Unique sleep-stage transitions determined by obstructive sleep apnea severity, age and gender

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Unique sleep-stage transitions determined by obstructive sleep apnea severity, age and gender

Running Head: Two-step transitions in obstructive sleep apnea

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Summary

In obstructive sleep apnea, patients' sleep is fragmented leading to excessive daytime sleepiness and comorbidities like arterial hypertension. However, traditional metrics are not always directly correlated with daytime sleepiness and the association between traditional sleep quality metrics like sleep duration and arterial hypertension is still ambiguous. In a development cohort, we analyzed hypnograms from mild (n=213), moderate (n=235) and severe (n=277) OSA patients as well as healthy controls (n=105) from the European Sleep Apnea Database (ESADA). We assessed sleep by the analysis of two-step transitions depending on OSA severity and anthropometric factors. Two-step transition patterns were examined for an association to arterial hypertension or daytime sleepiness. We also tested cumulative distributions of wake as well as sleep states for power laws (exponent α) and exponential distributions (decay time τ) in dependency on OSA severity and potential confounders. Independent of OSA severity and potential confounders, wake state durations followed a power-law distribution, while sleep state durations were characterized by an exponential distribution. Sleep stage transitions are influenced by OSA severity, age and gender. N2→N3→wake transitions were associated with high diastolic blood pressure. We observed higher frequencies of alternating (symmetric) patterns (e.g. N2→N1→N2, N2→wake→N2) in sleepy patients both in the development cohort and in a validation cohort (n=425). In conclusion, effects of OSA severity and potential confounders on sleep architecture are small, but transition patterns still link sleep fragmentation directly to OSA-related clinical outcomes like arterial hypertension and daytime sleepiness.

Keywords: sleep dynamics, sleep fragmentation, sleep-disordered breathing, power-law distribution, exponential distribution

Introduction

Transitions between wake and sleep and between NREM and REM sleep are based on mutually inhibitory neuronal circuits. The complex interactions of different neuronal networks result in stable and rapid transitions. (Saper et al., 2010). In obstructive sleep apnea (OSA), patients' sleep is increasingly fragmented, caused by arousals, leading to excessive daytime sleepiness (Bianchi et al., 2010; Penzel et al., 2005; Pataka and Riha, 2009). Apart from daytime sleepiness, manifold comorbidities and high economic costs are associated with the disorder (Parati et al., 2007; Al Ghanim et al., 2008).

The current polysomnographic evaluation of sleep quality is based upon absolute durations like total sleep time (TST) and sleep latencies. However, traditional metrics are not always directly correlated with daytime symptoms, such as daytime sleepiness usually assessed with the Epworth Sleepiness Scale (ESS), and they are incapable to detect sleep fragmentation in OSA patients (Chervin and Aldrich, 1999, Swihart et al., 2008, Bianchi et al., 2010). Furthermore, the association between traditional sleep quality metrics like sleep duration and OSA-related clinical outcomes like arterial hypertension is still ambiguous (Pepin et al., 2014).

Based on this, various approaches have been pursued to develop additional methods to understand sleep fragmentation and its clinical potential. Lo et al. described that, across different species, the distribution of the duration of wake states follows a power-law, while the distribution of sleep state durations follows an exponential law (Lo et al., 2004). Recent research showed that narcolepsy patients have an altered wake episode duration, while the

sleep episode duration was unaltered (Zhang et al., 2017). Furthermore, sleep dynamic analysis could distinguish narcolepsy type 1 from type 2 patients (Pizza et al., 2015), demarcate patients with chronic fatigue syndrome and those with fibromyalgia (Kishi et al., 2011) and disclosed a N2 vulnerability in insomnia (Wei et al., 2017). In OSA patients, transition analysis revealed more wake to NREM sleep and NREM sleep to wake transitions compared to subjects without OSA (Swihart et al., 2008). Furthermore, in children with OSA, a shorter mean N2 duration was seen compared to children without OSA (Chervin et al., 2009).

A new approach to portray sleep fragmentation is an analysis of transition patterns comprising multiple sleep states. A recent study from Schlemmer et al. introduced a two step-transition analysis suggesting that the transition probability of the present state is affected by the past two states. Two-step transitions comprise three sleep states (e.g. the pattern N1→wake→N1). They revealed a modulation of sleep-stage transitions dependent on age and sleep disorder. For example, N1→wake→N1 transitions are more frequent in older patients with sleep disorder (Schlemmer et al., 2015).

Based on these reflections, the purpose of our study has been three-fold. First, we have hypothesized that, with increasing OSA severity, modified typical transition patterns can be observed and that these transition patterns are associated with important OSA related outcomes like arterial hypertension or daytime sleepiness. Second, we have aimed to evaluate the influence of anthropometric factors on sleep-stage transitions in a large sleep apnea patient cohort. Finally, we wanted to confirm that, independent of OSA severity or potential confounders, wake states follow a power-law distribution, while sleep states follow an exponential distribution.

Methods

Participants

Our retrospective study is based on data assembled in the frame of the European Sleep Apnea Database (ESADA), a collaboration of 28 European sleep centers (Hedner et al., 2011). Clinical information (e.g. diagnosis of hypertension, systolic and diastolic blood pressure in sitting position) and sleep data from OSA patients are registered in a web-based report form. For the purpose of this study, 1877 patients from 11 sleep centers were assessed: Alexandroupolis, Antwerp, Berlin, Crete, Dublin, Kosice, Milano, Palermo, Paris, Thessaloniki and Warsaw. The following exclusion criteria were applied: age under 18 years, an apnea-hypopnea-index (AHI) < 5 events per hour, depression or other psychiatric disease, narcolepsy, restless legs syndrome, insomnia, opioid-induced central sleep apnea, bronchial asthma, fibromyalgia, malignancy or PAP-therapy. Furthermore, patients with missing values for ESS, diagnosis of hypertension, systolic or diastolic blood pressure were excluded. Based on these criteria, 703 patients (development cohort) from the sleep center of Antwerp remained, with a mean age of 49.7±11.0 years, mean BMI of 29.5±5.0 and a male predominance (81.1%). In 109 patients central sleep apnea (CSA) of different degrees was present. The control group consists of 105 subjects diagnosed as non-apneic snoring with an AHI<5/h recorded with polysomnography (PSG) in the sleep center of Antwerp. Furthermore, 425 patients (validation cohort) recorded in 10 different centers and registered to ESADA have been considered for comparison and validation (mean age 54.0±11.4, mean BMI 32.9±6.4, male 78.8%). Patients and controls were assessed according to the ESADA protocol (comprising anamnestic report about comorbidity and objective sleep time quantification in the night of polysomnography) and included in the study considering the

same exclusion criteria. Patients of the development cohort were categorized based on four variables: OSA severity, age, gender and BMI**.** The severity categories were controls (AHI<5/h), mild (5/h≤AHI<15/h), moderate (15/h≤AHI<30/h) and severe (≥30/h) disease, see also table 1. Based on age, patients were categorized into young (<40y), middle aged (40y≥age<60y), and elderly (≥60y). The BMI-group categories were normal weight (BMI<25 kg/m^2), overweight (25kg/m^2≤BMI<30 kg/m^2) and obesity (BMI≥30 kg/m^2). Furthermore, we considered gender differences. All patients and controls gave written informed consent. Approval was obtained from the Charité ethics committee (EA1/139/07).

