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ORIGINAL RESEARCH

Astroglial Mechanisms Underlying Chronic Insomnia Disorder: A Clinical Study

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Purpose: The objective of this study was to investigate whether the serum biomarkers S100 calcium binding protein B (S100B), glial fibrillary acidic protein (GFAP), brain-derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF) change in patients with chronic insomnia disorder (CID), and if this is the case, whether the altered levels of these serum biomarkers are associated with poor sleep quality and cognitive decline in CID.

Patients and Methods: Fifty-seven CID outpatients constituted the CID group; thirty healthy controls (HC) were also enrolled. Questionnaires, polysomnography, Chinese-Beijing Version of Montreal Cognitive Assessment (MoCA-C) and Nine Box Maze Test (NBMT) were used to assess their sleep and neuropsychological function. Serum S100B, GFAP, BDNF, and GDNF were evaluated using enzyme-linked immunosorbent assay.

Results: The CID group had higher levels of S100B and GFAP and lower levels of BDNF and GDNF than the HC group. Spearman correlation analysis revealed that poor sleep quality, assessed by subjective and objective measures, was positively correlated with S100B level and negatively correlated with BDNF level. GFAP level correlated positively with poor subjective sleep quality. Moreover, S100B and GFAP levels correlated negatively with general cognitive function assessed using MoCA-C. GFAP level correlated positively with poor spatial working memory (SWM) in the NBMT; BDNF level was linked to poor SWM and object recognition memory (ORcM) in the NBMT. However, principal component analysis revealed that serum S100B level was positively linked to the errors in object working memories, BDNF and GDNF concentrations were negatively linked with errors in ORcM, and GFAP concentration was positively correlated with the errors in the SWM and spatial reference memories.

Conclusion: Serum S100B, GFAP, BDNF, and GDNF levels were altered in patients with CID, indicating astrocyte damage, and were associated with insomnia severity or/and cognitive dysfunction.

Keywords: cognition, S100 calcium binding protein B, glial fibrillary acidic protein, brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor

Introduction

Insomnia is a common health problem characterized by subjective complaints about difficulties in initiating or maintaining sleep, early morning awakenings, and significant impairments in important areas of daytime functioning.[1] Roughly 50% of insomnia patients with more severe symptoms have a chronic course.[2] In most cases, chronic insomnia disorder (CID) is associated with medical and psychiatric disorders.[3,4] Even though the burden of CID on both individuals and society is significant, the neural underpinnings of insomnia are poorly understood.[5]

Accordingly, there is an urgent need to determine the neural mechanisms of CID, which would inform future attempts to alleviate or treat insomnia symptoms.

In previous studies, CID was considered a functionally injurious disease causing different functional impairments, such as fatigue, irritability, and cognitive impairment.[1,6] Patients with CID were more likely to report that cognitive impairments contributed to their reduced quality of life, highlighting the clinical significance of performance impairments.[7,8] However, we could not reasonably explain why functional impairment, especially the obvious residual cognitive function impairment, became a prominent residual symptom although other symptoms of insomnia disappeared after effective treatment. In addition, it is still unknown whether these functional impairments in patients with CID are a direct consequence of sleep loss or that of brain injury indirectly related to insomnia.

A growing body of studies has investigated insomnia-related functional or structural alterations in the brain. In terms of changes in gray matter, neuroimaging studies have linked insomnia to a decrease in gray matter in the left orbitofrontal cortex[9,10] and hippocampus.[11] In addition, diffusion tensor imaging has been used to evaluate differences in white matter tracts between insomniacs and matched controls.[12] Insomnia-related alterations in functional connectivity have also been investigated, but the results have been relatively inconsistent so far.[13–15] In summary, most studies on brain structure have evaluated insomnia-related differences in specific regions, but neuroimaging studies have not been able to clearly conclude what kind of damage insomnia causes and in which areas this damage occurs.[16] Furthermore, very few studies up till now have assessed the relevant microstructural changes in the brain, for example, at the cellular level.

Although CID can be assessed using objective means such as neuroimaging and polysomnography (PSG), which are regarded as standard methods for evaluation of objective sleep,[17,18] these methods are often impractical, as clinical screening and research tools are expensive. Simpler methods, such as those based on serological indicators, may provide information that is just as useful to clinicians. Therefore, there is increasing interest in the expanding field of serum markers for CID.

Astrocytes are the most predominant glial cells in the brain, and play a crucial role in sleep regulation under physiological conditions. Astrocytes can release adenosine and pro-inflammatory cytokines, with direct influence on sleep pressure, intensity, and duration. Moreover,

astrocytes determine the urge to sleep by clearing toxic substances through the glymphatic system.[19,20] Serum S100 calcium-binding protein B (S100B) levels are neurobiochemical markers and can reflect the severity of astrocyte damage. Our preliminary study showed that patients with CID had significantly increased serum S100B levels, while their concentrations did not change significantly after effective therapy.[21] Thus, we speculate that CID patients may have pathological changes in their astrocytes. Unfortunately, we did not control for potential non-cerebral sources of serum markers, and as various subtypes of leukocytes can also secrete S100B,[22] this was a limitation of the experimental design.

