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Original Article

Effects of white matter lesion grading on the cognitive function of patients with chronic alcohol dependence

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Abstract: Background: Alcohol dependence has become a major problem that poses a serious threat to public health. Long-term heavy alcohol consumption can lead to brain functional disorders. This study aimed to investigate the relationship of the severity of cerebral white matter lesions (WMLs), serum neurofilament light (NfL) and inflammatory factors, tumour necrosis factor alpha (TNF-α) and Interleukin-1β (IL-1β), with the cognitive function of patients with alcohol dependence. Methods: A total of 118 patients were enrolled in this prospective study, and divided into alcohol-dependent and non-alcohol-dependent groups. The severity of WMLs was assessed using the Fazekas scale based on magnetic resonance imaging analysis. The expression levels of NfL, TNF-α and IL-1β in the serum of the subjects were measured by enzyme-linked immunosorbent assay. The cognitive function and psychological status of the patients were assessed using the Minimum Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Hamilton Depression Rating Scale (HAMD) and Hamilton Anxiety Rating Scale (HAMA). The severity of WMLs and the expression levels of serum NfL, TNF-α and IL-1β in alcohol-dependent patients were analysed for their influence on cognitive function. This clinical trial was approved by China Clinical Trials Registry, and the trial number is ChiCTR2200066057 (http://www.chictr.org.cn/searchproj.aspx). Results: The score of Fazekas scale was higher, and the MMSE score and MoCA score were lower in the alcohol-dependent group than those in the non-alcohol-dependent group. Moreover, the Fazekas score of the alcohol-dependent group was negatively correlated with the MMSE and MoCA scores. The serum NfL, TNF-α and IL-1β levels were higher in the alcohol-dependent group than in the non-alcohol-dependent group, and the serum NfL, TNF-α and IL-1β levels in the alcohol-dependent group were negatively correlated with the MMSE and MoCA scores. Conclusion: Alcohol-dependent patients have more severe cerebral WMLs and significant cognitive impairment, particularly in visuospatial and executive functions, attention, calculation, abstraction, delayed recall and orientation. Serum NfL, TNF-α and IL-1β may be used as biomarkers to assess alcohol related cognitive decline.

Keywords: Alcohol dependence, white matter lesions (WMLs), NfL, inflammatory factors, cognitive function

Introduction

Alcohol dependence is a chronic and complex brain disorder associated with genetic factors, personality, upbringing, family factors, social stress etc. [1]. Neuropathology and imaging have confirmed that chronic permanent damage to the structure and function of brain tissue is found in over 75% of autopsies of excessive and persistent drinkers, which can manifest on imaging as white matter lesions (WMLs). Brain WMLs is also known as cerebral white matter hyper-signal or cerebral white matter sparing [2]. It has also been shown that 50% to 80% of alcohol-dependent patients have varying degrees of cognitive changes, such as mild cognitive impairment, executive decline and dementia [3, 4].

As research progresses, researchers have proposed many mechanisms of cognitive dysfunction due to alcohol dependence, while the

pathophysiological processes that lead to altered alcoholic cognitive function are not yet fully understood. There is now growing evidence that alcoholic cognitive impairment is associated with elevated inflammatory factors and neurotoxicity due to chronic inflammatory responses. Namely, persistent chronic inflammation, even at low levels, is thought to be an accelerator of biological ageing [5]. These findings have been supported by rodent studies [6], where, for example, elevated levels of the pro-inflammatory cytokines tumour necrosis factor alpha (TNF-α) and Interleukin-1β (IL-1β) were found in the brains of animals with chronic alcohol consumption. These studies have linked inflammation to alcoholic cognitive dysfunction.

Neurofilament light (NfL) is a subunit of neurofilaments, which are cylindrical proteins located in the cytoplasm of neurons that maintain the stability of neuronal structures. Under normal conditions, axons release low levels of NfL, with a gradual increase in NfL release with increasing age. Under pathological conditions, central nervous system (CNS) axonal damage due to inflammation, neurodegeneration, trauma or vascular injuries dramatically increases the release of NfL. The released NfL flows via the cerebrospinal fluid across the blood-brain barrier into the peripheral blood [7]. Therefore, NfL can be detected in both cerebrospinal fluid and plasma or serum following nerve injury and is considered a potential biomarker for axonal and neuronal injury, not only for accurate diagnosis but also for predicting disease progression and assessing therapeutic response [8]. In previous studies, NfL has been shown to correlate with white matter hyper-signals and severity of brain atrophy as well as diseases such as Alzheimer's disease and mild cognitive impairment [9, 10].

