

ORIGINAL RESEARCH ARTICLE

Analysis and exploration of peripheral T-cell subset characteristics and immune markers in patients with insomnia disorder

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Citation: Ding X, Song X, Wang G, *et al.* Analysis and exploration of peripheral T-cell subset characteristics and immune markers in patients with insomnia disorder. *J Clin Basic Psychosom.*
doi: 10.36922/JCBP026140017

Received: March 31, 2026**Revised:** July 22, 2026**Accepted:** August 7, 2026**Published online:** August 26, 2026

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Abstract

Chronic insomnia is closely associated with immune dysregulation; however, the immunometabolic characteristics of peripheral T-cell subsets and their relationship with sleep architecture remain largely unclear. This study investigated peripheral T-lymphocyte subsets and their immunometabolic characteristics in patients with chronic insomnia and identified immune indicators associated with sleep parameters. A total of 45 patients with chronic insomnia and 30 healthy controls were enrolled. Flow cytometry quantified T-cell subsets and two mitochondrial parameters: mitochondrial mass, reflecting mitochondrial content, and the percentage of cells with low mitochondrial membrane potential. A total of 37 patients underwent polysomnography. Multiple linear regression identified independent predictors after adjustment for age, sex, and anxiety/depression scores, while receiver operating characteristic analyses evaluated predictive performance. Compared with healthy controls, patients with insomnia showed imbalances in 21 of 30 immune indicators ($p < 0.05$), including increased frequencies of CD4⁺ CD45RA⁻ CD62L⁻ PD1⁺ effector memory T (Tem) cells, accompanied by increased mitochondrial mass and the percentage of cells with low mitochondrial membrane potential in this subset. Multivariate regression identified Tem cell percentage as an independent correlate of N1% ($\beta = 0.184, p = 0.014$) and Tem cell count as an independent correlate of sleep efficiency ($\beta = -7.47, p = 0.007$). Receiver operating characteristic analysis showed that Tem cell count predicted insomnia with an area under the curve of 0.858 (95% confidence interval: 0.761–0.955), a sensitivity of 95.6%, and a specificity of 66.7% at a cutoff of 18.015. Tem cell count may serve as an immunometabolic biomarker for insomnia assessment.

Keywords: Insomnia; Immunometabolism; Biomarker; Mitochondrial parameters; T cell subsets

1. Introduction

Insomnia disorder (ID) affects approximately 10%–30% of adults worldwide.¹ Immunological alterations, including changes in the CD4⁺/CD8⁺ ratio and regulatory T cell dysfunction, have been reported in insomnia², and immune dysregulation may contribute to sleep disturbances via inflammatory pathways.³ Upon antigen stimulation, T cells differentiate into short-lived effector T (Te) cells and long-lived effector memory T (Tem) cells, with the latter being highly sensitive to metabolic changes under chronic stress.⁴ Evidence indicates that mitochondrial dysfunction and metabolic inflexibility contribute to T cell exhaustion.⁵ Chronic stress impairs T cell mitochondria, disrupting immune homeostasis⁶, induces CD4⁺ T cell fragmentation and metabolic dysregulation, promotes anxiety-like behaviors⁷, and correlates with reduced mitochondrial mass and aberrant membrane potential in depression and bipolar disorder.^{8,9}

Programmed cell death protein 1 (PD-1) was selected as the exhaustion marker because it is the best-characterized inhibitory receptor in chronic stress-induced T cell exhaustion, with established roles in mitochondrial dysfunction and metabolic reprogramming^{10,11}; it is reliably detectable by flow cytometry; and other inhibitory receptors (e.g., T-cell immunoglobulin and mucin domain-containing protein 3, lymphocyte-activation gene 3, T cell immunoreceptor with Ig and ITIM domains) show variable expression across disease states and T cell subsets.¹² However, the metabolic characteristics of peripheral T cell subsets and their association with immune markers in insomnia remain poorly understood, despite their growing recognition as potential biomarkers. To address this knowledge gap, flow cytometry was employed to evaluate T cell subsets and mitochondrial parameters—PD-1 expression, mitochondrial mass (MM), and the percentage of cells with low mitochondrial membrane potential (MMPLow)—in patients with ID and healthy controls. This study aims to clarify the relationship between T cell mitochondrial status and insomnia and associated emotional symptoms, and to provide experimental evidence to support the identification of potential biomarkers.

2. Methods

2.1. Participant selection

Patients admitted to the Sleep Medicine Center of Wuhan First Hospital between September 2024 and July 2025 were recruited. Inclusion criteria for the ID group were: (i) a diagnosis of chronic ID, with comorbid anxiety and depressive disorders according to both the Third Edition of

the International Classification of Sleep Disorders and the Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders; and (ii) a Pittsburgh Sleep Quality Index (PSQI) score > 7 and a 23-item Hamilton Depression Rating Scale (HAMD-23) score ≥ 7.

Exclusion criteria included: (i) major neurological disorders (e.g., multiple sclerosis, stroke); (ii) severe psychiatric disorders (e.g., schizophrenia, bipolar disorder); (iii) other medical conditions affecting sleep (e.g., severe obstructive sleep apnea); (iv) ≤6 years of formal education. A total of 45 ID patients and 30 controls were enrolled. The study was approved by the hospital ethics committee, and all participants provided informed consent.

2.2. Measures

2.2.1. Data collection

All participants completed a standardized questionnaire collecting data on sex, age, years of education, and body mass index.

2.2.2. Assessment instruments

(a) Sleep quality assessment

The Pittsburgh Sleep Quality Index was used to assess subjective sleep quality across seven components—subjective sleep latency, sleep duration, habitual sleep efficiency (SE), sleep disturbances, use of sleep medications, and daytime dysfunction—with a total score of 0–21, with higher scores indicating worse sleep quality.

The Insomnia Severity Index (ISI) score ranges from 0 to 28, with higher scores indicating greater insomnia severity. All participants underwent overnight polysomnography (PSG) using the Graef system (Compumedics, Australia). Sleep staging followed the American Academy of Sleep Medicine Manual for the Scoring of Sleep and Associated Events (version 3.0). Parameters recorded included total sleep time (TST), SE, sleep latency, rapid eye movement (REM) sleep latency, arousal index, wake after sleep onset, and proportions of N1, N2, N3, and REM sleep.

