Decrease in sleep depth is associated with higher cerebrospinal fluid neurofilament light levels in patients with Alzheimer's disease

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Abstract

Study Objectives

The majority of studies investigating the association between sleep and Alzheimer's disease (AD) biomarkers have been performed in healthy participants. Our objective was to investigate the association between sleep and several biomarkers that reflect distinct aspects of AD physiopathology.

Methods

The cohort included 104 individuals with mild-moderate AD. The participants were submitted to one-night polysomnography, and cerebrospinal fluid was collected in the following morning to measure the selected biomarkers associated with amyloid deposition, tau pathology, neurodegeneration, axonal damage, synaptic integrity, neuroinflammation, and oxidative damage.

Results

There was a positive correlation between neurofilament light (NF-L) and the time spent in stage 1 of non-rapid eyes movement (NREM) (N1) sleep and a negative correlation between this marker and the time spent in stage 3 of NREM (N3) sleep. Accordingly, we observed that deep sleep was associated with lower levels of NF-L, whereas light sleep increased the probability of having higher levels of this marker. Furthermore, chitinase-3-like-1 (YKL-40) was negatively correlated with sleep efficiency, the time spent in stage 2 of NREM (N2) sleep, and the time spent in N3 sleep. Conversely, there was a positive correlation between N3 sleep and the oxidative protein damage markers N-ε-(carboxyethyl)lysine and N-ε-(malondialdehyde)lysine.

Conclusions

There were significant correlations between sleep parameters and AD biomarkers related to axonal damage and neuroinflammation, such as NF-L and YKL-40. A lack of deep sleep was associated with higher levels of NF-L. This highlights a potential role for NF-L as a biomarker of sleep disruption in patients with mild-moderate AD in addition to its role in predicting neurodegeneration and cognitive decline.

Introduction

Alzheimer's disease (AD) is a highly incapacitating and prevalent neurodegenerative disorder considered one of the largest public health and economic challenges of this century [1]. The main hallmarks of this disease are the deposition of amyloid-beta (Aβ) protein, the formation of tau protein neurofibrillary tangles (NFTs), and neurodegeneration [2]. These events precede the loss of cognitive function by years or decades. In addition, noncognitive signs, such as anxiety, depression, olfactory dysfunction, and sleep disturbances, which drastically affect patients' quality of life, may appear before cognitive symptoms [3–6].

The levels of AD biomarkers measured by positron emission tomography (PET) or assessed in cerebrospinal fluid (CSF), especially Aβ42 protein, total-tau (T-tau), and phospho-tau (P-tau), are strongly correlated with the levels in the brain [7–9]. Such assessments increase the possibility of an early diagnosis [10]. Accordingly, studies have suggested that biomarkers should be used to classify patients regardless of clinical symptoms or disease stage [11]. In addition, the study of biomarkers in recent years has shed light on distinct physiological events associated with the progression of the disease, such as disrupted sleep [10]. During the sleep–wake cycle, Aβ levels fluctuate in a circadian pattern such that there is an increase in the Aβ concentration during wakefulness and a decrease during sleep [12, 13]. Furthermore, animal studies have shown that Aβ levels are increased after acute sleep deprivation and infusion of orexin, a neurotransmitter that improves wakefulness [14]. This has been confirmed by some studies in humans [15], whereas others have failed to demonstrate the same results [16]. Nevertheless, Aβ clearance is demonstrably increased during sleep, especially in slow-wave sleep (SWS) [17]. A similar relationship between sleep and tau protein accumulation, the second pathological hallmark of AD, was recently proposed [18, 19]. However, studies on patients with AD are needed given that the above-mentioned investigations were predominantly performed in cognitively normal participants. In addition, a variety of molecules have recently emerged as potential AD biomarkers, but their relationship with sleep remains to be fully elucidated [20–23]. Furthermore, considering the modifiable nature of sleep and its influence on memory consolidation, the identification of markers for sleep disruption at the early stages of the disease could contribute to the implementation of sleep-based strategies aiming to prevent the cognitive decline.

