Sleep Treatment Improves Neuropsychological Symptoms and Reduces Blood Aβ42/pTau Protein in Alzheimer's Disease Patients: a Longitudinal Study

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Sleep treatment improves neuropsychological symptoms and reduces blood $A\beta_{42}$/pTau protein in Alzheimer's disease patients: a longitudinal study

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1. Abstract

Background: Amyloid β peptide-42 ($A\beta_{42}$) and phosphorylated Tau on Threonine 181 (Tau-pT181) are the core biomarkers in Alzheimer's disease. Accumulated evidence showed an aberrant elevation of these biomarkers due to sleep disturbance. However, it is not clear if improving sleep quality reduces $A\beta_{42}$ and Tau-pT181 in Alzheimer's disease patients.

Methods: A longitudinal study was conducted on 126 patients with mild-moderate dementia due to Alzheimer's disease. Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI). Behavioral and neuropsychological assessment was conducted using multiple self-reporting questionnaire score systems. $A\beta_{42}$ and Tau-pT181 levels in blood specimens were measured using an ELISA assay kit. All patients received Donepezil treatment for Alzheimer's disease. Sleep disorders were individually managed with either medication or physical therapy according to their symptom categories.

Results: Of the 126 cases, 93 (73.8%) patients were diagnosed with sleep disorders. The PSQI scores significantly correlated with depression and anxiety scores, as well as $A\beta_{42}$ and Tau-pT181 levels. Also, a significant correlation was found among depression, anxiety, $A\beta_{42}$ and Tau-pT181 in all patients. Sleep intervention drastically reduced PSQI score, as well as $A\beta_{42}$ and Tau-pT181 levels, in parallel with improved cognition, deterioration and anxiety scores. Dementia and depression scores were improved only in patients who completely recovered (PSQI < 7) after sleep treatment. There was a 35.9% reduction of $A\beta_{42}$ levels and a 32.8% reduction of Tau-pT181 levels in this subgroup of patients (56 cases). Conversely, the reduction extent was only 6.9% for $A\beta_{42}$ and 12.2% for Tau-pT181 in patients who did not completely recover (PSQI ≥ 7 post treatment, 37 cases). Multiple logistic regression analysis

revealed that $A\beta_{42}$ level is the strongest risk factor for sleep disorder incidence and incomplete recovery after sleep treatment.

Conclusion: Sleep quality score is associated with patient depression and anxiety status, as well as blood $A\beta_{42}$ and Tau-pT181 levels. A complete recovery is critical for a full improvement of all behavioral and neuropsychological assessments, which is also associated with a deep reduction of $A\beta_{42}$ and Tau-pT181 levels. $A\beta_{42}$ level is a prognostic factor for a diagnosis of sleep disorder and treatment responsiveness.

2. Introduction

Alzheimer's disease (AD) is a progressive neuronal degenerative disorder and nearly 50 million people live with dementia worldwide - 75% are Alzheimer's disease patients[1]. In the US, AD is the sixth leading cause of death accounting for more than 122K deaths in 2018, more than breast cancer and prostate cancer combined[2]. It is estimated that 5.8 million Americans age 65 and older are suffering from Alzheimer's disease this year and the number is projected to be 13.8 million by 2050 according to the Alzheimer's Association (www.alz.org). Therefore, there is a desperate need for medical breakthroughs to prevent, slow or cure Alzheimer's disease.

Alzheimer's disease has two pathophysiological hallmarks in the brain: interstitial deposition of insoluble amyloid-β (Aβ) peptides and intracellular aggregation of hyperphosphorylated Tau proteins[3]. Aβ deposition and Tau protein aggregation is a long-term and slow process starting 20 years or more before any noticeable symptoms, a so-called prodromal phase[4,5]. Aβ peptides are physiologically processed from its precursor protein mostly in neuronal cells after sequential proteolytic cleavage by β-secretase and γ-secretase[5,6]. There are four major isoforms of Aβ peptides (38, 40, 42 and 43), which are detectable in brain interstitial fluid (ISF), cerebrospinal fluid (CSF) and blood plasma[7]. $A\beta_{40}$ and $A\beta_{42}$ peptides are more abundant than others. The $A\beta_{42}$ peptide is less soluble due to two extra hydrophobic amino acid residues at the C-terminus, rendering it more prone to aggregation than $A\beta_{40}$[8]. Currently, the levels of $A\beta_{40}/A\beta_{42}$ peptides in patient CSF and blood are implicated as disease biomarkers in the clinic[7,9]. Tau protein is a key component of microtubule assembly in axons and its function is regulated by phosphorylation on multiple

residues including threonine 181 (Tau-pT181)[10]. Similar to Aβ peptides, Tau protein and its pT181 form are also detectable in CSF and blood specimens; their levels are associated with disease progression[11].

In recent years, sleep disorders are linked to Alzheimer's disease progression in addition to the typical symptoms of progressive loss of memory, speech and cognition[12,13]. A high prevalence (25-66%) of Alzheimer's disease patients was reported to exhibit various types of sleep disorders, including excessive daytime sleepiness (EDS), sundowning, nocturnal wandering, sunset syndrome, sleep breathing disorder (SBD) and insomnia[14]. In comparison, the incidence of sleep disorders was only 18.3-27.6% in elderly adults without cognition impairment[14]. These sleep behavior changes began in the prodromal phase of Alzheimer's disease while patients only suffered from mild cognitive impairment, possibly due to amyloid/Tau pathology before cognition decline[14]. Recent studies showed that sleep deprivation or disturbances increased Aβ peptides and Tau proteins in CSF solution compared to normal sleep controls[9,15-17] due to elevated production of Aβ peptides[18], which was supported by studies from transgenic mouse models of Alzheimer's disease[19]. On the other hand, a healthy sleep cycle was shown to facilitate Aβ clearance from the brain tissue[20]. These studies suggest a bidirectional relationship between sleep disorder and AD progression[14,21]. However, it is not clear if sleep quality improvement would reduce or slow cognition impairment in Alzheimer's disease patients.

The purpose of this study was to examine the effect of sleep quality improvement on behavioral and neuropsychological symptoms, as well as blood levels of Aβ peptides and Tau proteins in Alzheimer's disease patients with mild-moderate cognition impairments. We were

also interested in identifying potential risk factors that provide prognostic feasibility for sleep quality improvement in Alzheimer's disease patients. With a longitudinal approach, we compared the sleep quality (PSQI) score, behavioral and neuropsychological parameters (MoCA, GDS, CDR, HRSD-24 and HAMA), blood Aβ peptides and Tau proteins before and after a 6-month sleep treatment in 126 patients. Our results revealed that PSQI scores in Alzheimer's disease patients correlated with HRSD-24 and HAMA scores as well as $A\beta_{42}$ and Tau-pT181 levels, but not with MoCA, GDS or CDR scores. After sleep treatment, a significant improvement was achieved for PSQI, MoCA, GDS, HAMA scores and $A\beta_{42}$ and Tau-pT181 levels. Most interestingly, a complete recovery from sleep disorder was accompanied by improved GDS and HRSD-24 scores, as well as a deep reduction of $A\beta_{42}$ and Tau-pT181 levels. Multiple logistic regression analysis identified $A\beta_{42}$ peptide level as the sole predictor for poor sleep quality (OR = 1.013, 95% CI = 1.004-1.023, p = 0.006) and the strongest predicting factor for an incomplete recovery of sleep disorder after treatment (OR = 1.124, 95% CI = 1.065-1.186, p = 0.000) when considering all biometric parameters, neuropsychological assessments and blood biomarkers. Our study demonstrated for the first time, as the authors are aware, that sleep quality improvement is capable of slowing disease progression in patients with mild-moderate cognition impairment due to Alzheimer's disease.

