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

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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 polysomnographic metrics are not always straightforward correlated with daytime sleepiness and do not describe OSA patients with hypertension appropriately. We tested cumulative distributions of wake as well as sleep states for power-laws (exponent α) and exponential distributions (decay time λ) in dependency of OSA severity and potential confounders. We applied a new approach to assess 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 analyzed hypnograms from mild (n=213), moderate (n=235) and severe (n=277) OSA patients scored in a one center in the European Sleep Apnea Database (ESADA) and from 187 healthy controls (two nights per subject). 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 not only influenced by OSA severity, but even more by age and gender. In a single center cohort, only N2->N3->wake transitions could distinguish patients with an elevated diastolic blood pressure (RR≥90 mmHg). Patients with daytime sleepiness (ESS>10) had a higher total number of transitions, more N2->N1->N2 and N1->N2->N1 transitions. An analysis of two-step transitions links sleep fragmentation directly to clinical aspects like OSA-related 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 resulting in fast and complete 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). For example, OSA is associated with night-time and diastolic hypertension and often causes difficulties in diagnostic procedures (Baguet et al., 2005; Baguet et al., 2013).

The current polysomnographic evaluation of sleep quality is based upon absolute durations like total sleep time (TST) and sleep latencies. However, the metrics are not always straightforward correlated with daytime symptoms, such as day time sleepiness usually assessed with the Epworth Sleepiness Scale (ESS) (Chervin and Aldrich, 1999). Furthermore, the ability to appropriately describe subjects with OSA is limited. Transition analysis revealed that severe OSA patients have more wake to NREM sleep and NREM sleep to wake transitions compared to subjects without OSA (Swihart et al., 2008). In addition, a higher transition rate was associated with more superficial and restless sleep independent of traditional polysomnographic metrics (Laffan et al., 2010).

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 stage 2 vulnerability in insomnia (Wei et al., 2017). In adult OSA patients, an increased number of sleep-stage transitions has been reported, induced by a higher decay rate of NREM and REM sleep bouts (Bianchi et al., 2010). In children with OSA, a shorter mean stage 2 duration was seen compared to children without OSA (Chervin et al., 2009). Apart from the presence of a specific sleep disorder, several studies examined age as a potential confounder. Specifically, with increasing age, the exponential decay time λ decreases and older subjects have a higher number of awakenings (NASO) (Zhang et al., 2017; Klerman et al., 2004). A recent study from Schlemmer et al. introduced a two step-transition analysis, that revealed a modulation of sleep-stage transitions by age and sleep disorder and suggested typical patterns (Schlemmer et al., 2015).

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

## Methods

### Participants

Our retrospective study is based on the European Sleep Apnea Database (ESADA). This project is an association of 28 European sleep disorder centers and has the aim to implement standards in the diagnosis of OSA. Clinical information and sleep data from OSA patients are registered in a web-based report form (Hedner et al., 2011). In the context of this study, 1243 patients from the sleep center of Antwerp were assessed. Exclusion criteria were: age under 18, an apnea-hypopnea-index (AHI) <5, depression or other psychiatric disease, narcolepsy, restless legs syndrome, insomnia, opioid-induced central sleep apnea, bronchial asthma, fibromyalgia, malignancy or PAP-therapy. Based on these criteria, 725 patients remained with a mean age of 49.7±10.9 years and a male predominance (81.1%). 111 patients had additional or predominant central sleep apnea (CSA). Approval was obtained from the Charité ethics committee (EA1/139/07). The decision to analyze sleep stage transitions in one laboratory only was made to minimize the influence of possible differences in scoring practice. We classified the patients in categories based on four variables: OSA severity, age, gender and BMI**.** The severity categories were 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 old (≥60y) subjects. 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. For controls, the SIESTA project provided 374 nights of 187 healthy subjects (two nights per patient) recorded in eight European sleep laboratories and scored with increased effort (two individual scorers plus one consensus scorer for each subject) (Klosch

et al., 2001). The subgroup (or part of it) has already been analyzed in several preceding studies (Zhang et al., 2017; Lo et al., 2002; Danker-Hopfe et al., 2004). Registered subjects gave informed consent and had no neurological or psychiatric disease.