Based on this, we investigated the association between sleep and several CSF biomarkers in patients with mild-moderate AD. The investigated markers reflect different aspects of AD physiopathology: amyloid deposition (Aβ42), tau pathology (P-tau), neurodegeneration (T-tau), axonal damage (neurofilament light [NF-L]), synaptic integrity (neurogranin), microglial activation (soluble variant of the triggering receptor expressed on myeloid cells 2 [sTREM2]), neuroinflammation (chitinase-3-like-1 [YKL-40]), other types of neuronal injury (orexin and leptin), and protein oxidative damage (glutamic semialdehyde [GSA], aminoadipic semialdehyde [AASA], N-ε-(carboxyethyl)lysine [CEL], N-ε-(malondialdehyde)lysine [MDAL], and N-ε-(carboxymethyl)lysine [CML]).

Methods

Study population

This was an ancillary study of a prospective trial designed to evaluate the influence of obstructive sleep apnea on the cognitive evolution of patients with AD after a 1-year follow-up (NCT02814045). Patients were recruited from the Cognitive Disorders Unit at the Hospital Universitari Santa Maria (Lleida, Spain) for 4 years (2014–2018). Eligibility criteria included drug-naïve participants aged above

60 years who were diagnosed with AD according to the National Institute on Aging and Alzheimer's Association criteria [24]. Additionally, only patients with mild-moderate cognitive impairment (mini-mental state examination [MMSE]≥20) were included. The patient, the responsible caregiver, and the legal representative (when different from the responsible caregiver) signed an informed consent form.

The exclusion criteria were as follows: (1) the presence of visual and/or communication problems that could make adherence with the study procedures difficult; (2) the presence of a previously diagnosed sleep disorder; (3) the presence of excessive somnolence for unknown reasons; (4) comorbidities, such as cancer, severe depression, severe renal or hepatic insufficiency, severe cardiac, or respiratory failure; (5) excessive alcohol intake (>280 g/week); (6) MRI evidence of hydrocephalus, stroke, a space-occupying lesion, or any clinically relevant central nervous system disease other than AD; (7) the presence of mental disorders according to DSM-V-TR criteria; (8) any-time use of medication under investigation or the use of beta-blockers, antidepressants, neuroleptics, or hypnotics fewer than 15 days before the conduction of polysomnography (PSG); (9) the presence of untreated (or treated for less than 3 months prior to the screening visit) vitamin B12 or folate deficiency; and (10) the presence of untreated thyroid disease.

Study design

The patients arrived at the Cognitive Disorders Unit of Hospital Universitari Santa Maria (Lleida, Spain) and were assessed for eligibility. Eligible patients were submitted to overnight PSG, and in the following morning, CSF and blood were collected to determine the levels of the biomarkers. Only patients who underwent PSG and from whom CSF was collected were included in the study.

Neuropsychological assessment

The MMSE was used to include only patients with mild-moderate cognitive impairment. The MMSE includes questions to evaluate different domains, such as attention, time and place orientation, and word recall. The scores of this test range from 0 to 30, and a higher score indicates better cognitive function [25, 26].

Clinical variables

The following variables were collected: age, sex, years of education, toxic habits (alcohol consumption and smoking), vascular risk factors (hypertension, diabetes mellitus, dyslipidemia, stroke, and cardiopathy), personal psychiatric history, and family psychiatric and neurological history. Body mass index (BMI) was calculated as body weight (in kg)/height (in m2).

Polysomnography

To assess sleep–wake parameters, we performed a PSG during the night (Philips Respironics Alice 6 LDx, Somnomedics, Somnoscreen plus Versión 2.7.0, and ApneaLink Resmed). The measured PSG variables were sleep efficiency (in %, defined as the ratio between total sleep time and the time spent in bed), latency to stage 1 of non-rapid eyes movement (NREM) sleep (N1) (in minutes, defined as the time spent awake until the first sleep episode while in bed), latency to rapid eyes movement (REM) sleep (in minutes, defined as the time until the first REM sleep episode while in bed), the time spent in N1 stage (%, defined as the percentage of time spent in N1 while sleeping), the time spent in stage 2 of NREM sleep (N2) (%, defined as the percentage of time spent in N2 while sleeping), the time spent in stage 3 of NREM sleep (N3) (also known as SWS) (%, defined as the percentage of time spent in N3 while sleeping), the time in REM sleep (%, defined as the percentage of time spent in

REM sleep while sleeping), and the apnea-hypopnea index (AHI) (defined as the number of apnea and hypopnea events per hour during the time spent sleeping).