There is a need to investigate other astrocyte-related markers, such as glial fibrillary acidic protein (GFAP),[23] brain-derived neurotrophic factor (BDNF),[24] and glial cell line-derived neurotrophic factor (GDNF),[25] and study their role in CID. The aim of this study was to determine whether astrocyte-specific biomarker levels in patients with CID could serve as objective and accurate complementary diagnostic tools for the detection and assessment of insomnia severity.

Methods
Subjects

Fifty-seven outpatients with CID at the Clinics of Sleep Disorders of both the First Affiliated Hospital and the Affiliated Chaohu Hospital of Anhui Medical University were enrolled in this study. Patients were diagnosed with CID according to the International Classification of Sleep Disorders, third edition (ICSD-3).[26] Patients were excluded if they had craniocerebral injury, spinal cord injury, neurodegenerative neurological diseases, intracranial vascular disease or any other psychiatric disorders. Thirty healthy subjects were selected as healthy controls (HC), and the inclusion criteria were as follows: (1) with similar demographics, (2) without subjective complaint of insomnia and mood disorder with a Pittsburgh sleep quality index (PSQI) score <7,[27,28] a 17-item Hamilton depression rating scale (HAMD-17) score <7,[29] and (3) a Chinese-Beijing Version of Montreal Cognitive Assessment (MoCA-C) score ≥26.[30]

Ethics Approval and Informed Consent

This study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committees of the First Affiliated Hospital and the Affiliated Chaohu Hospital of Anhui Medical University

(Number 201,805-kyxm-01). All subjects signed a written informed consent form.

Baseline Data Collection

A researcher-developed questionnaire was used to collect demographic characteristics (ie, age, gender and educational information), medical history, and family history.

Depression, Anxiety and Comprehensive Cognition Evaluations

The HAMD-17 was employed to assess the severity of depressive symptoms.[29] The questionnaire contained 17 terms, and the total scores ranged from 0 to 52, where higher scores indicate greater depressive severity. In China, the corresponding grades to depressive states are as follows: no depression (0–7), mild depression (8–17), moderate depression (18–23), and severe depression (24–52).

The 14-item Hamilton anxiety rating scale (HAMA-14) was used to assess the severity of anxiety symptoms.[31] The 14 items are rated from 0 to 4, and they incorporate groups of symptoms (ie, symptoms related to anxiety mood, fears, feelings of tension, sleep, cognitive function, depressed mood, and behavior in conversation and autonomic, respiratory, somatic, cardiovascular, sensory, gastrointestinal, and reproductive and urinary symptoms). In China, the total scores corresponding to different anxiety states are as follows: <7, no anxiety; ≥7, probable anxiety; ≥14 mild anxiety; ≥21 moderate anxiety; ≥29, severe anxiety.

The MoCA-C scale is a cognitive screening test that has high levels of reliability and validity and is widely used in China.[30] It measures eight domains including visuospatial and executive functions, naming, attention, abstraction, short-term memory, delayed recall, language, and orientation. The maximum score is 30 points, and scores ≥26 are considered as normal cognitive function.

Subjective and Objective Insomnia Assessment

PSQI, a self-reported questionnaire, was employed to assess subjective quality of sleep.[28] It contains 7 domains including subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication and daytime dysfunction. The total score of PSQI ranges from 0 to 21, with a higher score corresponding to poorer sleep quality.

PSG was used to evaluate object sleep on two consecutive nights. The first night was used to acclimatize to novel environment. The second night was to assess the aspects of sleep including sleep continuity and sleep structure. The former involved the parameters of total sleep time (TST), sleep efficiency (SE) and sleep onset latency (SOL); the latter included percentages of sleep stage 1 (N1%), stage 2 (N2%), and slow wave sleep (SWS, N3%), time awake after sleep onset, and time (REMT) and percentage (REM%) of rapid eye movement sleep.[32]

Multi-Dimensional Memory Evaluation

The Nine Box Maze Test (NBMT) was modified to evaluate multi-dimensional memory, including spatial/object working memories (SWM/OWM), spatial/object reference memories (SRM/ORM), and object recognition memory (ORcM).[33,34] The experiment was conducted according to our previous studies.[21,35] The test included object-familiarization, training and testing periods. The numbers of errors were recorded as the performance of SWM, OWM, SRM, ORM, and ORcM, whereby a higher number of errors indicates poor memory.