(b) Assessment of anxiety and depressive symptoms

Anxiety and depressive symptoms were assessed with multiple instruments:

- (i) Hamilton Anxiety Rating Scale (HAMA): 14 items, each scored from 0 to 4, with a total score ranging from 0 to 56; higher scores indicate more severe anxiety; a score ≥ 14 indicates clinically significant anxiety.
- (ii) HAMD-23: 23 items with varying scoring; total score ranging from 0 to 74; higher scores indicate more severe depression; a score ≥ 20 indicates

clinically significant depressive symptoms.

- (iii) Generalized Anxiety Disorder-7 (GAD-7): 7 items, each scored from 0 to 3, with a total score ranging from 0 to 21; higher scores indicate more severe anxiety with cutoffs at 5, 10, and 15 for mild, moderate, and severe anxiety, respectively.
- (iv) Patient Health Questionnaire-9: 9 items, each scored from 0 to 3, with a total score ranging from 0 to 27; higher scores indicate more severe depression with cutoffs at 5, 10, 15, and 20 for mild, moderate, moderately severe, and severe depression, respectively.

2.2.3. Immune indicator

Fasting peripheral blood (8 mL) from each patient was collected in the morning after an 8–12 hour fast. Blood was allowed to stand for 30 min and then centrifuged (4,000 r/min for 10 min) to obtain serum, which was stored at -80°C . Flow cytometry (Navios, Beckman Coulter, USA) was used to quantify the frequencies of total CD4^{+} and CD8^{+} T cells, as well as CD4^{+} and CD8^{+} PD1^{+} T cells. T-cell subsets were defined based on CD45RA and CD62L expression as CD45RA^{+} CD62L^{-} (effector), CD45RA^{-} CD62L^{-} (effector memory), and their corresponding PD1^{+} subsets, CD45RA^{+} CD62L^{-} PD1^{+} (effector memory) and CD45RA^{-} CD62L^{-} PD1^{+} (effector memory), respectively, for both CD4^{+} and CD8^{+} T cells (UBBio, China).¹³ Mitochondrial parameters, including MM (reflecting mitochondrial content) and MMPLow (defined as the proportion of cells exhibiting low mitochondrial membrane potential), were acquired using the mitochondrial stain MitoDye-APC (UBBio, China).¹⁴

2.4. Statistical analysis

Statistical analyses were performed using SPSS version 27.0.1 (IBM, USA) and RStudio (Posit, USA). Data were first tested for normality. Continuous variables are presented as mean $\pm$ standard deviation, and categorical variables are presented as counts or percentages. Group comparisons were performed using independent-samples *t*-tests for normally distributed data with equal variances or the Mann–Whitney *U* test for non-normally distributed data. Within-group comparisons used paired *t*-tests or Wilcoxon signed-rank tests as appropriate. Associations between T-cell and mitochondrial indicators and clinical or PSG parameters were examined using Pearson or Spearman correlation analyses, as appropriate.

Multiple linear regression analysis was performed to identify immune indicators independently associated with insomnia-related sleep parameters, with adjustments for age, sex, and anxiety/depression scores. Age, sex, and

anxiety/depression scores were included as prespecified covariates, while immune indicators with a univariate *p*-value of <0.05 were considered for inclusion in the multiple linear regression model. The variance inflation factor (VIF) was calculated for all included predictors to assess multicollinearity. Receiver operating characteristic (ROC) curves were constructed to evaluate the ability of immune indicators to discriminate patients with ID from healthy controls. The Benjamini–Hochberg method was applied to control the false discovery rate (FDR) for multiple comparisons. A two-tailed *p*-value of <0.05 was considered statistically significant. Data visualization was performed with GraphPad Prism version 10.6.1 (GraphPad Software Inc., USA) and RStudio.

3. Results

3.1. Participant characteristics

As shown in Table 1, no significant differences were observed between the ID group and control group in general demographic characteristics, including sex, age, marital status, education level, and body mass index (all $p > 0.05$). The age of onset in patients with ID was concentrated in middle adulthood, and the majority were taking one or more insomnia medications. The ID group exhibited significantly higher scores on the PSQI, ISI, and anxiety/depression scales (HAMA, HAMD-23, GAD-7, and Patient Health Questionnaire-9) than the control group (all $p < 0.001$). The results of overnight PSG monitoring for the ID group are presented in Table 2.

3.2. Differences in peripheral T-cell subsets between the two groups

As shown in Table 3, significant differences were observed in the frequency and absolute count of 21 T-cell subsets between the two groups ($p < 0.05$). Compared with controls, the ID group showed increased CD4^{+} T cell count, CD4^{+} T cell frequency, and CD8^{+} T cell count, along with decreased CD8^{+} PD1^{+} T cell frequency (Figure 1A). Significant decreases were observed in the counts or frequencies of CD4^{+} CD45RA^{+} CD62L^{-} Te cells, CD4^{+} CD45RA^{+} CD62L^{-} Te cells, CD8^{+} CD45RA^{+} CD62L^{-} Te cells, CD4^{+} CD45RA^{+} CD62L^{-} PD1^{+} Te cells, CD4^{+} CD45RA^{+} CD62L^{-} PD1^{+} Te cells, CD8^{+} CD45RA^{+} CD62L^{-} PD1^{+} Te cells, and CD8^{+} CD45RA^{+} CD62L^{-} PD1^{+} Te cells (Figure 1B). The ID group showed increased counts of CD4^{+} CD45RA^{-} CD62L^{-} Tem cells, CD8^{+} CD45RA^{-} CD62L^{-} Tem cells, and CD4^{+} CD45RA^{-} CD62L^{-} PD1^{+} Tem cells, as well as increased CD4^{+} CD45RA^{-} CD62L^{-} PD1^{+} Tem cell frequency and CD8^{+} CD45RA^{-} CD62L^{-} PD1^{+} Tem cell count (Figure 1C). Significant differences in mitochondrial parameters, including MM and MMPLow, were also observed between the two groups (Figure 1D).