This study aimed to explore the effects of cerebral WML grading and the serum levels of NfL, TNF-α and IL-1β on the cognitive function of patients with chronic alcohol dependence.

Methods

Collection of subjects

In this prospective study, 118 patients who were admitted to the Department of Neurology and Department of Psychiatry of Hongqi Hospital affiliated with Mudanjiang Medical College from October 2020 to October 2021 were selected and enrolled in an alcohol-dependent group and a non-alcohol-dependent group. All subjects were informed of the purpose of the trail and provided their consent to participate in the study. This study was approved by the Hospital Ethics Committee of Hongqi Hospital, Affiliated to Mudanjiang Medical College (No. Y2020044). All patients completed the general data collection within 48 h, including age, sex, ethnicity, years of education, smoking, blood lipids, hypertension, diabetes, alcohol dependence (daily alcohol consumption, years of alcohol consumption, due to geographical situations, all patients who consumed 55% liquor, the daily alcohol consumption was calculated according to 1 tael = 50 g) as well as magnetic resonance imaging (MRI) results and neuropsychological scales (MMSE, MoCA, Hamilton Depression Rating Scale (HAMD) and Hamilton Anxiety Rating Scale (HAMA)).

Inclusion criteria for the alcohol-dependent group: (I) Patients who met the International Classification of diseases-10 (ICD-10) [11] diagnostic criteria for alcohol dependence; (II) Patients who were 18 years old or older; (III) Patients with a score of at least 5 in Michigan Alcoholism Screening Test (MAST) [12]. Inclusion criteria for the non-alcohol dependent group: (I) Those who did not meet the ICD-10 diagnostic criteria for alcohol dependence; (II) Those who were 18 years old or older; (III) Those with a score of less than 5 (the first item must be "yes") in MAST. Exclusion criteria for both groups: (I) Alcohol withdrawal patients with delirium tremens and psychotic symptoms who were unable to cooperate with cognitive function tests; (II) Patients with current or previous other psychoactive substance use disorders (except smoking); (III) Patients with diseases that may affect cognitive function (severe Parkinson's disease, Alzheimer's disease, stroke, epilepsy, traumatic brain injury, etc.); (IV) Patients with autoimmune diseases; (V) Patients with recent infections; (VI) Patients with severe heart, lung, liver or kidney diseases or tumours; (VII) Those who did not provide consent.

MRI data and Fazekas scale

MRI scans were performed using a 3.0 T scanner (Philips, Netherlands). MRI images were

analysed using the Fazekas scale to assess the extent of WMLs in the brain. PV: Fazekas = 0: absent; Fazekas = 1: cap or pencil-like thin layer; Fazekas = 2: smooth halo; Fazekas = 3: extension into deep white matter. DWM: Fazekas = 0: absent; Fazekas = 1: punctate lesions; Fazekas = 2: lesions beginning to confluence; Fazekas = 3: lesions confluent and united in sheets. The total score was calculated based on the site and the sum.

Serum collection and enzyme-linked immunosorbent assay (ELISA)

Within 48 h of hospitalisation, on the day of outpatient health check and on the day of social recruitment (it was informed in advance that 8 to 12 hours of fasting was required), 5 ml of blood was collected from all study subjects from the elbow vein in the morning for routine blood and biochemical tests. The blood samples were placed in an inert separator tube for 30 min and then centrifuged at 1000×g for 20 min. The supernatant was stored at -80°C. The serum levels of NfL, TNF-α and IL-1β were determined by using ELISA kits for NfL (HB727-Hu, Shanghai Hengyuan Biotechnology, China), TNF-α (MK0122A, Jiangsu Suaseke Biotechnology, China) and IL-1β (MK0181A, Jiangsu Suaseke Biotechnology), respectively.

Statistical analysis

SPSS 24.0 statistical software was used for statistical analysis in this study. Quantitative variables (continuous variables) were subjected to t-test or rank sum test according to the data distribution. Qualitative variables (discrete variables) were directly tested by chi-square test. General information, Fazekas score, cognitive scale test scores, NfL, TNF-α and IL-1β levels of the two groups were statistically processed. The measurement data were expressed as mean ± standard deviation or median (interquartile spacing), and count data were expressed as number of cases (%). Bivariate correlations (e.g., between cognitive function and severity of WMLs, serum NfL, TNF-α, IL-1β concentration levels, daily alcohol consumption, years of alcohol consumption in the alcohol-dependent group) were analysed using Pearson correlation. The receiver operating characteristic curves (ROC) was analysed and the area under the curve (AUC) was determined. A *P* value of less than 0.05 was considered statistically significant.