Blood Sample Collection

Blood samples were collected in the morning (between 07:30 h and 08:00 h) following the second PSG. Samples were separated immediately by centrifugation, and the serum was frozen at −80°C until assay. According to the manufacturer's instructions (Millipore Corporation; USA), a quantitative sandwich enzyme-linked immunosorbent assay was used to quantify serum levels of GFAP, BDNF, GDNF, and S100B.

Statistical Analysis

SPSS® 22.0 for Windows was used for statistical analyses. The Student's *t*-test was selected to analyze normally distributed data expressed as mean ± standard deviation (SD); the Mann−Whitney U-test was selected to analyze nonparametric data expressed as the 25th, 50th, and 75th percentiles (P25, P50, and P75, respectively). Spearman correlation analysis was used to analyze the correlations between serum S100B, GFAP, GDNF, and BDNF levels and sleep quality, general cognition, and NBMT performances. A multivariate data reduction technique of principal component analysis was performed to analyze the relationships among parameters from patients with CID. In addition, receiver operating characteristics (ROC) analysis was used to compare the diagnostic information provided by different biomarkers according to the calculated areas under the curves (AUCs), optimal cut-off

points, sensitivity, and specificity. Two-tailed P-values of ≤0.05 were considered statistically significant.

Results

Background Data

There were no significant differences in sex, age and educated levels between the two groups ($p > 0.05$, Table 1). Compared to the HC, patients with CID had significantly higher HAMD-17 and HAMA-14 ($p < 0.001$).

Subjective and Objective Insomnia

Individuals in CID group had significantly higher PSQI scores than the HC ($p < 0.001$, Table 1). Although limited PSG samples were available, the PSG results showed that the patients with CID had significantly longer SOL and significantly lower SE and TST than the HCs ($p < 0.01$, Table 2).

Comprehensive Cognition and Nine Box Maze Performance

Compared to the HC, patients with CID had significantly lower MoCA-C scores ($p < 0.01$) and more errors in tests of SWM, OWM, and ORcM ($p < 0.01$, Table 3).

Changes of Serum S100B, GFAP, GDNF and BDNF

Compared to the HC, patients with CID had higher S100B ($p < 0.001$) and GFAP ($p < 0.05$) levels and lower GDNF and BDNF ($p < 0.001$) levels (Figure 1AD).

Correlations Among Serum Biomarkers, Sleep Parameters and Cognitive Performances

The correlations between serum biomarkers of astrocyte damage and subjective sleep parameters are shown in

Table 1 Background Data, Subjective Sleep Quality

Variables	CID (N = 57)	HC (N = 30)	p-value
Male/Female	16/41	10/20	0.630
Age (yr)	46.58 ±12.26	46.50 ± 9.02	0.973
Years of education (yr)	9.84 ± 4.33	10.20 ± 3.71	0.702
HAMD (score)	6.00(6.00, 10.00)	3.00(2.00, 4.00)	<0.001
HAMA (score)	9.00(6.00, 11.00)	2.50(2.00, 4.00)	<0.001
PSQI (score)	14.00(12.00, 16.00)	4.00(3.00, 5.00)	<0.001

Notes: Normal distribution variables (Mean ± SD); non-normal distribution variables (P50 [P25, P75]).
Abbreviations: CID, chronic insomnia disorder; HC, healthy controls; HAMD, Hamilton depression rating scale (17 items); HAMA, Hamilton anxiety scale (14 items); PSQI, Pittsburgh sleep quality index.

Table 2 The Objective Sleep Quality

Variables	CID (N = 27)	HC (N = 10)	p-value
TST (min)	349.87 ± 88.04	453.80 ± 58.83	0.002
SOL (min)	17.00 (9.50, 49.50)	6.00 (2.37, 16.50)	0.002
SE (%)	68.98 ± 15.04	84.59 ± 8.96	0.004
REMT (min)	41.50 (22.50, 56.00)	38.50 (31.60, 61.13)	0.719
REMT% (%)	12.00 (6.90, 15.60)	9.65 (7.90, 25.40)	0.452
N3 (min)	32.00 (3.50, 71.50)	73.00 (44.63, 124.25)	0.080
N3% (%)	11.50 (1.20, 21.30)	17.50 (10.05, 28.68)	0.237

Notes: Normal distribution variables (Mean ± SD); non-normal distribution variables (P50 [P25, P75]).
Abbreviations: CID, chronic insomnia disorder; HC, healthy controls; TST, total sleep time; SOL, sleep onset latency; SE, sleep efficiency; REMT, rapid eye movement sleep time; REM%, percentage of the rapid eye movement sleep time; N3, sleep stage 3; N3%, percentage of the sleep stage 3.

Table 4. In patients with CID, S100B (r = 0.352, $p < 0.01$) and GFAP (r = 0.287, $p < 0.05$) levels correlated positively with PSQI score. Serum BDNF level (r = 0.369, $p < 0.01$) was negatively correlated with PSQI score.