Table 1. Baseline characteristics of patients with insomnia disorder and healthy controls

Characteristic	HC (<i>n</i> = 30)	Patients with ID (<i>n</i> = 45)	χ^2/Z	<i>p</i>
Sex (<i>n</i> [%])				
Male	9 (30.0%)	14 (31.1%)	0.01	0.919
Female	21 (70.0%)	31 (68.9%)	−0.579	0.563
Marital status (<i>n</i> [%])				
Single	9 (30.0%)	12 (26.7)	–	–
Have a partner	21 (70.0%)	33 (73.3%)	–	–
Medication status (<i>n</i> [%])				
Drug-naïve	–	10 (22.2%)	–	–
Benzodiazepines	–	3 (6.7%)	–	–
Non-benzodiazepines	–	6 (13.3%)	–	–
Benzodiazepines + others	–	8 (17.8%)	–	–
Non-benzodiazepines + others	–	11 (24.4%)	–	–
Others	–	7 (15.6%)	–	–
Age (mean ± SD)	42.8 ± 9.474	40.84 ± 16.209	−0.271	0.513
Body mass index (mean ± SD)	23.09 ± 3.33	22.91 ± 4.14	−0.579	0.563
Education (mean ± SD)	14.03 ± 2.16	13.56 ± 2.35	−0.711	0.477
Age at onset of illness (mean ± SD)	–	36.91 ± 15.88	–	–
PSQI (mean ± SD)	4.53 ± 1.78	14.38 ± 3.64	−7.273	<0.001
ISI (mean ± SD)	3.17 ± 2.53	15.29 ± 5.62	−6.814	<0.001
PHQ-9 (mean ± SD)	2.27 ± 1.74	13.13 ± 6.80	−6.432	<0.001
GAD-7 (mean ± SD)	2.37 ± 2.08	9.69 ± 5.83	−5.221	<0.001
HAMA (mean ± SD)	3.33 ± 2.38	21.36 ± 5.56	−7.269	<0.001
HAMD-23 (mean ± SD)	2.83 ± 2.34	19.49 ± 8.37	−6.543	<0.001

Abbreviations: GAD-7: Generalized Anxiety Disorder-7; HAMA: Hamilton Anxiety Rating Scale; HAMD-23: 23-item Hamilton Depression Rating Scale; HC: Healthy control; ID: Insomnia disorder; ISI: Insomnia Severity Index; PHQ-9: Patient Health Questionnaire-9; PSQI: Pittsburgh Sleep Quality Index; SD: Standard deviation.

Table 2. Characteristics of polysomnography-related parameters in patients with insomnia disorder

PSG	HC (<i>n</i> = 30, mean ± SD)	Patients with ID (<i>n</i> = 45, mean ± SD)	χ^2/Z	<i>p</i>
Total sleep time	–	420.54 ± 76.48	–	–
Sleep efficiency	–	76.14 ± 12.79	–	–
Sleep latency	–	34.70 ± 30.20	–	–
Wake during sleep	–	83.89 ± 67.47	–	–
Number of Awakenings	–	19.97 ± 10.00	–	–
Latency to stage REM	–	160.22 ± 85.83	–	–
Arousal index	–	12.46 ± 7.89	–	–
Percentage of N1 (%)	–	11.46 ± 4.93	–	–
Percentage of N2 (%)	–	58.08 ± 14.13	–	–
Percentage of N3 (%)	–	15.99 ± 13.69	–	–
Percentage of REM (%)	–	14.46 ± 7.26	–	–
N1 sleep time	–	46.35 ± 18.28	–	–
N2 sleep time	–	245.00 ± 77.79	–	–
N3 sleep time	–	66.72 ± 59.89	–	–
REM sleep time	–	62.48 ± 36.88	–	–

Abbreviations: HC: Healthy control; ID: Insomnia disorder; PSG: Polysomnography; REM: Rapid eye movement; SD: Standard deviation.