CSF biomarkers

CSF samples were collected between 8:00 am and 10:00 am to avoid variations related to the circadian rhythm. The samples were collected in polypropylene tubes, centrifuged at 2000 × g for 10 min at 4°C, immediately frozen, and stored within 4 hours in a −80°C freezer. Later, they were used for biomarkers analysis.

The concentration of neurogranin was measured using an in-house enzyme-linked immunosorbent assay (ELISA) as previously described in detail [27]. The CSF sTREM2 concentration was measured using an in-house immunoassay with electrochemiluminescent detection on a Meso Scale Discovery instrument (MSD, Rockville, MD) as previously described in detail [28]. The orexin concentration was measured using an in-house radioimmunoassay as previously described [29]. YKL-40, NF-L, and leptin were measured by commercial ELISA kits (Quidel, San Diego, CA; UmanDiagnostics, Sweden; and R&D Systems, Minneapolis, MN, respectively). The core AD biomarkers (Aβ42, T-tau, and P-tau) were measured using commercial kits (Innotest β-Amyloid1-42; Innotest hTAU Ag; and Innotest Phospho-TAU181P, Fujirebio-Europe, Gent, Belgium). All measurements were performed in one round of experiments using one batch of reagents by board-certified laboratory technicians who were blinded to the clinical data. The intra-assay coefficients of variation were lower than 10% for internal quality control samples (two per plate).

We measured five protein oxidation-derived markers. Two of them, GSA and AASA, are markers of direct oxidative damage to proteins, whereas CEL is a marker of indirect protein oxidation derived from carbohydrate oxidation/glycolysis. CML is a mixed oxidation marker derived from the oxidation of carbohydrates and lipids, and MDAL is a marker of indirect protein oxidation derived from the oxidation of lipids.

The concentration of these markers was measured as trifluoroacetic acid methyl ester derivates in acid hydrolyzed delipidated and reduced protein samples using gas chromatography/mass spectrometry as previously described [30].

Genetic analysis

DNA was extracted from buffy coat cells using a Maxwell RCS blood DNA kit (Promega, USA). Twenty microliters of DNA were used for apolipoprotein E (ApoE) genotyping by a polymerase chain reaction.

Statistical analysis

Descriptive statistics of the mean (standard deviation of the mean [SD]) and median (interquartile range [IQR]) were estimated for normally distributed and nonnormally distributed quantitative data, respectively. The absolute and relative frequencies were used for qualitative variables. The normality of the distribution was analyzed using the Shapiro–Wilk test. Associations between biomarkers and sleep parameters were assessed by Spearman's rank test. Partial correlations controlling for age, sex, and ApoE4 status were calculated. Principal component analysis (PCA) is a technique used to reduce the number of variables without losing information [31]. We performed this technique using the percentage of time on different sleep stages (N1, N2, N3, and REM) to characterize the sleep architecture of our cohort. The results indicated that the variability of our sample was mainly defined by the sleep depth. Accordingly, there were three main profiles of patients in terms of sleep architecture: (1) deep sleepers (individuals with a propensity to deepen their sleep, reaching the later stages of sleep [N3 and REM sleep]); (2) moderate sleepers (individuals who exhibited intermediate

sleep); and (3) light sleepers (individuals who spent most of the time in the lighter sleep stage [N1]). The probability of having high values of biomarkers (using the median as the cutoff) was assessed in relation to sleep depth using logistic regression models. R statistical software version 3.3.1 was used for all analyses [32]. All the tests were two-tailed, and p-values < 0.05 were considered statistically significant.