The correlations between serum biomarkers and objective sleep parameters are displayed in Figure 2AE. The levels of S100B negatively correlated with the TST, SE, N3 and N3% in the CID (r = −0.485, −0.454, r = −0.433 and −0.423; $p < 0.05$). The BDNF positively correlated with the TST (r = 0.386, $p < 0.001$).

The correlations between serum biomarkers and cognitive parameters are shown in Table 4. S100B and GFAP concentrations (r = −0.301, $p < 0.05$ and r = −0.307, $p < 0.05$, respectively) were negatively correlated with MoCA-C scores in the CID group. Regarding the performance in the NBMT, the CID group had significant positive correlations between serum GFAP level and SWM errors (r = 0.317, $p < 0.05$) and negative correlations between serum BDNF level and the errors of SWM and ORcM (r = −0.456, $p < 0.01$ and r = −0.410, $p < 0.01$, respectively).

Principal Component Analysis for CID

The Kaiser–Meyer–Olkin value, as a measure of sampling adequacy, was 0.501, and p was lower than 0.001 in Bartlett's test of sphericity, suggesting that these measures selected in the CID group (the number of patients who completed the PSG evaluation is 27) are appropriate for principle components analysis. Thus, five significant factors were obtained, and the component loadings of variables on the rotated factors are shown in Table 5. The PSG parameters of sleep (TST, SE, and REMT), PSQI score, OWM, and S100B level loaded highly on factor 1. REM%, ORcM, and GDNF and BDNF level loaded on factor 4; GFAP level, MoCA-C score, SRM, and SWM loaded on factor 5.

Table 3 The Cognitive Performance

Variables	CID (N = 57)	HC (N = 30)	*p*-value
MoCA-C (score)	25.00(23.00, 27.00)	26.00(26.00, 27.00)	0.006
ORM (number of errors)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.222
SRM (number of errors)	0.00 (0.00, 1.00)	0.00 (0.00, 0.00)	0.065
OWM (number of errors)	1.00 (0.00, 1.50)	0.00 (0.00, 0.25)	0.002
SWM (number of errors)	3.00 (2.00, 4.00)	1.00 (1.00, 2.00)	<0.001
ORcM (number of errors)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.022

Notes: Normal distribution variables (Mean ± SD); nonnormal distribution variables (P50 [P25, P75]).
Abbreviations: CID, chronic insomnia disorder; HC, healthy controls; MoCA-C, Chinese-Beijing Version of Montreal Cognitive Assessment; ORM, object reference memory; SRM, spatial reference memory; OWM, object working memory; SWM, spatial working memory; ORcM, object recognition memory.

Appropriateness of Serum Indexes as Diagnostic Biomarkers for CID

The results of the ROC analysis are listed in Table 6. The AUCs of S100B, GFAP, GDNF, and BDNF levels were all larger than 0.70, which were greater for predicting CID compared to HC. The optimal cut-off values for S100B and GFAP were 323.9 pg/mL and 888.5 pg/mL, respectively, indicating that the subject should be considered as having CID other than health sleeper when their serum biomarker levels exceed the corresponding cut-off values. The optimal cut-off values for GDNF and BDNF were 24.5 pg/mL and 26.7 ng/mL, indicating that the subject should be considered as having CID other than health sleeper when their serum biomarker levels are lower than the corresponding cut-off values.

Discussion

CID Seems to Be an Organic Condition Characterized by Prominent Dysfunction of Astrocytes

S100B, GFAP, BDNF, and GDNF are deemed to be astrocyte-related biomarkers, as shown in Table 7. In the
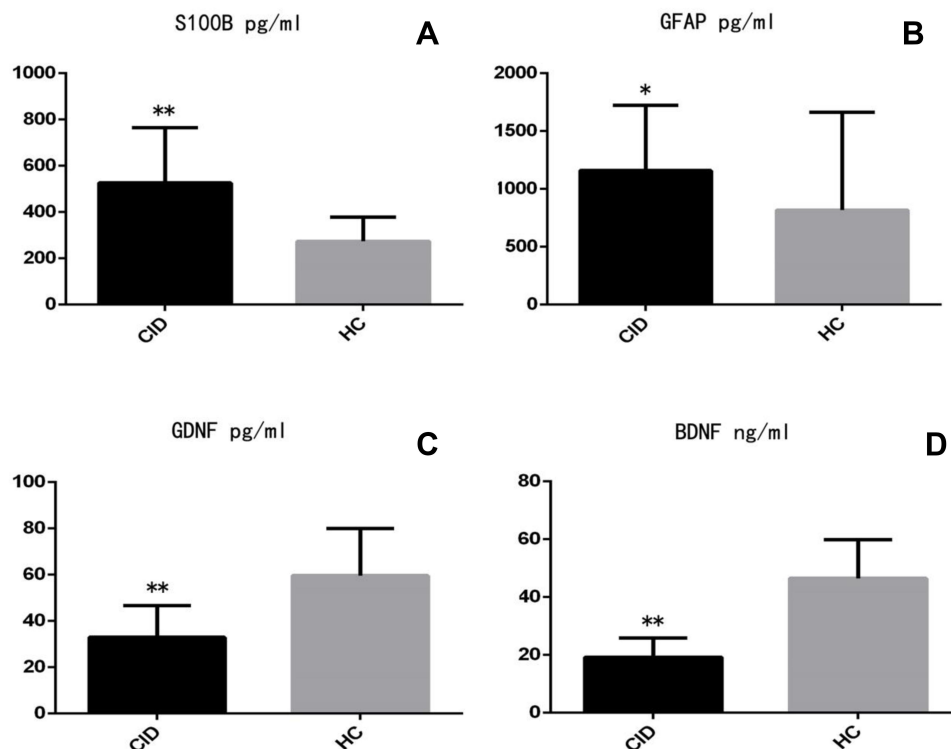