3. Methods

3.1 Study design and patients

A prospective longitudinal study was designed to investigate the effect of sleep improvement on neuropsychological behaviors and the changes of blood biomarkers in

patients with mild and moderate cognition impairment due to Alzheimer's disease. A total of 146 patients were recruited at the Memory & Sleep Clinic at the Jianghan Oilfield General Hospital from February 2017 to December 2019. The diagnosis was made based on the core diagnostic criteria for AD dementia developed by the National Institute on Aging-Alzheimer's Association in 2011[22]. The diagnostic criteria for sleep disorders in AD patients were based on the definition of "Dementia-related sleep disorders" in the International Classification of Sleep Disorders (ICSD-3) guidelines[23] and the clinical diagnostic criteria for Alzheimer's disease-related sleep disorders[24]. Other inclusion criteria include no serious dysfunctions or lesions of the heart, lung, liver, kidney and other important organs and the ability to complete relevant neuropsychological assessment and auxiliary examination. Exclusion criteria included: (1) severe dementia; (2) other types of cognitive impairment, such as vascular dementia, Parkinson's disease, frontotemporal dementia, lewy body dementia, etc; (3) a history of severe mental illness; (4) association with severe debilitating disease, infectious disease, painful condition or other diseases that may affect the quality of sleep, such as chronic obstructive pulmonary disease, stroke, heart failure, kidney failure, severe cerebrovascular disease, epilepsy; (5) severe physical movement disorder.

3.2 Ethical Approval and Consent to participate

The study protocol was reviewed and approved by the Ethics Committee of the Jiangshan Oilfield General Hospital. All the participated patients and their immediate family members were informed with a written consent form, and the patient's signatures were obtained prior to enrollment. This study was conducted according to the principles stated in the Declaration of Helsinki[25].

After diagnosis and recruitment, all patients were managed by dedicated research nurses, who were responsible for collecting demographic data, medical history, physical examination, behavioral and neuropsychological assessment, biochemical specimen harvest and regular follow-up[26]. Patient medical history, gender, age, onset age of the disease, course of the disease, years of education, marital status, living situation at home and patient support level were assessed by structured questionnaires on the enrollment day. Patients were requested to spend one night at the hospital where peripheral blood specimens were harvested in the morning before daytime activity.

3.3 Sleep quality evaluation

A Chinese version of the Pittsburgh Sleep Quality Index (PSQI) questionnaire was used to evaluate the overall sleep status of the patients[27,28]. There were 18 items in the scale that were divided into 7 sub-items: the subjective sleep quality, time to sleep, sleep time, sleep efficiency, night sleep disturbance, sleep drug use and daytime dysfunction. The score of each item is 0-3 points, and the total score is 0-21 points. According to the Chinese Classification and the Diagnose Criterion of Mental Disorder (3rd edition in 2011, developed by the Psychiatry Branch of Chinese Medical Association), a PSQI score at or above 7 was set as the cutoff value for AD-associated sleep disorder (AD-SD), while patients with a PSQI score less than 7 were considered as AD without sleep disorder (AD-NonSD). This PSQI cutoff value of 7 had a sensitivity of 98.3% and a specificity of 90.2% for predicting sleep disorder in the Chinese population[28].

3.4 Assessment of behavioral and neuropsychological status

The Montreal Cognitive Assessment Scale (MoCA) was used to assess patient cognitive

function[29]. There were 7 cognitive domains: visuospatial and executive function, naming, delayed recall, attention, language, abstraction and orientation. A total score less than 26 was considered as cognitive impairment.

The Clinical Dementia Assessment Scale (CDR) was used to provide a global evaluation of the severity of dementia[30]. A CDR score of 1 was classified as mild, 2 as moderate and 3 as severe.

The Global Deterioration Scale (GDS) was used to assess the extent and progress of dementia[31], which provides an overview of patient suffering from a degenerative dementia. The GDS scale was divided into seven levels and was completed by interviewing the patients and their caregivers. A GDS score of 1 indicated no cognitive impairment, 2 indicated a very mild cognitive impairment, 3 as mild, 4 as moderate, 5 as severe, 6 as very severe and 7 as worst impairment.

The 24-item Hamilton Rating Scale for Depression (HRSD-24) was utilized to assess patient depressive symptoms[32]. In the HRSD-24 version, a total score of 8 or less was classified as no depression, 8-20 as suspicious depression, 21-35 as moderate or mild depression, and above 35 as severe depression.

Hamilton Anxiety Scale (HAMA) was used to evaluate patient anxiety and there were 14 items in this scale[33]. The scale adopts a 5-point scoring method ranging from 0 to 4 points. A total score less than 7 indicated no anxiety, 7-13 were classified as likely to have anxiety, 14-20 was definitely anxious, 21-28 was classified as significantly anxious, above 29 was classified as severe anxiety.

3.5 Measurement of blood/plasma levels of amyloid peptides and Tau-pT181 proteins

$A\beta_{42}$, $A\beta_{40}$ and Tau-pT181 levels in blood specimens were measured using the enzyme-linked immunosorbent assay (ELISA) methods. Preassembled ELISA kits for human $A\beta_{42}$ and $A\beta_{40}$ peptides (catalog #27711/27713) were purchased from IBL (Gunma, Japan). The ELISA kits for Tau-pT181 proteins (Catalog #KHO0631) were obtained from Invitrogen (Carlsbad, CA). All blood specimens were collected between 6:00 and 9:00 a.m. in a fasted state. Heparin anticoagulant blood was collected by a vacuum tube, centrifuged at 3000 rpm for 10 min for plasma separation. The samples were then aliquoted and stored at -80C. ELISA assays were conducted within one week at three repeats for each specimen.

3.6 Intervention of sleep disorders

All patients enrolled in this study received a standard anti-dementia medicine Donepezil (5-10 mg, QN). Patients with sleep disorders were managed individually based on their symptoms of sleep disorders. Detailed intervention information was summarized in Tab 2.