Results

Cohort characteristics

The cohort included 104 participants, most of whom were women (56.7%), with a median [IQR] age of 76 [72.0;80.0] years and a BMI of 27.8 [25.6; 31.2]. The most frequently associated comorbidities were hypertension (63.5%), dyslipidemia (42.3%), diabetes mellitus (19.2%), and heart diseases (19.2%). Fifty percent of the participants were ApoE4-positive. Table 1 shows all of the demographic characteristics, including sample characteristics, cognitive status, and self-reported and objective sleep measurements.

Table 1.Descriptive characteristics of patients with mild-moderate AD

Global	
n (%), mean (SD), or median [IQR]	
Sociodemographic data	
Women	59 (56.7%)
Age, years	76.0 [72.0;80.0]
BMI, kg·m-2	27.8 [25.6;31.2]
Comorbidities	
Hypertension	66 (63.5%)
Diabetes mellitus	20 (19.2%)
Dyslipidemia	44 (42.3%)
Heart diseases	20 (19.2%)
Stroke	4 (3.85%)
AD parameters	
MMSE	23.5 [22.0;25.0]
Aβ42	69 (75.0%)
T-tau	55 (59.8%)
P-tau	62 (67.4%)
ApoE4	52 (50.0%)

PSG parameters

Epworth sleepiness scale	5.00 [2.00;8.00]
Time in bed, minutes	412 (47.2)
Total sleep time, minutes	266 (81.8)
Sleep efficiency, %	67.3 [53.9;78.2]
N1 stage, %	11.6 [7.14;18.4]
N2 stage, %	23.0 [16.6;31.1]
N3 stage, %	17.5 [9.15;25.2]
REM sleep, %	6.99 [2.98;11.4]
Latency to N1, minutes	32.1 (36.7)
Latency to REM sleep, minutes	171 (84.1)
AHI	30.1 (22.8)
Arousal index	39.8 [26.2;51.3]

Correlations between sleep parameters and CSF biomarkers

Unadjusted and adjusted correlations between sleep parameters and all of the studied CSF biomarkers are presented in Table 2. The majority of these correlations were related to NF-L and YKL-40. Sleep efficiency and the percentage of time spent in N2 and N3 were negatively correlated with the levels of YKL-40 (rho = −0.287, p = 0.006; rho = −0.240, p = 0.022; rho = −0.254, p = 0.015, respectively). In addition, there was a positive correlation between NF-L and the percentage of time spent in N1 (rho = 0.253, p = 0.016) and a negative correlation between this marker and the percentage of time spent in N3 (rho = −0.268, p = 0.010). Aβ42 was negatively correlated with N1 latency only (rho = −0.221, p = 0.039) and demonstrated a tendency to be related to REM sleep latency (rho = −0.214, p = 0.056) that was lost after adjusting for age, sex and ApoE4 status. No correlation was observed with T-tau, P-Tau, neurogranin, or sTREM2. Regarding oxidative damage markers, there was a positive correlation between CML and REM sleep latency (rho = 0.242, p = 0.040) and a negative correlation between this marker and the percentage of time spent in REM sleep (rho = −0.240, p = 0.029). In addition, CEL showed a negative correlation with N1 duration (rho = −0.266, p = 0.016).

Table 2.Correlations between sleep parameters and CSF biomarkers

Sleep efficiency		N1 stage	N2 stage	N3 stage	REM sleep	Latency to N1
stage	Latency to REM sleep		Arousal index			
Biomarkers	Rho	Rho adjusted	Rho	Rho adjusted	Rho	Rho adjusted
	Rho	Rho adjusted	Rho	Rho adjusted	Rho	Rho adjusted
	Rho	Rho adjusted	Rho	Rho adjusted		