**Polysomnography (PSG)**

Our analysis was hypnogram based**.** In an hypnogram, wake and sleep stages (Wake, N1, N2, N3, and REM) were scored visually in 30 seconds epochs. Hypnograms were exported from the original PSG report. For the system BrainLab RT (OSG, Rumst, Belgium) a guidance was provided. All patient PSG's were based on the AASM scoring rules from 2007 (Hedner et al., 2011; Iber et al., 2007). PSG's of the control group were based on the recommendations of Rechtschaffen & Kales where we summarized NREM3 and NREM4 to N3 (Rechtschaffen and Kales, 1968). To minimize the influence of recording- and scoring practice, we decided to compute 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) directly from the hypnogram. Consequently, we ignored wake times at the beginning and at the end. 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, and whether sleep-state durations followed an exponential distribution. 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 -α representing power-laws and a semi-logarithmic plot with slopes -1/ λ representing exponential decays. Sleep states have only been presented in a semi-logarithmic plot. The exponential decay time λ corresponds to the mean sleep episode duration. The analyzed interval for wake durations was from 1 to 30 minutes and for sleep durations from 3 to 90 minutes. We used the ratio of fitting errors ($\chi p/\chi e$) 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 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-four 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. Referring to sleep-stage transitions, their analysis suggested that the transition probability of the present state is affected by the past two states. 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 and to compare frequencies of two-step transitions in the control group and the apnea severity groups (level of significance: $p<0.01$). We examined differences in exponential decay times by consideration of standard deviations. The influence of OSA severity and potential confounder on transition pattern was tested by multivariate analysis of variance (MANOVA) restricted on OSA patients (n=725), with the 25 most common two-step-transition patterns as dependent variables and four categories (apnea-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 $\eta^2$ was considered as indicator, how much variance of the dependent variable (transition) is explained by the independent variable (group). Furthermore, we considered Cohen's d as declaration of effect size. Multiple comparisons were performed by Tukey-Test (level of significance: $P<0.05$). Subsequently, we performed t-test's (level of significance: $p<0.05$) to examine differences in polysomnographic data (traditional metrics and two-step transitions) between patients with diagnosed and without diagnosed arterial hypertension and between patients with daytime sleepiness (ESS>10) and

normal (ESS≤10). Regarding arterial hypertension, we focussed on patients with high systolic blood pressure (RRsyst.≥140 mmHg) and diastolic blood pressure (RRdiast.≥90 mmHg), irrespectively of diagnosis or cardiovascular medication. ESS values were missing in 6 patients and blood pressure was missing in 18 patients. To validate our findings, we repeated testing in 433 patients scored in 10 different centers registered to ESADA.

## Results

### Patients

Clinical data as well as sleep data of OSA severity categories and a healthy control group are presented in table 1. The highest values for age and BMI were found in severe OSA patients. Traditional sleep parameters were homogenous between severe OSA patients and controls. Variations could be observed between controls and moderate as well as mild OSA. Healthy subjects spent more time in N1 and N2, whereas time in N3 and REM was reduced. The total number of transitions was highest in controls, but the number of awakenings was significantly higher in severe OSA patients. However, neither TST nor SE showed significant differences amongst subgroups. Interestingly, the ESS did not differ between OSA severity categories.

**Table 1.** Clinical groups and sleep data (means). Bold numbers represent significance compared to controls (Level of significance <0.01 according to an ANOVA and Tukey-test for multiple comparisons) 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.
***** No significant differences between OSA severity categories after performing an ANOVA with level at significance at 0.05.

| | Controls | Mild OSA | Moderate OSA | Severe OSA |
|---|---|---|---|---|
| Count | 187 | 213 | 235 | 277 |
| Age (years) | 50.3 | 47.4 | 49.5 | 51.6 |
| BMI (kg/m$^2$) | 24.4 | 27.4 | 29.0 | 31.4 |
| Gender (males/females) | 86/101 | 159/54 | 185/50 | 244/33 |
| ESS* | n.a. | 9.3 | 9.3 | 9.8 |
| AHI (#/h) | n.a. | 9.6 | 22.4 | 51.0 |
| TST (min) | 388.2 | 389.5 | 379.4 | 378.8 |
| SE (% TST) | 85.7 | 87.8 | 85.7 | 85.8 |
| N1 (% TST) | 11.5 | **7.1** | **8.3** | 10.4 |
| N2 (% TST) | 54.9 | **49.8** | **50.5** | 55.2 |
| N3 (% TST) | 14.8 | **21.7** | **20.6** | 16.3 |
| REM (% TST) | 18.8 | **21.3** | **20.6** | 18.1 |
| WASO (min) | 64.4 | 54.3 | 63.7 | 62.1 |
| NASO | 22.9 | 21.1 | 23.9 | **26.6** |
| TRANS | 142.1 | **84.4** | **94.7** | **103.5** |