3.7 Data collection and statistical analysis

Data of biometrics, behavioral and neuropsychological assessment, and biochemical tests were conducted at the enrollment prior to treatment and at the end of the 6-month treatment. Statistical analysis was performed using SPSS software (version 28.0, Chicago, IL) and GraphPad Prism. Statistical comparison among multiple groups were conducted using two-tail ANOVA analysis. The longitudinal comparison of the parameters between pre- and post-treatment was analyzed using paired *t*-test. Spearman correlation analysis was used to determine the association among all analyzed parameters. Multiple logistic regression analysis was conducted to identify potential risk factors that significantly affected sleep

quality or the efficacy of sleep recovery after intervention.

3.8 Availability of data and materials

All clinical data and questionnaire data will be available upon request and there was no specific materials generated from this study.

4. Results

4.1 Patients Population and Clinical Parameters

A total of 146 patients were initially enrolled in this study and 20 patients (13.7%) were excluded before the final assessment due to unrelated death (2 cases), stroke (3 cases), acute heart attack (1 case), acute pancreatitis (1 case), femoral neck fracture (2 cases), lost contact (2 cases) and incomplete clinical data (9 cases). All biometric, behavioral and neuropsychological assessments and biochemical parameters for the final 126 patients were summarized in Tab 1. A schematic illustration for the entire study was shown in Fig S1.

Sleep quality is associated with depression, anxiety, $A\beta_{42}$ and Tau-pT181.

In this study, all cases had a CDR score of 1-2 (mild-moderate dementia), GDS score of 2-4 (mild-moderate cognition impairment) and MoCA score of 12-26 (cognition impairment). The majority of cases had a HRSD-24 score 7-12 (88.9%, suspicious depression) and HAMA score 8-15 (89.7%, likely anxiety), as shown in Fig S2. Spearman correlation analysis revealed that PQSI score was significantly associated with HRSD-24 and HAMA scores, but not with MoCA, GDS, CDR (Tab 3), as well as clinical parameters including age, sex, education, course (disease length), BMI and onset age of the disease (Supplemental data Tab S1). These data indicate that sleep disorder is associated with depression and anxiety in Alzheimer's disease patients.

Blood levels of $A\beta_{42}$ peptides and Tau-pT181 protein have been considered as reliable biomarkers for Alzheimer's disease[11]. In this study, two amyloid peptides ($A\beta_{42}$ and $A\beta_{40}$) and Tau-pT181 protein were assessed in all patients to evaluate their correlation with sleep quality status. Our results showed a significant correlation of $A\beta_{42}$ and Tau-pT181 but not $A\beta_{40}$ levels with PSQI, HRSD-24 and HAMA scores (Tab 3). Similarly, a strong correlation was noticed either between $A\beta_{42}$ and Tau-pT181 levels or between $A\beta_{40}$ levels and GDS scores. Interestingly, a negative correlation was observed between $A\beta_{40}$ levels and MoCA scores. These data strongly suggest that blood levels of $A\beta_{42}$ and Tau-pT181 are associated with patient sleep quality, depression and anxiety and that $A\beta_{40}$ levels are associated with cognition impairment (MoCA and GDS scores).

4.2 Sleep treatment improves cognition and reliefs anxiety.

Based on PSQI scores, 93 cases (73.8%) were enrolled in the sleep disorder (AD-SD) group (PSQI $\geq$ 7)[28] to receive sleep treatment in addition to Donepezil. Other 33 cases (PSQI < 7, AD-NonSD group) were treated with Donepezil only. Between these two groups, significant differences were noticed in HRSD-24 and HAMA scores, as well as Tau-pT181 and $A\beta_{42}$ levels (Tab 1). Significant correlations (Spearman analysis) were also observed among PSQI, HRSD-24, HAMA, $A\beta_{42}$ and Tau-pT181, as well as $A\beta_{40}$ with MoCA or GDS (Supplemental Tab S2), consistent with the correlation data for the entire group (Tab 3).

The symptoms for sleep disorders included insomnia, daytime sleepiness, sleep rhythm disturbance, sleep disordered breathing and sunset syndrome (Tab 2). Patients with sleep disorders were treated with medical or physical therapy for 6 months in addition to the standard anti-Alzheimer's disease medicine Donepezil[34]. At the end of sleep treatment,

patients were re-assessed for MoCA, GDS, CDR, HRSD-24 and HAMA scores.

After sleep treatment, 89 out of 93 (95.7%) patients achieved a significant reduction of PSQI scores compared to their initial scores and only 4 cases remained unchanged (Fig 1A). Meanwhile, a significant improvement of MoCA, GDS and HAMA score was observed after sleep treatment (Fig 3B-3F). The improved incidence for MoCA, GDS and HAMA were 90.3% (84 cases), 94.6% (88 cases) and 86% (80 cases), respectively. However, the changes for CDR and HRSD-24 scores were not significantly different when assessed as a whole group.

We divided the cases into two subgroups based on their post-treatment PSQI scores: PSQI < 7 as the complete recovery group and PSQI ≥ 7 as the incomplete recovery group. There were 56 (60.2%) cases completely recovered from sleep disorders and 37 (39.8%) cases whose PSQI scores were still ≥ 7. As shown in Tab 4, all the parameters were significantly improved in the complete recovery subgroup. Conversely, the changes for CDR and HRSD-24 scores were not significantly different between pre- and post-treatment scores in the incomplete recovery subgroups. These data clearly suggest that sleep treatment improves cognition and reliefs anxiety and that a complete recovery of sleep disorder (PQI < 7) is critical for improving dementia and depression status.

4.3 Sleep treatment reduces blood levels of amyloid peptides and Tau proteins.

We assessed if sleep treatment resulted in significant changes of blood biomarkers, Aβ peptides and Tau-pT181 proteins. Our results showed that $A\beta_{42}$, $A\beta_{40}$ and Tau-pT181 levels were significantly reduced after sleep treatment (Fig 1G-1I), of which Tau-pT181 showed the most dramatical changes. Individually, there were 82 (88.2%) cases that showed $A\beta_{42}$

reduction: 76 (81.7%) cases for $A\beta_{40}$ and 88 (94.6%) cases for Tau-pT181 levels (Fig 1J). We calculated the reduction in net change (ng/L) and percentage (net change/pre-treatment level x %) per individual cases between pre- and post-treatment levels and the results showed a reduction of 51.6 ± 5.46 ng/L (24%) for $A\beta_{42}$, 14.6 ± 3.61 ng/L (4%) for $A\beta_{40}$ and 18.1 ± 1.76 ng/L (25%) for Tau-pT181 (Supplemental Fig S3). These data demonstrated a clear reduction of blood amyloid peptides and phosphorylated Tau protein after sleep treatment.

We compared the changes of blood biochemical markers between the complete recovery and incomplete recovery subgroups. As shown in Tab 4, both $A\beta_{42}$ and Tau-pT181 were significantly reduced after sleep treatment in both subgroups, while $A\beta_{40}$ reduction was not statistically significant ($p = 0.089$) in the incomplete recovery subgroup. Further analysis revealed that post-treatment levels of $A\beta_{42}$ and Tau-pT181 in the incomplete recovery subgroup were significantly higher than that in the complete recovery subgroups while their pre-treatment levels were compatible (Fig 2). These data indicate that the reducing effect on $A\beta_{42}$ and Tau-pT181 levels are due to sleep intervention.