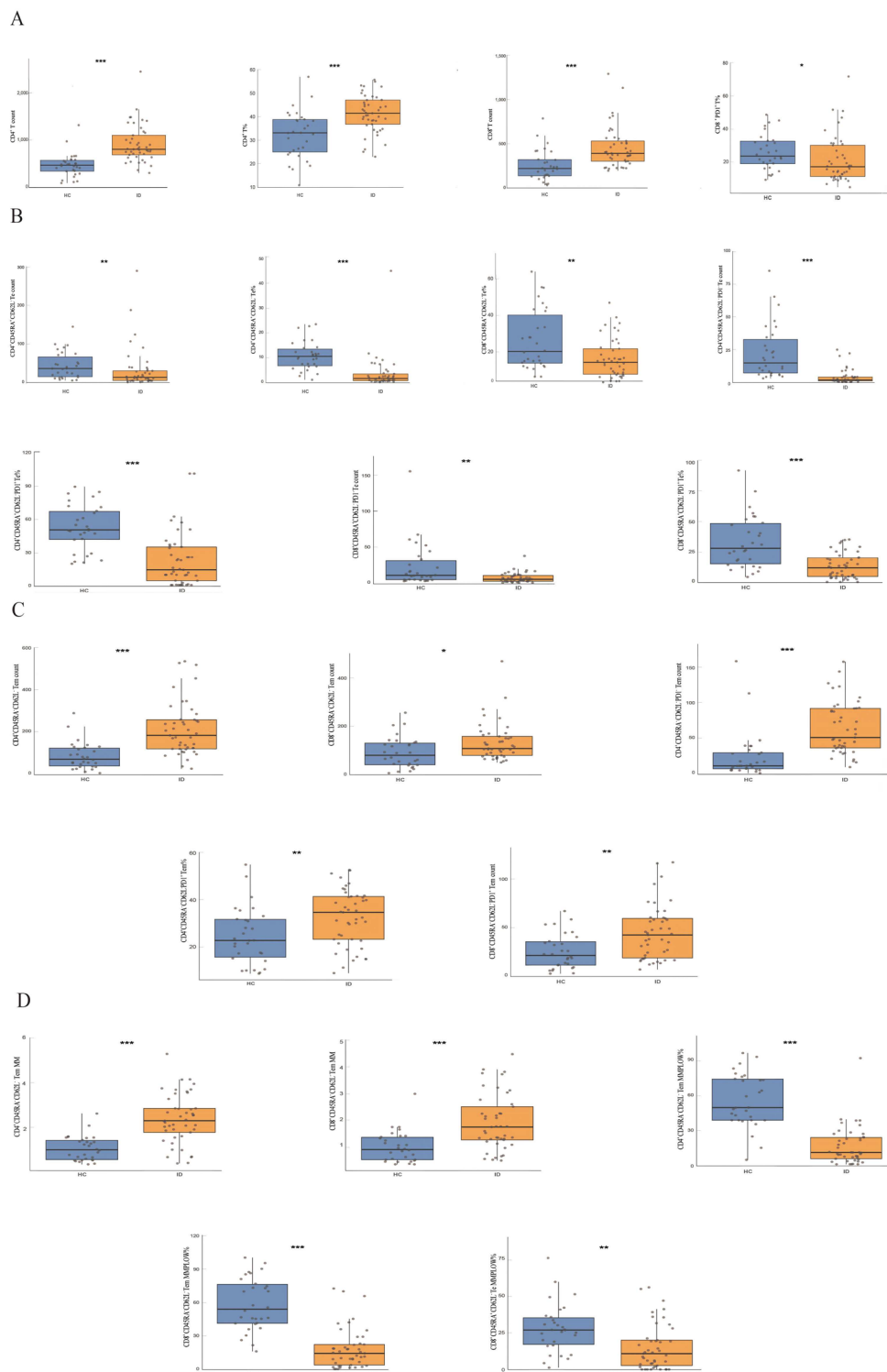


Figure 1. Peripheral T-cell subset and mitochondrial parameters in patients with ID and HC subjects. (A) CD4⁺ and CD8⁺ T-cell counts and frequencies. (B) CD4⁺ and CD8⁺ Te-cell counts and frequencies. (C) CD4⁺ and CD8⁺ Tem-cell counts and frequencies. (D) Mitochondrial parameters, including MM and MMPLow. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ indicate statistical significance.

Abbreviations: HC: Healthy control; ID: Insomnia disorder; MM: Mitochondrial mass; MMPLow: The percentage of cells with low mitochondrial membrane potential; Te: Effector T cell; Tem: Effector memory T cell.

Table 3. Comparison of T-cell-related parameters between patients with insomnia disorder and healthy controls

T cell	HC (<i>n</i> = 30, mean ± SD)	Patients with ID (<i>n</i> = 45, mean ± SD)	<i>p</i>	FDR-adjusted <i>p</i>
CD4 ⁺ T cell				
Cell count	424.39 ± 249.28	869.18 ± 397.12	<0.001	0.03
Cell frequency (%)	32.34 ± 10.16	41.32 ± 7.97	<0.001	0.015
CD8 ⁺ T cell				
Cell count	255.46 ± 174.16	456.16 ± 233.04	<0.001	0.01
Cell frequency (%)	19.22 ± 7.75	22.50 ± 6.26	0.058	0.07
CD4 ⁺ CD45RA ⁺ CD62L ⁻ Te cell				
Cell count	44.17 ± 34.63	32.77 ± 53.65	0.003	0.005
Cell frequency (%)	10.83 ± 5.73	3.75 ± 6.93	<0.001	0.008
MM	2.50 ± 0.74	2.45 ± 1.57	0.275	0.306
MMPLow	13.89 ± 7.96	19.92 ± 22.52	0.897	0.897
CD8 ⁺ CD45RA ⁺ CD62L ⁻ Te cell				
Cell count	80.18 ± 97.06	69.89 ± 53.64	0.341	0.366
Cell frequency (%)	20.55 ± 16.45	16.36 ± 10.65	0.006	0.009
MM	1.96 ± 0.84	2.04 ± 0.98	0.711	0.853
MMPLow	28.02 ± 16.79	15.04 ± 15.59	0.001	0.003
CD4 ⁺ CD45RA ⁺ CD62L ⁻ Tem cell				
Cell count	86.85 ± 63.34	213.43 ± 126.15	<0.001	0.002
Cell frequency (%)	21.02 ± 8.95	24.29 ± 9.35	0.136	0.157
MM	1.07 ± 0.55	2.39 ± 1.11	<0.001	0.003
MMPLow	55.89 ± 23.01	16.74 ± 16.38	<0.001	0.003
CD8 ⁺ CD45RA ⁺ CD62L ⁻ Tem cell				
Cell count	91.03 ± 63.82	133.42 ± 78.51	0.009	0.012
Cell frequency (%)	37.51 ± 15.40	31.12 ± 12.31	0.05	0.063
MM	1.01 ± 0.58	1.92 ± 1.04	<0.001	0.003
MMPLow	58.00 ± 23.49	17.98 ± 18.28	<0.001	0.003
CD4 ⁺ PD1 ⁺ T cell%	19.11 ± 8.55	20.28 ± 15.24	0.538	0.538
CD8 ⁺ PD1 ⁺ T cell%	26.03 ± 10.72	21.72 ± 14.27	0.022	0.029
CD4 ⁺ CD45RA ⁺ CD62L ⁻ PD1 ⁺ Te cell				
Cell count	22.55 ± 20.76	3.86 ± 5.75	<0.001	0.006
Cell frequency (%)	51.28 ± 20.25	22.27 ± 24.52	<0.001	0.005
CD8 ⁺ CD45RA ⁺ CD62L ⁻ PD1 ⁺ Te cell				
Cell count	23.24 ± 31.60	6.98 ± 6.87	0.003	0.005
Cell frequency (%)	32.65 ± 21.47	13.12 ± 10.41	<0.001	0.004
CD4 ⁺ CD45RA ⁺ CD62L ⁻ PD1 ⁺ Tem cell				
Cell count	24.86 ± 33.25	64.07 ± 36.53	<0.001	0.002
Cell frequency (%)	25.78 ± 17.78	45.03 ± 27.91	0.002	0.003
CD8 ⁺ CD45RA ⁺ CD62L ⁻ PD1 ⁺ Tem cell				
Cell count	31.62 ± 12.89	35.06 ± 16.08	0.358	0.37
Cell frequency (%)	49.98 ± 23.71	22.23 ± 20.86	<0.001	0.002

Notes: FDR-adjusted *p*-values were calculated using the Benjamini–Hochberg method for multiple comparisons (total number of tests = 30). An FDR-adjusted *p*-value < 0.05 was considered statistically significant.

Abbreviations: FDR: False discover rate; HC: Healthy control; ID: Insomnia disorder; MM: Mitochondrial mass; MMPLow: The percentage of cells with low mitochondrial membrane potential; Te: Effector T cell; Tem: Effector memory T cell.