Aβ42	0.173	0.194	0.047	−0.033	0.173	0.171
	0.097	0.172	0.051	0.051	−0.221 *	−0.179
	−0.214	−0.133	0.177	0.090		
T-Tau	−0.124	−0.112	−0.019	−0.056	−0.151	−0.152
	−0.109	−0.060	0.087	0.086	−0.001	0.018
	−0.029	0.005	0.032	−0.003		
P-Tau	−0.107	−0.079	−0.044	−0.088	−0.111	−0.091
	−0.070	0.001	0.049	0.053	−0.022	−0.001
	0.163	0.209	0.033	−0.030		
NF-L	−0.182	−0.190	0.253 *	0.240 *	−0.003	−0.022
	−0.268*	−0.266*	−0.079	−0.083	−0.117	−0.112
	−0.205	−0.223*	0.035	0.057		
YKL-40	−0.287**	−0.298**	0.096	0.123	−0.240*	−0.245*
	−0.254*	−0.277**	−0.019	−0.022	0.064	0.048
	−0.083	−0.108	−0.013	0.027		
Leptin	0.109	0.136	−0.071	−0.211*	0.045	0.019
	0.054	0.193	0.083	0.069	−0.160	−0.068
	0.110	0.200	0.085	−0.049		
Orexin	0.100	0.131	0.096	−0.087	−0.009	−0.025
	−0.003	0.167	0.165	0.139	−0.211*	−0.098
	−0.227*	−0.066	0.074	−0.079		
Neurogranin	−0.107	−0.086	0.073	0.003	−0.057	−0.056
	−0.122	−0.041	0.043	0.041	−0.031	0.002
	0.009	0.055	0.094	0.027		
sTREM2	0.001	−0.005	0.056	0.049	−0.014	−0.032
	−0.037	−0.045	−0.002	−0.006	0.021	0.027
	0.023	0.013	−0.031	−0.013		
AASA	−0.045	−0.014	−0.044	−0.144	−0.120	−0.119
	0.069	0.179	−0.095	−0.089	−0.011	0.065
	0.109	0.187	−0.049	−0.140		
CEL	0.016	0.027	−0.266*	−0.295**	−0.137	−0.141
	0.191	0.226 *	−0.003	−0.001	0.185	0.213
	0.045	0.106	−0.075	−0.113		
CML	0.034	0.040	−0.182	−0.191	−0.035	−0.035
	0.120	0.134	−0.240*	−0.240*	0.168	0.179
	0.242 *	0.272 *	−0.126	−0.139		
GSA	0.036	0.056	−0.174	−0.243*	0.050	0.040
	0.076	0.160	−0.140	−0.131	0.144	0.194
	0.079	0.137	−0.187	−0.247*		

MDAL	0.042	0.063	−0.087	−0.149	−0.083	−0.080
	0.179	0.243 *	−0.063	−0.063	0.068	0.106
	−0.024	0.046	0.011	−0.056		

Spearman correlations between sleep parameters and CSF biomarkers. The represented values are unadjusted (Rho) or adjusted for age, sex, and ApoE4 status (Rho adjusted). The values in bold represent statistically significant correlations: *p < 0.05 and **p < 0.01.

After adjusting all the parameters for age, sex, and ApoE4 status, there was a similar pattern of correlations in relation to YKL-40, NF-L, and CML. Sleep efficiency (rho = −0.298, p = 0.003), the percentage of time spent in N2 (rho = −0.245, p = 0.018), and the percentage of time spent in N3 (rho = −0.277, p = 0.007) remained negatively correlated with YKL-40. Additionally, NF-L was positively correlated with the percentage of time spent in N1 (rho = 0.240, p = 0.021) and was negatively correlated with the percentage of time spent in N3 (rho = −0.266, p = 0.010), as previously demonstrated. In addition, there was a positive correlation between CML and REM sleep latency (rho = 0.272, p = 0.019) and a negative correlation between this marker and the percentage of time spent in REM sleep (rho = −0.240, p = 0.028). The percentage of time spent in N3 was positively correlated with CEL (rho = 0.226, p = 0.039) and MDAL (rho = 0.243, p = 0.025).