**Cumulative distributions of wake and sleep states**

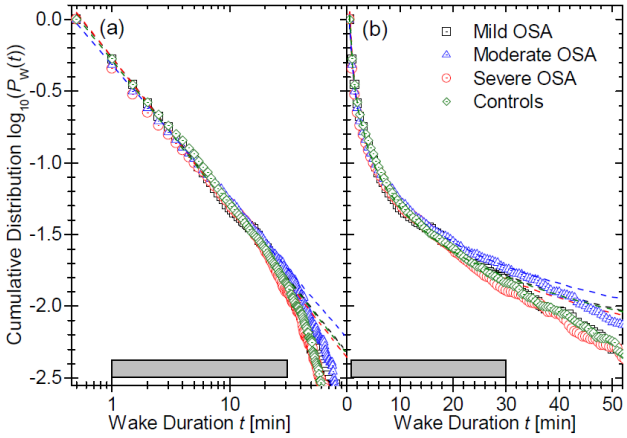


Figure 1. Cumulative distributions of wake duration for each OSA group and for controls. Grey bars indicate the analyzed interval from 1 to 30 min. In (a) dashed lines represent the best fits for a power law in a double logarithmic plot. In (b) dashed lines represent the best fits for an exponential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

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.

**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 λ (min).

| | Wake States | | Sleep States | |
|---|---|---|---|---|
| Group | α | $X_p/X_e$ | λ (min) | $X_p/X_e$ |
| Male | 1.01 | 0.05 | 24.42 | 107.9 |
| Female | 1.01 | 0.06 | 24.12 | 62.7 |
| <40y | 0.97 | 0.04 | 26.81 | 72.8 |
| 40y to 60y | 1.03 | 0.05 | 24.17 | 133.9 |
| 60y and Older | 0.99 | 0.11 | 22.29 | 78.7 |
| Mild OSA | 1.03 | 0.03 | 24.14 | 167.7 |
| Moderate OSA | 0.95 | 0.01 | 24.56 | 101.9 |
| Severe OSA | 1.05 | 0.24 | 24.36 | 28.9 |
| Normal Weight | 1.06 | 0.04 | 23.48 | 87.6 |
| Overweight | 0.97 | 0.02 | 24.66 | 96.7 |
| Obesity | 1.03 | 0.14 | 24.39 | 73.8 |
| Controls | 1.08 | 0.03 | 28.97 | 82.2 |

The power law exponent α showed salience not only regarding severity (0.95 in moderate and 1.05 in severe), but also in terms of BMI -groups and age -groups. 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 167.7 in mild OSA, see table 2. The exponential decay was nearly the same (λ≈24 min) for almost all groups, except for age and controls. With increasing age, the exponential decay decreased. Young patients had λ=26.81 min (68% confidence interval=26.73, 26.89), middle aged 24.17 min

(68% confidence interval=24.12,24.22), and elderly 22.29 min (68% confidence interval=22.23,22.35).

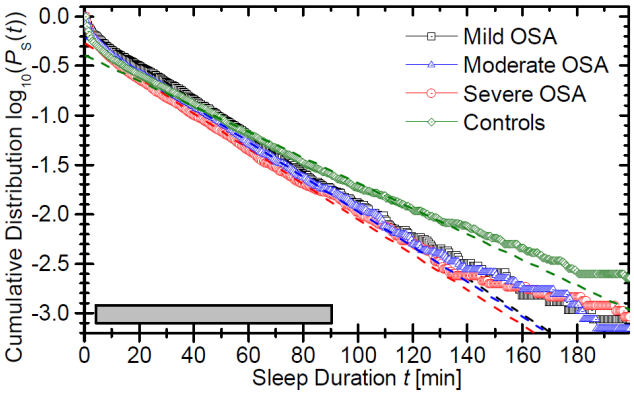


Figure 2. Cumulative distribution of sleep duration for each OSA group and for controls are plotted against their best fit for exponential distribution in this semi logarithmic plot. The grey bar indicates the analyzed interval from 3 to 90 min.

**Comparison between a healthy cohort and different degrees of OSA severity**

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.

