

# Are Anti-Inflammatory Cytokines Associated with Cognitive Impairment in Patients with Insomnia Comorbid with Depression? A Pilot Study

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**Background:** To distinguish insomnia comorbid with depression (ICD) from chronic insomnia disorder (CID) by exploring the relationship between serum levels of frequently overlooked anti-inflammatory cytokines and cognitive function.

**Methods:** A total of 42 ICD patients, 63 CID patients, and 42 healthy control subjects were enrolled in the study. The Pittsburgh Sleep Quality Index and Hamilton Depression Rating Scale were used to assess sleep quality and depression severity, respectively. The Chinese-Beijing version of Montreal Cognitive Assessment scale (MoCA-C) and Nine-Box Maze Test (NBMT) were used to assess cognitive function. Serum levels of anti-inflammatory interleukins (IL-1RA, IL-4, IL-5, IL-10, IL-13, and IL-28A), transforming growth factor (TGF)- $\beta$ 1, granulocyte-macrophage colony-stimulating factor, interferon- $\gamma$ , and the chemokine regulated upon activation, normal T cell expressed and secreted (RANTES) were measured by enzyme-linked immunosorbent assay.

**Results:** The ICD group had significantly more errors in the spatial reference task ( $H=2.55$ ,  $P_s=0.03$ ) and spatial working memory task ( $H=5.67$ ,  $P_s<0.01$ ) of the NBMT, as well as lower levels of IL-1RA ( $H=-2.85$ ,  $P_s=0.01$ ), IL-4 ( $H=-3.28$ ,  $P_s<0.01$ ), IL-5 ( $H=-3.35$ ,  $P_s<0.01$ ), IL-10 ( $H=-4.46$ ,  $P_s<0.01$ ), and IL-28A ( $H=-2.75$ ,  $P_s=0.02$ ) than control subjects. Compared with the CID group, the ICD group had significantly more errors in the spatial reference memory task ( $H=-2.84$ ,  $P_s=0.01$ ) of the NBMT, and lower levels of IL-5 ( $H=3.41$ ,  $P_s<0.01$ ), IL-10 ( $H=5.30$ ,  $P_s<0.01$ ), IL-13 ( $H=3.89$ ,  $P_s<0.01$ ), and GM-CSF ( $H=2.72$ ,  $P_s=0.02$ ). A partial correlation analysis showed that the level of one or more of IL-4, IL-5, IL-10, IL-13, and TGF- $\beta$ 1 was positively correlated with cognitive function (MoCA-C score and/or performance in spatial memory task) in ICD patients.

**Conclusion:** ICD is a distinct condition that can be distinguished from CID based on immune dysfunction and specific types of cognitive dysfunction.

**Keywords:** insomnia, depression, cytokine, cognition

## Introduction

Depression is a chronic mental disorder that frequently co-occurs with other medical conditions or psychiatric illnesses; it has a high prevalence and is associated with high rates of recurrence and disability.<sup>1</sup> Although depression typically manifests as low mood and cognitive impairment, chronic insomnia disorder (CID) is a frequent complaint.<sup>2,3</sup> Insomnia is not only a risk factor for depression but is also a residual symptom following its treatment,<sup>4,5</sup> highlighting a bidirectional relationship between insomnia and depression.<sup>6,7</sup> Cognitive deficits are present in

both disorders and exacerbate cognitive impairment in patients with depression.<sup>8–10</sup> In the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) and the International Classification of Sleep Disorders, Third Edition (ICSD-3), insomnia comorbid with other mental disorders must be excluded and diagnosed separately from CID.<sup>11,12</sup>

Inflammation has been proposed as a mechanism underlying multiple chronic diseases including depression, insomnia, and mild cognitive impairment.<sup>13–19</sup> Among patients who received antidepressant treatment, patients with a low level of inflammation had better prognosis than those with a high level.<sup>20</sup> Depression is thought to be related to activation of the immune response leading to the increased secretion of cytokines,<sup>21</sup> which can cause cognitive impairment including changes in attention, learning, and memory.<sup>22</sup> Additionally, inflammation plays an important role in sleep regulation and disturbance. Sleep promotes immune homeostasis through the modulation of inflammatory mediators such as cytokines, which regulate normal sleep–wake behavior.<sup>23</sup> Insomnia is associated with several chronic infectious and inflammatory diseases.<sup>24</sup> Thus, inflammation links depression, insomnia, and cognitive impairment, although it is unclear whether insomnia comorbid with depression (ICD) has a distinct immunologic profile.