3.3. Correlations between immune indicators, PSG parameters, and clinical scales in patients with insomnia disorder

3.3.1. Correlations between immune indicators and PSG parameters

Significant associations were observed between 10 T-cell immune indicators and PSG parameters (Figure 2). CD4⁺ T cell frequency was significantly negatively correlated with REM sleep time ($r = -0.35$, $p < 0.05$). CD4⁺ T cell count was significantly positively correlated with N1% ($r = 0.34$, $p < 0.05$). CD4⁺ CD45RA⁺ CD62L⁻ Te cell count was significantly positively correlated with wake after sleep onset ($r = 0.34$, $p < 0.05$). CD8⁺ CD45RA⁻ CD62L⁻ Tem cell count was significantly positively correlated with the number of awakenings ($r = 0.34$, $p < 0.05$). CD8⁺ CD45RA⁺ CD62L⁻ PD1⁺ Te cell frequency was significantly negatively correlated with N2 sleep time ($r = -0.36$, $p < 0.05$) and N2% ($r = -0.39$, $p < 0.05$). CD4⁺ CD45RA⁻ CD62L⁻ PD1⁺ Tem cell frequency was significantly positively correlated with N1% ($r = 0.33$, $p < 0.05$). CD4⁺ CD45RA⁺ CD62L⁻ PD1⁺ Te cell count was significantly negatively correlated with SE ($r = -0.35$, $p < 0.05$) and TST ($r = -0.38$, $p < 0.05$). CD4⁺ CD45RA⁻ CD62L⁻ PD1⁺ Tem cell count was significantly negatively correlated with N2 sleep time ($r = -0.43$, $p < 0.01$), TST ($r = -0.42$, $p < 0.05$), and SE ($r = -0.38$, $p < 0.05$), and significantly positively correlated with N1% ($r = 0.44$, $p < 0.01$). CD8⁺ CD45RA⁻ CD62L⁻ PD1⁺ Tem cell count was significantly negatively correlated with N2 sleep time ($r = -0.40$, $p < 0.05$) and TST ($r = -0.33$, $p < 0.05$). CD4⁺ CD45RA⁻ CD62L⁻ Tem MM was significantly negatively correlated with SE ($r = -0.34$, $p < 0.05$).

3.3.2. Correlations between immune indicators and clinical scales

Significant associations were observed between 20 T-cell immune indicators and clinical scale scores (Figure 3). Notably, CD8⁺ CD45RA⁻ CD62L⁻ Tem cell count, CD4⁺ CD45RA⁻ CD62L⁻ PD1⁺ Tem cell frequency, and CD8⁺ CD45RA⁻ CD62L⁻ PD1⁺ Tem cell count showed significant positive correlations with anxiety and depressive symptom scales only ($r = 0.25$ – 0.35 ; $p < 0.05$). In contrast, CD4⁺ T cell frequency, CD4⁺ T cell count, CD8⁺ T cell count, and CD4⁺ and CD8⁺ Tem-cell subsets exhibited significant positive correlations with both sleep and anxiety and depressive symptom scale scores ($r = 0.24$ – 0.56 ; $p < 0.05$). CD4⁺ and CD8⁺ Te-cell subsets showed significant negative correlations with both sleep and anxiety and depressive symptom scale scores ($r = -0.23$ to -0.68 ; $p < 0.05$).

3.4. Multiple linear regression analysis to identify independently associated immune indicators

After adjusting for potential confounders such as age, sex, and anxiety/depression scores, multiple linear regression analyses were conducted with objective sleep indicators (PSG parameters) and subjective sleep indicators (PSQI and ISI) as dependent variables and immune indicators that showed statistically significant associations in the univariate analyses (Table 3) as independent variables.

To minimize the risk of overfitting and multicollinearity, a two-step variable selection strategy was adopted: candidate immune indicators with $p < 0.05$ in univariate analyses were entered into the multivariate models, and VIF values were calculated for each final model to assess multicollinearity. The refined models revealed that CD4⁺ CD45RA⁻ CD62L⁻ PD1⁺ Tem-cell frequency was independently associated with N1% ($\beta = 0.184$, 95% confidence interval [CI]: 0.039–0.329, $p = 0.014$, VIF = 1.765), with $R^2 = 0.517$ and adjusted $R^2 = 0.401$, whereas CD4⁺ CD45RA⁻ CD62L⁻ PD1⁺ Tem-cell count was independently associated with SE ($\beta = -7.47$, 95% CI: -12.73 to -2.21, $p = 0.007$, VIF = 2.106), with $R^2 = 0.442$ and adjusted $R^2 = 0.318$.

Notably, the previously observed significant association between CD8⁺ T-cell count and N2% was attenuated and no longer reached statistical significance after covariate adjustment, suggesting that this association was not independently supported in the multivariable model. All VIF values in the final models were below 5 (range: 1.765–2.106), indicating no evidence of significant multicollinearity. The adjusted R^2 values (0.318–0.401) were lower than the corresponding crude R^2 estimates, as expected after adjustment for model complexity. Given the exploratory nature of the study and the limited sample size, these findings should be interpreted cautiously. After FDR adjustment, these associations remained statistically significant ($p < 0.05$) (Figure 4).

3.5. Analysis of the diagnostic performance of immune indicators for insomnia disorder

Based on the immune indicators identified as independently associated with sleep parameters in the multiple regression analyses, ROC curves were constructed to assess the diagnostic performance of relevant immune indicators for ID. All predictive values were calculated assuming a prevalence of 60% in the study cohort (45 patients with ID and 30 controls).



Figure 2. Correlation analysis between polysomnography parameters and T-cell and mitochondrial parameters (MM and MMPLow). * $p < 0.05$ and ** $p < 0.01$ indicate statistical significance.

Abbreviations: MM: Mitochondrial mass; MMPLow: The percentage of cells with low mitochondrial membrane potential; REM: Rapid eye movement; Te: Effector T cell; Tem: Effector memory T cell.

For CD4⁺ CD45RA⁻ CD62L⁻ PD1⁺ Tem-cell frequency, which was identified as an independent factor associated with N1%, the area under the curve (AUC) for distinguishing patients with ID from controls was 0.690 (95% CI: 0.568–0.813, $p = 0.005$). At the optimal cutoff value of 28.88, determined by the maximum Youden index (0.334), the sensitivity was 66.7%, specificity was 66.7%, positive predictive value was 75.0%, and negative predictive value was 57.2%.

For CD4⁺ CD45RA⁻ CD62L⁻ PD1⁺ Tem-cell count, which was identified as an independent factor associated with SE, the AUC was 0.858 (95% CI: 0.761–0.955, $p < 0.001$). At the optimal cutoff value of 18.015, determined by the maximum Youden index (0.623), the sensitivity was 95.6%, specificity was 66.7%, positive predictive value was 81.2%, and negative predictive value was 91.0% (Figure 5).