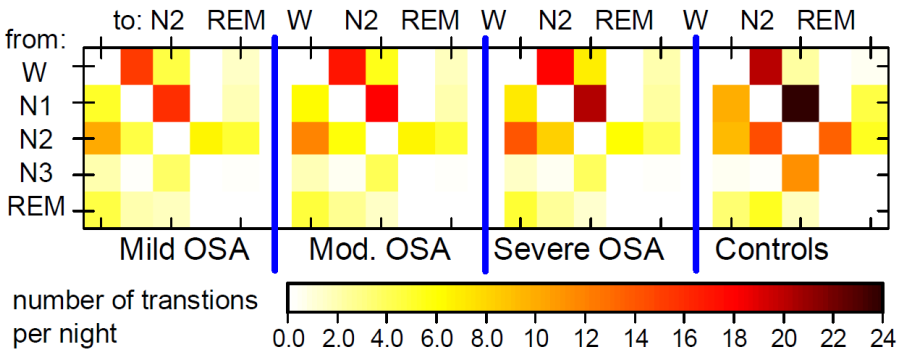


Figure 3. Average number of transitions per night between wake and sleep states for each OSA severity group and the control group. Transitions are from the states noted on the left to the states noted on the top. The average number of transitions is color coded for each square. In healthy controls transitions between N1 and N2 and between N2 and N3 were more frequent due to the increased effort in hypnogram scoring.

In all groups, the most frequent transitions happened between wake and light sleep. In healthy controls, transitions between N1 and N2 and between N2 and N3 were more salient. These findings are confirmed and refined by the analysis of two-step transitions. We considered the frequencies of the 25 most frequent two-step transitions for the control group and different degrees of OSA severity (Figure 4). In controls, the frequencies of the transition patterns N1->N2->N1 (12.2±10.6), N2->N1->N2 (12.7±9.9), N2->N3->N2 (10.1±7.4) and N3->N2->N3 (8.6±6.7) were significantly more frequent compared to all OSA severity categories ($p<0.001$). On the other hand, wake->N2->wake (0.9±1.7) transitions and N2->wake->N2 (1.6±2.4) transitions were significantly ($p<0.001$) less frequent in controls compared to both moderate and severe OSA patients. Frequencies of all significant transitions are presented in the supplement (S1).

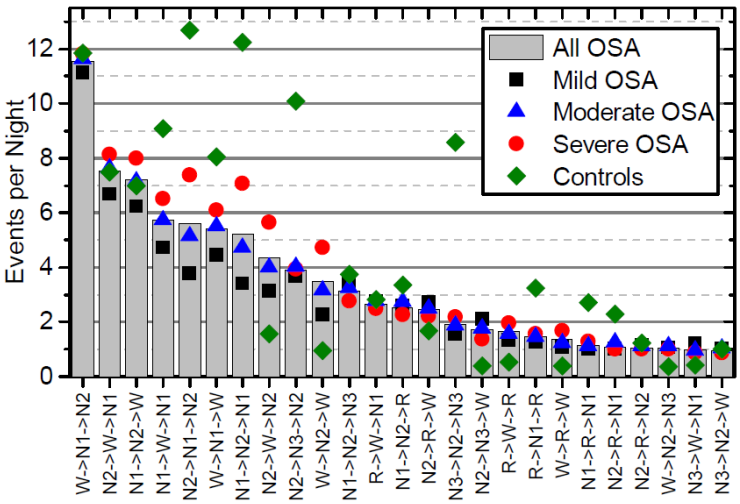


Figure 4. Representation of the 25 most frequent two-step transitions in descending order for each OSA severity group and for the control group. Grey bars indicate the average number in all OSA groups and determine the order in the horizontal axis.

**The influence of OSA severity and potential confounders on two-step transitions**

To evaluate the influence of OSA severity and potential confounders on transition patterns, we performed a MANOVA restricted to OSA patients (n=725). In our model, we focused on main effects of our independent variables AHI -group, Age -group, BMI -group and gender. The 25 most frequent two-step transitions were our dependent variables. Pillai's trace was significant for each of the four groups. The transition N2->wake->N2 (p=0.001, partial $\eta^2$=0.021) was associated with OSA severity, see table 3. Severe OSA patients had significantly more N2->wake->N2 transitions (5.6±10.2) than moderate (4.0±4.8, p=0.028) and mild disease (3.1±3.9, p<0.001). Furthermore, severe OSA patients had more N1->N2->N1 (7.1±15.5) transitions compared to moderate (4.7±7.8, p=0.042) and mild disease (3.4±5.1, p=0.001), whereas N2->N1->N2 were only significantly higher in severe (7.4±15.4) compared to mild OSA (3.8±5.3, p=0.001) disease. However, the strongest relation was found for the transitions N1->wake->N1 (p<0.001, partial $\eta^2$=0.044) and wake->N1->wake (p<0.001, partial $\eta^2$=0.053) regarding age. Older patients had significantly more wake>N1>wake transitions (8.2±8.2) than middle aged (4.9±5.0, p<0.001) and young patients (4.2±5.1, p<0.001). Likewise, N1->wake->N1 was significantly higher in patients 60 years or older. Furthermore, gender showed a relation to the pattern N2->N3->wake (p<0.001, partial $\eta^2$ = 0.044). Women had significantly (p<0.001) more transitions (2.5±2.2) 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=0.004, partial $\eta^2$=0.015), 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 (S2). It is of interest to mention, that Cohen's d was weak for all significant transitions.