Cytokines are polypeptides produced by immune cells that regulate cellular functions; they can be divided into six types according to their source—namely, interleukins (ILs), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN), tumor necrosis factor (TNF), growth factor, and chemokines such as regulated upon activation, normal T cell expressed and secreted (RANTES).<sup>25</sup> There are two functional subtypes of cytokine: proinflammatory (IL-1, IL-1 $\beta$ , IL-2, IL-6, IFN- $\gamma$ , TNF- $\alpha$ , TNF- $\beta$ , etc) and anti-inflammatory (IL-1RA, IL-4, IL-5, IL-10, IL-13, etc), which promote and inhibit immune activation, respectively.<sup>26–29</sup> Although the cytokine profile of depression has not been clearly established, patients with depression exhibit cytokine abnormalities such as elevated levels of IL-1 $\beta$ , IL-6, and TNF.<sup>30–32</sup> Cognitive dysfunction is associated with elevated plasma levels of C-reactive protein and IL-6.<sup>22,33</sup> In addition, IL-1 and TNF are known to modulate sleep, while sleep deficiency induces immune system changes.<sup>23</sup> Thus, while proinflammatory cytokines have been widely studied in the contexts of sleep disorder and depression, the roles of

other inflammatory factors such as TGF, GM-CSF, IFN, and RANTES remain unclear.

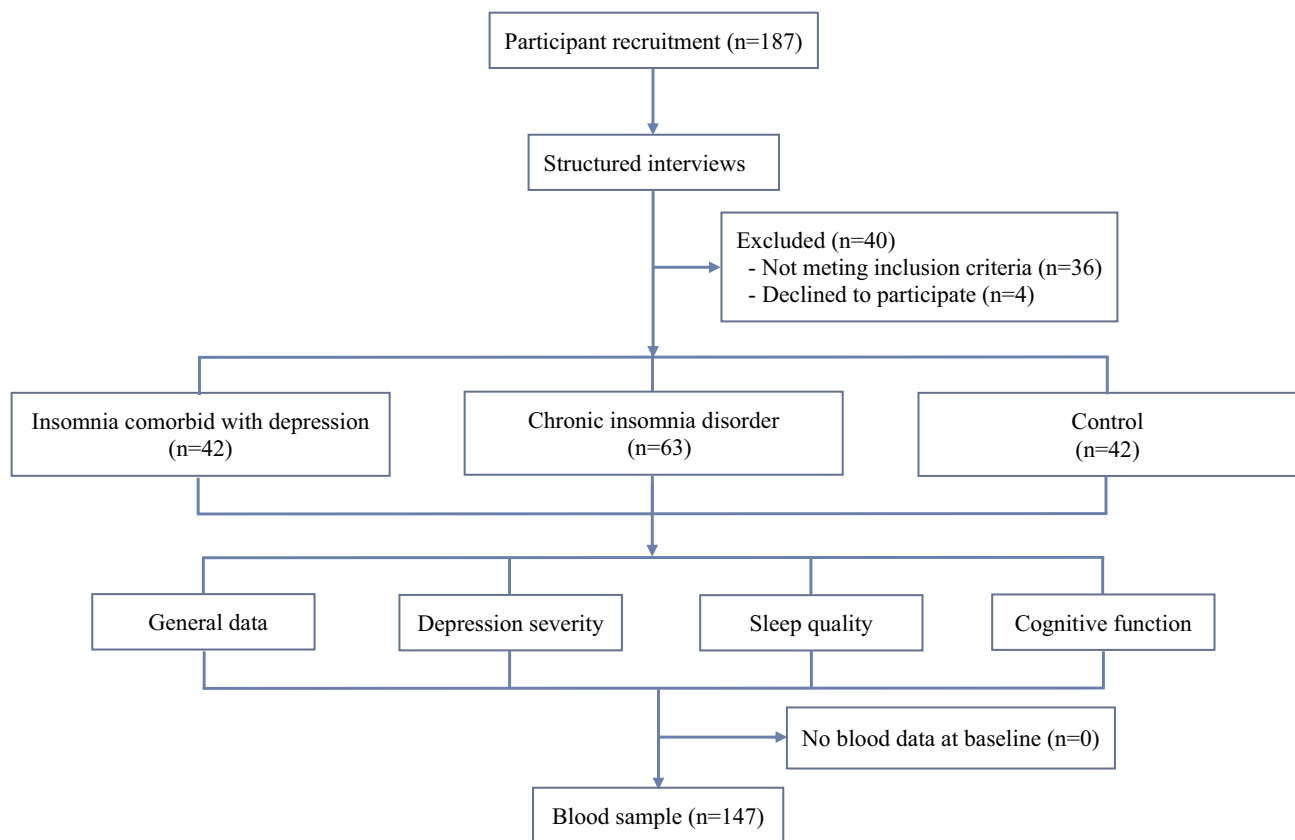
The aims of this study were to 1) examine the correlation between immunologic profile and cognitive function in ICD and 2) distinguish ICD from CID by investigating the roles of the abovementioned cytokines (anti-inflammatory ILs, TGF, GM-CSF, IFN, and RANTES) and cognitive function in ICD and CID.