Figure 1 (**A–D**). Comparison of serum levels between chronic insomnia disorder patients (CID) and healthy controls (HC).
Notes: *t*-test for independent samples; **p < 0.01. *p < 0.05.
Abbreviations: S100B, S100 calcium binding protein B; GFAP, glial fibrillary acidic protein; GDNF, glial derived neurotrophic factor; BDNF, brain derived neurotrophic factor.

**Table 4** The Correlations Between S100B/GFAP/GDNF/BDNF and Subjective Sleep Quality, Cognitive Performance

Variables	PSQI (Score)	MoCA-C (Score)	ORM (Score)	SRM (Score)	OWM (Score)	SWM (Score)	ORcM (Score)
S100B (pg/mL)	**0.352****	**−0.301***	−0.073	0.126	−0.027	0.180	0.102
GFAP (pg/mL)	**0.287***	**−0.307***	0.099	0.055	0.197	**0.317***	0.051
GDNF (pg/mL)	−0.185	0.089	−0.074	−0.079	0.018	−0.178	−0.234
BDNF (ng/mL)	**−0.369****	−0.029	0.069	0.058	−0.047	**−0.456****	**−0.410****

Notes: *Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level (2-tailed).
Abbreviations: S100B, S100 calcium binding protein B; GFAP, glial fibrillary acidic protein; GDNF, glial derived neurotrophic factor; BDNF, brain derived neurotrophic factor; PSQI, Pittsburgh sleep quality index; MoCA-C, Chinese-Beijing Version of Montreal Cognitive Assessment; ORM, object reference memory; SRM, spatial reference memory; OWM, object working memory; SWM, spatial working memory; ORcM, object recognition memory.