**Table 3.** Significant transitions regarding OSA severity after performing MANOVA. Bold numbers indicate significance to severe OSA. Level of significance was set at 0.05. 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 | OSA | | | | |
|---|---|---|---|---|---|
| | Mild N=213 | Moderate N=235 | Severe N=277 | p | partial $\eta^2$ |
| N2-wake-N2 | **3.1±3.9** | **4.0±4.8** | 5.6±10.2 | 0.001 | 0.021 |
| N1-N2-wake | **6.2±4.3** | 7.2±5.1 | 8.0±5.5 | 0.001 | 0.019 |
| wake-N2-wake | **2.1±3.1** | **2.9±4.1** | 4.5±9.6 | 0.001 | 0.018 |
| N2-N1-N2 | **3.8±5.3** | 5.1±7.8 | 7.4±15.4 | 0.002 | 0.017 |
| N2-N3-wake | **2.1±1.9** | **1.7±1.6** | 1.4±1.5 | 0.002 | 0.017 |
| N1-N2-N1 | **3.4±5.1** | **4.7±7.8** | 7.1±15.5 | 0.003 | 0.017 |
| N1-N2-N3 | **3.4±1.9** | **3.2±2.0** | 2.8±2.0 | 0.003 | 0.016 |
| N1-N2-REM | 2.6±1.6 | **2.8±1.8** | 2.3±1.7 | 0.014 | 0.012 |
| N2-REM-wake | **2.7±1.7** | 2.5±1.8 | 2.2±1.7 | 0.012 | 0.012 |
| N2-REM-N1 | 0.9±1.1 | 1.3±1.6 | 1.0±1.4 | 0.017 | 0.011 |
| N3-wake-N1 | **1.2±1.3** | 0.9±1.2 | 0.8±1.2 | 0.039 | 0.009 |
| N2-wake-N1 | **6.5±4.3** | 7.4±5.2 | 7.9±5.2 | 0.043 | 0.009 |

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

We analyzed traditional polysomnographic metrics and in dependency of OSA severity significant two-step transitions regarding hypertension and an association to sleepiness. Hypertensive patients (n=223) had a shorter TST (370.1±66.9 min vs. 387.5±62.6 min, p=0.01), a lower SE (83.8±11.5 % vs. 87.5±9.7 %, p=0.007) and more WASO (71.7±53.2 min vs. 55.3±42.6 min, p=0.005) compared to non-hypertensive ones (n=502). The total number

of transitions 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. 203 patients with high systolic pressure (104 were diagnosed as hypertensive) showed no differences in both, traditional metrics and two-step transitions. In 160 patients with high diastolic blood pressure (68 were diagnosed as hypertensive), it was striking that only the transition pattern N2->N3->wake differed significantly (p=0.017). Patients with high diastolic pressure had fewer transitions (1.4±1.6) compared to patients without high diastolic pressure (1.8±1.7). To consider sleepiness, we simplified the ESS into normal (ESS ≤ 10, n=432) and daytime sleepiness (ESS > 10, n=287). Patients with daytime sleepiness had a higher total number of transitions (100.4±48.3 vs. 91.3±39.2, p=0.008). In addition, transition patterns of N2->N1->N2 (6.9±13.4 vs. 4.8±9.0, p=0.019) and N1->N2->N1 (6.4±13.2 vs. 4.5±9.3, p=0.034) were higher in patients with daytime sleepiness. Interestingly, TST (391.4±62.8 min vs. 375.9±65.1 min, p=0.002) and SE (87.5±10.1 % vs. 85.6±10.6 %, p=0.018) were also higher in patients with a daytime sleepiness.