4. Discussion

This study elucidated the characteristics of peripheral T-cell subsets in patients with ID and identified immune markers with high diagnostic performance through a comprehensive analysis that integrated phenotypic features, mitochondrial functional status, PSG parameters, and insomnia-related scales. The results revealed extensive T-cell subset imbalances in patients with ID, notably an increased frequency of CD4⁺ CD45RA⁻ CD62L⁻ PD1⁺

Tem cells. Importantly, the increased Tem-cell subset also exhibited elevated MM and reduced MMPLow. Multivariate regression analysis, controlling for confounders such as age, sex, anxiety, and depression, identified two immune indicators independently associated with objective sleep parameters. ROC curve analysis further assessed the predictive performance of these key indicators. Together, these findings reveal a multi-level association pattern between insomnia and peripheral T cells and provide novel evidence from the perspective of cellular metabolism for understanding the immunological pathophysiology of insomnia, thereby supporting further investigation of peripheral immune markers as potential biomarkers of insomnia.

In this study, patients with ID exhibited widespread peripheral T-cell subset imbalances, consistent with prior reports of sleep-associated immune dysfunction¹⁵, and providing direct immunophenotypic support for the view that insomnia is accompanied by a systemic low-grade inflammatory state.¹⁶ Prior work has shown that sleep deprivation can alter T cell subset distributions, particularly affecting memory T cell differentiation and maintenance.^{17,18} Tem cells are long-lived lymphocytes formed after antigen exposure and are responsible for rapid secondary responses.¹⁹ PD-1, an immune checkpoint molecule, is typically upregulated in chronic

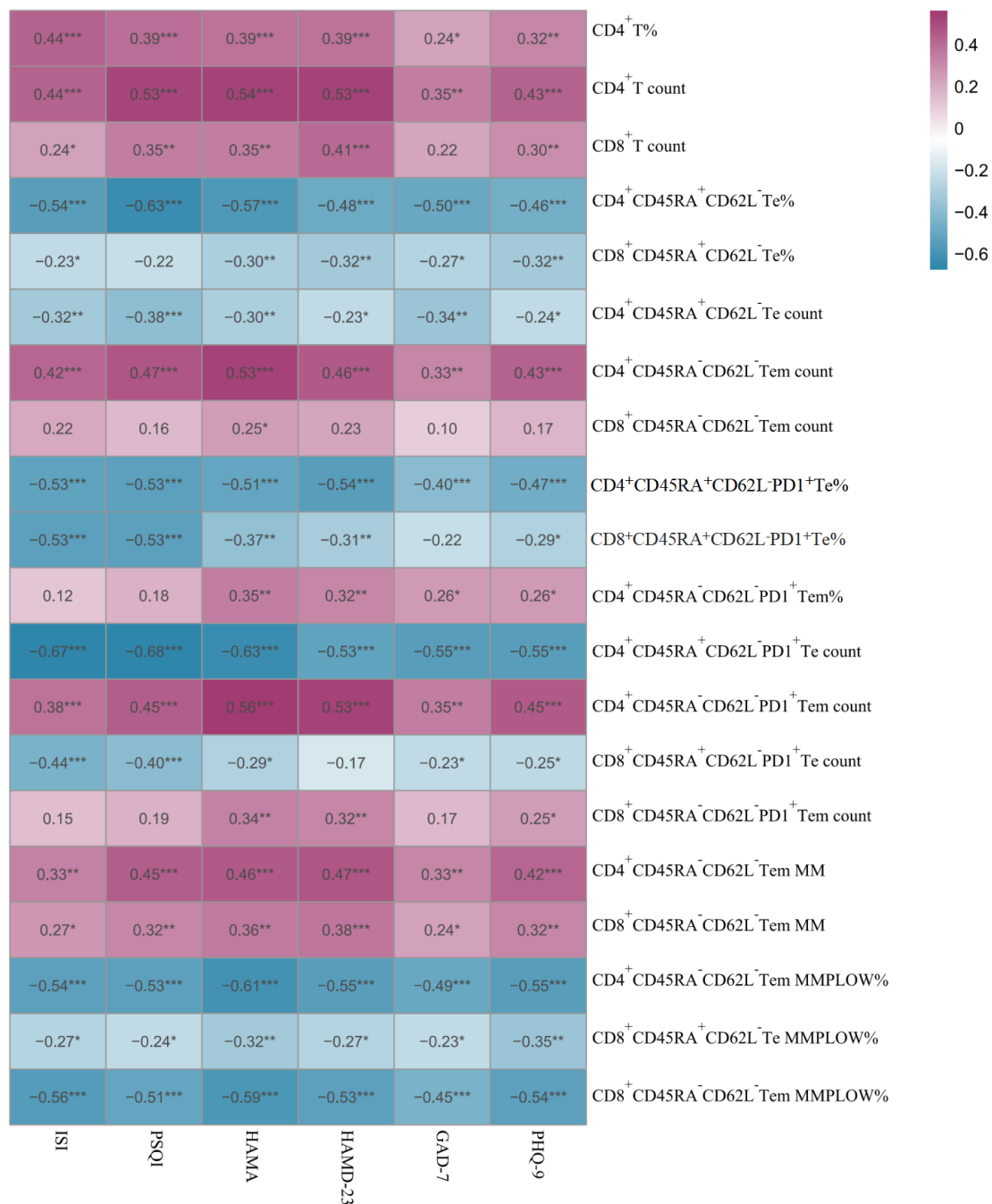


Figure 3. Correlation analysis between sleep- and emotion-related scale scores and T-cell and mitochondrial parameters (MM and MMPLOW). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ indicate statistical significance.

Abbreviations: GAD-7: Generalized Anxiety Disorder-7; HAMA: Hamilton Anxiety Rating Scale; HAMD-23: 23-item Hamilton Depression Rating Scale; ISI: Insomnia Severity Index; MM: Mitochondrial mass; MMPLOW: The percentage of cells with low mitochondrial membrane potential; PHQ-9: Patient Health Questionnaire-9; PSQI: Pittsburgh Sleep Quality Index; Te: Effector T cell; Tem: Effector memory T cell.