After an in-depth analysis, of the complete recovery subgroup, $A\beta_{42}$ levels were reduced in 55 (98.2%) cases with an average of 35.9% reduction, Tau-pT181 proteins were reduced in 50 (89.3%) cases with an average of 32.9% reduction, and $A\beta_{40}$ peptides were reduced in 48 (85.7%) cases with only 5.1% reduction in average. In contrast, the incomplete recovery group after sleep treatment showed an increased level of $A\beta_{42}$, $A\beta_{40}$ and Tau-pT181 in 11 (29.7%), 10 (27%) and 4 (10.8%) cases, respectively. There was only about a 6.9%, 2.5% and 13.2% reduction in average levels for $A\beta_{42}$, $A\beta_{40}$ and Tau-pT181, respectively, after sleep treatment. The reduction extent, either in net changes or percentage, for $A\beta_{42}$ and Tau-pT181

levels was significantly greater in the complete recovery group compared to that in the incomplete recovery group after sleep treatment (Fig 3). However, $A\beta_{40}$ reduction had no significant difference between these two subgroups. These data further demonstrated that $A\beta_{42}$ and Tau-pT181 reduction are tightly associated with sleep quality improvement.

4.4 $A\beta_{42}$ level is the strongest predicting factor for sleep disorder development and complete recovery

Finally, we determined any of behavioral and neuropsychological scores or blood biomarkers as the risk factor for sleep disorder or sleep treatment responsiveness using multiple logistic regression analysis. The first analysis included the pre-treatment values from all 126 patients and sleep disorder (pre-treatment PSQI ≥ 7 *vs* < 7) was set as the dependent variate. After controlling for biometrics, behavioral and neuropsychological assessments, and blood biomarkers as the independent covariates, $A\beta_{42}$ was identified as the sole risk factor (odd ratio = 1.013, 95% CI = 1.004-1.023, p = 0.006). A receiver operating characteristic (ROC) curve analysis revealed that blood $A\beta_{42}$ level was a moderate prognose factor (ROC area curve = 0.667 ± 0.064, p = 0.009) compared to Tau-pT181 level (Fig 4A), HRSD-24 and HAMA scores (FIG 4B) for sleep disorder diagnosis. The second analysis only considered patients with sleep disorders (93 cases) and an incomplete recovery (post-treatment PSQI score < 7 *vs* ≥ 7) was set as the dependent variate. $A\beta_{42}$ was identified as the strongest risk factor for incomplete recovery after sleep treatment and the odd ratio was 1.124 (95% CI = 1.065-1.186, p = 0.000). ROC curve analysis indicated that $A\beta_{42}$ levels has the highest specificity (ROC curve area = 0.919 ± 0.034, p = 0.000) for treatment recovery compared to Tau-pT181 and $A\beta_{40}$ (Fig 4C), as well as HRSD-24, HAMA and MoCA scores (Fig 4D).

These data indicate that $A\beta_{42}$ level is the strongest risk factor for sleep disorder development and its complete recovery after treatment.

5. DISCUSSION

In this study, our main findings are listed below: (1) sleep quality (PSQI score) correlates with depression (HRSD-24 score), anxiety (HAMA score), blood $A\beta_{42}$ and Tau-pT181 levels; (2) $A\beta_{42}$ and Tau-pT181 levels also correlate with HRSD-24 and HAMA individually; (3) sleep treatment improves cognition (MoCA and GDS scores), reliefs anxiety (HAMA scores), and reduces blood $A\beta_{42}$ and Tau-pT181 levels; (4) a complete recovery after sleep treatment is critical for an improved dementia (CDR score) and depression (HRSD-24 score); (5) blood $A\beta_{42}$ level is the sole risk factor among the biometrics and neuropsychological parameters for sleep disorder development and a complete recovery after sleep treatment. As the authors are aware, this is the first clinical study showing a significant improvement of neuropsychological symptoms and a drastic reduction of amyloid peptides and Tau protein levels after sleep intervention in mild-moderate Alzheimer's disease.

There are many types of sleep quality assessments with different degrees of sensitivity and specificity[35]. In this study, patient sleep quality was assessed utilizing the PSQI questionnaire method[27]. The Chinese version of the PSQI questionnaire was established in 1996[36] and has been adopted as the standard procedure by the Psychiatry Branch of Chinese Medical Association. Based on this Chinese version of the PSQI score system, 73.9% (93/126) of cases were diagnosed with sleep disorders in our cohort of AD patients, which was supported by previous reports showing a significantly higher incidence of sleep

disturbance in Alzheimer's disease patients compared to control population[12,37].

Accumulating evidence has demonstrated sleep disorder as a clinical contributing factor in patients with mild-moderate Alzheimer's disease at both disease development and progression[12]. However, very few studies were reported about the correlation of sleep quality (PSQI score) with behavioral and neuropsychological symptoms that occur at any stage in Alzheimer's disease[38]. In this study, we assessed patient sleep quality with the PSQI score system in parallel with behavioral and neuropsychological symptoms using MoCA (cognition), CDR (dementia), GDS (global deterioration scale), HRSD-24 (depression) and HAMA (anxiety) scoring systems. Our results revealed that PSQI scores correlated significantly with HRSD-24 and HAMA scores but not with MoCA, GDS and CDR scores. These results were supported by a cross-section study showing an inverse correlation between sleep length and anxiety symptoms in Alzheimer's disease patients[39] and by a recent study from a Chinese cohort showing a close correlation between PSQI scores and depression in Alzheimer's disease patients[37]. So far there is a paucity of literature about the correlation of sleep quality (PSQI scores) with MoCA, GDS and CDR scores, although a weak linear correlation (coefficient of multiple correlation at 0.307-0.34) between sleep quality and GDS score was reported in Alzheimer's disease patients with moderate to severe dementia (CDR 2-3)[40], indicating a possible connection of sleep disturbance with global deterioration scale at a late stage of AD patients. In addition, lower MoCA scores were found in patients with obstructive sleep apnea hypopnea syndrome (OSAHS) compared to non-insomnia patients[41,42], indicating a potential effect of sleep disturbance on cognition impairment in patients without Alzheimer's disease. Therefore, further investigation is warranted to

determine the clinical significance of sleep disorder on behavioral and neuropsychological symptoms during different phases of Alzheimer's disease.