Results

Comparison of general information between the alcohol-dependent and non-alcohol-dependent control groups

In this study, 59 patients who met the criteria for alcohol dependence were enrolled in the alcohol-dependent group and 59 healthy adults who met the criteria for non-alcohol dependence were selected as the control group. Significant differences were found between the two groups in terms of marital status, occupation, hypertension and anxiety status ($P < 0.05$). There were no statistically significant differences in sex, age, ethnicity, years of education, smoking, blood lipids, diabetes and depressive status ($P > 0.05$). In the alcohol-dependent group, the alcohol-dependent patients drank 225±112.86 g of alcohol per day and had been drinking for 33.05±10.77 years (**Table 1**).

Comparison of Fazekas score, serum NfL, TNF-α, IL-1β levels and cognitive function between the two groups

The total Fazekas, PV lesion and DWM lesion scores in the alcohol-dependent group were significantly higher than those in the non-alcohol-dependent group, and the differences were statistically significant (all $P < 0.001$) (**Table 2**). The levels of serum NfL, TNF-α and IL-1β were significantly higher in the alcohol-dependent group than in the non-alcohol-dependent group, with statistically significant differences between the two groups ($P < 0.05$) (**Figure 1**). Compared with those in the non-alcohol-dependent group, the total scores on the MMSE and MoCA scales decreased, and the scores on visuospatial and executive function, attention and delayed recall also decreased in the alcohol-dependent group, with statistically significant differences ($P < 0.001$) (**Figure 2** and **Table 2**). There was no statistically significant difference between the two groups in the naming, calculation, language, abstraction and orientation univariate scores ($P > 0.05$) (**Table 2**).

Correlation of Fazekas score and serum NfL, TNF-α, IL-1β levels with overall neurocognitive function assessment scores in the alcohol-dependent group

Analysis of the correlation of Fazekas scores and serum NfL, TNF-α, IL-1β levels with overall

Table 1. Comparison of general information between the alcohol-dependent and non-alcohol-dependent control groups

	AlcoholDependence group (n = 59)	Non-alcohol dependent group (n = 59)	t/χ^2	P
Sex			0.12	0.729
Male, n (%)	55 (93.2)	54 (91.5)		
Female, n (%)	4 (6.8)	5 (8.5%)		
Age (years)	59.61±8.70	59.02±9.05	0.363	0.717
Ethnicity			0.902	0.342
Han Chinese, n (%)	52 (88.1)	55 (93.2)		
Ethnic Minority, n (%)	7 (11.9)	4 (6.8)		
Years of education (years)	8 (6, 9)	9 (6, 10)	-1.535	0.125
Marital status			9.37	0.009*
Unmarried, n (%)	2 (3.4)	0 (0)		
Divorced and widowed, n (%)	20 (33.9)	8 (13.6)		
Married, n (%)	37 (62.7)	51 (86.4)		
Occupation			12.231	0.002*
Brain work, n (%)	17 (28.8)	35 (59.3)		
Manual work, n (%)	40 (67.8)	24 (40.7)		
Unemployed, n (%)	2 (3.4)	0		
Smoking history			4.674	0.097
Smoking, n (%)	43 (72.9)	32 (54.2)		
Quit smoking, n (%)	7 (11.9)	14 (23.7)		
Non-smoker, n (%)	9 (15.2)	13 (22.1)		
Blood lipids				
TC (mmol/L)	4.85±1.11	4.82±1.09	0.131	0.896
TG (mmol/L)	1.53 (1.09, 2.21)	1.41 (1.1, 2.07)	0.396	0.692
LDL (mmol/L)	2.36±0.70	2.43±0.70	-0.528	0.599
Hypertension, n (%)	40 (67.8)	25 (42.4)	7.707	0.006*
Diabetes, n (%)	12 (20.3)	11 (18.6)	0.054	0.816
Anxiety status, n (%)	35 (59.3)	19 (32.2)	8.741	0.003*
Depressive state, n (%)	0	0	-	-
Daily alcohol consumption (g)	225±112.86	NA	-	-
Years of alcohol consumption (years)	33.05±10.77	NA	-	-