Polysomnography (PSG)

Our analysis was hypnogram based**.** In a hypnogram, wake and sleep stages (Wake, N1, N2, N3, and REM) were scored visually in 30 seconds epochs based on the AASM scoring rules from 2007 (Hedner et al., 2011; Iber et al., 2007). Hypnograms were exported manually from the original PSG reports in text or Excel file format (depending on the recording system) and then converted into an identical text file format (one number for each epoch) using adjusted GNU AWK (Aho et al., 1988) scripts for each sleep laboratory. Afterwards, three additional GNU AWK scripts were written and applied to (i) calculate the sleep parameters for each hypnogram, (ii) count the frequencies of single-step (e.g. N1→N2) and two-step (e.g., N1→N2→N3) transitions, and (iii) determine the distributions of wake-state and sleep-state durations (see below). The results were further analyzed with Excel [Microsoft] and SPSS [IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp]; plots were created with Origin [www.originlab.com/Origin]. We had to deal with some missing sleep parameter values in ESADA. To ensure consistent calculations we decided to compute all parameters of interest for all patients directly from the hypnogram:

total sleep time (TST), sleep efficiency (SE), percentages of sleep stages, wake after sleep onset (WASO) in min, number of awakenings (NASO) and total number of transitions (TRANS). We ignored wake times at the beginning and at the end of the hypnogram, since light off and light on times were not available consistently. An apnea was defined by a termination of airflow with a minimum length of 10 seconds. A hypopnea was determined either by a decrease of airflow ≥50% for at least 10 seconds accompanied by ≥3% oxygen desaturation or an arousal or by a decrease of airflow ≥30% for at least 10 seconds accompanied by ≥4% oxygen desaturation (Hedner et al., 2011).

Cumulative Distributions

For each subgroup, we examined whether wake-state durations followed a power-law distribution ($P(t)=t^{-\alpha}$), and whether sleep-state durations followed an exponential distribution ($P(t)=\exp(-t/\tau)$). The exponential decay time τ corresponds to the mean sleep episode duration, whereas the power-law exponent α was observed to be the same in different species (Lo, 2004). We simplified each hypnogram into wake bouts and sleep bouts (NREM and REM combined) and plotted the cumulative distributions $P_i(t)$, i= W,S of wake and sleep versus the duration t. To test qualitatively for superiority of power-law versus exponential decay, wake-states have been presented both in a double-logarithmic plot with slopes $-\alpha$ representing power-laws and a semi-logarithmic plot with slopes $-1/\tau$ representing exponential decays. Sleep states have only been presented in a semi-logarithmic plot. The analyzed interval for wake durations was from 1 to 30 minutes and for sleep durations from 3 to 90 minutes. We fitted the data with both, a power-law distribution and an exponential distribution and calculated the corresponding fitting errors χp and χe as root-mean-square

deviations of the data from the fit. Then we used the ratio of fitting errors (χp/χe) as an indicator, which distribution law was superior. A ratio larger than unity indicated superiority for the exponential fit, while a ratio smaller than unity indicated superiority for the power-law fit. For example, if χp (e.g. χp = 0.05) is larger than χe (e.g. χe = 0.005), the ratio of fitting errors would be over unity (χp/χe = 10), indicating superiority for exponential distribution. For further information about this method, we refer to the work of Zhang et al (Zhang et al., 2017).

One and Two-step transitions

The transition analysis was based on symbolic dynamics. Symbolic dynamics is a technique that creates patterns by transforming previously confusing information into symbols. This process is already established in many scientific fields, e.g. neuroscience or cardiovascular physiology (Porta et al., 2015). The classification of sleep in separate sleep stages (Wake, N1, N2, N3, REM) is already a depiction in symbols and can be used for further analysis (Schlemmer et al., 2015). Frequencies of transitions between wake and sleep states are displayed in form of transition matrices for each OSA severity category group and the control group, where frequencies are marked by color (variation from zero up to twenty transitions). Schlemmer et al. identified two-step transitions as suitable for further analysis, after they worked with Markov chains (Schlemmer et al., 2015). A Markov process is a stochastic process, based on the assumption that future events are not affected by the past. Two-step transitions comprise three symbols (e.g. the pattern wake→N1→wake). We present the frequencies of the 25 most common transition patterns per night for each disease severity group and our healthy control group in descending order.

Statistical Analysis

An analysis of variance (ANOVA) with post-hoc Tukey Test was performed to test for differences in clinical and traditional sleep data regarding OSA severity and controls. We examined differences in exponential decay times by consideration of standard deviations. The influence of OSA severity and potential confounders on transition patterns was tested by multivariate analysis of variance (MANOVA) restricted to subjects investigated in Antwerp (n=808), with the 25 most common two-step-transition patterns as dependent variables and four categories (OSA severity, age-group, gender, BMI-group) as independent variables. Analysis was limited to main effects. Pillai's trace was chosen to evaluate significance and partial η^2 was considered as indicator, how much variance of the dependent variable (transition) is explained by the independent variable (group). Effect sizes were interpreted based on benchmarks for small (η^2=0.01), medium (η^2=0.06) and large (η^2=0.14) effects provided by Cohen (Cohen, 1988). Multiple comparisons were performed by a Tukey-test. Subsequently, we performed t-tests to examine differences in traditional metrics and two-step transitions (only significant transitions regarding OSA severity) between patients with diagnosed and without diagnosed arterial hypertension and between patients with (ESS>10) and without (ESS≤10) daytime sleepiness. Effect sizes were indicated by Cohen's d, based on benchmarks for small (d=0.2), medium (d=0.5) and large (d=0.8) effects provided by Cohen. Cohen's d is calculated based on standardized mean differences and a pooled standard deviation. (Cohen, 1988; Lakens, 2013). Regarding arterial hypertension, we focussed on patients with high systolic (RRsyst.≥140 mmHg) and diastolic blood pressure (RRdiast.≥90 mmHg), irrespectively of diagnosis or cardiovascular medication. We repeated

the testing for daytime sleepiness and diastolic blood pressure in a multicenter cohort (validation cohort). Level of significance was always set at $p<0.05$.

Results

Patients from the development cohort

Clinical data as well as sleep data of OSA severity categories and a control group are presented in table 1. The highest values for age and BMI were found in severe OSA patients. Severe OSA patients spent more time in N1 and N2, whereas time in N3 and REM was reduced. The total number of transitions was higher in moderate and severe OSA patients compared with controls. However, neither TST nor SE showed significant differences amongst subgroups. Interestingly, the average ESS results of OSA patients did not differ from controls.

Cumulative distributions of wake and sleep states

The cumulative distributions of wake episode durations with respect to OSA severity are presented in figure 1, both in a double logarithmic and a semi logarithmic plot. For all three OSA groups and the control group, the decay was very well described by power-law fits up to durations of approximately 30 minutes (represented by straight lines in panel a). The ratios of fitting errors were under unity for all subgroups, indicating superiority for power-law fits, see table 2. The power law exponent α was nearly the same for all groups ($\alpha = 1$). Yet, minor deviations were observed not only in relationship to OSA severity (0.95 in moderate and 1.05 in severe OSA), but also in terms of BMI and age. The cumulative distributions of sleep state durations are presented in figure 2. In this semi- logarithmic plot, data of all groups were well

described by exponential fits up to durations of approximately 90 minutes (represented by dashed lines). The ratios of fitting errors showed a strong advantage of exponential distributions compared to power-law distributions for all groups. Specifically, ratios of fitting errors varied from 28.9 in severe OSA and 152.8 in mild OSA, see table 2. The exponential decay was nearly the same ($\tau \approx 24$ min) for almost all groups, except for age. With increasing age, the exponential decay decreased. Young patients had $\tau = 27.28$ min (68% confidence interval=27.20, 27.36), middle aged 23.84 min (68% confidence interval=23.79,23.89), and elderly 22.55 min (68% confidence interval=22.49,22.61).