Aberrant accumulation of amyloid peptides and aggregation of phosphorylated Tau protein are the major pathogenic factors in the development and progression of Alzheimer's disease[3,8]. Studies in mouse models of Alzheimer's disease and in humans have convincingly demonstrated a tight association between sleep disorder and Alzheimer's disease in terms of amyloid peptides and Tau proteins[14,15,43-46]. Increased cerebrospinal fluid and blood levels of amyloid peptides and phosphorylated Tau protein were reported in patients with sleep disturbances[9,16,17,47-49] and have been considered as biomarkers for disease progression[11]. In this study, our data also showed a strong correlation between PSQI scores and blood levels of $A\beta_{42}$ and Tau-pT181, either before or after sleep intervention. Interestingly, we also found that both $A\beta_{42}$ and Tau-pT181 correlated with HRSD-24 and HAMA scores while $A\beta_{40}$ correlated with GDS and MoCA scores. Although it is hard to postulate their causative relationship among these correlated factors based on these preliminary data, they are the first clue for a further investigation to determine the effect of different amyloid peptides or Tau protein on cognition/dementia or depression/anxiety during Alzheimer's disease progression.

In the clinic for Alzheimer's disease, a fundamental challenge is to determine if sleep intervention improves behavioral and neuropsychological status and reduces amyloid peptides or Tau proteins burden in patients[21]. Our data demonstrated for the first time that sleep intervention resulted in a 24-25% reduction of blood $A\beta_{42}$ and Tau-pT181 levels. More specifically, for those patients who completely recover (PSQI < 7 post treatment), the reduction increased to 35% for $A\beta_{42}$ and 32% for Tau-pT181. In contrast, for patients who did

not completely recover (PSQI ≥ 7 post treatment), the reduction was only 6.9% for $A\beta_{42}$ and 13.2% for Tau-pT181. In parallel to these reductions of $A\beta_{42}$ and Tau-pT181, MoCA, GDS and HAMA scores were also greatly improved after sleep treatment in most complete and incomplete recovery cases while CDR and HRSD-24 scores were improved in the complete recover group. Our data are supported by other clinical studies showing a 30% increase of $A\beta_{42}$ peptides in human cerebrospinal fluid[18] and a 50% increase of phosphorylated Tau proteins in human blood[16]. These results clearly indicate a potential causative relationship between sleep disturbance and accumulation of $A\beta_{42}$ and Tau-pT181 in Alzheimer's disease. Aberrant accumulation of amyloid peptide and Tau protein occurs due to an imbalanced process of production and clearance as reported recently[14,21,45]. A high quality sleep-wake cycle is the key for a successful clearance of these pathogenic molecules[19,20,50,51]. Therefore, it is plausible that sleep intervention at the preclinical stage of Alzheimer's disease has a strong potential to prevent or slowdown disease progression[52].

Current clinical treatments for Alzheimer's disease are symptomatic but not disease intervention. Thus, it is important to identify individuals who are at high risk for developing dementia due to Alzheimer's disease for preventive intervention. For this purpose, we performed multiple logistic regressive analysis and identified blood $A\beta_{42}$ level as the sole risk factor that significantly associates with sleep disorder after controlling for all other covariates. In addition, $A\beta_{42}$ is also identified as the strongest risk factor associated with a complete recovery after sleep treatment. These results suggest that a higher $A\beta_{42}$ level in patients with mild-moderate dementia due to Alzheimer's disease is a critical risk factor in developing sleep disorder and in achieving a complete recovery from sleep disorder, although

further clinical validation is needed in a large-scale clinical study.

There were four major limitations in our study. First, we utilized the most reliable and economic biomarkers, $A\beta_{42}$, $A\beta_{40}$ and Tau-pT181[11], although more precise and accurate methods are available in the field, such as HPLC measurement of these biomarkers in CSF or blood specimens[53] and florbetapir (^{18}F)-positron emission tomography (PET) for amyloid peptide burden in brain tissue[54]. With advanced imaging technologies like functional MRI and ^{18}F (or ^{11}C-PIB)-PET, more precise changes in the brain tissue after sleep treatment will be clearly understood for mechanistic analysis. Second, we used the subjective assessment of sleep quality with PSQI score system, as well as MoCA, GDS, CDR, HRSD-24, HAMA score systems for cognition and neuropsychological evaluation because they have been widely used in the field and also as common tools in our clinical practice[55]. Although the objective self-assessment system by older subjects might not reflect their actual sleep structure and quality compared to a subjective measurement like polysomnography (PSG)[56], the changes of PSQI scores (sleep quality) between pre- and post-treatment were analyzed using a paired t-test (longitudinal self-comparison) so that the derivations among individuals were minimized. It is plausible that objective measurements with advanced technology for sleep quality monitoring will greatly strengthen our conclusion. Third, we noticed a few outliers of blood $A\beta_{42}$, $A\beta_{40}$ and Tau-pT181 values and excluded any possibility of technical error. Recent studies reported that a genetic variant of the *APOE* ε4 allele is linked to increased risk of Alzheimer's disease[14]. A separate project is ongoing by our team to conduct genetic profiling to determine this genetic variant in our cohort. Lastly, there 37 cases who did not recover completely after a 6-month treatment. We are still monitoring these cases for

another 6-month continuous sleep intervention or a longer follow-up till a possible complete recovery within 2 years.

In conclusion, this is a longitudinal study with 126 cases of Mild-moderate Alzheimer's disease and 93 cases were accompanied with sleep disorders. Their sleep quality scores significantly correlated with depression and anxiety scores, as well blood $A\beta_{42}$ and Tau-pT181 levels. Sleep treatment largely improved their sleep quality, enhanced cognition and relieved anxiety, as well as a drastically decreased blood $A\beta_{42}$ and Tau-pT181 levels. A complete recovery of sleep quality is critical to achieve a reduced dementia and depression status. Among all biometrics, neuropsychological parameters and blood biomarkers, blood $A\beta_{42}$ level was identified as the strongest risk factor for sleep disorder incidence and treatment responsiveness. Our data for the first time provided a clear clue that sleep treatment is feasible to reduce neuropsychological symptoms and might be able to slow disease progression when implicated in the early phase of Alzheimer's disease.

6. Authors' Contributors

HH designed the study and acquired funding as the principal investigator of the project. HH, ML, MZ, HC, XM, QC and JP were involved in patient recruitment, clinical examination, the eligibility evaluation, the consent form and the questionnaire assessment. JQ, JP and MZ collected blood specimens and conducted ELISA analyses. HH and BL performed statistical analysis and data interpretation. TL and BL drafted the manuscript including data tables and graphs.

7. Consent for publication

All authors agreed with the decision of this submission at current formation.

8. Competing interests

The funder of this project had no role in the study design, data collection and analysis, result interpretation and report preparation. All authors had full access to the data obtained from the study and the corresponding authors had the final responsibility for the decision of submission and publication.

9. Funding

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11. Authors' information

No specific

12. References

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13. Figure legends

Fig 1. **Sleep treatment reduces behavioral and neuropsychological scores, as well as $A\beta_{42}$ and Tau-pT181 levels**. A total of 93 cases with sleep disorders were subjected to various sleep intervention according to their symptoms of sleep disorder, as listed in Tab 2. All data were plotted as pairs of pre- and post-treatment scores or values (panel A-I). P values were derived from a paired two-tail *t*-test. Panel J is a bar graph for the net changes of blood levels of $A\beta_{42}$, $A\beta_{40}$ and Tau-pT181 between pre- and post-treatment (pre-treatment level minus post-treatment level individually).