## Methods

### Subjects

A total of 42 ICD patients were recruited continuously at the Clinic of Sleep and Memory Disorder of the Affiliated Chaohu Hospital of Anhui Medical University between January 2019 and April 2020. The flowchart of the study participants is presented in Figure 1. Besides simultaneously meeting ICSD-3 and DSM-5 diagnostic criteria for ICD, inclusion criteria for patients were as follows: 1) age between 18 and 75 years; 2) at least 9 years of education without problems in comprehension; 3) not taking antidepressants or other drugs that could potentially interfere with sleep, cognitive function, or endocrine function in the 3 months prior to enrollment; 4) Pittsburgh Sleep Quality Index (PSQI)  $>7$ ;<sup>34–36</sup> 5) 17-Item Hamilton Depression Rating Scale (HAMD-17) score  $\geq 7$ ;<sup>37</sup> and 6) participating voluntarily in the study after signing the informed consent form. The exclusion criteria were as follows: 1) any other somatic comorbidity (including immunologic, endocrine, cardiovascular, neurologic, liver, or kidney disease or organic brain disease); 2) history of substance abuse; 3) recent infection or inflammation (within 2 weeks of the start of the study); 4) taking drugs that could affect sleep, mood, immune function, and cognition; 5) shift-work subjects; and 6) pregnant or lactating women.

The study also enrolled 63 CID patients (insomnia lasting  $\geq 6$  months; HAMD-17 score  $<17$ ; no other disease comorbid with insomnia) and 42 control subjects (PSQI and HAMD-17 scores  $<7$ ; no insomnia or related medical history during the same period). All the enrolled patients were the first to visit who did not take antidepressants or other drugs that could potentially interfere with sleep, cognitive function, or endocrine function in the 3 months prior to enrollment. The study was approved by the Affiliated Chaohu Hospital of Anhui Medical University Ethics Committee (approval no. 201805-kyxm-01).



**Figure 1** Flowchart of the study participants.

## General Data Collection

General information was collected on each participant including sex, age, education level, body mass index (BMI), individual history, daily life history, medical history, and family medical history.

## Assessment of Depression Severity

To ensure the consistency of enrolled patients, the Chinese version of Mini International Neuropsychiatry Interview 5.0 (Mini 5.0) was independently conducted by Master's- and doctoral-level evaluators with background and training in psychology.<sup>38</sup> Depression severity was assessed with the HAMD-17, which comprises 17 items relating to depressed mood, feelings of guilt and suicide, sleep, work, and activities.<sup>37</sup> A score <7 indicates a normal state while scores of 7–17, 18–24, and >24 correspond to mild, moderate, and severe depression, respectively.

## Evaluation of Sleep Quality

Sleep quality was assessed with the PSQI, which has seven components including subjective sleep quality, sleep

latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleep medication, and daytime dysfunction during the previous month scored on a 4-point rating scale ranging from 0 (none) to 3 ( $\geq 3$  times per week).<sup>34</sup> In China, a score  $\geq 7$  has high diagnostic sensitivity and specificity for distinguishing patients with poor sleep from normal subjects.<sup>36</sup> Total PSQI scores range from 0 to 21, with a higher score corresponding to worse sleep quality.<sup>34</sup>

## Cognitive Assessment

The Chinese-Beijing Version of the Montreal Cognitive Assessment scale (MoCA-C) is a widely used cognitive screening test with good reliability and validity.<sup>39</sup> The MoCA-C has eight dimensions: visual space and executive function, naming, attention, language, abstraction, short-term memory, delayed recall, and orientation.<sup>40</sup> The maximum score is 30 points, and a score  $\geq 26$  indicates normal cognitive function.<sup>39</sup>

The Nine-Box Maze Test (NBMT), which was originally designed to assess spatial memory, was modified to

evaluate multiple aspects of memory function including spatial/object working memory (SWM/OWM), spatial/object reference memory (SRM/ORM), and object recognition memory (ORcM).<sup>41,42</sup> The experiment was conducted according to our previous study.<sup>43</sup> The test comprises object familiarization, training, and testing phases, and the final result is represented by the number of errors. A higher number of errors indicated poor memory.

## Serum Cytokine Profiling

After the intervention and all other assessments had been completed, a 3-mL sample of blood was obtained from each subject the next morning between 7:30 a.m. and 8:00 a.m., with fasting and avoidance of strenuous activity and mental stimulation. On this condition, all serum cytokines measured showed stable values according to each individual's condition. The sample was allowed to stand at room temperature for 30 min and then centrifuged at 3000 rpm for 5 min; the serum was stored with anticoagulant and frozen at  $-80^{\circ}\text{C}$ . TGF- $\beta$ 1, GM-CSF, IFN- $\gamma$ , and RANTES levels in the serum were measured using enzyme-linked immunosorbent assay kits (Shanghai Jianglai Co, Shanghai, China) and a microplate reader (Labsystems Multiskan MS 352; Thermo Fisher Scientific, Waltham, MA, USA), with a sensitivity  $>84\%$  and specificity  $>98\%$ . All samples were prepared in duplicate and average values were used for analysis. Intra- and inter-assay coefficients of variation were  $<9\%$  and  $<11\%$ , respectively.