Biomarker levels as a function of sleep depth

To evaluate the relationship between sleep structure and CSF biomarkers in more detail, we first selected the biomarkers that correlated with at least one sleep stage (the percentage of time spent in N1, N2, N3, and/or REM sleep) (see Table 2). We performed a PCA, which indicated that N1 and N3 stages were the sleep variables that most captured the total variability of the data (Figure 1). Based on this, we observed that our population was distributed according to the sleep depth, as deep sleepers (Figure 1, right side), moderate sleepers (Figure 1, middle), and light sleepers (Figure 1, left side). The cluster that represented the light sleepers exhibited an increased percentage of time in the N1 stage, whereas the deep sleepers cluster exhibited an increased percentage of time in N3 (p < 0.001 for both) (Supplementary Table S1).

Distribution of patients with mild-moderate AD according to the PCA. The PCA indicated that time in the N1 and N3 stages were the sleep variables that most captured the total variability of the data. Based on this, the participants were distributed in three different populations defined by tertiles: individuals who exhibited lighter sleep (light sleepers), individuals who exhibited intermediate sleep (moderate sleepers), and individuals who exhibited deep sleep (deep sleepers).

The evaluation of biomarker levels as a function of sleep depth is presented in Table 3 (for the analysis performed exclusively with Aβ42 positive individuals, see Supplementary Table S2). CSF levels of NF-L increased as the depth of sleep decreased (Figure 2). Accordingly, moderate sleep increased the probability of higher levels of NF-L with an odds ratio (OR) of 1.694 (95% confidence interval (CI): 0.609 to 4.857), but this relationship did not reach statistical significance (p = 0.316). However, light sleepers presented an OR of 3.273 (95% CI: 1.150 to 9.845; p = 0.029), which was maintained after adjusting for age, sex, and ApoE4 status (OR: 3.125; 95% CI: 0.992 to 10.442; p = 0.056). Considering that the apnea-hypopnea index was different among the groups (Supplementary Table S1), we performed an additional model including OSA as a possible confounding factor (Supplementary Table S3). Similarly, due to the influence of cardiovascular risk factors on the biomarkers herein studied, we included a model considering cardiovascular disease-associated variables (hypertension, heart disease, and stroke). Both models generated similar outcomes

compared with the previous ones, with light sleepers presenting an increased risk of higher levels of NF-L (OR: 3.171; 95% CI: 0.986 to 10.847; p = 0.057, with OSA as a confounding factor; and OR: 3.485; 95% CI: 0.991 to 13.262; p = 0.057, with cardiovascular disease-associated variables as a confounding factor).

Table 3.Biomarker levels according to sleep depth

Model 1	Model 2		Model 3			
Biomarkers	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
NF-L						
Moderate sleep	1.694 (0.609 to 4.857)	0.316	1.847 (0.646 to 5.479)	0.257	1.855 (0.646 to 5.533)	0.256
Light sleep	3.273 (1.15 to 9.845)	0.029	3.165 (1.008 to 10.552)	0.053	3.125 (0.992 to 10.442)	0.056
YKL-40						
Moderate sleep	1.255 (0.456 to 3.498)	0.66	1.34 (0.477 to 3.838)	0.58	1.343 (0.475 to 3.872)	0.579
Light sleep	1.846 (0.662 to 5.294)	0.245	1.885 (0.613 to 5.993)	0.272	1.85 (0.598 to 5.91)	0.289
Leptin						
Moderate sleep	0.609 (0.215 to 1.678)	0.341	0.841 (0.273 to 2.594)	0.762	0.843 (0.272 to 2.608)	0.766
Light sleep	0.409 (0.14 to 1.149)	0.094	0.825 (0.244 to 2.864)	0.757	0.839 (0.246 to 2.942)	0.78
CEL						
Moderate sleep	0.643 (0.212 to 1.892)	0.426	0.71 (0.229 to 2.149)	0.546	0.705 (0.227 to 2.138)	0.539
Light sleep	0.454 (0.144 to 1.368)	0.166	0.478 (0.138 to 1.582)	0.231	0.469 (0.135 to 1.561)	0.222
CML						
Moderate sleep	0.872 (0.293 to 2.563)	0.803	0.854 (0.281 to 2.563)	0.779	0.859 (0.282 to 2.579)	0.786
Light sleep	0.543 (0.175 to 1.63)	0.28	0.524 (0.154 to 1.718)	0.29	0.531 (0.155 to 1.749)	0.302
GSA						
Moderate sleep	0.679 (0.227 to 1.987)	0.481	0.807 (0.256 to 2.519)	0.711	0.822 (0.26 to 2.578)	0.736