The influence of OSA severity and potential confounders on transition patterns

Transition matrices showing frequencies of one-step transitions between wake and sleep states for each OSA severity category and the control group are presented in figure 3, where frequencies are marked by color. In all groups, the most frequent transitions happened between wake and light sleep. In healthy controls, transitions between N1 and N2 were less salient. To evaluate the influence of OSA severity and potential confounders on transition patterns, we performed a MANOVA (n=808). In our model, we focused on main effects of our independent variables OSA severity, age-group, gender, BMI-group. The 25 most frequent two-step transitions were our dependent variables (Figure 4). Pillai's trace was significant for each of the four groups. The transition N1→N2→wake ($p<0.001$, partial $\eta^2=0.025$) was associated with OSA severity, see table 3. In controls, N1→N2→wake transitions were less frequent (5.6±3.6) compared to both moderate (7.1±5.1; $p=0.048$) and severe (8.0±5.5; $p<0.001$) OSA patients. Furthermore, severe OSA patients had more N2→wake→N2, wake→N2→wake, N2→N1→N2 and N1→N2→N1 transitions compared to controls.

However, the strongest relation was found for the transitions wake→N1→wake ($p<0.001$, partial $\eta^2=0.045$) and N1→wake→N1 ($p<0.001$, partial $\eta^2=0.037$) regarding age. Older patients had significantly more wake>N1>wake transitions (8.0±8.2) than middle aged (4.8±4.9, $p<0.001$) and young patients (4.1±5.2, $p<0.001$). Likewise, N1→wake→N1 transitions were more frequent in patients 60 years or older. Furthermore, gender showed a relation to the pattern N2→N3→wake ($p<0.001$, partial $\eta^2 = 0.039$). Women had significantly ($p<0.001$) more transitions (2.5±2.1) than men (1.5±1.5). The influence of BMI on transition patterns was extremely mild. The largest effect was observed for the transition REM→wake→N1 ($p=0.010$, partial $\eta^2=0.011$), which was the lowest in obese patients. However, gender had an even stronger effect (partial $\eta^2=0.024$). All significant transitions regarding age, BMI and gender are presented in the supplement (S1).

Two-step transitions in hypertensive patients and in patients with daytime sleepiness

We analyzed traditional polysomnographic metrics and two-step transitions in OSA patients (n=703) with hypertension and sleepiness. Hypertensive patients (n=220) had a shorter TST (370.3±67.1 min vs. 387.2±62.6 min, $p=0.001$, $d=0.260$), a lower SE (83.8±11.6 % vs. 87.5±9.7 %, $p<0.001$, $d=0.346$), more WASO (72.0±53.4 min vs. 55.1±42.5 min, $p<0.001$, $d=0.350$) and more NASO (25.7±15.7 vs. 23.3±12.7, $p=0.042$, $d=0.168$) compared to normotensive ones (n=483). The total number of one-step and two-step transitions did not differ in hypertensive patients. Subsequently, we focused separately on high systolic (RRsyst.≥140 mmHg) and diastolic (RRdiast.≥90 mmHg) blood pressure. 201 patients with high systolic pressure (103 were diagnosed as hypertensive) showed no differences in both traditional metrics and two-step transitions. In 159 patients with high diastolic blood pressure (67 were diagnosed as hypertensive), N2→N3→wake transitions were less frequent

(1.4±1.6, p=0.018, d=0.242) compared to patients with normal diastolic pressure (1.8±1.7). In patients with excessive daytime sleepiness (n=281) the total number of transitions (100.9±48.5 vs. 91.1±39.4, p=0.005, d=0.222) was elevated compared to non-sleepy patients (n=422). In addition, transition patterns of N2→N1→N2 (7.0±13.5 vs. 4.7±9.0, p=0.016, d=0.200), N1→N2→N1 (6.5±13.3 vs. 4.5±9.3, p=0.028, d=0.174) were more frequent in patients with daytime sleepiness. Interestingly, TST (390.7±63.1 min vs. 376.0±64.7 min, p=0.003, d=0.230) and SE (87.3±10.2 % vs. 85.7±10.6 %, p=0.039, d=0.154) were also higher in patients with daytime sleepiness.

Validation cohort

To validate the association between sleep-stage transitions and the outcome parameters like daytime sleepiness and elevated diastolic blood pressure, we repeated the analysis in 425 OSA patients from 10 different centers. Compared to the development cohort (single center), heterogeneity in anthropometrics and sleep data was observed. Patients in the multicenter cohort were older (54.0±11.4 y vs. 49.7±11.0 y, p<0.001), had a higher AHI (42.3±25.1/h vs. 29.7±21.1/h, p<0.001) and a shorter TST (358.6±101.6 min vs. 381.9±64.4 min, p<0.001). A complete comparison between the development cohort and the validation cohort is presented in the supplement (S2). In the multicenter cohort, N2→N1→N2, N1→N2→N1, N2→N3→N2, and N3→N2→N3 transitions were more frequent compared to the single center cohort and to the control group (Figure 5). In 152 patients of the multicenter cohort with high diastolic blood pressure we identified higher transition rates for N2→N1→N2 (p=0.023, d=0.239), N1→N2→N1 (p=0.016, d=0.254), N1→N2→wake (p=0.030, d=-0.231), and N2→REM→N1

(p=0.020, d=0.231) and an increased percentage of N1 (p<0.001, d=0.403) compared to normotensive patients. Fewer N2→REM→wake transitions (p=0.006, d=0.208) and reduced N3 (p=0.001, d=0.331) and REM (p=0.013, d=0.255) were also observed. The transition pattern N2→N3→wake did not differ compared to normotensive patients. In 194 patients of the multicenter cohort with daytime sleepiness we observed more N2→wake→N2 (p<0.001, d=0.406) and wake→N2→wake (p<0.001, d=0.402) transitions and an increased percentage of N2 (p=0.014, d=0.246) compared with non-sleepy individuals. In addition, fewer N1→N2→N3 (p=0.020, d=0.235), N1→N2→REM (p=0.001, d=0.322) and N2→REM→N1 (p=0.010, d=0.240) could be observed. The transition patterns N2→N1→N2 and N1→N2→N1, identified in the single center cohort, did not differ significantly.

Discussion

In our study we identified three major findings. First, independent of OSA severity and potential confounders, wake state durations followed a power-law distribution, while sleep state durations were characterized by an exponential distribution. Second, sleep dynamics are not only influenced by OSA severity, but also age and gender confound sleep-stage transitions in unique ways. Third, we describe an association between sleep-stage transition patterns and relevant OSA-related problems like arterial hypertension and daytime sleepiness.