The data are shown as mean ± standard deviation or n (%). TC, Total cholesterol; TG, Triglycerides; LDL, Low-Density Lipoprotein. Note: P < 0.05 is denoted by *.

neurocognitive function assessment scores in the alcohol-dependent group revealed that: (1) Fazekas score was negatively correlated with MMSE (**Figure 3A**) and MoCA (**Figure 3B**) scores, with statistically significant differences, r = -0.936, P = 0.0001; r = -0.702, P = 0.0001. (2) Serum NfL level was negatively correlated with MMSE (**Figure 3C**) and MoCA (**Figure 3D**) scores, with statistically significant differences, r = -0.991, P = 0.0001; r = -0.701, P = 0.0001, respectively. (3) Serum TNF-α level was negatively correlated with MMSE (**Figure 3E**) and MoCA (**Figure 3F**) scores, with statistically significant differences, r = -0.386, P = 0.003; r = -0.46, P = 0.0001, respectively. (4) Serum IL-1β level was negatively correlated with MMSE (**Figure 3G**) and MoCA (**Figure 3H**) scores, with statistically significant differences, r = -0.266, P = 0.042; r = -0.343, P = 0.008.

Correlation analysis of Fazekas score, serum NfL, TNF-α, IL-1β levels and neurocognitive univariate score in alcohol-dependent group

Visuospatial and executive functions, attention, calculation, abstraction, delayed recall and orientation scores were negatively correlated with Fazekas score in the alcohol-depen-

Table 2. Comparison of Fazekas score, serum NfL, TNF-α, IL-1β and cognitive function between alcohol-dependent and non-alcohol-dependent control groups

	Alcohol Dependence group (n = 59)	Non-alcohol dependent group (n = 59)	t/χ^2	P
Fazekas total score	4 (3, 5)	1 (0, 1)	8.132	0.0001**
PV score	2 (2, 2)	0 (0, 1)	8.084	0.0001**
DWM score	2 (1, 2)	0 (0, 1)	7.658	0.0001**
NfL, ng/L	14.54±2.53	7.51±2.31	9.368	0.0001**
TNF-α, nmol/L	4.18±0.32	4.05±0.07	2.884	0.005*
IL-1β, nmol/L	5.73±0.09	5.58±0.23	4.616	0.0001**
MMSE score	22.29±4.01	27.42±2.41	-8.435	0.0001**
MoCA score	18.63±3.80	25.32±2.87	-10.793	0.0001**
Visuospatial and executive ability	2 (1, 3)	5 (4, 5)	-8.192	0.0001**
naming	3 (2, 3)	3 (3, 3)	-1.664	0.096
attention	3 (2, 3)	3 (3, 3)	-3.317	0.002*
calculation	3 (2, 3)	3 (2, 3)	-0.856	0.392
language	2 (1, 3)	2 (2, 3)	-0.629	0.53
abstraction	1 (0, 2)	1 (0, 2)	-1.612	0.107
Delayed recall	0 (0, 1)	4 (3, 5)	-8.980	0.0001**
orienteering	5 (4, 6)	6 (4, 6)	-1.380	0.167

Data are expressed as mean ± standard deviation or median (interquartile spacing). *P < 0.05, **P < 0.001. PV, Periventricular; DWM, Deep White Matter; NfL, Neurofilament Light; TNF-α, Tumour Necrosis Factor Alpha; IL-1β, Interleukin-1β; MMSE, Minimum Mental State Examination; MoCA, Montreal Cognitive Assessment.