Light sleep	0.718 (0.235 to 2.152)	0.555	1.25 (0.365 to 4.451)	0.725
	1.32 (0.381 to 4.784)	0.664		
MDAL				
Moderate sleep	0.989 (0.332 to 2.922)	0.984	1.056 (0.344 to 3.237)	
	0.923	1.022 (0.324 to 3.205)	0.971	
Light sleep	0.47 (0.15 to 1.417)	0.184	0.623 (0.184 to 2.079)	0.44
	0.553 (0.157 to 1.897)	0.347		

Logistic regression models assessing the probability of having high levels of biomarkers based on sleep depth. Model 1, unadjusted analysis; model 2, adjusted for age and sex; model 3, adjusted for age, sex, and ApoE4 status.

Regarding YKL-40, there was also a progressive increase in the CSF levels as the depth of sleep decreased (Figure 2). Moderate sleepers presented an OR of 1.343 (95% CI: 0.475 to 3.872), and deep sleepers presented an OR of 1.850 (95% CI: 0.598 to 5.910) after adjustment. However, none of these relationships reached statistical significance ($p = 0.579$ and $p = 0.289$, respectively). A similar outcome was observed for the marker GSA. In moderate sleepers (OR: 0.822; 95% CI: 0.260 to 2.578; $p = 0.736$) and light sleepers (OR: 1.320; 95% CI: 0.381 to 4.784; $p = 0.664$) the OR increased as GSA levels rose, but this relationship did not reach statistical significance after adjusting for the conditional factors. No significant differences were observed for leptin, CEL, CML, or MDAL.

Discussion

In the present study, we investigated the relationship between sleep structure and several CSF biomarkers that reflect distinct aspects of AD pathophysiology. Our findings suggest significant correlations between sleep and molecules such as NF-L and YKL-40. We observed that this cohort of patients exhibited three different profiles according to the sleep depth, which we classified as light, moderate, and deep sleep. Interestingly, light sleepers demonstrated an increased probability of high levels of NF-L, a marker that predicts cognitive decline and is strongly correlated with T-tau. Despite the reduced statistical power, a similar outcome was observed for YKL-40 levels.

Several CSF and plasma biomarkers have been studied in recent years to achieve early and/or accurate diagnosis of the disease. Among these biomarkers, NF-L, a biomarker of axonal damage and neurodegeneration, plays a prominent role in AD as well as in other neurodegenerative and nondegenerative diseases [33, 34]. In AD, this molecule is also a marker of cognitive decline and predicts clinical progression [35]. In the present study, we observed a positive correlation between CSF NF-L levels and N1 sleep and a negative correlation between this marker and N3 sleep. Furthermore, individuals who spent more time in the lighter phases of sleep (light sleepers) had an increased probability of having high NF-L levels compared with that of individuals who spent more time in N3 sleep (deep sleepers). This is in line with studies reporting the importance of SWS for different biological processes, including Aβ42 clearance [17, 36]. Based on this, it can be speculated that a decrease in the time spent in N3 affects the elimination of NF-L in a similar manner as that demonstrated for Aβ42. In addition, the decreased clearance of toxic metabolites due to poor sleep quality leads to neuronal damage and axonal injury, which in turn can increase NF-L levels [37]. Regardless, our findings suggest a possible role for NF-L as a marker of sleep disruption in AD.

Accordingly, Zhang and collaborators [38] reported an important role for NF-L in predicting both self-reported and objective sleep quality in a non-AD population with chronic insomnia disorder.