Regarding the distributions of wake and sleep durations, our findings confirm that wake states follow a power-law distribution, while sleep states follow an exponential distribution, independent of OSA severity or potential confounders. Power-law behavior is common in

nature and implies a long-term memory. Specifically, the duration of the current wake-state is influenced by the durations of all previous wake episodes (Lo et al., 2004; Newman, 2005). The corresponding control system seems to work unimpaired in OSA patients and independent of OSA severity, while it is altered in narcolepsy patients (Zhang et al., 2017). For the durations of sleep states, we observed exponential distributions with characteristic time scales ($\tau \approx 24$ min). With age, the exponential decay time decreases (from $\tau = 27.28$ min in young to $\tau = 22.55$ min in elderly). These findings agree with results from Zhang et al. (Zhang et al., 2017). However, we noticed tremendous variations of the accuracy of the fits regarding all groups, but especially in relationship to OSA severity. These deviations could indicate limitations in describing wake states by power-law and sleep states by exponential distributions. An alternative approach favors multi-exponential processes for both wake and sleep durations (Bianchi et al., 2010). Based on this, Bianchi et al. introduced a Markov model, based on multi-exponential stage dynamics and probabilistic transitions, to quantify sleep fragmentation (Bianchi et al., 2012). This model was limited, because it solely considered exit rates to determine the mean time spent in any state and disregarded the influences of previous transitions. Our approach to analyze two-step transition could be extended and incorporated into this Markov sleep model.

We observed unique two-step transitions dependent on OSA severity, age and gender. Severe OSA patients had notably more N1→N2→wake, N2→wake→N2 and N2→N1→N2 transitions compared with controls, which agrees with results obtained by Schlemmer et al. and implies a N2 vulnerability (Schlemmer et al., 2015). A N2 vulnerability was also found in insomnia patients (Wei et al., 2017). In patients over 60, we have seen significantly higher frequencies for N1→wake→N1 and wake→N1→wake transitions. Women had more

N2→N3→wake transitions than men. The findings regarding potential confounders of sleep apnea coincide with results obtained by Redline et al., who stated a greater influence on sleep architecture by age and gender compared to sleep disordered breathing and a weak influence of BMI (Redline et al., 2004). However, the effects must be interpreted as small (partial η^2 always <0.06) (Cohen, 1988). Yet, some references recommend not using Cohen's benchmarks to interpret effect sizes. Instead, they suggest relating results to similar literature and explaining practical consequences. (Olejnik and Algina, 2003, Thompson, 2007, Lakens, 2013). Chervin et al. observed a shorter mean N2 duration in children with OSA with a large effect size (d of approximate 1.15 at baseline and 0.97 at a one year follow up), which is impressive, suggesting the potential of dynamic parameters (Chervin et al., 2009). Admittedly, we observed smaller effect sizes regarding transition patterns and their association to OSA severity and potential confounders. Nevertheless, based on these transition patterns with small effect sizes, we could link sleep fragmentation directly to OSA-related clinical outcomes.

In the development cohort, the transition pattern N2→N3→wake was the only polysomnographic sleep parameter distinguishing OSA-patients with high diastolic blood pressure from patients with normal diastolic blood pressure. Interestingly, the same pattern was significantly lower in men (male predominance with 84.9% in the group of OSA patients with high diastolic blood pressure). In the validation cohort, we observed higher transition rates for N2→N1→N2 and N1→N2→N1 and a reduced time in N3. These findings could result from a distinct N2 vulnerability due to more severe disease (AHI/h: 42.3±25.1 vs. 29.7±21.1, p<0.001) in the validation cohort. However, small effect sizes (d always under 0.5) and heterogeneity of the hypertensive subgroups regarding diagnosis and medication

limit this observation, especially since different β-blockers influence sleep in various manner (Yilmaz et al., 2008). In this context, it is further necessary to consider the possible influences of scoring differences in the validation cohort. A rather low scoring agreement in OSA patients (Danker-Hopfe et al., 2004; Penzel et al., 2003), aggravated by pooling scorings from different centers, could impair an analysis of two-step transitions. Future studies could compare our results with recordings scored by computerized algorithms. Despite these unresolved issues, this association of sleep fragmentation and diastolic hypertension is impressive, since OSA-related arterial hypertension is predominantly diastolic (Baguet, 2005).

In patients of the development cohort with daytime sleepiness, a higher frequency of N1→N2→N1 and N2→N1→N2 transitions could be observed. Interestingly, TST and SE were higher in sleepy patients, which agrees with previous results (Roure et al., 2008). The highest effect sizes (still small according to Cohen's benchmarks) were observed for N2→wake→N2 (d=0.406) and wake→N2→wake transitions (d=0.402) in the validation cohort. Nevertheless, we observed higher frequencies of alternating (symmetric) patterns in sleepy patients both in the development cohort (e.g. N2→N1→N2) and in a validation cohort (e.g. N2→wake→N2). Interestingly, asymmetry in sleep stage transitions was described to be fundamental in sleep dynamics. Furthermore, decreased asymmetry was observed in OSA patients (Lo et al., 2013). We observed not only higher frequencies of symmetric patterns (e.g. N2→wake→N2 and N2→N1→N2) in severe OSA patients but also an association between these alternating transition patterns to sleepiness. Admittedly, we simplified sleepiness very strongly by just comparing patients with ESS > 10 and ≤ 10. However, in a previous study by Laffan et al., using two 5-point Likert scales on sleep depth

and restfulness, lighter and restless sleep was associated with a higher sleep-stage transition rate (Laffan et al., 2010). In combination with our results, it may be suggested that sleep-stage transition analysis and particularly the analysis of alternating patterns related to simplified measurements of sleepiness could help to evaluate sleep continuity.

Our retrospective study has several strengths and limitations. Our study is based on ESADA. This large and standardized database enabled both a single-center analysis to minimize a possible influence of scoring differences and a multicenter analysis for validation. We could ensure a large patient cohort with different OSA degrees, strict exclusion criteria and without any missing values for ESS or arterial hypertension. As limitation we have to state that patients were analyzed only for a single night under laboratory conditions. Since light off and light on times were not available consistently, we could not assess sleep latency and had to exclude sleep latency when calculating sleep efficiency. Despite an exclusion of patients with psychiatric disease, a few patients used antidepressants or another medication which may impact sleep and sleep stage transitions (Wilson and Argyropoulos, 2005). Furthermore, patients of the development cohort and validation cohort were not matched for age, BMI or gender.

Conclusion

Our analysis of sleep architecture revealed that wake states follow a power-law distribution, while sleep states follow an exponential distribution, independent of OSA severity or potential confounders. Effects of OSA severity and potential confounders on sleep architecture are small, but transition patterns still link sleep fragmentation directly to OSA-related clinical outcomes like arterial hypertension and daytime sleepiness.

