Sleep-stage sequencing of sleep-onset REM periods in MSLT predicts treatment response in patients with narcolepsy

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SUMMARY

Current treatment recommendations for narcolepsy suggest that modafinil should be used as a first-line treatment ahead of conventional stimulants or sodium oxybate. In this study, performed in a tertiary sleep disorders centre, treatment responses were examined following these recommendations, and the ability of sleep-stage sequencing of sleep-onset rapid eye movement periods in the multiple sleep latency test to predict treatment response. Over a 3.5-year period, 255 patients were retrospectively identified in the authors' database as patients diagnosed with narcolepsy, type 1 (with cataplexy) or type 2 (without) using clinical and polysomnographic criteria. Eligible patients were examined in detail, sleep study data were abstracted and sleep-stage sequencing of sleep-onset rapid eye movement periods were analysed. Response to treatment was graded utilizing an internally developed scale. Seventy-five patients were included (39% males). Forty (53%) were diagnosed with type 1 narcolepsy with a mean follow-up of 2.37 $\pm$ 1.35 years. Ninety-seven percent of the patients were initially started on modafinil, and overall 59% reported complete response on the last follow-up. Twenty-nine patients (39%) had the sequence of sleep stage 1 or wake to rapid eye movement in all of their sleep-onset rapid eye movement periods, with most of these diagnosed as narcolepsy type 1 (72%). The presence of this specific sleep-stage sequence in all sleep-onset rapid eye movement periods was associated with worse treatment response ($P = 0.0023$). Sleep-stage sequence analysis of sleep-onset rapid eye movement periods in the multiple sleep latency test may aid the prediction of treatment response in narcoleptics and provide a useful prognostic tool in clinical practice, above and beyond their classification as narcolepsy type 1 or 2.

INTRODUCTION

Narcolepsy represents the classic hypersomnia of central origin. It is a disabling syndrome characterized by the symptom tetrad of excessive daytime sleepiness (EDS), hypnagogic hallucinations, sleep paralysis and cataplexy (American Academy of Sleep Medicine, 2014). However, only 10–15% of patients have all four features. Nocturnal polysomnography (NPSG) and the multiple sleep latency test (MSLT) remain important diagnostic tools for the diagnosis of narcolepsy. In the recently published third edition of the International Classification of Sleep Disorders (ICSD), NPSG and MSLT are mandatory for the diagnosis of narcolepsy with (N + C) or without cataplexy (N – C). With these recent changes, narcolepsy is now categorized into type 1 (NT1) if cataplexy and a positive MSLT or cerebrospinal fluid hypocretin (CSF-hcrt-1) deficiency is present, and type 2 (NT2) if a positive MSLT is associated with absence of cataplectic features and normal CSF-hcrt-1 if measured (American Academy of Sleep Medicine, 2014).

The MSLT is, however, a biological test with a high false-positive and false-negative rate (Aldrich *et al.*, 1997; Mignot *et al.*, 2006), and the diagnosis of narcolepsy can rest mostly on clinical grounds, as the major differential for NT2 is behaviourally induced inadequate sleep syndrome (BIISS; Leschziner, 2014). Recently, an analysis of sleep-stage

sequence of sleep-onset rapid eye movement (REM) periods (SOREMPs) in the MSLT and of the first REM period (FREMP) in the NPSG demonstrated that FREMPs or SOREMPs arising from sleep stage 1 (N1) or wake (W) appear to be more specific to narcolepsy than BIISS, idiopathic hypersomnia with long sleep period or periodic limb movement disorder (PLMD), and a feature of sleepiness severity (Drakatos *et al*., 2013a,b; Marti *et al*., 2009). Furthermore, this sequence was much more common in NT1 compared with NT2, implying a heterogeneity in the latter group, expressed by differences in phenotyping.