## Statistical Analysis

SPSS v.20 was used for statistical analyses. Continuous normally distributed data are presented as mean $\pm$ SD and were evaluated with the Student's *t*-test to compare differences between groups and one-way analysis of variance to determine the main effects, with the least significant difference (LSD) test used for multiple comparisons. Non-normally distributed data are expressed as *P*50 (*P*25, *P*75), and differences between groups were analyzed with the rank-sum test for two independent samples with completely randomized design (Mann–Whitney *U*). The Kruskal–Wallis *H*-test was used for multiple-group comparisons by adjusting the significance level to  $P<0.0167$  (Bonferroni correcting:  $0.05/3$ ), and pairwise comparisons were conducted with the Student's *t*-test by manual calculation. Categorical data were analyzed with the chi-squared test. To control the influence of confounding factors to the variables, partial correlation analysis was used for the

relationships among continuous variables. In partial correlation analysis, the confounder between serum cytokines and HAMD-17 (controlling for sex, age, education, BMI, and PSQI), serum cytokines and PSQI (controlling for sex, age, education, BMI, and HAMD-17), and serum cytokines and MoCA-C scores and spatial memory (controlling for sex, age, education, BMI, HAMD-17, and PSQI) are different. Two-sided *P* values  $\leq 0.05$  were considered statistically significant.

## Results

### General Characteristics of the Study

#### Subjects

There were no significant differences in sex ratio ( $\chi^2=2.7$ ,  $P=0.3$ ), age ( $F=0.3$ ,  $P=0.7$ ), education level ( $H=3.1$ ,  $P=0.2$ ), or BMI ( $t=0.02$ ,  $P=0.98$ ) among the three groups (Table 1).

#### Depression Severity

HAMD scores of the three groups differed significantly ( $H=108.55$ ,  $P<0.001$ ) (Table 1). A pairwise comparison revealed that the ICD and CID groups had significantly higher scores than control subjects ( $P_s<0.001$ ). Additionally, the score for the ICD group was nearly two times higher than that of the CID group ( $P<0.001$ ).

#### Sleep Quality

PSQI scores differed significantly across groups ( $H=95.34$ ,  $P<0.001$ ). Both the ICD and CID groups had significantly higher scores than control subjects ( $P_s<0.001$ ), although there was no significant difference between them (Table 1).

#### Cognitive Function

There were no significant differences in MoCA scores among the 3 groups ( $H=1.85$ ,  $P=0.40$ ) (Table 1). For performance in the NBMT, significant differences were observed in SRM ( $H=9.36$ ,  $P=0.01$ ), SWM ( $H=37.60$ ,  $P<0.001$ ), and ORcM ( $H=8.79$ ,  $P=0.01$ ). The ICD group had more errors related to SRM than the CID and control groups ( $P=0.01$ ;  $P=0.03$ ), with no difference between the latter two groups ( $P=1.00$ ). The ICD and CID groups had more errors related to SWM than controls ( $P_s<0.001$ ) but there were no differences between them ( $P=0.56$ ). Only the CID group had significantly more errors related to ORcM than controls ( $P=0.01$ ) (Table 2).

**Table 1** Demographic Characteristics, Depression Level, and Sleep Quality of the Study Subjects

Variable	Insomnia Comorbid with Depression	Chronic Insomnia Disorder	Healthy Controls	Statistic	P value	P value in Multiple Comparisons		
						ICD vs CID	ICD vs CON	CID vs CON
Number of cases	42	63	42					
Male/female	17/25	20/43	22/20	$C^2=2.7$	0.3			
Age, years	39.6±12.1	41.3±12.3	41.4±14.1	F=0.3	0.7			
Education, years	12.0 (6.0, 16.0)	12.0 (9.0, 16.0)	15.0 (9.0, 16.0)	H=3.1	0.2			
BMI	21.7±3.1	21.7±2.6	–	$t=0.02$	0.98			
HAMD-17	19.0 (13.8, 22.0)* <sup>#</sup>	10.0 (7.0, 13.0)*	2.0 (1.0, 4.3)	H=104.0	<0.001	<0.001	<0.001	<0.001
PSQI	17.0 (15.0, 19.0)*	15.0 (13.0, 17.0)*	2.0 (0.0, 3.3)	H=92.2	<0.001	0.13	<0.001	<0.001

**Notes:** Normally distributed variables are shown as mean±SD; non-normally distributed variables are shown as P50 (P25, P75). \* $P<0.05$  vs healthy controls. <sup>#</sup> $P<0.01$  vs chronic insomnia disorder.

**Abbreviations:** BMI, body mass index; CID, chronic insomnia disorder; CON, control; HAMD-17, Hamilton Depression Rating Scale (17 items); ICD, insomnia comorbid with depression; PSQI, Pittsburgh Sleep Quality Index.