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List of abbreviations

OSA= obstructive sleep apnea

TST= total sleep time

ESS= Epworth sleepiness scale

NASO= number of awakenings

ESADA= European Sleep Apnea Database

AHI= apnea-hypopnea index

PAP-therapy= positive airway pressure therapy

CSA= central sleep apnea

BMI= body mass index

PSG= polysomnography

AASM= American Academy of Sleep Medicine

SE= sleep efficiency

WASO= wakefulness after sleep onset

NASO= number of awakenings

TRANS= total number of transitions

ANOVA= analysis of variance

MANOVA= multivariate analysis of variance

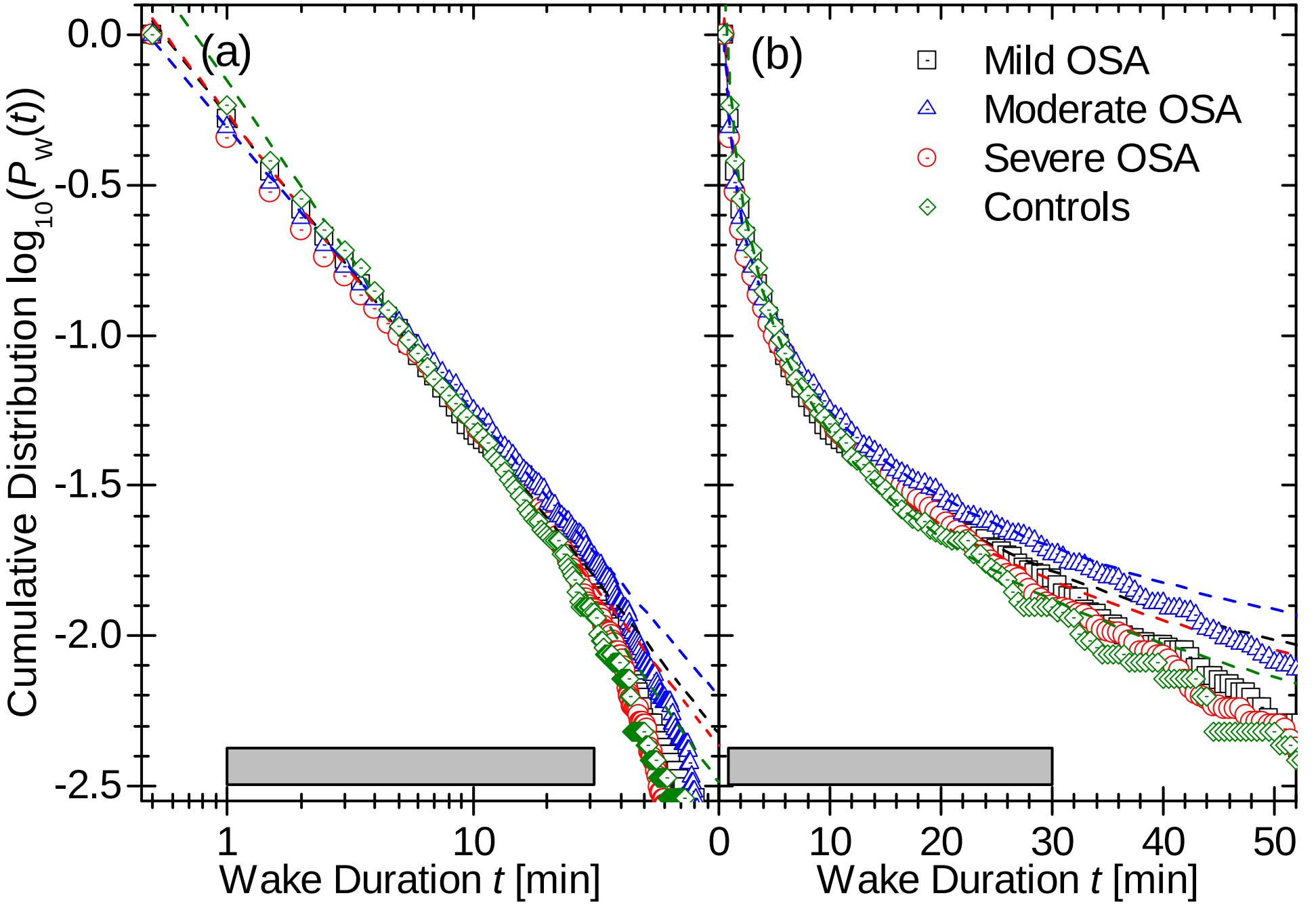
Figure 1. Cumulative distributions of wake durations for each OSA group and for controls. Grey bars indicate the analyzed interval from 1 to 30 min. In (a) dashed lines represent the best fits for a power law in a double logarithmic plot. In (b) dashed lines represent the best fits for an exponential distribution in a semi logarithmic plot. Independent of OSA severity, wake durations rather followed power law than exponential distributions.

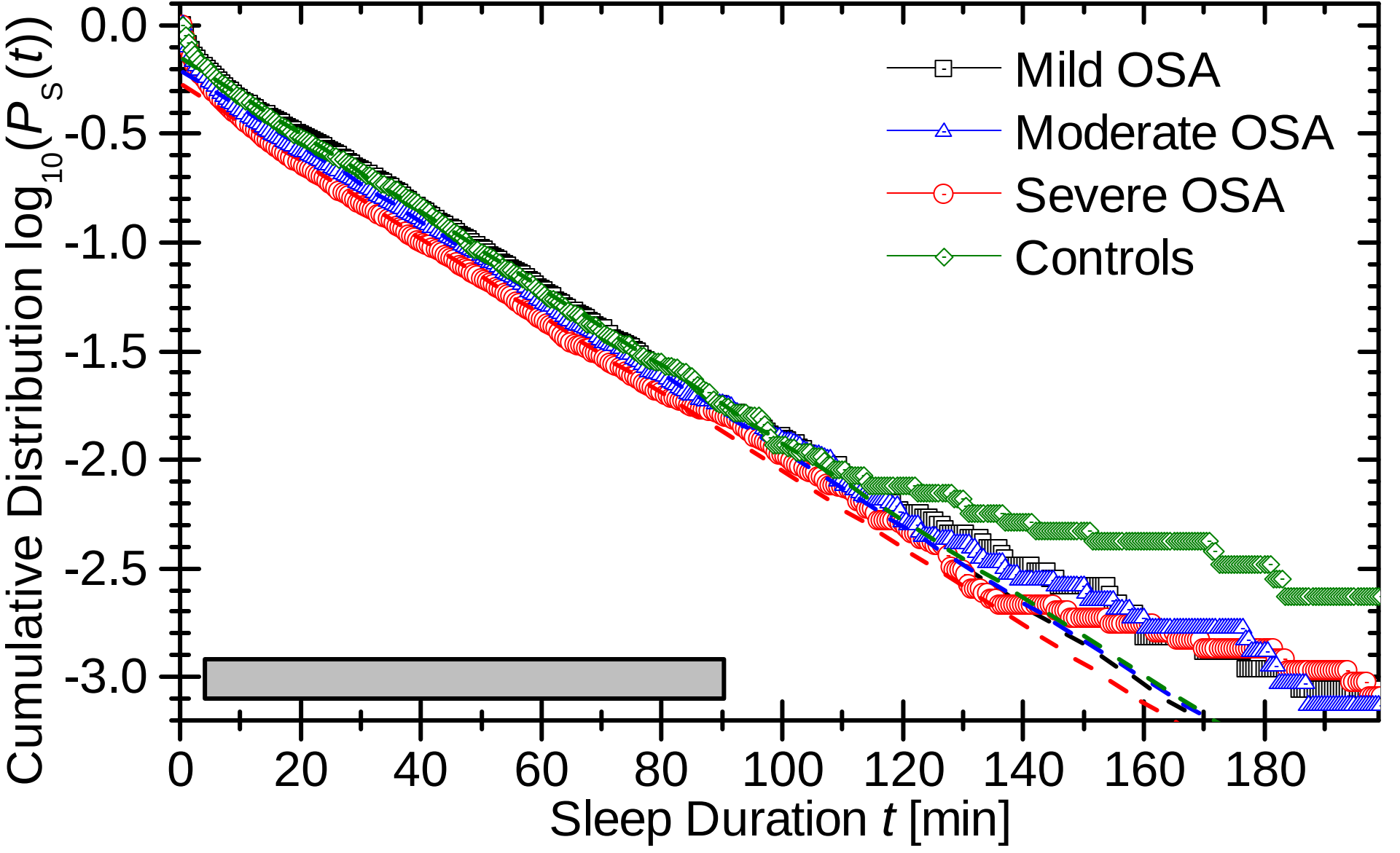
Figure 2. Cumulative distribution of sleep durations for each OSA group and for controls are plotted with their best fit for exponential distribution in this semi logarithmic plot. The grey bar indicates the analyzed interval from 3 to 90 min.