The only mandatory, and typically most disabling, feature of narcolepsy is EDS, and the treatment goal should be to produce the fullest possible return to normal function for patients at work, home, school and socially. Pharmacotherapy with stimulants remains the major treatment option for these patients; scheduled naps have proven to be beneficial to combat EDS, but seldom suffice as primary therapy for narcolepsy (Billiard *et al*., 2006; Morgenthaler *et al*., 2007). Currently, modafinil may be considered the first-line treatment of EDS (Billiard *et al*., 2006; Morgenthaler *et al*., 2007). There are existing randomized, double-blind, placebo-controlled clinical trials of modafinil showing its effectiveness, but the authors are unaware of any direct comparison between it and traditional stimulants (Beusterien *et al*., 1999; Billiard *et al*., 1994, 2006; Broughton *et al*., 1997; Group, U. M. I. N. M. S, 1998, 2000; Mitler *et al*., 2000; Moldofsky *et al*., 2000; Morgenthaler *et al*., 2007). Sodium oxybate has become more popular and has slowly emerged as a first-line treatment, challenging the conventional stimulants (amphetamines, methylphenidate), especially in the USA (Billiard *et al*., 2006; Morgenthaler *et al*., 2007). As with modafinil, several studies support its efficacy, but direct comparative studies remain lacking (Billiard *et al*., 2006; Black and Houghton, 2006; Group, X. I. S Xyrem International Study Group, 2002, 2003, 2005; Mamelak *et al*., 2004; Morgenthaler *et al*., 2007; Scrima *et al*., 1989).

The apparent utility of sleep-stage sequencing of SOREMPs in the MSLT and FREMPs as a diagnostic tool in narcolepsy, providing further evidence of possible pathophysiological heterogeneity within the NT2 group, has led to explore this technique as a possible predictor of treatment response and potentially a useful prognostic tool in clinical practice.

MATERIALS AND METHODS

Retrospectively, patients diagnosed with narcolepsy were identified on the basis of NPSG followed by MSLT at Guy's and St Thomas' Sleep Disorders Centre between January 2009 and March 2013. All patients had been evaluated by an experienced sleep physician prior to their sleep study, and were required to complete a sleep diary or 2 weeks of actigraphy prior to the study. Appropriate approval from the institutional review board on human research was obtained (project number 4262).

The NPSG montage included frontal (F3 and F4), central (C3 and C4) and occipital (O1 and O2) electrodes with auricular reference electrodes, two electrooculographic channels, two submental electromyographic channels, electrocardiography, electromyographic channel on anterior tibialis bilaterally, pulse oximetry, nasal cannula, and respiratory inductance plethysmography with chest and abdominal belts. Sleep stages were scored using 30-s epochs according to standard American Academy of Sleep Medicine criteria (Berry *et al*., 2012).

The MSLTs were performed according to standard guidelines using central (C3 and C4) and occipital (O1 and O2) electrodes for the montage, with auricular reference electrodes, two electrooculographic channels, two submental electromyographic channels and electrocardiography (Carskadon *et al*., 1986). Patients took four or five naps under standard MSLT conditions, each lasting 20 min, at 2-h intervals on the following day.

Patients were subsequently reviewed with the results of the NPSG and MSLT, and the diagnoses of NT1 and NT2 were made *de novo* in accordance with ICSD-3 (American Academy of Sleep Medicine, 2014). Other sleep disorders (e.g. PLMD, sleep disorder breathing) could be present, but were either already treated or of minor clinical significance. Exclusion criteria were diagnostic doubt (e.g. possible influence of psychiatric disease); incomplete clinical information; less than 6 h of sleep during NPSG or technical issues; failure to stop all medications that affect sleep; failure to comply with treatment for previously diagnosed sleep disorders.

Detailed clinical notes were available for all patients and were reviewed. In line with a recently published study of idiopathic hypersomnia (Ali *et al*., 2009), treatment response regarding EDS was assessed subjectively by utilizing a three-group response categorization based on the language used by physicians or patients to report progress during the follow-up visits. These three categories consisted of: 'complete response', which correlated with adjectives such as 'excellent', 'great' or 'entirely satisfactory', provided there was no change in pharmacotherapy; 'partial response', which correlated with phrases such as 'doing better' or 'improved', but in the setting of increased dose adjustment of stimulant medications; and 'no or poor response', which associated with phrases such as 'still sleepy' or 'discontinued due to development of side-effects' and with a subsequent switch to another medication. In order to assess the validity of the outcome measurement scale, an inter-rater agreement analysis was performed on scoring of two blinded independent raters (PD, BK) for 20 patients. The agreement was found to be high (Cronbach's $\alpha = 0.937$; Cronbach, 1951), and the residual disagreements between raters were discussed further until agreement was reached.