We demonstrated associations between sleep structure and other biomarkers, such as YKL-40, that were not observed when we stratified our population into three clusters according to sleep profiles. This suggests that the relationship between these markers and sleep may be particular to specific sleep stages. In fact, the YKL-40 level is negatively correlated with the percentage of time spent in N2 and N3. YKL-40 has been reported to be a promising indicator of glial inflammation in AD [39], and poor-quality sleep is associated with an increase in microglial activation and neuroinflammation [40, 41]. Accordingly, previous studies have reported that CSF YKL-40 levels predict poor sleep in Aβ-positive older adults [42]. Similarly, worse self-reported sleep quality is associated with higher levels of this marker in non-AD participants with and without a family history of AD [43]. This may explain the negative association between the time spent in N3 and YKL-40 levels observed in the current study.

Studies have reported that Aβ levels decrease due to an increased rate of metabolite clearance during sleep and that this event is especially dependent on SWS [17, 36]. Accordingly, sleep deprivation and chronic sleep restriction increase the levels of this marker [14]. Similarly, specific sleep events, such as sleep spindles, are able to predict T-tau concentrations in healthy individuals [19, 44]. Despite this, we did not observe the expected association between sleep and the classical biomarkers of AD (Aβ, T-tau, and P-Tau). In fact, such association was not observed in a study with patients with AD [45]. Based on this, we hypothesize that the relationship between sleep and AD classical biomarkers is present up to a specific point of the disease's progression. However, as the levels of these markers increase, such correlation is no longer observed. At this stage, other markers, such as NF-L, may better represent sleep quality.

Evidence from both animal and human studies have linked sleep deprivation to increased oxidative stress and reduced antioxidant defenses [46, 47]. In addition, studies have suggested that diverse oxidative pathways contribute to AD pathogenesis [48, 49]. In fact, Pamplona and collaborators [30] reported increased levels of the same oxidative stress markers that we measured in this study in postmortem tissue from patients with AD. However, whether sleep disturbances and poor sleep quality increase oxidative damage in patients with AD is unknown. Here, we observed that all oxidative damage markers, with the exception of AASA and CML, demonstrated an unexpected association with sleep variables. Both CEL and MDAL were positively correlated with the time spent in N3, whereas GSA was negatively correlated with the time spent in N1. Accordingly, we observed correlations with specific sleep stages but failed to observe an association with sleep depth. Further studies using oxidative damage-related approaches will improve our understanding and elucidate the relationship between oxidative stress and sleep structure in patients with AD.

It is important to address some limitations of our study. First, the patients were enrolled from a cognitive unit, not from a population-based community. Second, patients with severe AD were not included. Also, due to the sample size, the data herein presented were not adjusted for multiple comparisons. In addition, although statistically significant, the observed correlations were relatively weak and the sleep–wake schedule was not monitored in the days before the study. Considering this, our findings should be carefully considered. Furthermore, it is not possible to establish whether there was a causal relationship between sleep parameters and biomarker levels due to the cross-sectional design of the study. On the other hand, this study has some strengths. All sleep data were generated by PSG in a population of patients with mild-moderate AD, which has been accomplished in very few studies. To our knowledge, this is the first time that the association between sleep and several AD biomarkers that reflect distinct aspects of the disease has been investigated in individuals with AD.

In conclusion, we demonstrated significant correlations between different sleep parameters and AD biomarkers, such as NF-L and YKL-40. In addition, we observed that our population of patients with mild-moderate AD is divided into three different clusters according to their sleep profiles: light, moderate, and deep sleepers. Furthermore, a lack of sleep depth was associated with higher levels of NF-L, a marker of neurodegeneration that demonstrably predicts cognitive decline. This highlights the potential role of NF-L as a marker of sleep disruption in patients with mild-moderate AD. Further studies using different approaches and performed in distinct cohorts of patients will be necessary to confirm this. In case of a positive outcome, sleep-based interventions could be considered to prevent the axonal damage and possibly the cognitive decline.

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Conflict of interest statement. All authors declare that they have no conflict of interest.

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