**Single center vs. multicenter cohort**

To validate the association between sleep stage transitions and daytime sleepiness and elevated diastolic blood pressure, we repeated the analysis in 433 OSA patients from 10 different centers. Compared to the single-center cohort heterogeneity in anthropometrics and sleep data was observed. Patients in the multicenter cohort were older (54.0±11.3 vs. 49.7±10.9, p<0.001), had a higher AHI/h (42.0±25.2 vs.29.6±20.9, p<0.001) and less TST (359.4±101.1 min vs. 382.2±64.4 min, p<0.001). Thus, transition frequencies differed from the single center cohort (Figure 5.). Especially, N1->N2->N1 and N2->N1->N2 transitions are

more frequent in the multicenter cohort. This confirms previous findings that elevated severity of OSA is associated with more transitions. Accordingly, in 151 patients with high diastolic blood pressure the transition pattern N2->N3->wake differed not compared to patients with normal diastolic blood pressure. Instead, we a higher transition rate for N2->N1->N2 (p=0.021), N1->N2->N1 (p=0.015) and a reduced time in N3 (p=0.001). Interestingly, in 197 patients with daytime sleepiness the transition patterns N2->N1->N2 and N1->N2->N1 differed not significantly. Instead, we observed more transitions for N2->wake->N2 (p<0.001), wake-N2->wake (p<0.001) and a higher percentage of N2 (p=0.011) compared with non-sleepy individuals.

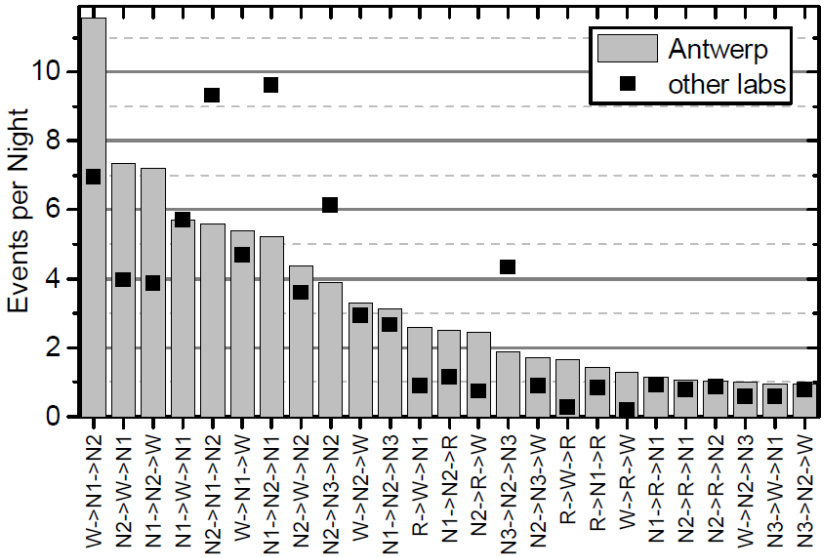


Figure 5. Representation of the 25 most frequent two-step transitions in descending order for the laboratory of Antwerp and a multicenter cohort. Grey bars indicate the average number in all OSA groups and determine the order in the horizontal axis.

## Discussion

In this retrospective study, our results suggest that sleep dynamics are not only influenced by OSA severity, but age and gender confound sleep-stage transitions in unique ways. Furthermore, two-step transitions are associated to patients with an elevated diastolic blood pressure and patients with daytime sleepiness in single center cohort. The transition pattern N2->N3->wake was the only polysomnographic sleep parameter, distinguishing OSA-patients with an elevated diastolic blood pressure. However, 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 patients with daytime sleepiness, a higher frequency of N1->N2->N1 and N2->N1->N2 transitions could be observed. Interestingly, TST and SE were also significantly higher in sleepy patients, which agrees with results obtained by Roure et al. (Roure et al., 2008). For this analysis, we simplified sleepiness very strongly, by just comparing patients with ESS > 10 and ≤ 10. In a previous study by Laffan et al., using two 5-point Likert scales onsleep depth and restfulness, lighter and restless sleep were associated with a higher sleep-stage transition rate (Laffan et al., 2010).

In healthy patients, we observed more transitions into deep sleep. Furthermore, N1->N2->N1 and N2->N1->N2 transitions were considerably more frequent in healthy patients, questioning the association of these transitions to sleepiness. But in this context, it is necessary to consider the very careful sleep scoring in the control group (two individual scorers plus one consensus scorer for each subject) and that scoring was according to Rechtschaffen & Kales. The new American Academy of Sleep Medicine (AASM) standards improved scoring compared to the previous Rechtschaffen & Kales criteria, but nevertheless

discrepancies remained. Particularly, the scoring of N2 has not improved and the scoring agreement of N1 is still low compared to other sleep stages (Danker-Hopfe et al., 2009).