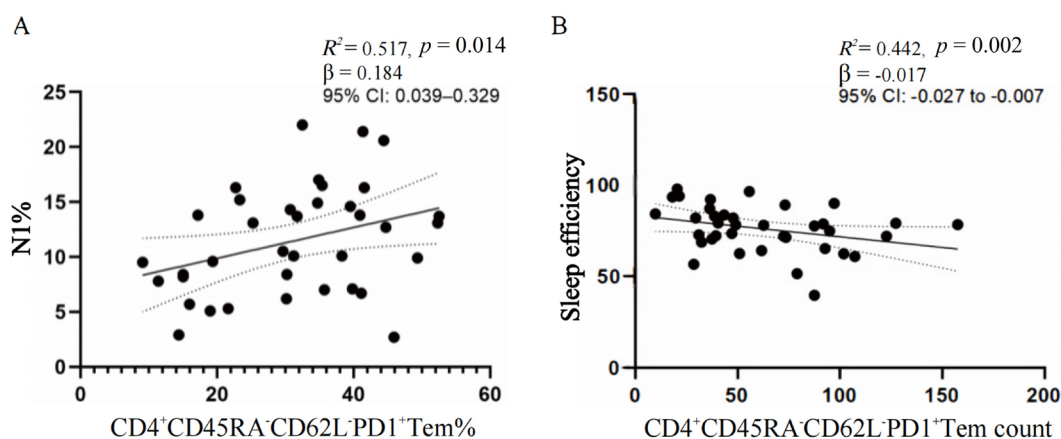


Figure 4. Correlations between peripheral immune parameters and polysomnography parameters after adjustment for age, sex, and anxiety/depressive symptoms. (A) CD4⁺ PD1⁺ Tem-cell frequency vs. N1% and (B) CD4⁺ PD1⁺ Tem-cell count versus sleep efficiency. Solid lines represent regression lines, and gray-shaded areas indicate 95% CIs.

Abbreviations: CI: Confidence interval; Te: Effector T cell; Tem: Effector memory T cell.

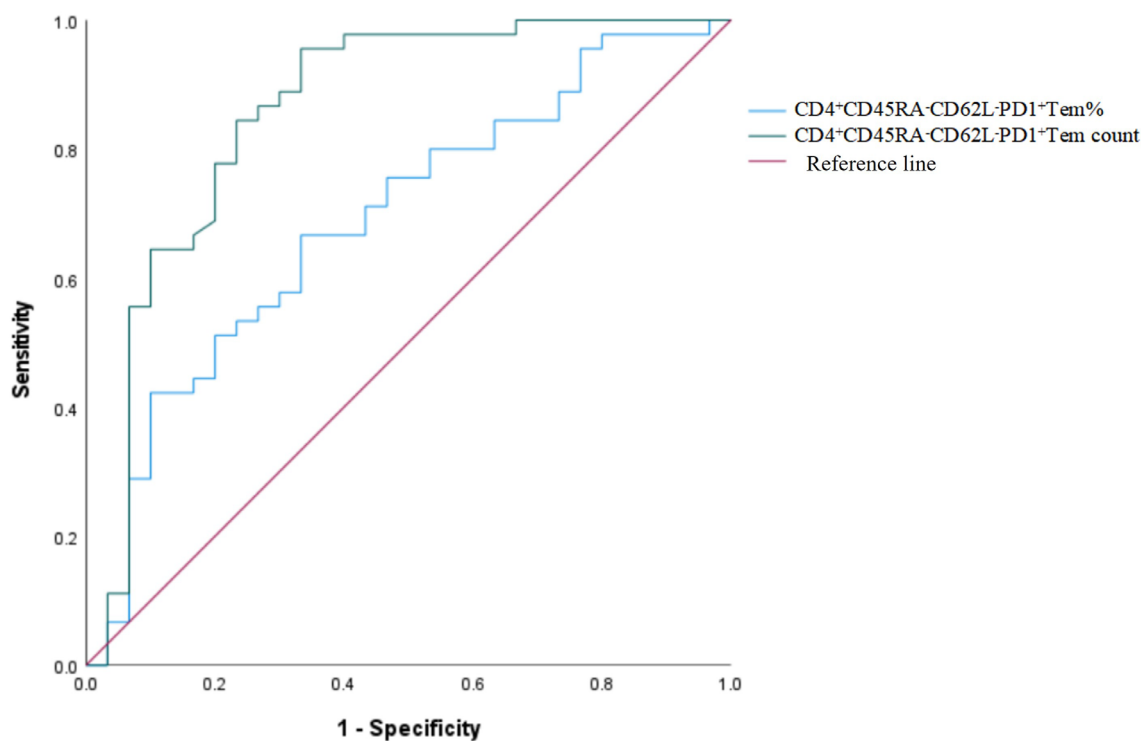


Figure 5. Receiver operating characteristic curves of peripheral blood T-cell immune markers for discriminating patients with insomnia disorder from healthy controls

Abbreviation: Tem: Effector memory T cell.

T cell activation.²⁰ Given that insomnia is accompanied by prolonged psychological stress and circadian cortisol disruptions due to hypothalamic–pituitary–adrenal axis dysfunction, cortisol signaling via the glucocorticoid receptor may influence T cell differentiation and function.²¹ Importantly, in our cohort, the increase in Tem cells accompanied by elevated MM and reduced MMPLow suggests a potential metabolic adaptation of this cell population to sustained immune activation demands. Mitochondria, as central regulators of T cell energy metabolism, profoundly influence effector function and cellular survival.²² Such metabolic adaptations may represent a potential mechanism involved in maintaining immune homeostasis, while potentially contributing to altered immune signaling that may affect central nervous system function.

Correlation analyses showed that the majority of immune indicators that achieved statistical significance were significantly associated with clinical scale scores, whereas relatively few immune indicators correlated with objective PSG sleep structure parameters. This pattern suggests that, without controlling for potential confounders, observed immune alterations may more closely reflect the overall psychophysiological stress state of individuals with insomnia than a direct effect on sleep architecture. This finding aligns with prior findings that the relationship between subjective sleep perception and inflammatory markers can be independent of objective sleep metrics.²³

Figure 2 displays the correlation matrix between immune indicators and objective sleep parameters, highlighting significant associations between specific T-cell subsets and metrics such as SE, awakenings, and N1%. Figure 3 depicts correlations between immune indicators and clinical scales (ISI, PSQI, HAMD-23, and GAD-7), with some indicators showing moderate correlations. These results indicate an intrinsic link between peripheral immune status and both clinical manifestations and sleep structure disruptions in insomnia. Notably, the association between T cell mitochondrial parameters (MM and MMPLow) and sleep parameters provides new insight into the metabolic–immune interaction mechanisms in ID.