present study, we found that serum levels of S100B and GFAP in CID patients were significantly higher than those in healthy individuals, while levels of GDNF and BDNF in CID were significantly lower when compared to healthy individuals. Therefore, it is reasonable to assume that patients with CID have some degree of astrocyte damage.
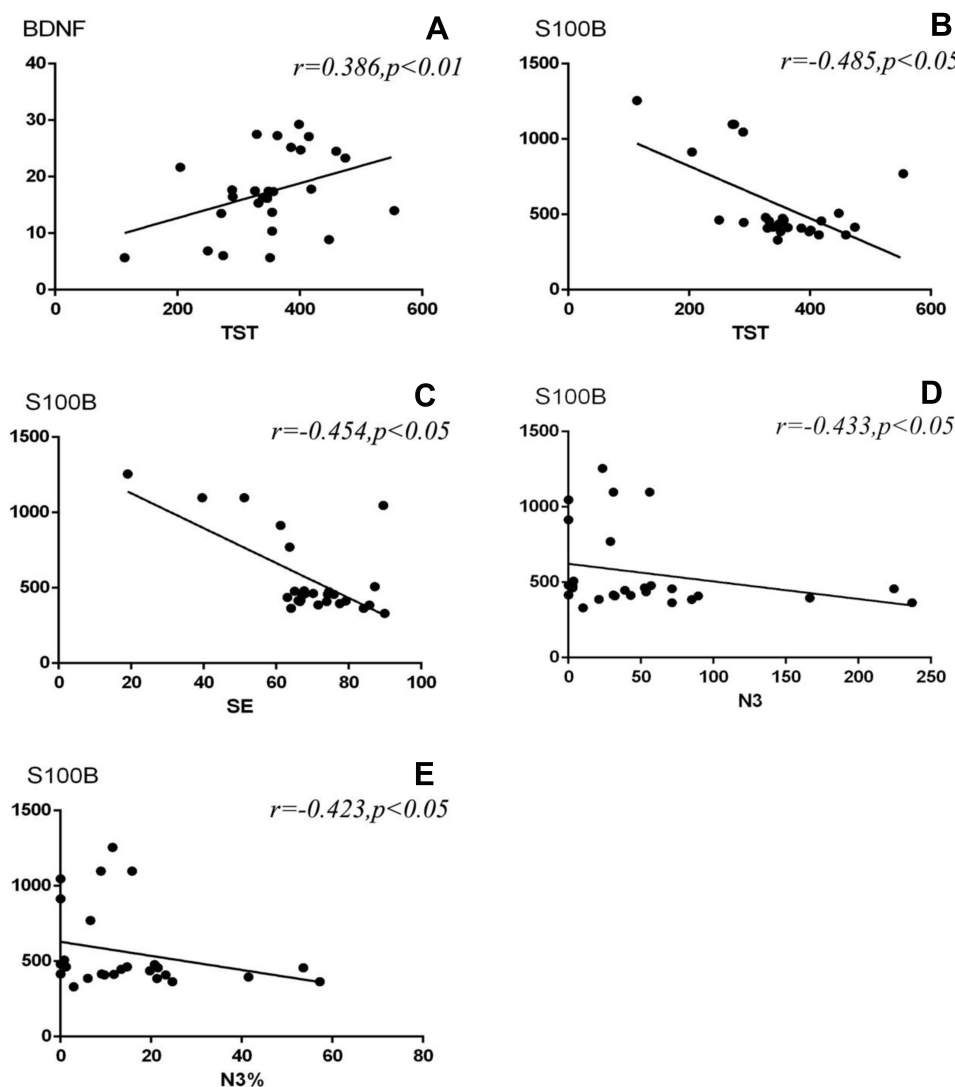
Figure 2 (**A–E**). Scatter plots: Correlation between BDNF and TST in the CID; Correlation between S100B and TST, SE, N3, N3% in the CID.
Abbreviations: BDNF, brain derived neurotrophic factor; TST, total sleep time; S100B, S100 calcium binding protein B; SE, sleep efficiency; N3, sleep stage 3; N3%, percentage of the sleep stage 3.

Table 5 Principal Component Analysis for CID

Variables	Measures	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Sleep parameters	PSQI	−0.767[a]	−0.061	0.047	−0.062	−0.148
	TST	0.705[a]	−0.139	0.364	0.012	0.273
	SE	0.693[a]	−0.075	0.090	−0.069	0.252
	SOL	0.039	0.083	−0.808[a]	0.051	0.053
	N1	0.184	−0.795[a]	0.336	0.033	0.056
	N1%	0.060	−0.830[a]	0.179	0.077	−0.124
	N3	0.260	−0.789[a]	0.343	−0.014	−0.032
	N3%	0.231	−0.773[a]	0.367	0.033	−0.092
	REMT	0.622[a]	0.114	0.262	0.394	0.098
	REM%	0.221	0.206	0.235	0.697[a]	−0.015
Cognitive performances	MoCA-C	0.233	−0.122	0.104	0.065	0.692[a]
	ORM	−0.154	−0.119	−0.797[a]	0.011	−0.024
	SRM	−0.119	0.323	0.038	0.352	−0.587[a]
	OWM	−0.651[a]	0.111	0.453	−0.068	0.182
	SWM	0.019	0.619[a]	0.169	−0.423	−0.531[a]
	ORcM	0.214	0.234	0.065	−0.780[a]	−0.026
Serum biomarkers	S100B	−0.918[a]	0.077	−0.031	−0.063	0.065
	GFAP	−0.390	0.025	0.239	0.220	−0.563[a]
	GDNF	0.160	0.074	−0.263	0.588[a]	0.163
	BDNF	0.447	−0.470	−0.061	0.506[a]	−0.036
Eigenvalues		4.646	3.236	2.355	2.008	1.386
Variance accounted for (%)		23.318	16.178	11.776	10.040	6.930

Note: [a]Loadings higher than 0.5.
Abbreviations: PSQI, Pittsburgh sleep quality index; TST, total sleep time; SE, sleep efficiency; SOL, sleep onset latency; N1, sleep stage 1; N1%, percentage of the sleep stage 1; N3, sleep stage 3; N3%, percentage of the sleep stage 3; REMT, rapid eye movement sleep time; REM%, percentage of the rapid eye movement sleep time; MoCA-C, Chinese-Beijing Version of Montreal Cognitive Assessment; ORM, object reference memory; SRM, spatial reference memory; OWM, object working memory; SWM, spatial working memory; ORcM, object recognition memory. S100B, S100 calcium binding protein B; GFAP, glial fibrillary acidic protein; GDNF, glial derived neurotrophic factor; BDNF, brain derived neurotrophic factor.