Fig 2. **An incomplete recovery after sleep treatment is associated with higher levels of $A\beta_{42}$ and Tau-pT181**. All data were scatter-plotted in different groups and the error bars indicate the MEAN and 95% CI (confidence interval). P values were derived from a two-tail Student *t*-test.

Fig 3. **Complete recovery after sleep treatment is associated with a drastic reduction of $A\beta_{42}$ and Tau-pT181 levels.** (A) The percentage of reduction was calculated as follow: (pre-treatment minus post-treatment)/pre-treatment x 100%. (B) Net reduction was calculated as follow: pre-treatment minus post-treatment. A positive value indicates a decrease while a negative value indicates an increased post-treatment level compared to pre-treatment level. *P* values were derived from a two-tail Student *t*-test.

Fig 4. **Blood $A\beta_{42}$ level is a strong risk factor for sleep disorder incidence and**

intervention responsiveness. (A & B) Pre-treatment values for all listed parameters from all 126 cases were utilized for ROC curve analysis. Pre-treatment PSQI score < 7 or ≥ 7 was set as the dependent variate. (C & D) Pre-treatment values for all listed parameters from all 93 cases who received sleep intervention were entered for ROC curve analysis. Post-treatment PSQI score < 7 or ≥ 7 was set as the dependent variate.

14. Supplemental data:

Fig S1. **A schematic illustration of the study design and protocol**. A total of 146 patients were enrolled initially and 20 cases were excluded. Of the 126 cases, 93 patients were diagnosed with sleep disorders and were subjected to various sleep intervention in addition to Donepezil treatment. Other 33 cases received Donepezil only. After a 6-month treatment, patients were re-assessed and divided into different groups based on PSQI scores.

Fig S2. Percentage distribution of the scores for behavioral and neuropsychological assessments.

Fig S3. **Sleep treatment reduces blood levels of $A\beta_{42}$, $A\beta_{40}$ and Tau-pT181**. (A) Net changes were calculated individually as follows: pre-treatment minus post-treatment. (B) Percentage changes were calculated individually as follow: (pre-treatment minus post-treatment)/pre-treatment x 100%. All data were scatter-plotted, and the error bars indicate the MEAN and 95% CI (confidence interval).

Tab S1. The results of Spearman correlation analysis for all 126 cases.

Tab S2. The results of Spearman correlation analysis for 93 cases who received sleep treatment.

Figures

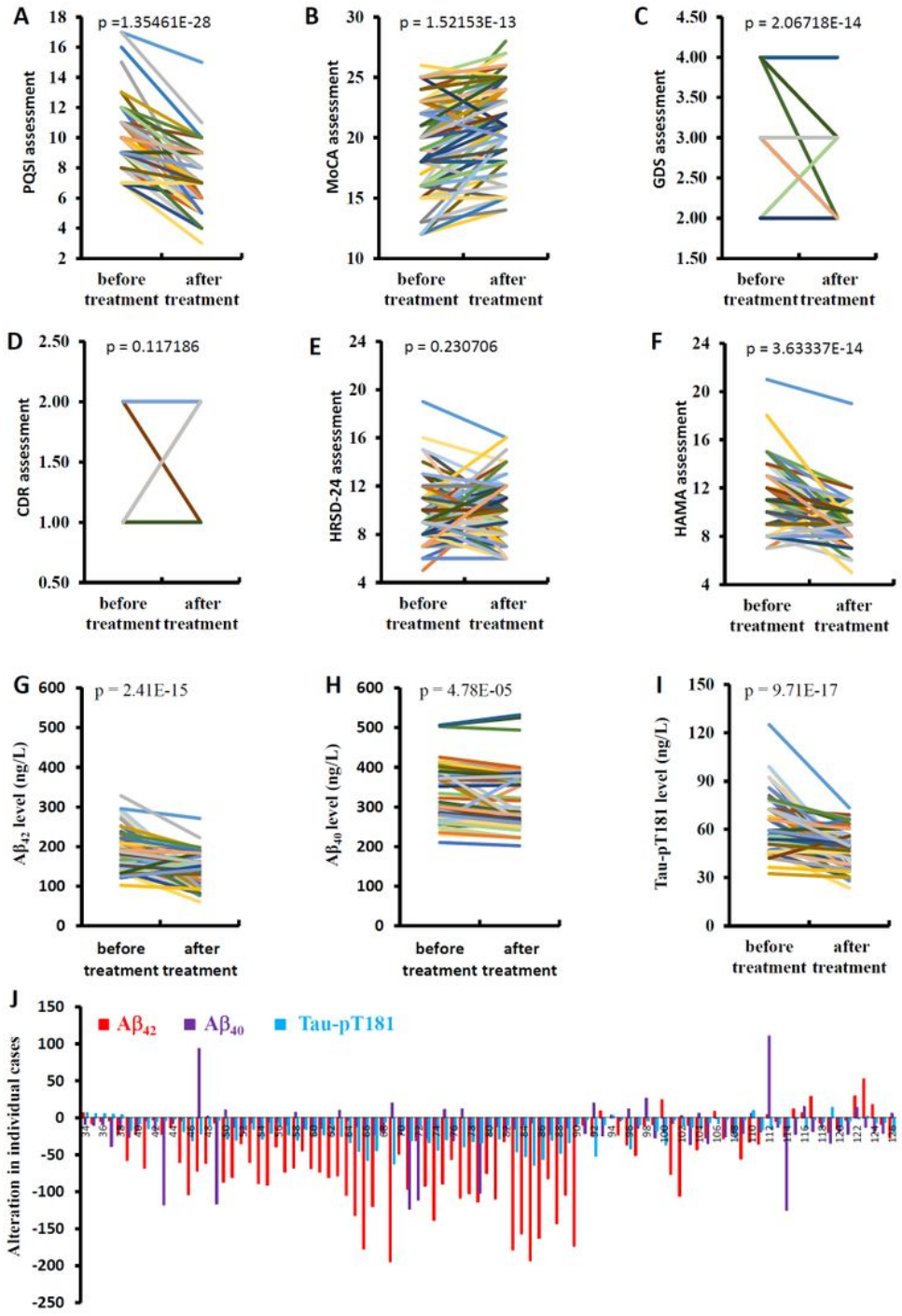


Figure 1

Sleep treatment reduces behavioral and neuropsychological scores, as well as Aβ42 and Tau-pT181 levels. A total of 93 cases with sleep disorders were subjected to various sleep intervention according to their symptoms of sleep disorder, as listed in Tab 2. All data were plotted as pairs of pre- and post-

treatment scores or values (panel A-I). P values were derived from a paired two-tail t-test. Panel J is a bar graph for the net changes of blood levels of Aβ42, Aβ40 and Tau-pT181 between pre- and post-treatment (pre-treatment level minus post-treatment level individually).