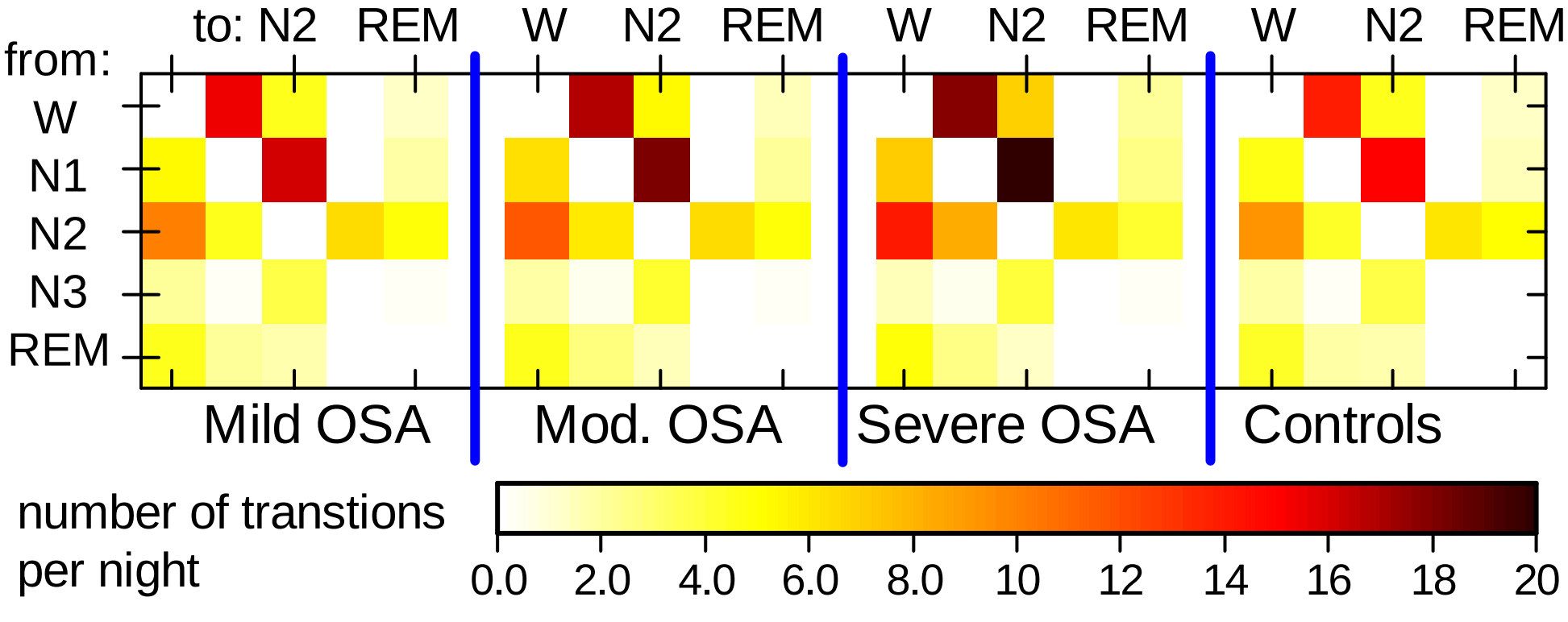
Figure 3. Average number of transitions per night between wake and sleep states for each OSA severity group and the control group. Transitions are from the states noted on the left to the states noted on the top. The average number of transitions is color coded for each square. In controls, transitions between N1 and N2 were less salient.

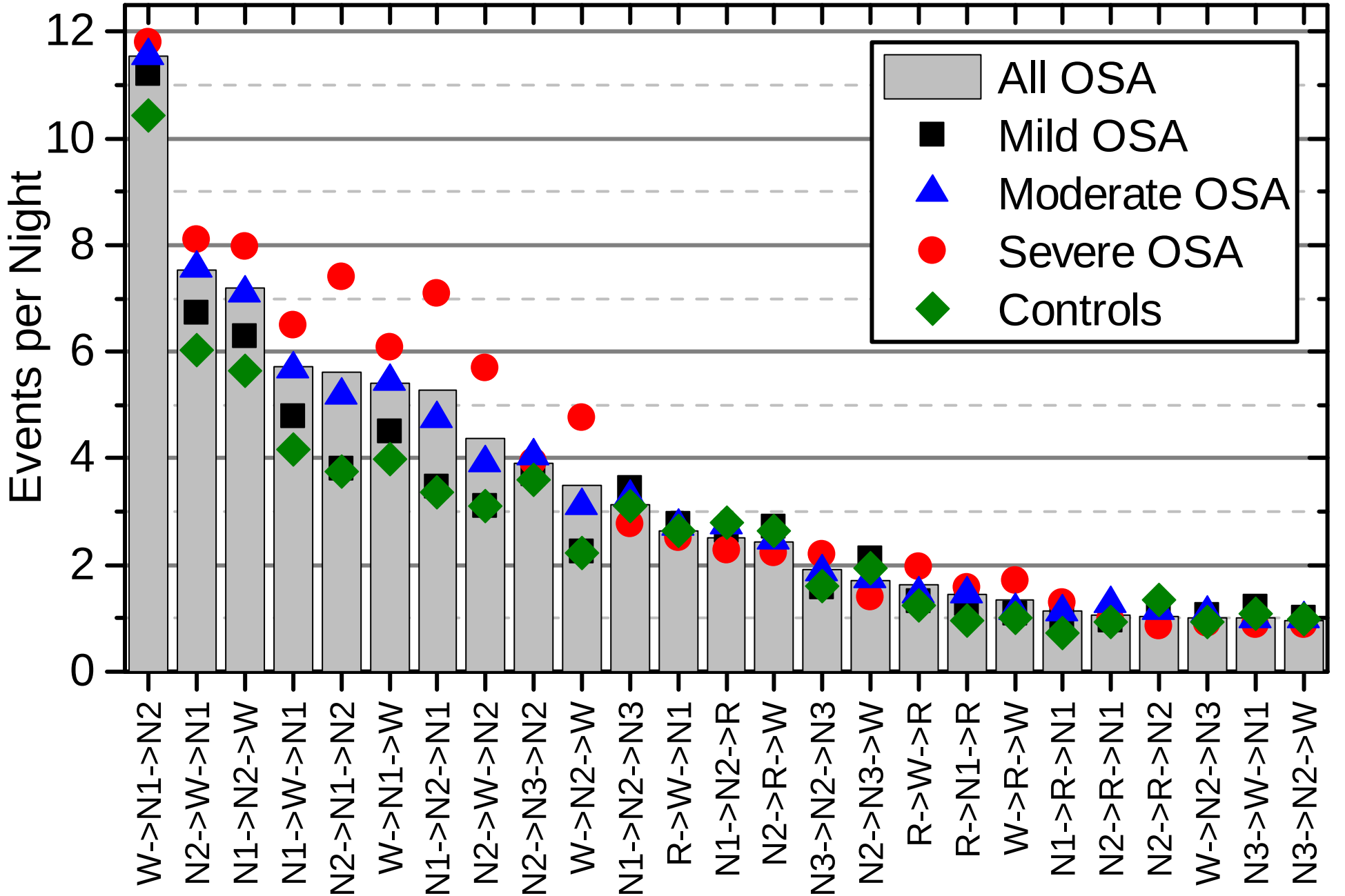
Figure 4. Representation of the 25 most frequent two-step transitions in descending order for each OSA severity group and for the control group. Grey bars indicate the average number in all OSA groups and determine the order in the horizontal axis.