Table 6 Characteristics of Potential Blood Biomarkers for CID in ROC Analysis*

	AUC	Cut-off	Sensitivity	Specificity	95% CI
S100B (pg/mL)	0.889	≥323.9	0.70	1.00	(0.808,0.970)
GFAP (pg/mL)	0.735	≥888.5	0.72	0.67	(0.606,0.805)
GDNF (pg/mL)	0.867	≤24.5	0.67	1.00	(0.781,0.954)
BDNF (ng/mL)	0.993	≤26.7	0.86	1.00	(0.982,1.000)

Note: *The CID group compared to the HC group.
Abbreviations: AUC, area under the receiver operating curve; CI, confidence interval. S100B, S100 calcium binding protein B; GFAP, glial fibrillary acidic protein; GDNF, glial derived neurotrophic factor; BDNF, brain derived neurotrophic factor.

S100B is crucial for the maintenance of proper neuronal function, particularly under pathological conditions. Our preliminary study[21] showed that patients with CID had significantly increased S100B serum levels when compared to healthy individuals; these were found to reflect the poor subjective and objective sleep quality (TST, SE, N3 and N3%) in patients with CID. In addition, higher serum S100B concentrations were associated with poor general cognition, as assessed using MoCA-C, and poor SRM, as indicated by the number of errors. In the present study, our findings also indicate that elevated S100B levels were negatively correlated with MoCA-C score in patients with CID. However, higher serum S100B concentrations were linked to poor OWM, rather than poor SRM. These conflicting findings may be due to several factors such as sample size, as a bigger sample was used in the current study; severity of impaired cognitive function; duration of illness; education level; depressive symptoms; among

Table 7 List of the Astrocyte Associated Proteins, Normal Function, and Association with Disease

Factors	Normal Function	Association with Disease
S100B	S100B is an astrocyte-enriched calcium binding protein. It can regulate several intracellular activities by interacting with effector proteins. S100B can also interact in a paracrine (eg, microglia) or autocrine (eg, astrocytes) manner with trophic or apoptotic consequences depending on the concentration attained.[36]	S100B is one of the most promising biomarkers of extensive brain injury. It is a suitable surrogate marker for the diagnostic or prognostic assessment of neurodegenerative diseases, brain damage, mood disorders and schizophrenia.[37–40]
GFAP	GFAP is a monomeric intermediate filament protein found in the astroglial cytoskeleton.[41] It has been linked to a variety of processes implicated in the signaling and structural properties of astrocytes, ranging from resistance to mechanical stress, mitosis, anchoring of transporters, motility of cells, mobility of vesicles and resistance to oxidative and electrophilic stress.[41]	GFAP has been proposed as a candidate biomarker for diagnosing traumatic brain injury.[42,43] This protein is only released into the blood stream after cell death, which suggests that GFAP is CNS-specific and indicative of definite astroglial injury.[44]
BDNF	BDNF belongs to the neurotrophin family and is produced by astrocytes. It can regulate survival, growth, and differentiation of neurons during development as well as activity-dependent synaptic plasticity and processes of learning and memory in the mature CNS.[45]	BDNF has become increasingly recognized as a central mediator of the effects of stress on neuronal plasticity and for its implications in psychopathology.[46,47] BDNF also participates both in the pathogenesis of mental disorders and the regulation of cognition and sleep.[48,49]
GDNF	GDNF is exclusively expressed by neurons. It is identified as a neurotrophic factor crucially involved in the survival of dopaminergic neurons of the nigrostriatal pathway.[50]	GDNF is a well-known neuroprotective therapeutic candidate for Parkinson's disease. It has been implicated in neuropsychiatric disorders, neurodegenerative diseases, brain injury and neuroinflammation.[51–53]

Abbreviations: S100B, S100 calcium binding protein B; GFAP, glial fibrillary acidic protein; BDNF, brain derived neurotrophic factor; GDNF, glial derived neurotrophic factor; CNS, central nervous system.

others. Since S100B serum levels cannot be used as a specific marker of astrocytes damage,[22] we selected other astrocyte-related serum biomarkers to test this conclusion.

GFAP is only released into the blood stream after cell death, which suggests that GFAP is specific to the central nervous system (CNS) and indicative of definite astroglial injury.[44] To the best of our knowledge, the current study is the first to explore the correlation between serum GFAP levels and sleep quality and cognitive impairment in patients with CID. Our results show that serum GFAP levels positively correlate with subjective sleep quality, while they had no correlations with objective sleep parameters. GFAP serum levels were also associated with both general cognition dysfunction and spatial memory impairment, including SWM and SRM. The reason may be that astrocytes are actively involved in normal memory functions and in the abnormal processes, including astroglial injury, leading to cognitive impairment in pathological conditions.[53] Even a few hours of sleep deprivation led to astrocytic processes extending closer to the synaptic cleft. These structural changes potentially represent the importance of positioning astrocytes adjacent to synapses

for the clearance of neurotransmitters via astrocytic transporters or for the transmission of glial-derived signals to support synaptic plasticity.[54]