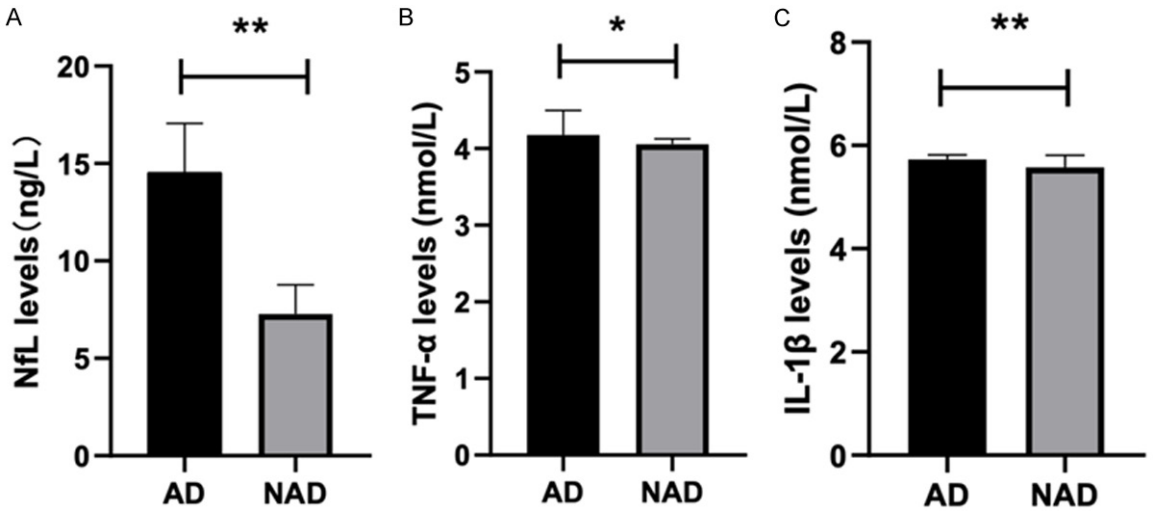


Figure 1. The differences in serum NfL, TNF-α and IL-1β levels between the alcohol-dependent and non-alcohol-dependent groups. A. Serum NfL levels in the alcohol-dependent and non-alcohol-dependent groups. B. Serum TNF-α levels in the alcohol-dependent and non-alcohol-dependent groups. C. Serum IL-1β levels in the alcohol-dependent and non-alcohol-dependent groups. *P < 0.05, **P < 0.001. AD, Alcohol-Dependent; NAD, Non-Alcohol-Dependent; NfL, Neurofilament Light; TNF-α, Tumour Necrosis Factor Alpha; IL-1β, Interleukin-1β.

dent group, with statistically significant differences (P < 0.05). It is suggested that visuospatial and executive function, attention, calculation, abstraction, delayed recall, and orientation are closely related to the Fazekas score in alcohol-dependent patients (**Table 3**).

Visuospatial and executive function, naming, attention, calculation, abstraction, delayed recall and orientation scores were negatively correlated with serum NfL level in the alcohol-dependent group, with statistically significant differences (P < 0.05). It is suggested that the

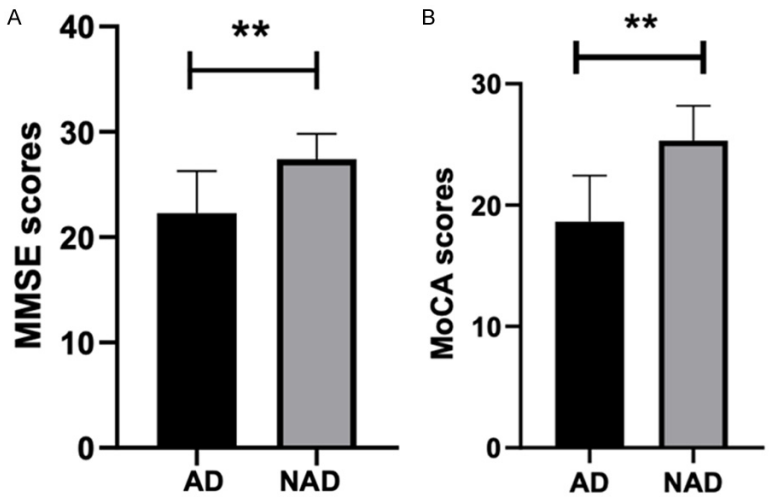
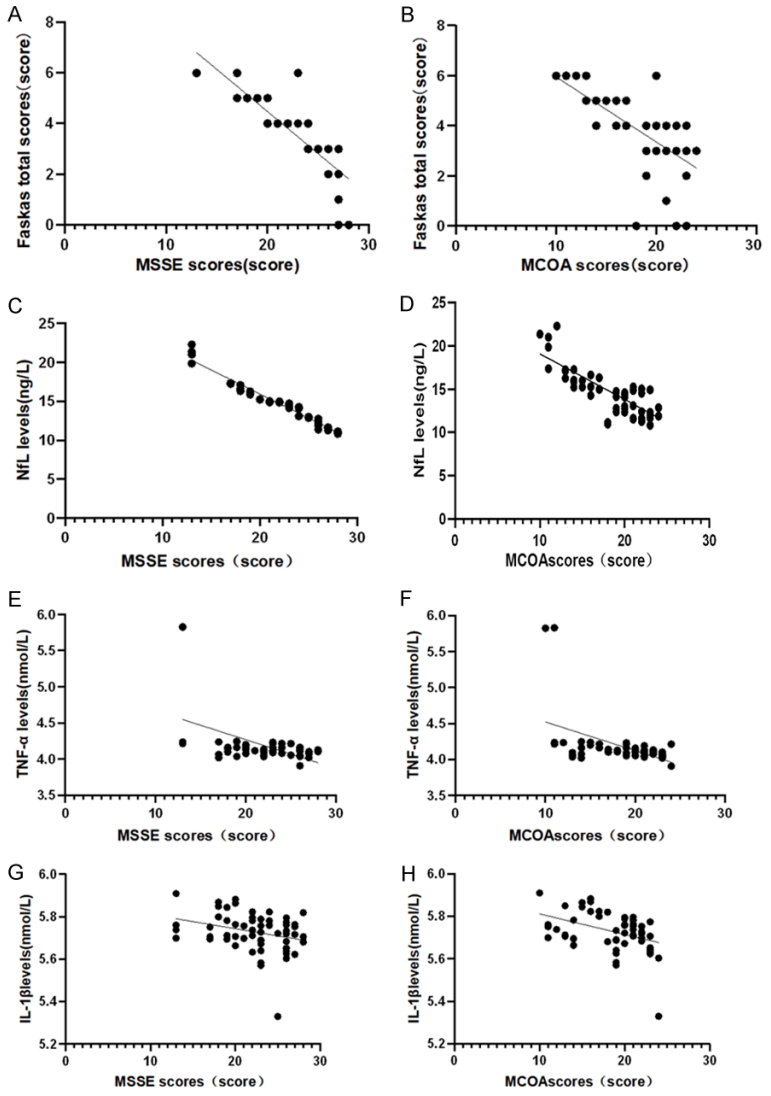