Additionally, high frequencies of symmetric patterns in controls face results obtained by Lo et al., who observed decreasing asymmetry with increasing OSA severity (Lo et al., 2013). Our results in severe OSA patients support this, because the transition N2->wake->N2 is accompanied by significantly increased transition pattern wake->N2->wake. Likewise, N1->N2->N1 and N2->N1->N2 transitions are increased in severe OSA. Thus, in our multicenter cohort with elevated severity, these transition patterns were more frequent. In addition, N2->N3->N2 and N3->N2->N3 transitions were more frequent, which was unexpected, because elevated severity was associated with reduced time in N3 previously (see table 1.). One possible explanation could be a rather low scoring agreement in OSA patients (Danker-Hopfe et al., 2004; Penzel et al., 2003), aggravated by pooling scorings from different centers. Regardless, both the presence of elevated severity and the possibility of scoring differences in a multicenter cohort compromise a validation of the association of two-step transitions to clinical aspects and reveal the susceptibility of a two-step transition analysis compared to total durations or percentual values.

Regarding age, we observed more awakenings in the elderly, in agreement with Klerman et al. (Klerman et al., 2004). In patients over 60, we have seen significantly higher frequencies for N1->wake->N1 and wake->N1->wake. As for gender, the highest influence was found on the transition pattern N2->N3->wake with significantly more transitions for females than males. Interestingly, the same pattern distinguished patients with an elevated diastolic blood pressure. 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).

As for the distribution 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. These findings are consistent with previous results (Lo et al., 2004; Zhang et al., 2017). Especially, the decreasing exponential decay (from 26.81 min in young to 22.29 min in older patients) was salient and agrees with previous results (Zhang et al., 2017). Interestingly, Arnadottir et al. observed in a group of healthy humans that age has no significant effect on λ and that the distribution of wake states follows a power-law only at ages 34-56 years (Arnadottir et al., 2010). We noticed tremendous variations of the accuracy of the fits regarding all groups, but especially in terms of OSA severity. These deviations could indicate a limitation of 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 not the influence of past transitions. Our approaches to analyze two-step transition could be extended and incorporated to this Markov sleep model.

Our retrospective study has several limitations. We had to deal with some missing data in our ESADA database. We computed polysomnographic sleep data directly from the hypnogram and, due to some missing lights off and on times, we ignored wake times at the beginning and end of the recordings. Our patients were not matched and were analyzed a single night

under laboratory conditions. Even if we excluded patients with psychiatric disease, 91 patients used antidepressants or another medication with may impact sleep and sleep stage transitions (Wilson and Argyropoulos, 2005).

**Conclusion**

We want to emphasize, that sleep architecture is influenced by OSA severity and even more by confounders. Wake states follow a power-law distribution, while sleep states follow an exponential distribution independent of OSA severity or potential confounders. An analysis of two-step transitions deepens the understanding of the complexity of sleep fragmentation and establishes approaches to link sleep fragmentation directly to clinical aspects like OSA-related 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

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**S1.** Two-Step transition comparison between a healthy control group (SIESTA) and different degrees of OSA severity (Mean±SD). Bold numbers indicate significance compared to the control group. Level of significance was set at p<0.01.

| Two-step transitions | Controls N=374 | Mild N=213 | Moderate N=235 | Severe N=277 |
|---|---|---|---|---|
| Wake-N1-wake | 8.1±7.2 | **4.4±4.8** | **5.5±6.3** | **6.1±6.3** |
| Wake-N2-wake | 0.9±1.7 | 2.1±3.1 | **2.9±4.1** | **4.5±9.6** |
| Wake-N2-N3 | 0.4±0.8 | **1.1±1.5** | **1.1±1.6** | **0.9±1.4** |
| Wake-REM-wake | 0.4±0.9 | 1.0±2.2 | **1.2±2.3** | **1.6±3.9** |
| N1-wake-N1 | 9.1±7.9 | **4.7±5.1** | **5.7±6.2** | **6.5±6.8** |
| N1-N2-wake | 7.0±4.2 | 6.2±4.3 | 7.2±5.1 | 8.0±5.5 |
| N1-N2-N1 | 12.2±10.6 | **3.4±5.1** | **4.7±7.8** | **7.1±15.5** |
| N1-N2-N3 | 3.7±1.8 | 3.4±1.9 | 3.2±2.0 | **2.8±2.0** |
| N1-N2-REM | 3.4±1.9 | **2.6±1.6** | **2.8±1.8** | **2.3±1.7** |
| N1-REM-N1 | 2.7±3.4 | **1.0±2.5** | **1.1±3.3** | **1.3±3.1** |
| N2-wake-N2 | 1.6±2.4 | 3.1±3.9 | **4.0±4.8** | **5.6±10.2** |
| N2-N1-N2 | 12.7±9.9 | **3.8±5.3** | **5.1±7.8** | **7.4±15.4** |
| N2-N3-wake | 0.4±0.7 | **2.1±1.9** | **1.7±1.6** | **1.4±1.5** |
| N2-N3-N2 | 10.1±7.4 | **3.7±3.1** | **4.0±3.9** | **3.9±6.8** |
| N2-REM-wake | 1.7±1.5 | **2.7±1.7** | **2.5±1.8** | **2.2±1.7** |
| N2-REM-N1 | 2.3±1.9 | **0.9±1.1** | **1.3±1.6** | **1.0±1.4** |
| N3-wake-N1 | 0.4±0.7 | **1.2±1.3** | **0.9±1.2** | **0.8±1.2** |
| N3-N2-N3 | 8.6±6.7 | **1.6±2.3** | **1.9±3.0** | **2.2±6.2** |
| REM-wake-REM | 0.5±1.2 | **1.3±2.4** | **1.6±2.5** | **2.0±4.0** |
| REM-N1-REM | 3.2±3.5 | **1.2±2.8** | **1.5±3.6** | **1.6±3.5** |