Figure 5. Representation of the 25 most frequent two-step transitions in descending order for the laboratory of Antwerp (development cohort), a multicenter cohort (validation cohort) and controls. Grey bars indicate the average number in all OSA groups and determine the order in the horizontal axis.


(a)
(b)
Mild OSA
Moderate OSA
Severe OSA
Controls
Cumulative Distribution $\log_{10}(P_W(t))$
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
1
10
0
10
20
30
40
50
Wake Duration t [min]
Wake Duration t [min]


Cumulative Distribution $\log_{10}(P_S(t))$
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
0
20
40
60
80
100
120
140
160
180
Sleep Duration t [min]
Mild OSA
Moderate OSA
Severe OSA
Controls


to: N2 REM W N2 REM W N2 REM W N2 REM
from:
W
N1
N2
N3
REM
Mild OSA
Mod. OSA
Severe OSA
Controls
number of transtions per night
0.0 2.0 4.0 6.0 8.0 10 12 14 16 18 20


Events per Night
0
2
4
6
8
10
12
All OSA
Mild OSA
Moderate OSA
Severe OSA
Controls
W->N1->N2
N2->W->N1
N1->N2->W
N1->W->N1
N2->N1->N2
W->N1->W
N1->N2->N1
N2->W->N2
N2->N3->N2
W->N2->W
N1->N2->N3
R->W->N1
N1->N2->R
N2->R->W
N3->N2->N3
N2->N3->W
R->W->R
R->N1->R
W->R->W
N1->R->N1
N2->R->N1
N2->R->N2
W->N2->N3
N3->W->N1
N3->N2->W

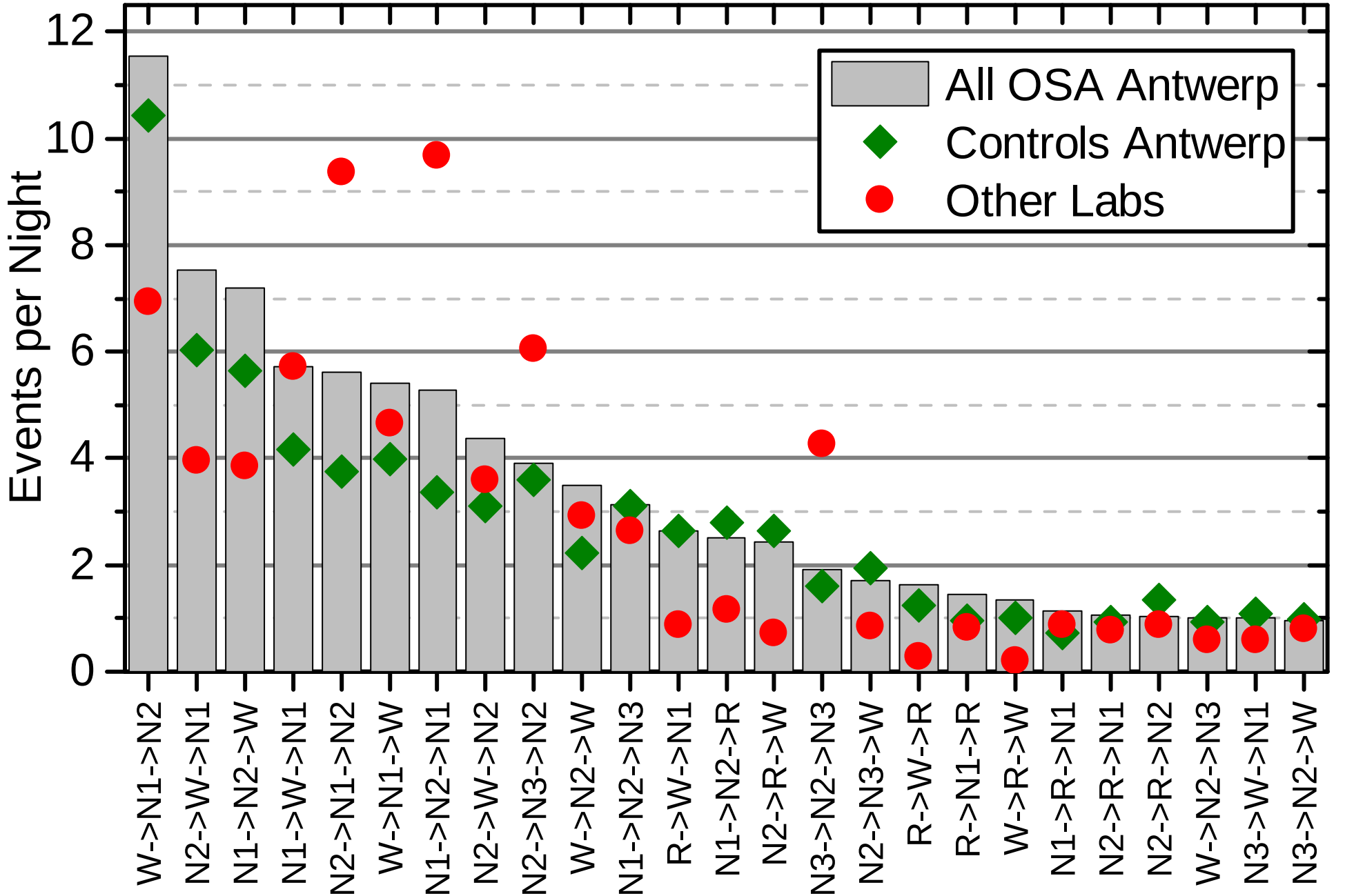

Events per Night
0
2
4
6
8
10
12
All OSA Antwerp
Controls Antwerp
Other Labs
W->N1->N2
N2->W->N1
N1->N2->W
N1->W->N1
N2->N1->N2
W->N1->W
N1->N2->N1
N2->W->N2
N2->N3->N2
W->N2->W
N1->N2->N3
R->W->N1
N1->N2->R
N2->R->W
N3->N2->N3
N2->N3->W
R->W->R
R->N1->R
W->R->W
N1->R->N1
N2->R->N1
N2->R->N2
W->N2->N3
N3->W->N1
N3->N2->W

Table 1. Clinical groups and sleep data (mean).				
	Controls	Mild OSA	Moderate OSA	Severe OSA
Number	105	209	222	272
Age (years)	43.6	**47.4**	**49.7**	**51.6**
BMI (kg/m^2)	26.7	27.5	**29.1**	**31.5**
Gender* (males/females)	64/41	156/53	175/47	239/33
AHI (#/h)	2.4	**9.6**	**22.4**	**51.1**
TST (min)	381.9	389.1	378.7	379.0
SE (% TST)	87.4	87.7	85.5	85.9
N1 (% TST)	6.8	7.2	8.2	**10.4**
N2 (% TST)	51.0	49.8	50.6	**55.2**
N3 (% TST)	21.0	21.8	20.8	**16.3**
REM (% TST)	21.3	21.3	20.4	**18.1**
WASO (min)	54.4	54.6	64.4	61.6
NASO	19.8	21.2	23.5	**26.7**
TRANS	79.8	84.9	**93.9**	**103.7**
ESS	9.1	9.3	9.3	9.8

Notes: BMI=body mass index; ESS=Epworth sleepiness scale; AHI=apnea-hypopnea index; TST=total sleep time; SE=sleep efficiency; WASO= wake after sleep onset; NASO= number of awakenings after sleep onset; TRANS=number of transitions.