Statistical analysis

Statistical analysis was performed using the SPSS statistical analysis program (IBM, SPSS 20.0, US). Data are reported

as mean ± SD, if not otherwise indicated. Following testing for normality, the similarity of two means in demographics and sleep parameters was compared using the Student's *t*-test and χ^2-statistics in the case of normal distribution; when more than two groups were included in the analysis, the Kruskal–Wallis test was performed with Dunn's Multiple Comparison Test when needed. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Over a 4-year period, a total of 255 patients were identified for analysis. Forty-one patients had their sleep studies performed prior to 2009, with PSG data and notes unavailable on the digital database, and were subsequently excluded. Sixty-seven patients that did not meet all ICSD-3 (American Academy of Sleep Medicine, 2014) criteria (e.g. less than 6 h of sleep on the NPSG preceding the MSLT, or on medications affecting MSLT results) for the diagnosis of narcolepsy, and 24 patients who did not comply with treatment for other previously diagnosed sleep disorders or who were not adequately treated for co-morbidities likely to affect the reliability of the MSLT results (e.g. psychiatric conditions), were excluded. Twenty-three patients were excluded due to loss of follow-up or with total follow-up duration of less than 6 months. Finally, due to lack of clinical information or technical issues related to their studies, another 25 patients were excluded from the analysis.

Ultimately, 75 drug-naïve patients were categorized into firm diagnostic categories of NT1 ($n = 40$) and NT2 ($n = 35$). The two groups did not differ significantly regarding their age and body mass index. With regard to sleep architecture, the NT1 group demonstrated a higher percentage of N1 (8.91 ± 4.56 versus 5.81 ± 3.37, $P = 0.002$) when compared with NT2. As expected, the NT1 group had the shortest REM latency (REML; 36.52 ± 38.17 versus 56.84 ± 32.73, $P = 0.016$) and mean sleep latency (MSL; 2.68 ± 1.90 versus 4.03 ± 2.21, $P = 0.006$) compared with NT2 (Table 1).

The mean duration of follow-up was 2.37 ± 1.35 years. Forty-three (58%) patients reported a total response, 19 (25%) a partial response and 13 (17%) a poor response to treatment, with statistically significant changes in Epworth Sleepiness Score (ΔESS) across response groups ($P = 0.0028$; Table 2). The mean total daily doses of prescribed medications are shown in Table 3. Forty-one (55%) patients tried one treatment and 34 (45%) at least two. The number of patients commenced on and receiving a particular medication at the last follow-up, either as monotherapy or in combination, is presented in Table 3.

Modafinil was the most common first-line agent used (73/75, 97%), as per current recommendations (Billiard *et al.*, 2006; Morgenthaler *et al.*, 2007). Of those, 50 (68%) patients were on modafinil at the last follow-up, with 42

Table 1 Demographics and sleep parameters of NT1 and NT2

Demographics	*NT1 (*n = *40)*	*NT2 (*n = *35)*	P-*value*
Gender (M/F)	15/25	14/21	NS*
Age	32.50 ± 11.65	33.87 ± 11.91	NS
Body mass index	30.05 ± 7.14	28.34 ± 7.33	NS
Sleep parameters			
Sleep onset	7.72 ± 7.54	9.13 ± 14.66	NS
REML	36.52 ± 38.17	56.84 ± 32.73	0.016
MSL	2.68 ± 1.90	4.03 ± 2.21	0.006
Total sleep time	394.23 ± 44.74	402.38 ± 71.41	NS
Sleep efficiency (%)	83.56 ± 8.65	86.38 ± 10.66	NS
Apnea–hypopnoea index	2.77 ± 4.57	4.21 ± 7.84	NS
Periodic limb movement index	14.74 ± 25.63	10.18 ± 18.30	NS
REM duration	82.35 ± 29.59	84.75 ± 30.90	NS
N1% (of sleep period)	8.91 ± 4.56	5.81 ± 3.37	0.002
N2%	35.55 ± 7.79	37.56 ± 10.13	NS
N3%	22.22 ± 7.30	25.54 ± 8.46	NS
REM%	17.68 ± 6.45	18.14 ± 5.29	NS
Arousal index	19.10 ± 8.93	18.04 ± 9.94	NS

Analysis was performed with *t*-test, two-tailed.
MSL, mean sleep latency; N1, stage 1 sleep; N2, stage 2 sleep; N3, stage 3 sleep; REM, rapid eye movement; REML, rapid eye movement latency. NS, not significant ($P > 0.05$).
*Analysis was performed with Chi-square test.