## Serum Cytokine Levels

There were significant intergroup differences in serum levels of IL-1RA (H=9.85,  $P=0.007$ ), IL-4 (H=15.85,  $P<0.001$ ), IL-5 (H=14.77,  $P=0.001$ ), IL-10 (H=31.58,  $P<0.001$ ), IL-13 (H=14.37,  $P=0.001$ ), IL-28A (H=17.55,  $P<0.001$ ), and GM-CSF (H=18.37,  $P<0.001$ ). The results of multiple comparisons indicated that the ICD group had significantly lower levels of IL-1RA

( $P=0.01$ ), IL-4 ( $P=0.03$ ), IL-5 ( $P=0.02$ ), IL-10 ( $P<0.001$ ), and IL-28A ( $P=0.02$ ) than control subjects, and lower levels of IL-1RA ( $P=0.02$ ), IL-5 ( $P=0.002$ ), IL-10 ( $P<0.001$ ), IL-13 ( $P=0.001$ ), and GM-CSF ( $P=0.02$ ) than the CID group. Moreover, the CID group had elevated IL-13 and GM-CSF ( $P=0.03$ ;  $P<0.001$ ) and reduced IL-4 and IL-28A ( $P=0.001$ ;  $P<0.001$ ) levels relative to controls (Table 3).

**Table 2** Cognitive Performance of the Study Subjects

Terms	ICD	CID	CON	H	P value	P value in Multiple Comparisons			
						ICD vs CID	ICD vs CON	CID vs CON	
MoCA-C (scores)	26.5 (25.0, 28.0)	27.0 (25.0, 29.0)	27.0 (26.0, 28.0)	1.9	0.4				
Nine-box maze	ORM (errors)	0.0 (0.0, 1.0)	0.0 (0.0, 1.0)	0.0 (0.0, 1.0)	0.8	0.7			
	SRM (errors)	1.0 (0.0, 2.0)* <sup>#</sup>	0.0 (0.0, 2.0)	1.0 (0.0, 1.0)	9.4	0.01	0.01	0.03	1.00
	OWM (errors)	0.0 (0.0, 1.0)	0.0 (0.0, 0.0)	0.0 (0.0, 1.0)	2.4	0.3			
	SWM (errors)	4.0 (3.0, 8.3)*	4.0 (2.0, 5.0)*	1.0 (1.0, 3.0)	37.6	<0.001	0.56	<0.001	<0.001
	ORcM (errors)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)*	0.0 (0.0, 0.0)	8.8	0.01	0.25	0.95	0.01

**Notes:** Normally distributed variables are shown as mean±SD; non-normally distributed variables are shown as P50 (P25, P75). \* $P<0.05$  vs healthy controls. <sup>#</sup> $P<0.01$  vs chronic insomnia disorder.

**Abbreviations:** CID, chronic insomnia disorder; CON, control; ICD, insomnia comorbid with depression; MoCA-C, Chinese-Beijing Version of Montreal Cognitive Assessment Scale; ORcM, object recognition memory; ORM, object reference memory; OWM, object working memory; SRM, spatial reference memory; SWM, spatial working memory.



## Correlations Among Serum Cytokine Levels, Depression Severity, Sleep Quality, and Cognitive Function

In the ICD group, partial correlation analysis between serum cytokine levels and cognitive performance showed that IL-4 level was positively correlated with the number of errors in OWM ( $r=0.39$ ,  $P=0.04$ ) while IL-5 level was correlated with number of errors in OWM ( $r=0.39$ ,  $P=0.04$ ) and ORcM ( $r=0.58$ ,  $P<0.01$ ), IL-10 level was also positively correlated with the number of errors in 3 tasks—ie, ORM ( $r=0.40$ ,  $P=0.03$ ), SRM ( $r=0.57$ ,  $P<0.01$ ), and ORcM ( $r=0.47$ ,  $P=0.01$ ), and IL-13 ( $r=0.51$ ,  $P<0.01$ ) and TGF- $\beta$ 1 ( $r=0.51$ ,  $P<0.01$ ) after controlling for sex, age, education, BMI, and PSQI and HAMD-17 scores (Table 4). In the CID group, the identical partial correlation analysis showed that serum concentration of IL-1RA was positively correlated with ORM errors ( $r=0.34$ ,  $P=0.01$ ), whereas IL-5 ( $r=0.37$ ,  $P<0.001$ ), IL-10 ( $r=0.28$ ,  $P=0.03$ ), and IL-13 ( $r=0.40$ ,  $P<0.001$ ) levels were positively correlated with OWM errors.

## Discussion

### ICD Patients Had Decreased Serum Levels of Anti-Inflammatory Cytokines and GM-CSF

Pro-inflammatory and anti-inflammatory mediators as the two classed of molecules so-exist and cause effects jointly in actual pathological process.<sup>44</sup> Immune changes in depression and insomnia resulting from the activation of proinflammatory cytokines.<sup>45–47</sup> However, other cytokines including anti-inflammatory ILs, TGF, GM-CSF, IFN, and RANTES have received less attention. In the current study, we tried to examine serum cytokine profiles (focusing on the abovementioned previously overlooked cytokines) in ICD to evaluate their change and its relationship with cognitive impairments. Our findings showed that patients with ICD had lower serum levels of anti-inflammatory cytokines and GM-CSF than patients with CID and control subjects. The results indicate that ICD as a comorbidity disease may present lower serum levels of anti-inflammatory cytokines and GM-CSF. Considering an imbalance between pro- and anti-inflammatory cytokines triggers the onset of depression<sup>48</sup>; we proposed that such an imbalance—caused by decreased anti-inflammatory and increased proinflammatory cytokine levels—exists in ICD. The reason may be the activation of serine kinases by ATP

on inflammatory response.<sup>49</sup> By interacting with the purinergic receptor P2X7, eATP triggers the secretion of pro-inflammatory cytokines. Additionally, it may inhibit the inflammatory response through an interaction with the receptor P2Y and its downstream cAMP-PKA pathway.<sup>50,51</sup>