Bold numbers represent significance compared to controls (Level of significance <0.05 according to an ANOVA and Tukey-test for multiple comparisons).

*Pearson Chi^2: $p<0.001$

Table 2. Results for power-law and exponential distribution fits in each subset. The power-law exponent α is presented in column 1. For wake states, the ratio of fitting errors (X_p/X_e) indicates an advantage for power laws, whereas sleep states are better described by exponential distributions. Further sleep states are presented with the exponential decay time τ(min).

	Wake States		Sleep States	
Group	α	X_p/X_e	τ (min)	X_p/X_e
Male	1.02	0.05	24.39	114.4
Female	1.04	0.07	24.33	118.6
<40y	1.01	0.03	27.28	85.2
40y to 60y	1.05	0.05	23.84	153.5
60y and Older	0.99	0.11	22.55	84.2
Mild OSA	1.03	0.03	23.92	152.8
Moderate OSA	0.95	0.01	24.78	99.7
Severe OSA	1.05	0.27	24.39	28.9
Normal Weight	1.09	0.06	23.87	156.4
Overweight	0.99	0.02	24.47	111.8
Obesity	1.04	0.13	24.52	79.2
Controls	1.17	0.05	24.42	93.8

Table 3. Significant transitions regarding OSA severity after performing MANOVA. Bold numbers indicate significance to controls. Partial Eta-Squared states, how much variance of the dependent variable (transition) is explained by the independent variables (group). Transitions are presented in descending order dependent on partial eta-squared.

2-step transition		OSA				
	Controls N=105	Mild N=209	Moderate N=222	Severe N=272	p	partial η^2
N1-N2-wake	5.6±3.6	6.3±4.3	**7.1±5.1**	**8.0±5.5**	<0.001	0.025
N2-wake-N2	3.1±6.4	3.1±4.0	3.9±4.8	**5.7±10.3**	<0.001	0.022
N2-N1-N2	3.8±5.5	3.8±5.3	5.2±7.9	**7.4±15.5**	0.001	0.020
wake-N2-wake	2.3±6.5	2.1±3.1	2.9±4.0	**4.6±9.7**	0.002	0.019
N1-N2-N1	3.3±5.5	3.5±5.1	4.7±7.9	**7.1±15.6**	0.002	0.019
N2-N3-wake	1.9±1.8	2.1±1.9	1.7±1.6	**1.4±1.5**	0.005	0.016
N1-N2-N3	3.0±1.7	3.4±1.9	3.3±2.0	2.8±2.0	0.005	0.016
N2-REM-N1	1.0±1.4	0.9±1.1	1.3±1.7	1.0±1.4	0.008	0.015
N2-REM-wake	2.7±2.1	2.7±1.7	2.5±1.7	2.2±1.8	0.013	0.013
N2-wake-N1	6.0±3.7	6.6±3.4	7.3±5.2	**7.9±5.3**	0.016	0.013
N1-N2-REM	2.8±1.7	2.6±1.6	2.7±1.8	**2.3±1.7**	0.034	0.011

Note: Level of significance was set at 0.05.

S1. Significant transitions regarding age, BMI and gender after performing MANOVA. Bold numbers indicate significance respectively age 60 and older and obesity. Partial Eta-Squared states, how much variance of the dependent variable (transition) is explained by the independent variables (group). Transitions are presented in descending order, dependent on partial eta-squared and for each group separately.

2-step transition	Age-Group				
	Under 40 N=168	40 to 60 N=499	60 and older N=141	p	partial η^2
wake-N1-wake	**4.1±5.2**	**4.8±4.9**	8.0±8.2	<0.001	0.045
N1-wake-N1	**4.6±5.7**	**5.1±5.2**	8.1±8.3	<0.001	0.037
REM-wake-N1	2.2±1.8	2.7±1.9	2.6±2.2	0.001	0.019
N2-REM-wake	2.2±1.6	2.6±1.9	2.3±1.7	0.002	0.016
wake-REM-wake	**0.7±1.5**	**1.2±2.8**	1.9±4.1	0.002	0.015
N2-REM-N1	**1.3±1.5**	1.0±1.4	0.8±1.2	0.005	0.013
N2-wake-N2	3.0±3.6	4.8±8.5	3.5±5.2	0.006	0.013
REM-wake-REM	**1.1±1.8**	1.6±3.0	2.2±4.2	0.010	0.012
N3-wake-N1	1.0±1.3	**0.9±1.1**	1.2±1.4	0.007	0.012
N1-N2-N3	3.2±1.9	**3.0±1.9**	3.5±2.3	0.017	0.010
N2-REM-N2	**1.3±1.8**	**1.1±2.0**	0.7±1.2	0.026	0.009
wake-N2-wake	2.0±2.9	3.6±8.0	2.9±4.5	0.026	0.009
wake-N1-N2	**10.6±5.9**	11.4±5.3	12.5±6.3	0.044	0.008

2-step transition	BMI-Group				
	Normal Weight N=154	Overweight N=352	Obese N=302	p	partial η^2
REM-wake-N1	2.7±2.0	**2.8±2.1**	2.3±1.8	0.010	0.011
N1-N2-N3	**3.1**±1.8	**3.3±2.0**	2.9±1.9	0.030	0.009

2-step transition	Gender-Group			
	Male N=634	Female N=174	p	partial η^2
N2-N3-wake	1.5±1.5	2.5±2.1	<0.001	0.039
REM-wake-N1	2.7±2.0	2.1±1.9	<0.001	0.024
wake-N2-N3	0.9±1.3	1.4±1.8	<0.001	0.022
N2-REM-wake	2.6±1.8	2.2±1.8	0.002	0.012
N3-wake-N1	0.9±1.1	1.3±1.5	0.004	0.010
N1-N2-N3	3.0±1.9	3.5±2.1	0.010	0.008
N1-wake-N1	5.8±6.4	4.3±4.4	0.009	0.009
wake-N1-wake	5.5±6.2	4.1±4.1	0.009	0.009

Note: Level of significance was set at 0.05.

S2. Clinical and sleep data (mean±SD) of the development cohort and the validation cohort.		
	Development cohort	Validation cohort
Number	703	425
Age (years)	49.7±11.0	**54.0±11.4**
BMI (kg/m^2)	29.5±5.0	**32.9±6.4**
Gender* (males/females)	570/133	335/90
AHI (#/h)	29.7±21.1	**42.3±25.1**
TST (min)	381.9±64.4	**358.6±101.6**
SE (% TST)	86.3±10.5	86.9±12.0
N1 (% TST)	8.8±7.7	**14.8±13.8**
N2 (% TST)	52.1±11.3	54.1±19.5
N3 (% TST)	19.4±10.0	17.8±15.1
REM (% TST)	19.8±6.3	**13.3±7.6**
WASO (min)	60.4±46.8	**52.9±50.2**
NASO	24.0±13.7	**15.8±16.9**
TRANS	95.0±43.5	**83.5±85.0**
ESS	9.5±5.1	**10.1±5.1**

Notes: BMI=body mass index; ESS=Epworth sleepiness scale; AHI=apnea-hypopnea index; TST=total sleep time; SE=sleep efficiency; WASO= wake after sleep onset; NASO= number of awakenings after sleep onset; TRANS=number of transitions.

Bold numbers represent significance compared to Development Cohort (Level of significance <0.05 according to t-test).

*Pearson Chi^2: p=0.356

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