Table 2 Response to treatment

	Required criteria			
Response	*Comments from notes*	*Changes to medication*	*Patients, no. (%)*	*ΔESS (no. of pt available)**
Complete response	'Great, excellent, entirely satisfactory, very well indeed'	None	43 (58)	−9.55 ± 4.92 (20)
Partial response	'Reasonably well controlled, doing better, better overall'	Dose increase	19 (25)	−4.36 ± 5.16 (11)
Poor response	'Still sleepy, residual sleepiness, has not done well'	Medication changed	13 (17)	−1.66 ± 2.73 (6)

ESS, Epworth Sleepiness Scale.
*$P = 0.0028$. Analysis was performed using Kruskal–Wallis with Dunn's multiple comparison test. Complete response versus poor response, $P < 0.01$. Δ, delta. Data are presented as mean ± SD.

Table 3 Treatment response according to drug used

Medication	*Pt exposed to drug at any time, no. (% total)*	*Pt started on drug, no. (% total)*	*Pt on drug at last visit, as in combination no. (% total exposed)*	*Pt on drug at last visit, as monotherapy no. (% total exposed)*	*Pt on drug at last visit, no. (% total exposed)*	*Pt with complete/ partial/poor response, no. (% of exposed)*	*ΔESS as monotherapy in last visit (no. with ESS)*	*Dose (mg)*
Modafinil	73/75 (97)	73/75 (97)	8/73 (11)	42/73 (58)	50/73 (68)	31 (43) 12 (16) 30 (41)	9.8 ± 4.9 (20)	298.9 ± 120.1
Concerta/ methylphenidate	29/75 (39)	2/75 (3)	6/29 (21)	12/29 (41)	18/29 (62)	9 (31) 7 (24) 13 (45)	7.5 ± 2.8 (7)	39.45 ± −28.95
Dexamphetamine	13/75 (16)	0	1/13 (8)	11/13 (85)	12/13 (92)	2 (15) 4 (31) 7 (54)	3 ± 3.9 (6)	24 ± 15.04
Sodium oxybate	8/75 (11)	0	3/8 (37)	2/8 (25)	5/8 (62)	4 (50) 1 (12.5) 3 (37.5)	7 (1)	7.3 ± 1.9 (g)

Data are presented as mean ± SD. Poor responses includes the patients that tried and discontinued the drug at any point either because of lack of efficacy or development of limiting side-effects.
ESS, Epworth Sleepiness Scale; no., number; Pt, patients.

Table 4 Patients categorized according to SOREMPs sleep-stage sequencing in MSLTs and analysis of their treatment response is presented

Groups based on SOREMPs sleep-staging sequence	*No. (% of all)*	*NT1/NT2*	*Treatment response (complete/partial/poor)*
ALL SOREMPs from N1/W (Group N1/W), $n = 29$	29/75 (39%)	21/8*	8/13/8†,‡
Mixed SOREMPs (Group Mixed), $n = 23$	23/75 (31%)	12/11	17/3/3
ALL SOREMPs from N2 (Group N2), $n = 23$	23/75 (31%)	7/16	18/3/2
Group Mixed + Group N2 = Group 4, $n = 23 + 23$	46/75 (61%)	19/27	35/6/5

Analysis was performed using Chi-square test.
SOREMP, sleep-onset rapid eye movement period.
*Group N1/W versus Group 4, $P = 0.0037$.
†Group N1/W versus Group 4, $P < 0.0001$.
‡Group N1/W versus Group Mixed, N2, $P < 0.0016$.

(58%) receiving it as monotherapy and eight (11%) in combination. Thirty-one (43%) of exposed patients reported a complete response, 12 (16%) a partial and 30 (41%) a poor response.

Twenty-nine (39%) patients were commenced on methylphenidate during the study period, and 18 of them (62%) were receiving it at the last follow-up; 12 of the 29 (41%) as monotherapy. Treatment responses to modafinil and methylphenidate were similar ($P > 0.05$). Treatment with modafinil was associated with a significantly greater improvement in ESS when compared with dexamphetamine (9.8 ± 4.9 versus 3 ± 3.9, $P < 0.05$).

Dexamphetamine was prescribed for 13/75 (16%) patients, 12 of whom (92%) were receiving it at the last follow-up. Another eight patients tried sodium oxybate, with five of them (62%) remaining on it at the last follow-up.