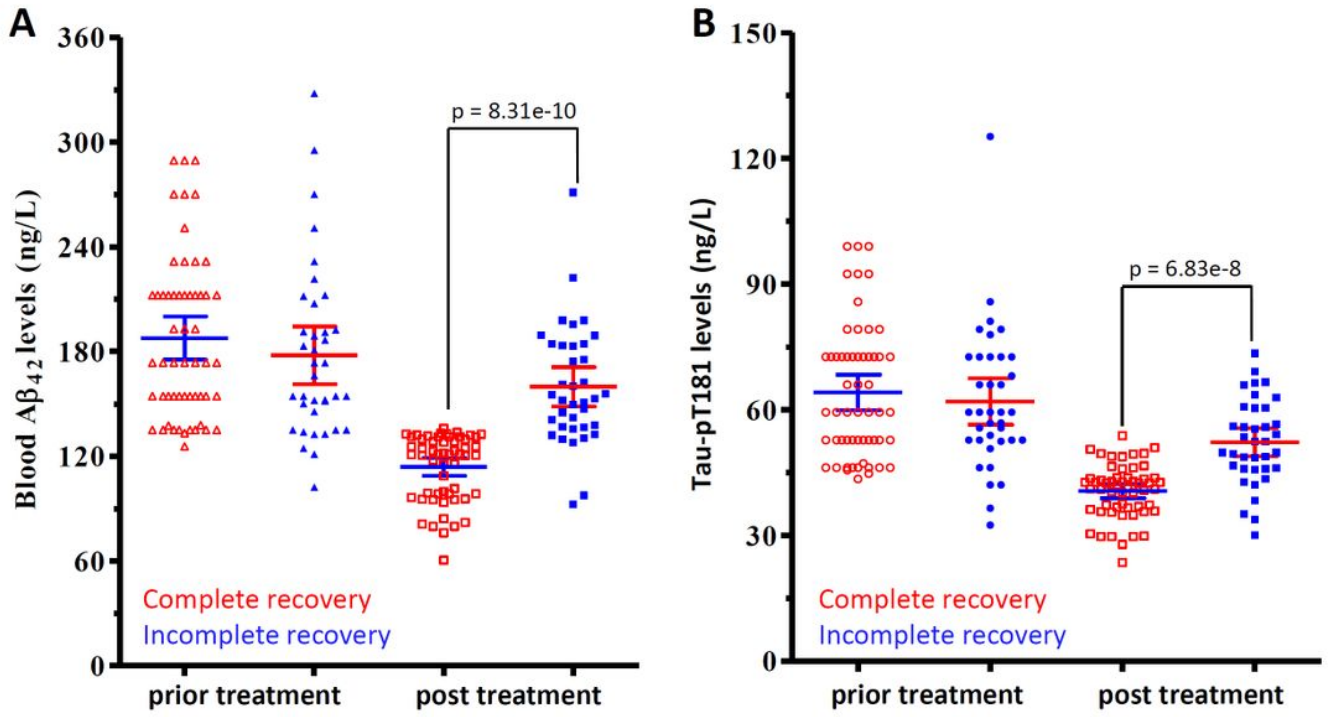


Figure 2

An incomplete recovery after sleep treatment is associated with higher levels of Aβ42 and Tau-pT181. All data were scatter-plotted in different groups and the error bars indicate the MEAN and 95% CI (confidence interval). P values were derived from a two-tail Student t-test.

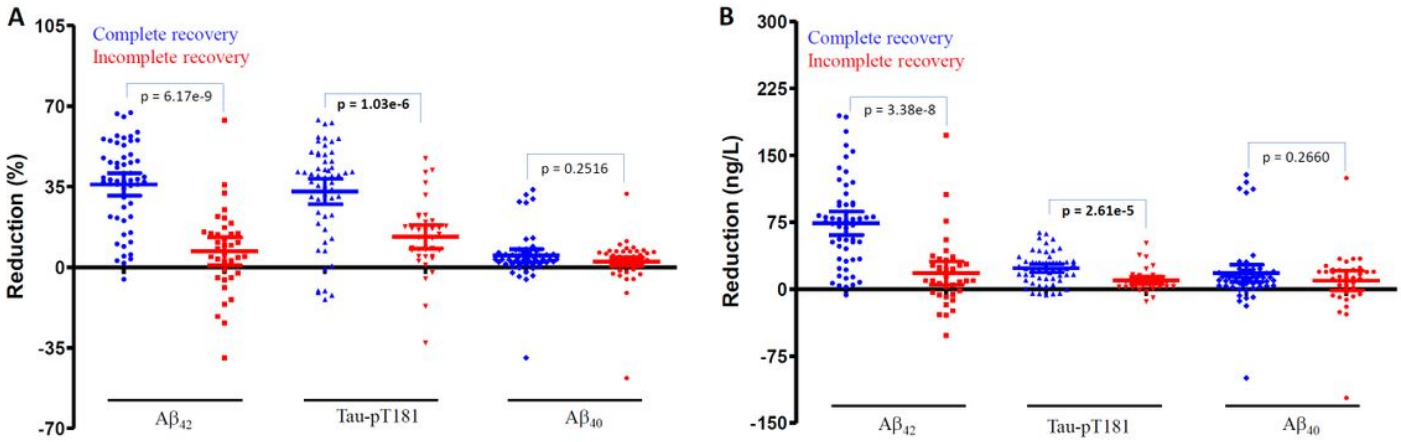
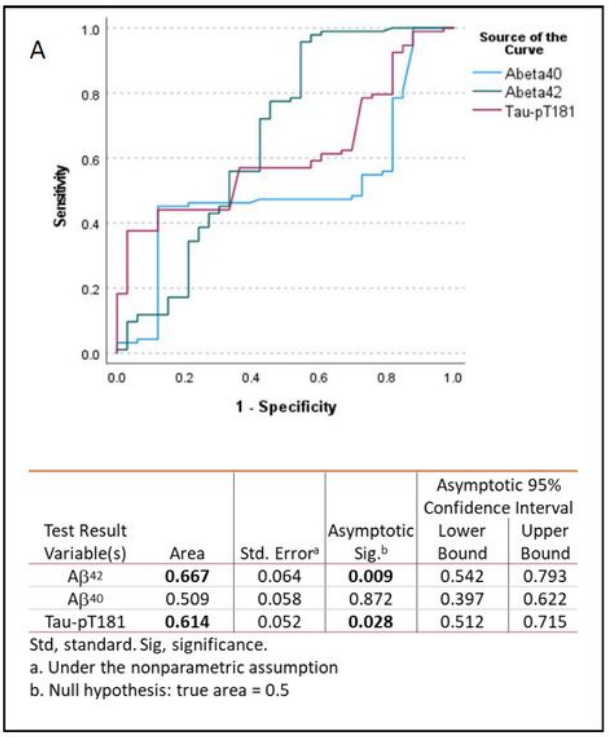