BDNF is a critical growth factor involved in the maturation of the CNS.[45] Giese's pilot study suggested that peripheral BDNF could be regarded as a biomarker for subjective insomnia, and that the more severe the insomnia, the lower the serum BDNF level.[55] In our study, decreased BDNF levels directly correlated with poor subjective and objective sleep quality in patients with CID. However, a recent study suggests that BDNF is related to subjective sleep perception but not to objective sleep continuity.[56] This discrepancy may be attributed to some factors such as genetic background, the duration of illness, and comorbidities. BDNF mediates long-term potentiation, a form of synaptic plasticity that is associated with enhanced learning and memory.[57–59] A recent study in China found that decreased peripheral BDNF might mediate impaired neuropsychological performances (including brief visuospatial memory, spatial span, managing emotions, fluency, and continuous performance) in insomniacs with short sleep duration.[60] Our results indicate that decreased serum BDNF concentration was linked

to poor SWM and ORcM. Interestingly, both SWM and ORcM are categories of visual memory, and SWM has similar components to the brief visuospatial memory detected in the aforementioned study.[60]

GDNF has a potential neuroprotective effect in the adult brain. In the present study, patients with CID had a significant reduction of serum GDNF when compared to healthy controls, and it had no correlation with sleep quality. Moreover, decreased serum concentrations of GDNF were linked to poor ORcM in patients with CID. GDNF may play an important role in cognitive abilities. For instance, GDNF heterozygous mutant mice show impaired water-maze learning performance.[61–63] Furthermore, GDNF exposure in hippocampal astrocytes led to improved spatial cognitive abilities in aged, cognitively impaired rats.[64] These studies are consistent with our findings.

According to our results, we consider that patients with CID have some degree of astrocytes dysfunction. The nature of the relationship remains unclear, that is, whether astrocyte dysfunction causes insomnia or insomnia causes astrocyte dysfunction. The important role of astrocytes in sleep is to regulate sleep homeostasis and clean the brain parenchyma. They release ATP and increase levels of adenosine to stimulate sleep in the brain.[65–69] In addition, astrocytes, through regulation of extracellular glutamate, are involved in triggering a slow neuronal rhythm in the brain.[70] Moreover, experimental activation of astrocytes, and thus induction of calcium transients, results in slow oscillations which have been shown to be important in sleep and memory formation.[71–73] Therefore, it is plausible that astrocyte dysfunction observed in CID impairs astrocytes sleep-regulating capabilities. On the other hand, sleep has been proposed to be "the brain's housekeeper", serving to restore and repair the brain. The restorative function of sleep may be a consequence of the enhanced removal of potentially neurotoxic waste products that accumulate in the awake CNS.[74] There is little evidence that CID presents organic brain damage.[20,21] Therefore, insomnia may have a direct impact upon dysfunction of astrocytes, which may begin at the early course or even the prodromal stage of the illness.

In addition, stress can induce structural and functional modifications of astrocytes.[75,76] At the same time, current consensus is that exposure to stressful events impairs normal sleep function.[77,78] Therefore, it is plausible that, at least in some individuals, the mechanism leading to insomnia may be accumulating stress events across the lifetime that could result in the dysfunction of astrocytes, in turn causing the onset and development of insomnia.

In summary, we conclude that patients with CID have pathological astrocyte-related changes, which may be a trait of CID patients. Astrocyte dysfunction correlates with poor sleep quality, on parameters such as decrease of total sleep time, sleep efficiency, and N3 sleep. Furthermore, astrocyte dysfunction may be involved in the underlying mechanism of cognitive impairment in CID.

The Possible Diagnostic and Prognostic Value of These Serum Indexes

ROC analysis was used to calculate the cut-off values of different serum biomarkers to discriminate between true and false CID diagnosis as established by the ICSD-3 criteria. The results showed that the AUCs of all four proteins were over 0.7 between patients with CID and HC. The optimal cut-off values for S100B and GFAP indicated that subjects should be considered to have CID if their serum biomarkers levels were over the corresponding cut-off values. The optimal cut-off values for GDNF and BDNF indicated that subjects should be considered to have CID if their serum biomarkers levels were lower than the corresponding cut-off values. These results might provide strong cues for considering CID relative to healthy sleep, implying a possibility that these serum indexes act as objective diagnostic markers of CID. Combining these four indicators may provide a more specific and sensitive method to better manage and offer more accurate prognosis of patients with CID.

Conclusion

Patients with CID have altered serum levels of astrocyte-related biomarkers, with increased S100B and GFAP and decreased BDNF and GDNF serum levels, indicating pathological changes involving astrocytes. In addition, these serum markers of astrocyte injury were associated with insomnia severity and/or cognitive dysfunction.

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