Analysis of sleep-stage sequencing in MSLTs revealed that the occurrence of all SOREMPs arising from N1 or W occurred more frequently in NT1 ($P = 0.0037$; Table 4). For the purpose of assessing response to treatment, patients with all SOREMPs from the MSLT arising from N1 or W were designated Group N1/W ($n = 29$; 38%); patients with SOREMPs arising from a mix of W, N1 and N2 were designated Group Mixed ($n = 23$; 31%); and patients with SOREMPs arising solely from N2 were designated Group N2 ($n = 23$; 31%).

Patients in Group N1/W had a poorer response overall compared with those in Groups Mixed and N2 ($P = 0.0016$; Fig. 1; Table 4). The results were similar when FREMPs and SOREMPs were taken into consideration ($P < 0.0023$). Subgroup analysis of Groups N1/W, N2 and Mixed regarding treatment response demonstrated no statistically significant

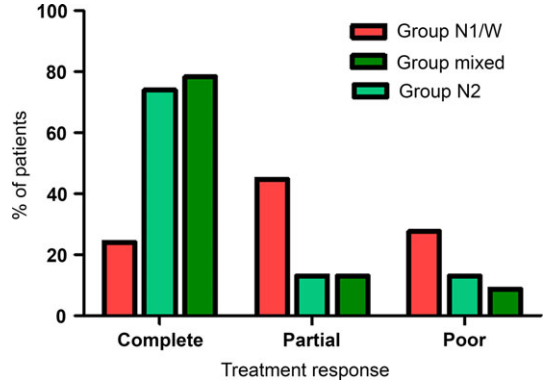

Figure 1. Treatment response of the Groups N1/W, N2 and Mixed. Group N1/W, sleep-onset rapid eye movement periods (SOREMPs) only from N1/W; Group Mixed, mixed SOREMPs; Group N2, SOREMPs only from N2. Group N1/W versus Groups Mixed, N2, $P < 0.0016$ (analysis was performed using Chi-square test).

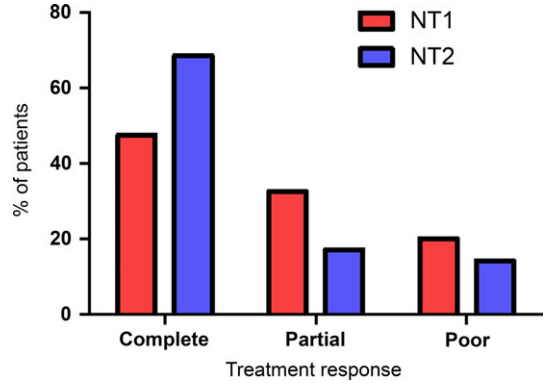

Figure 2. Treatment response of NT1 and NT2.

difference between NT1 and NT2 ($P = 0.08$, $P = 0.59$ and $P = 0.41$, respectively). Furthermore, no statistically significant difference in treatment response was found overall between NT1 and NT2 ($P = 0.17$; Fig. 2).

Demographics and sleep parameters of the Groups N1/W, N2 and Mixed are presented in Table 5. Those in Group N1/W were younger than Group Mixed (29.29 ± 9.17 versus 37.54 ± 13.05, $P < 0.05$) and demonstrated a shorter REML (31.60 ± 37.53 versus 67.48 ± 23.94, $P < 0.05$), a decreased sleep efficiency and an increased percentage of sleep period as N1 when compared with Group N2 ($P < 0.05$). Both Groups N1/W and Mixed were characterized by a shorter MSL compared with Group N2 ($P < 0.0001$; Table 5). Eight out of 75 patients had a fifth nap; three, one and four from Groups N1/W, Mixed and N2, respectively. Their MSLs (3.06 ± 2.85 min, 2.5 min and 5.75 ± 2.98 min) were similar to those of the main groups as presented in Table 5.

Comparison of MSL in NT1 versus NT2 patients demonstrated no significant difference in Groups N1/W and N2, although there was a small difference in Group Mixed (1.76 ± 1.21 min versus 3.12 ± 1.39 min, $P = 0.024$).

All NT1 patients had cataplectic events ranging from several/day to 1/month. Selective serotonin reuptake inhibitors (fluoxetine, citalopram), serotonin–norepinephrine reuptake inhibitors (venlafaxine), tricyclic antidepressants (clomipramine) and norepinephrine reuptake inhibitors (reboxetine) were used to effectively control these events.

Two out of 75 patients overall had their CSF-hcrt measured, which precluded us from further analysis on the basis of CSF-hcrt levels.