### ICD Patients Have Cognitive Impairment Compared with CID Patients

Cognitive dysfunction is a frequent complaint of patients with depression or insomnia.<sup>8</sup> Cognitive impairment associated with depression includes reduced daytime functioning and deficits in attention, memory, psychomotor processing, and problem solving.<sup>9</sup> Individuals who have insomnia show poor performance in objective measures of perceptual function, manipulation and retention capacity in working memory, complex attention, alertness, episodic memory, and problem solving relative to control subjects.<sup>52</sup> Our preliminary study showed that ICD group had more errors in all five measures of memory (SRM, SWM, ORM, and ORcM) compared to controls.<sup>42</sup> However, in the CID group, there had some difference between our previous researches about spatial memory evaluation. In the present study, patients with ICD only showed impairment in SRM and SWM. Consistent with Chen's study, we found here that patients with CID had more errors in SWM and ORcM than control subjects.<sup>43</sup> The results clearly implying that the nature of cognitive impairment differs between ICD and CID, which may help us distinguish ICD from CID. This discrepancy may be attributable to factors such as the illness duration, the existence of heterogeneity in the current study, sample size, and differences in the severity of insomnia.<sup>53–56</sup> Further studies are needed to reach a definitive conclusion.

### Relationship Between Serum Cytokine Levels and Sleep Quality and Cognitive Function

Inflammation in acute and chronic diseases has been shown to contribute to poor sleep quality.<sup>57,58</sup> The sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis are the main systems linking sleep and immune function.<sup>59</sup> The increased excitability of the SNS leading to overexpression of inflammation-related genes, overproduction of inflammatory cytokines, and increased systemic inflammation.<sup>60</sup> Different levels of anti-inflammatory cytokines such as IL-10 and IL-13 have

**Table 3** Cytokine Levels in the Study Subjects

Cytokine	Insomnia Comorbid with Depression	Chronic Insomnia Disorder	Healthy Controls	H	P value	P value in Multiple Comparisons		
						ICD vs CID	ICD vs CON	CID vs CON
Anti-inflammatory interleukin	IRA (ng/mL)	2.13 (1.70, 3.07)	2.68 (1.49, 3.68)	9.76	0.01	0.03	0.01	1.00
	4 (pg/mL)	20.43* (10.31, 31.34)	37.78 (21.61, 59.90)	15.85	<0.001	1.00	0.003	0.001
	5 (pg/mL)	19.47* <sup>#</sup> (0.00, 46.62)	55.43 (31.62, 89.41)	14.77	<0.001	0.002	0.002	1.00
	10 (pg/mL)	0.21* <sup>#</sup> (0.00, 5.55)	8.38 (3.53, 18.26)	8.73 (3.49, 14.32)	31.58	<0.001	<0.001	1.00
	13 (pg/mL)	74.41 <sup>#</sup> (63.63, 0.86)	89.07* (73.48, 114.41)	77.87 (68.80, 91.13)	14.37	<0.001	0.001	0.03
	28A (pg/mL)	14.89* (9.48, 25.26)	13.16* (0.00, 21.45)	22.10 (14.80, 36.34)	17.55	<0.001	0.76	<0.001
TGF-β1 (ng/mL)	5.16 (4.01, 7.15)	6.16 (3.97, 8.74)	5.40 (4.74, 6.72)	2.16	0.34			
GM-CSF (pg/mL)	123.00 <sup>#</sup> (103.29, 141.16)	148.38* (115.72, 186.62)	111.19 (81.54, 136.80)	18.37	<0.001	0.02	0.60	<0.001
IFN-γ (pg/mL)	391.48 (282.45, 578.49)	382.04 (257.50, 563.65)	380.28 (266.21, 504.80)	0.48	0.79			
RANTES (ng/mL)	4.21 (3.84, 4.57)	4.24 (3.92, 4.77)	4.64 (3.80, 5.30)	5.55	0.06			

**Notes:** \*p<0.05 vs healthy controls. <sup>#</sup>p<0.01 vs chronic insomnia disorder.

**Abbreviations:** CID, chronic insomnia disorder; CON, control; ICD, insomnia comorbid with depression; IFN-γ, interferon gamma; GM-CSF, granulocyte-macrophage colony-stimulating factor; RANTES, regulated upon activation normal T cell expressed and secreted; TGF-β1, transforming growth factor beta.