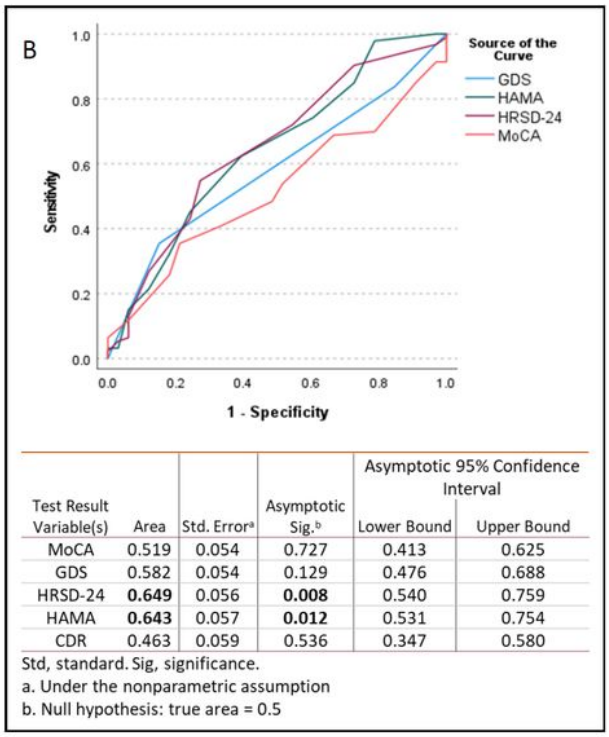
Figure 3

Complete recovery after sleep treatment is associated with a drastic reduction of Aβ42 and Tau-pT181 levels. (A) The percentage of reduction was calculated as follow: (pre-treatment minus post-treatment)/pre-treatment x 100%. (B) Net reduction was calculated as follow: pre-treatment minus post-treatment. A positive value indicates a decrease while a negative value indicates an increased post-treatment level compared to pre-treatment level. P values were derived from a two-tail Student t-test.



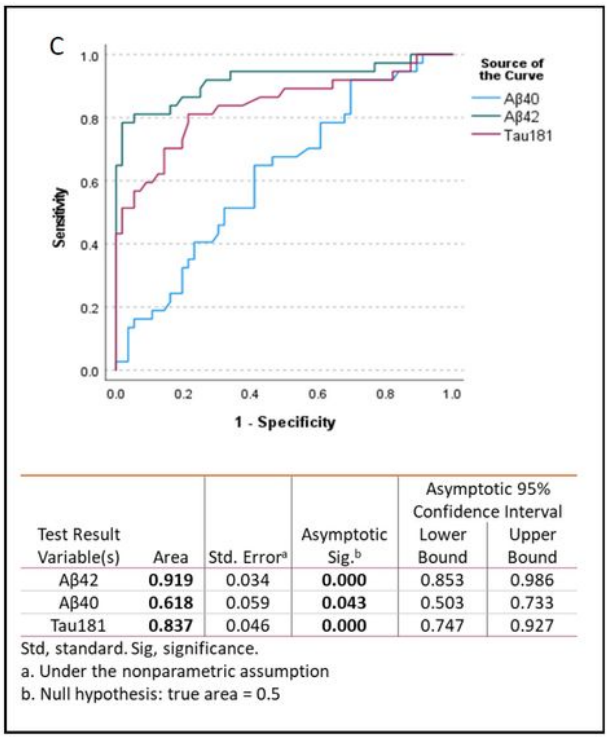
Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval Lower Bound	Upper Bound
Aβ42	**0.667**	0.064	**0.009**	0.542	0.793
Aβ40	0.509	0.058	0.872	0.397	0.622
Tau-pT181	**0.614**	0.052	**0.028**	0.512	0.715

Std, standard. Sig, significance.
a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5



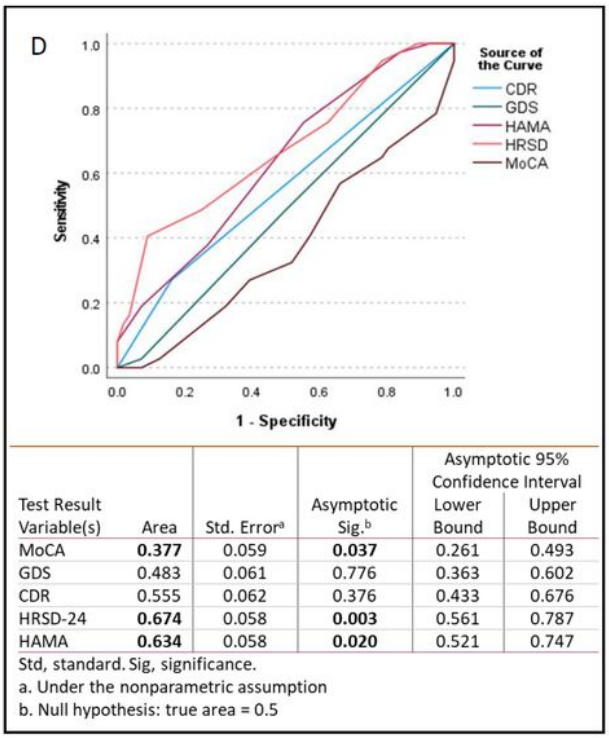
Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval Lower Bound	Upper Bound
MoCA	0.519	0.054	0.727	0.413	0.625
GDS	0.582	0.054	0.129	0.476	0.688
HRSD-24	**0.649**	0.056	**0.008**	0.540	0.759
HAMA	**0.643**	0.057	**0.012**	0.531	0.754
CDR	0.463	0.059	0.536	0.347	0.580

Std, standard. Sig, significance.
a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5



Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval Lower Bound	Upper Bound
Aβ42	**0.919**	0.034	**0.000**	0.853	0.986
Aβ40	**0.618**	0.059	**0.043**	0.503	0.733
Tau181	**0.837**	0.046	**0.000**	0.747	0.927

Std, standard. Sig, significance.
a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5



Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval Lower Bound	Upper Bound
MoCA	**0.377**	0.059	**0.037**	0.261	0.493
GDS	0.483	0.061	0.776	0.363	0.602
CDR	0.555	0.062	0.376	0.433	0.676
HRSD-24	**0.674**	0.058	**0.003**	0.561	0.787
HAMA	**0.634**	0.058	**0.020**	0.521	0.747

Std, standard. Sig, significance.
a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Figure 4

Blood Aβ42 level is a strong risk factor for sleep disorder incidence and intervention responsiveness. (A & B) Pre-treatment values for all listed parameters from all 126 cases were utilized for ROC curve analysis. Pre-treatment PSQI score < 7 or ≥ 7 was set as the dependent variate. (C & D) Pre-treatment values for all listed parameters from all 93 cases who received sleep intervention were entered for ROC curve analysis. Post-treatment PSQI score < 7 or ≥ 7 was set as the dependent variate.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tab01.jpg
- Tab02.jpg
- Tab03.jpg
- FigS01.jpg
- FigS02.jpg
- FigS03.jpg
- supplementaldata.xlsx