DISCUSSION

These findings suggest that sleep-stage sequencing of SOREMPs in the MSLT in narcoleptics may provide prognostic information and predict treatment response in clinical practice, beyond their classification as NT1 or NT2. The difference in response to treatment furthermore implies that differences in sleep-stage sequence progression in SOREMPs in some way reflect biological/pathophysiological differences between the groups.

According to current recommendations (Billiard *et al.*, 2006; Morgenthaler *et al.*, 2007), modafinil is considered the first-line treatment option. Ninety-seven percent of the cohort patients were initiated on this wake promoter, with 23 (32%) withdrawing from it, either because of lack of effectiveness or side-effects, in keeping with previously published data (Beusterien *et al.*, 1999; Group, U. M. I. N. M. S, 1998; Mitler *et al.*, 2000). Due to financial limitations that apply in the UK, sodium oxybate was utilized least frequently in the cohort, although a number of subjects prescribed sodium oxybate were excluded from analysis due to having their initial course of NPSG, MSLT prior to 2009 and failing to withdraw from their medication prior to the performance of more recent course of studies. Methylphenidate and amphetamines were the most common second-line treatment option in the current study. No stimulant used was found to be significantly more efficacious, but this is not a comparative study and no conclusions could be drawn; the study reflects clinical practice in that more refractory patients are likely to escalate to more 'potent' stimulant drugs. Previously published work has demonstrated that SOREMPs arising from N1 and W in MSLT are strongly associated with NT1 (Drakatos *et al.*, 2013b), and this current analysis confirms this to be the case, even in the presence of co-existent adequately treated sleep disorders, extending this finding into an arena more consistent with clinical practice.

It remains uncertain if differences in SOREMP sleep-stage sequence are purely a function of severity of sleepiness, a consequence of REM instability or other differences in sleep architecture. This study, however, demonstrates that the presence of the N1/W-REM sequence is associated with a worse treatment response, irrespective of the presence of cataplexy or severity of objectively determined sleepiness, as seen in treatment response differences between Groups N1/W and Mixed; these groups demonstrated similar MSLs (Tables 4 and 5). The proportions of patients with NT2 in Groups N1/W and Mixed were 27.5 and 48%, respectively. The difference in treatment response between groups N1/W

Table 5 Demographics and sleep parameters of narcoleptics with SOREMPs in MSLT either all from 1/W (Group N1/W), N2 (Group N2) or in between (Group Mixed)

Demographics	*Group N1/W (n = 29)*	*Group Mixed (n n = 23)*	*Group N2 (n = 23)*	P-*value*
Age	29.29 ± 9.17*	37.54 ± 13.05	33.62 ± 12.01	0.0236
Body mass index	29.34 ± 6.97	31.57 ± 7.77	26.84 ± 6.50	NS
Gender (M/F)	12/17	6/17	11/12	NS[1]
NT1/NT2	21/8	12/11	7/16	0.01[1]
Sleep parameters				
Sleep onset	6.21 ± 6.33	7.02 ± 6.47	12.47 ± 17.79	NS
REML	31.60 ± 37.53†	42.98 ± 38.15	67.48 ± 23.94	0.0024
MSL	2.71 ± 1.87†	2.44 ± 1.45†	5.04 ± 2.03	< 0.0001
Total sleep time	393.1 ± 64.10	403.9 ± 43.67	398.3 ± 65.44	NS
Sleep efficiency (%)	81.55 ± 8.73†	86.21 ± 8.18	87.75 ± 11.23	0.0011
Apnea–hypopnoea index	1.95 ± 2.23	3.59 ± 5.29	5.23 ± 9.67	NS
Periodic limb movement index	7.36 ± 7.34	7.64 ± 7.39	4.41 ± 5.50	NS
REM duration	73.72 ± 29.52	93.36 ± 29.91	86.37 ± 28.27	NS
N1 (% of sleep period)	10.10 ± 4.65*,†	6.05 ± 3.56	5.44 ± 2.55	0.0002
N2 (%)	33.67 ± 7.86	38.84 ± 9.91	37.86 ± 8.75	NS
N3 (%)	23.27 ± 6.91	22.15 ± 7.49	26.03 ± 9.45	NS
REM (%)	15.44 ± 6.15	19.86 ± 5.14	19.12 ± 5.36	NS
Arousal index	18.60 ± 6.73	19.98 ± 10.86	17.30 ± 10.84	NS
Potentially affecting co-morbidities				
Obstructive sleep apnoa[2]	3	5	5	
PLMD/restless legs syndrome[3]	3	4	1	
Anxiety[4]	1	0	6	
Other[5]	2 REM behaviour disorder, 1 hypothyroidism	3 hypothyroidism	1 diabetes mellitus, 1 chronic fatigue syndrome	

