13**(3):419–425.
13. Pizza F, *et al*. Validation of multiple sleep latency test for the diagnosis of pediatric narcolepsy type 1. *Neurology*. 2019;**93**(11):e1034–e1044.
14. Littner MR, *et al*. Practice parameters for clinical use of the multiple sleep latency test and the maintenance of wakefulness test. *Sleep*. 2005;**28**(1):113–121.
15. Aurora RN, *et al*. Practice parameters for the non-respiratory indications for polysomnography and multiple sleep latency testing for children. *Sleep*. 2012;**35**(11):1467–1473.
16. Reiter J, *et al*. Usefulness of a nocturnal SOREMP for diagnosing narcolepsy with cataplexy in a pediatric population. *Sleep*. 2015;**38**(6):859–865.
17. Andlauer O, *et al*. Nocturnal rapid eye movement sleep latency for identifying patients with narcolepsy/hypocretin deficiency. *JAMA Neurol*. 2013;**70**(7):891–902.
18. Chemelli RM, *et al*. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell*. 1999;**98**(4):437–451.
19. Bastianini S, *et al*. Sleep related changes in blood pressure in hypocretin-deficient narcoleptic mice. *Sleep*. 2011;**34**(2):213–218.
20. Olesen AN, *et al*. A comparative study of methods for automatic detection of rapid eye movement abnormal muscular activity in narcolepsy. *Sleep Med*. 2018;**44**:97–105.
21. Khalil A, *et al*. Loss of rapid eye movement sleep atonia in patients with REM sleep behavioral disorder, narcolepsy, and isolated loss of REM atonia. *J Clin Sleep Med*. 2013;**9**(10):1039–1048.
22. Vandi S, *et al*. Cardiovascular autonomic dysfunction, altered sleep architecture, and muscle overactivity during nocturnal sleep in pediatric patients with narcolepsy type 1. *Sleep*. 2019;**42**(12). doi:10.1093/sleep/zsz169
23. Bin-Hasan S, *et al*. Nocturnal REM sleep without atonia is a diagnostic biomarker of pediatric narcolepsy. *J Clin Sleep Med*. 2018;**14**(2):245–252.
24. Silvani A, *et al*. Muscle activity during sleep in human subjects, rats, and mice: Towards translational models of REM sleep without atonia. *Sleep*. 2017;**40**(4). doi:10.1093/sleep/zsx029
25. Ferri R, *et al*. Improved computation of the atonia index in normal controls and patients with REM sleep behavior disorder. *Sleep Med*. 2010;**11**(9):947–949.
26. Cesari M, *et al*. Comparison of computerized methods for rapid eye movement sleep without atonia detection. *Sleep*. 2018;**41**(10). doi:10.1093/sleep/zsy133
27. Vandi S, *et al*. A standardized test to document cataplexy. *Sleep Med*. 2019;**53**:197–204.
28. Plazzi G, *et al*. Nocturnal aspects of narcolepsy with cataplexy. *Sleep Med Rev*. 2008;**12**(2):109–128.
29. Berry RB, *et al*. *The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Version 2*. 2nd ed. Darien, IL: American Academy of Sleep Medicine; 2015.
30. Vergara IA, *et al*. StAR: a simple tool for the statistical comparison of ROC curves. *BMC Bioinformatics*. 2008;**9**:265.
31. Maski K, *et al*. Defining disrupted nighttime sleep and assessing its diagnostic utility for pediatric narcolepsy type 1. *Sleep*. 2020. doi:10.1093/sleep/zsaa066