**Table 4** Partial Correlations Among Serum Cytokines and HAMD-17, PSQI, and MoCA Scores and Spatial Memory in Patients with Insomnia Comorbid with Depression and Chronic Insomnia Disorder

Disorder	Cytokine		HAMD-17 <sup>a</sup>	PSQI <sup>b</sup>	MoCA-C <sup>c</sup>	Spatial Memories <sup>c</sup>				
						ORM	SRM	OWM	SWM	ORcM
ICD	Anti-inflammatory interleukin	1RA (ng/mL)	-0.06	0.04	0.11	-0.09	-0.20	-0.02	-0.12	0.09
		4 (pg/mL)	-0.13	0.30	0.24	0.04	0.08	0.39*	-0.07	0.29
		5 (pg/mL)	-0.05	0.09	0.01	0.20	0.35	0.39*	-0.20	0.58 <sup>#</sup>
		10 (pg/mL)	-0.19	0.10	-0.17	0.40*	0.57 <sup>#</sup>	0.21	-0.25	0.47*
		13 (pg/mL)	0.09	-0.16	0.51 <sup>#</sup>	-0.30	-0.15	-0.18	-0.13	-0.01
		28A (pg/mL)	0.01	-0.09	-0.15	0.17	0.14	0.33	0.00	0.30
	TGF-β1 (ng/mL)		0.12	-0.12	0.51 <sup>#</sup>	-0.26	-0.16	-0.29	0.06	0.10
	GM-CSF (pg/mL)		0.10	-0.10	0.24	0.07	0.11	-0.24	0.16	0.17
	IFN-γ (pg/mL)		-0.21	-0.13	0.16	-0.32	-0.20	-0.24	0.15	-0.04
	RANTES (ng/mL)		0.21	0.24	0.27	0.23	0.20	0.15	-0.35	0.10
CID	Anti-inflammatory interleukin	1RA (ng/mL)	0.02	0.21	-0.05	0.34*	0.11	0.15	-0.12	-0.03
		4 (pg/mL)	-0.15	-0.04	0.01	-0.10	-0.06	0.15	-0.11	-0.12
		5 (pg/mL)	-0.14	0.08	0.10	-0.01	-0.05	0.37 <sup>#</sup>	-0.19	0.07
		10 (pg/mL)	-0.18	0.13	0.11	-0.10	-0.06	0.28*	-0.20	0.10
		13 (pg/mL)	-0.02	0.05	-0.25	-0.08	-0.01	0.40 <sup>#</sup>	0.03	0.04
		28A (pg/mL)	0.04	-0.20	-0.05	-0.11	-0.20	0.00	0.18	-0.02
	TGF-β1 (ng/mL)		-0.01	0.04	-0.02	-0.07	0.15	0.21	-0.05	0.10
	GM-CSF (pg/mL)		-0.16	0.08	-0.15	-0.07	0.14	0.23	0.04	0.00
	IFN-γ (pg/mL)		0.01	0.03	-0.16	-0.10	0.10	0.23	-0.10	-0.03
	RANTES (ng/mL)		0.11	0.15	-0.05	-0.10	-0.19	-0.03	-0.18	0.02

**Notes:** <sup>a</sup>Controlling for sex, age, education, BMI, PSQI; <sup>b</sup>controlling for sex, age, education, BMI, HAMD-17; <sup>c</sup>controlling for sex, age, education, BMI, HAMD-17, and PSQI. \*P<0.05 vs healthy controls. <sup>#</sup>P<0.01 vs chronic insomnia disorder.

**Abbreviations:** CID, chronic insomnia disorder; GM-CSF, granulocyte-macrophage colony-stimulating factor; HAMD-17, Hamilton Depression Rating Scale (17 items); ICD, insomnia comorbid with depression; IFN-γ, interferon gamma; MoCA-C, Chinese-Beijing Version of Montreal Cognitive Assessment Scale; ORcM, object recognition memory; ORM, object reference memory; OWM, object working memory; PSQI, Pittsburgh Sleep Quality Index; RANTES, regulated upon activation normal T cell expressed and secreted; SRM, spatial reference memory; SWM, spatial working memory; TGF-β1, transforming growth factor beta 1.

been observed cause the reduction at non-rapid eye movement (NREM) sleep in both humans and animal models.<sup>61–63</sup> In the present study, we did not find a significant correlation between anti-inflammatory cytokine levels and subjective sleep quality as indicated by PSQI score in patients with ICD. Our results were consistent with Petrov's clinical research about subjective sleep quality and inflammation in healthy subjects.<sup>64</sup> Additionally, individuals with insomnia tend to overestimate time to fall asleep time in comparison to PSG measures.<sup>65</sup> Thus, subjective sleep measures for insomnia are associated with unsatisfactory diagnostic accuracy.<sup>66</sup> Considering the relationship between serum cytokines and subjective sleep quality still controversial and our results cannot conclusively prove that anti-inflammatory cytokines correlated with subjective sleep quality,<sup>67,68</sup> we

consider the possible reason may be attributable to the existence of comorbidities, various degree of insomnia severity, lack of objective tools for evaluating sleep quality (eg, polysomnography), and classification of different types of insomnia.<sup>69–71</sup>