Mitochondria regulate not only energy metabolism but also calcium signaling, reactive oxygen species production, and pro-inflammatory cytokine release^{24,25}, which can influence sleep-regulatory brain networks via multiple pathways.²⁶ Notably, when key emotional comorbidities such as depression and anxiety were included as covariates, the analysis results changed: most immune indicators previously associated with subjective scales lost statistical significance, whereas some T-cell indicators not strongly

linked to objective sleep parameters emerged as robust correlates of sleep structure. This “filtering–emergence” phenomenon highlights the potential confounding role of emotional comorbidity in the insomnia–immune association and supports the hypothesis that insomnia may affect the immune system through dual pathways: an emotion-mediated pathway driving broad, non-specific immune activation, and a sleep-direct pathway involving specific T-cell subsets that may be more closely related to core sleep physiology.²⁷

After rigorous adjustment for age, sex, and anxiety/depression scores, multivariate regression analyses using a two-step variable selection strategy with VIF assessment (all VIFs < 5) identified CD4⁺ CD45RA[−] CD62L[−] PD1⁺ Tem-cell frequency as an independent factor associated with N1% ($\beta = 0.184$, 95% CI: 0.039–0.329, $p = 0.014$, $R^2 = 0.517$, adjusted $R^2 = 0.401$) and Tem-cell count as an independent factor associated with SE ($\beta = -7.47$, 95% CI: −12.73 to −2.21, $p = 0.007$, $R^2 = 0.442$, adjusted $R^2 = 0.318$). All VIF values were below 5 (range: 1.765–2.106), indicating no significant multicollinearity, and both associations remained significant after FDR correction.

An elevated N1 proportion is a marker of reduced sleep depth and sleep instability, often linked to hyperarousal states.¹⁵ PD1⁺ Tem cells are known to secrete pro-inflammatory cytokines such as tumor necrosis factor- α and interferon- γ ²⁸, and animal studies have shown that these cytokines can act on the central nervous system to promote light sleep and disrupt sleep architecture.²⁹ The negative association between Tem-cell count and SE further suggests that a higher burden of these activated memory T cells may be associated with impaired sleep continuity, potentially through systemic inflammatory pathways that affect sleep-regulatory circuits. In contrast, the previously observed association between CD8⁺ T-cell count and N2% was attenuated and no longer significant after this rigorous adjustment, suggesting that this original finding may have been confounded by emotional comorbidities or may have reflected model overfitting in the initial analysis. Taken together, these findings underscore the necessity of comprehensive confounder control in immune–sleep research and highlight that specific T cell parameters—particularly the frequency and absolute count of the Tem subset—may be more robustly linked to objective sleep structure than broader phenotypic markers.

Using the independent factors associated with sleep parameters identified in multivariate analyses, ROC curves were constructed to evaluate the discriminative performance of immune indicators for insomnia. CD4⁺ CD45RA[−] CD62L[−] PD1⁺ Tem-cell frequency yielded an AUC of 0.690 (95% CI: 0.568–0.813, $p = 0.005$), with a

sensitivity of 66.7% and specificity of 66.7% at the optimal cutoff of 28.88. CD4⁺ CD45RA⁻ CD62L⁻ PD1⁺ Tem-cell count demonstrated superior discriminative ability, with an AUC of 0.858 (95% CI: 0.761–0.955, $p < 0.001$), a sensitivity of 95.6%, and a specificity of 66.7% at the optimal cutoff of 18.015. The markedly higher AUC of Tem-cell count suggests its superior capacity to distinguish patients with ID from healthy controls. The excellent sensitivity (95.6%) and negative predictive value (91.0%) suggest potential value for identifying individuals unlikely to have insomnia at lower values, whereas its moderate specificity (66.7%) implies that a positive result alone may be insufficient for definitive classification. Collectively, these findings suggest that absolute cell counts may confer greater discriminative value than relative percentages, potentially because they more accurately reflect the total circulating burden of Tem cells. The high sensitivity of Tem-cell count supports its potential application as a candidate screening biomarker for insomnia, while its suboptimal specificity underscores the value of combining it with additional clinical or immunological markers to optimize overall predictive performance.

5. Limitations

Several limitations should be acknowledged. First, nearly 78% of patients were receiving sedative-hypnotic drugs at baseline, with heterogeneous regimens (benzodiazepines, non-benzodiazepines, or combinations). These agents can modulate immune function via GABA-A receptors and mitochondrial translocator protein pathways.³⁰ Due to the limited sample size, medication status was not included as a covariate, nor were stratified analyses performed; thus, potential confounding cannot be excluded. Second, as a single-center, cross-sectional study with a small sample, the findings of the present study may lack generalizability, and causality cannot be established. Accordingly, the observed immune–sleep associations and mitochondrial alterations should be interpreted as correlational rather than causal. Third, subgroup analyses based on HAMD/HAMA scores were not feasible due to sample size constraints; however, HAMA, HAMD-23, age, and sex were adjusted in multivariate models, and the independent associations remained significant. Fourth, ROC analyses were performed without external validation; thus, the reported AUC values may be optimistically biased due to potential overfitting. Therefore, the clinical utility of these immune parameters—particularly MM—as biomarkers remains preliminary and requires prospective validation in independent cohorts. Fifth, our mitochondrial assessment was limited to MM and MMPLow, without direct measurements of oxygen consumption rate or ATP production, precluding a comprehensive evaluation of

bioenergetic function. Future work should incorporate longitudinal designs, multicenter studies with larger samples, and mechanistic experimental studies to further elucidate the role of T-cell immunometabolism in the onset and progression of insomnia.

6. Conclusion

Patients with ID exhibit significant T cell subset imbalances, characterized by an expansion of CD4⁺ CD45RA⁻ CD62L⁻ PD1⁺ Tem cells accompanied by concomitant alterations in mitochondrial parameters (MM and MMPLow). Multivariate regression identified Tem-cell frequency as an independent correlate of N1% and Tem-cell count as an independent correlate of SE. ROC analysis further demonstrated superior discriminative ability of Tem-cell count for insomnia (AUC = 0.858). Collectively, these findings provide novel immunometabolic insights into the pathophysiology of insomnia and identify the absolute count of Tem cells as a promising candidate biomarker that warrants further investigation.

Acknowledgments

None.

Funding

This work was supported by the Hubei Province Natural Science Funding (Grant Number: 2025YFC109), the 2025 Municipal Natural Science Foundation (Grant Number: 2025020701020233), and the Hubei Provincial Administration of Traditional Chinese Medicine Research Project (Grant Number: ZY2025L039).

Conflict of interest

The authors declare no competing interests.

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Ethics approval and consent to participate

This study was reviewed and approved by the Ethics Committee of Wuhan First Hospital (Approval number:

[2022]22). All participants provided written informed consent prior to their inclusion in the study.

Consent for publication

Written informed consent was obtained from all participants involved in this study for the release of their data in this publication.

Availability of data

The data can be obtained upon request from the corresponding author.

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