**S2.** Significant transitions regarding age, BMI and gender after performing MANOVA. Bold numbers indicate significance respectively age 60 and older and obesity. Level of significance was set at 0.05. 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=134 | 40 to 60 N=452 | 60 and older N=139 | p | partial $\eta^2$ |
| wake-N1-wake | **4.2±5.1** | **4.9±5.0** | 8.2±8.2 | <0.001 | 0.053 |
| N1-wake-N1 | **4.6±5.7** | **5.2±5.2** | 8.3±8.3 | <0.001 | 0.044 |
| wake-REM-wake | **0.7±1.7** | 1.3±2.9 | 1.9±4.1 | 0.004 | 0.015 |
| N2-wake-N2 | 3.1±3.8 | 5.0±8.4 | 3.5±5.2 | 0.006 | 0.014 |
| REM-wake-N1 | 2.2±1.7 | 2.7±2.0 | 2.6±2.2 | 0.008 | 0.014 |
| N2-REM-N1 | **1.3±1.5** | 1.1±1.4 | 0.8±1.2 | 0.010 | 0.013 |
| N3-wake-N1 | 1.0±1.4 | **0.9±1.1** | 1.2±1.4 | 0.013 | 0.012 |
| wake-N2-wake | 2.0±3.0 | 3.8±7.8 | 2.9±4.5 | 0.021 | 0.011 |
| N1-N2-N3 | 3.2±1.9 | **3.0±1.9** | 3.4±2.3 | 0.023 | 0.010 |
| N2-REM-N2 | 1.1±1.6 | **1.1±2.0** | 0.6±1.2 | 0.025 | 0.010 |
| REM-wake-REM | **1.2±1.9** | 1.6±3.0 | 2.2±4.2 | 0.023 | 0.011 |

| 2-step transition | BMI-Group | | | | |
|---|---|---|---|---|---|
| | Normal Weight N=121 | Overweight N=315 | Obese N=288 | p | partial $\eta^2$ |
| REM-wake-N1 | **2.9±2.0** | **2.8±2.1** | 2.3±1.8 | 0.004 | 0.015 |
| wake-N2-N3 | 1.4±1.9 | 0.9±1.3 | 1.0±1.5 | 0.023 | 0.010 |
| N2-N3-wake | **2.2±2.0** | 1.6±1.4 | 1.6±1.8 | 0.037 | 0.009 |
| N2-REM-wake | **2.8±1.8** | 2.5±1.8 | 2.2±1.6 | 0.045 | 0.009 |

| 2-step transition | Gender-Group | | | |
|---|---|---|---|---|
| | Male N=588 | Female N=137 | p | partial $\eta^2$ |
| N2-N3-wake | 1.5±1.5 | 2.5±2.2 | <0.001 | 0.044 |
| wake-N2-N3 | 0.9±1.4 | 1.6±1.9 | <0.001 | 0.028 |
| REM-wake-N1 | 2.7±2.0 | 2.0±1.9 | <0.001 | 0.024 |
| N1-wake-N1 | 6.0±6.5 | 4.3±4.1 | 0.002 | 0.013 |
| N2-REM-wake | 2.5±1.8 | 2.1±1.6 | 0.002 | 0.013 |
| wake-N1-wake | 5.7±6.3 | 4.1±3.9 | 0.002 | 0.013 |
| N1-N2-N3 | 3.0±1.9 | 3.6±2.1 | 0.007 | 0.010 |
| N3-wake-N1 | 0.9±1.1 | 1.3±1.6 | 0.008 | 0.010 |