Cytokines have been implicated in a variety of cognitive processes such as learning, memory, and executive function.<sup>72,73</sup> A recent study about insomnia patients indicates the role of brain-derived neurotrophic factor (BDNF) gene polymorphism in cognitive impairment.<sup>74</sup> Systemic changes in cytokine levels have been linked to several types of cognitive disorder including Alzheimer disease and mild cognitive impairment.<sup>75</sup> Proinflammatory cytokines modulate hippocampal function by stimulating the production of oxygen free radicals, leading to cognitive impairment.<sup>76</sup> Meanwhile, anti-inflammatory cytokines



such as IL-4, IL-10, and IL-13 can inhibit the activation of microglia to suppress inflammation.<sup>76</sup> Thus, when the levels of anti-inflammatory cytokines are reduced, the deleterious effects of proinflammatory cytokines on cognitive function cannot be mitigated.<sup>77,78</sup> In the present work, we found that elevated levels of IL-4, IL-5, IL-10, IL-13, and TGF- $\beta$ 1 had positive effects on cognitive function (as evaluated by MoCA and a spatial memory task) in ICD patients. Thus, anti-inflammatory cytokines may actively participate in normal memory functions and conversely, their reduction could lead to cognitive deficits in pathologic states such as ICD.<sup>79</sup> Notably, our results also demonstrated that the levels of another set of anti-inflammatory cytokines (IL-1RA, IL-5, IL-10, and IL-13) were positively correlated with object memory (ORM or OWM) in CID patients, which was similar with the cognitive domains associated with anti-inflammatory cytokines in ICD. In view of both OWM and ORM belong to object memory, our finding suggested that anti-inflammatory cytokines are positively correlated with object memory and the inhibition of these cytokines may be a potential treatment strategy to relieve cognitive dysfunction.<sup>80</sup> The possible mechanisms may be due to the presence of high levels of pro-inflammatory cytokines, the production or action of anti-inflammatory cytokines are suppressed, abolishing the beneficial effects of anti-inflammatory cytokines on learning and memory.<sup>81</sup>

## Limitations

The current study had several limitations. First, given this was a cross-sectional study, we could not draw causal inference and the inflammatory factors were only measured at initial presentation rather than being tracked during the treatment. Therefore, the relationship between ICD and anti-inflammatory cytokines with cognitive impairments could not be determined casually. For the same reason, the analysis of cytokines that we do not account for the improvement in depression and lack of a control group only with depression without insomnia, which would be another limitation. Second, there is still controversy about whether insomnia is a specific disease or a symptom comorbid with other diseases. The latest diagnostic of ICSD-3 has stressed the existence of insomnia as a specific disease.<sup>12</sup> However, for patients comorbid with depression and insomnia, it is hard to distinguish the causal relationship between depression and insomnia and whether insomnia is a specific disease or a depressive symptom. Third, a small sample size, little data on

baseline characteristics and a lack of objective instrument to evaluate sleep quality, which may affect the results. Further prospective studies are therefore required to overcome these limitations and further verify the generalization of the results in this study.

## Conclusion

Individuals with ICD showed decreased levels of anti-inflammatory ILs, suggesting immune regulation as a possible mechanism underlying this disorder. Additionally, ICD patients had cognitive impairment—specifically, related to spatial memory, and the cognitive deficits were associated with altered serum cytokine levels. Our results suggest that ICD is a distinct condition that can be distinguished from CID based on serum cytokine profile and specific types of cognitive dysfunction.

## Abbreviations

ATP, adenosine triphosphate; BDNF, brain-derived neurotrophic factor; BMI, body mass index; CID, chronic insomnia disorder; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; eATP, extracellular ATP; GM-CSF, granulocyte-macrophage colony-stimulating factor; HAMD-17, 17-Item Hamilton Depression Rating Scale; HPA, hypothalamic-pituitary-adrenal; ICD, insomnia comorbid with depression; ICSD-3, International Classification of Sleep Disorders, Third Edition; IFN, interferon; IL, interleukin; Mini 5.0, Mini International Neuropsychiatry Interview 5.0; MoCA, Montreal Cognitive Assessment; MoCA-C, Chinese-Beijing Version of Montreal Cognitive Assessment; NBMT, Nine-Box Maze Test; NREM, non-rapid eye movement; ORcM, object recognition memory; ORM, object reference memory; OWM, object working memory; PSQI, Pittsburgh Sleep Quality Index; RANTES, regulated upon activation, normal T cell expressed and secreted; SNS, sympathetic nervous system; SRM, spatial reference memory; SWM, spatial working memory; TGF- $\beta$ 1, transforming growth factor beta 1; TNF, tumor necrosis factor.

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## Disclosure

The authors declare no conflicts of interest in this work.

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