Analysis was performed using Kruskal–Wallis one-way ANOVA with Dunn's multiple comparison test.
MSL, mean sleep latency; PLMD, periodic limb movement disorder; REM, rapid eye movement; REML, rapid eye movement latency.
[1]Analysis was performed using Chi-square test. *$P < 0.05$ versus Group Mixed; †$P < 0.05$ versus Group N2.
[2]All patients were treated successfully with continuous positive airway pressure machine.
[3]Patients were treated successfully with dopamine agonists (pramipexol, ropinirole), pregabalin/gabapantin and iron supplementation when required.
[4]Patients were on selective serotonin reuptake inhibitors, serotonin–norepinephrine reuptake inhibitors or tricyclic antidepressants, which were discontinued 2 weeks prior to their sleep studies.
[5]Conditions have been assessed and appropriately treated.

and Mixed is unlikely to be explained by the difference in proportions of NT1 and NT2 patients in the two groups, as there was no statistically significant difference in treatment response between NT1 and NT2 overall ($P = 0.17$; Fig. 2).

The ICSD-3 categorizes patients with narcolepsy into two groups (American Academy of Sleep Medicine, 2014), but clinical experience and literature imply the existence of three groups: N + C with CSF-hcrt-1 deficiency; N − C without CSF-hcrt-1 deficiency; and N − C with CSF-hcrt-1 deficiency. The classification of patients with cataplexy or CSF-hcrt-1 deficiency as type 1 narcolepsy suggests a shared pathophysiology and the possibility that those without cataplexy and deficient in CSF-hcrt-1 will go on to develop cataplexy in the future (Gelardi and Brown, 1967; Hartse *et al.*, 1988). The findings of the current study add more weight to the relevance of sleep sequence staging, and suggest a role in both the phenotyping of narcolepsy and prognostication. From previous studies, it has been hypothesized that sleep-stage sequencing may permit further, clinically relevant, dissection of the type 2 narcolepsy diagnostic category, although larger prospective studies are required, as are analyses of sleep-stage sequencing against HLA type or CSF-hcrt-1 (Drakatos *et al.*, 2013a,b). Sleep-stage sequencing of SOREMPs in MSLT may therefore represent a useful tool for sleep clinicians in clinical practice, for the prediction of response to stimulant drugs in the absence of knowledge of CSF-hcrt status. A recently published study reported that sleep-stage transitions reliably identify NT1 with measured CSF-hcrt-1 deficiency among central disorders of hypersomnolence (Pizza *et al.*, 2015), thus CSF-hcrt-1 deficiency could be the interconnecting factor with the treatment response results presented here. In routine clinical practice, most patients do not currently undergo CSF-hcrt testing, whereas almost all patients with narcolepsy will undergo PSG and MSLT; sleep staging of SOREMPs and FREMPs is thus likely to be a more uniformly applicable technique to guide management and predict response to treatment, which seems also to be irrespective of the clinical NT1/NT2 classification.

Limitations

There is an age discrepancy between the three groups, which could be affecting MSL and treatment response, even though the relatively similar age between Groups N1/W and N2 makes this difference seems coincidental (Table 5). Lack of the age of onset of clinical features in this study, as a possible contributor to treatment response, is also acknowledged, and future studies would address this and many of the limitations of the current retrospective analysis, including prospectively ascertained response to medication, and validated measures of alertness and quality of life. The fifth nap in some of the patients could have potentially affected their MSL and their propensity to exhibit only N1-SOREMPs, but this was not seen in this study, particularly because of their small number. The MSLT is a biological test and thus prone to false-negative results that *a priori* would affect the reliability of sleep-stage sequencing of SOREMPs.

CONCLUSION

The utilization of sleep-stage sequencing of SOREMPs in the clinical setting in patients with narcolepsy may identify patients likely to be refractory to treatment with stimulant drugs.

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AUTHOR CONTRIBUTIONS

PD: analysis, concept, writing, design and interpretation. KP & CT: data acquisition, analysis, writing and discussion. AW: concept, writing and interpretation. BDK: design, writing and interpretation. GL: concept, design, writing, interpretation.

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