Figure 2. Total scores of the MMSE and MoCA scales in the two groups. A. MMSE scores (points). B. MoCA scores (points). *P < 0.05, **P < 0.001. AD, Alcohol-Dependent; NAD, Non-Alcohol-Dependent; MMSE, Minimum Mental State Examination; MoCA, Montreal Cognitive Assessment.

Figure 3. Correlation analysis. Correlation of Fazekas score with MMSE (A) and MoCA (B) scores. Correlation of serum NfL levels with MMSE (C) and MoCA (D) scores. Correlation of serum TNF-α levels with MMSE (E) and MoCA (F) scores. Correlation of IL-1β levels with MMSE (G) and MoCA (H) scores. Spearman's analysis showed that Fazekas score, serum NfL, TNF-α and IL-1β were negatively correlated with the overall alcohol neurocognitive function assessment scores in the alcohol dependent group (P < 0.05). AD, Alcohol-Dependent; NAD, Non-Alcohol-Dependent; NfL, Neurofilament Light; TNF-α, Tumour Necrosis Factor Alpha; IL-1β, Interleukin-1β; MMSE, Minimum Mental State Examination; MoCA, Montreal Cognitive Assessment.



neurocognitive single factors of alcohol-dependent patients are closely related to serum NfL level (**Table 4**).

Visuospatial and executive function, and delayed recall scores were negatively correlated with the TNF-α level in the alcohol-dependent group, with statistically significant differences (P < 0.05). It is suggested that visuospatial and executive function and delayed recall in alcohol-dependent patients are closely correlated with the levels of inflammatory factors (**Table 4**).

Visuospatial and executive function and attentional function in the alcohol-dependent group were negatively correlated with the IL-1β level, and the differences were statistically significant (P < 0.05). It is suggested that the visuospatial and executive function and attentional function of alcohol-dependent patients are closely correlated with the levels of inflammatory factors (**Table 4**).

Table 3. Correlation between Fazekas score, PV score, DWM score and neurocognitive univariate scores in the alcohol dependent group (P < 0.05)

	Fazekas score		PV score		DWM score	
	r	p	r	p	r	p
Visuospatial and executive functions	-0.434	0.001*	-0.356	0.006*	-0.393	0.002*
naming	-0.243	0.064	-0.234	0.074	-0.174	0.187
attention	-0.359	0.005*	-0.357	0.006*	-0.324	0.012*
calculation	-0.270	0.039*	-0.213	0.105	-0.279	0.032*
language	-0.181	0.170	-0.152	0.251	-0.077	0.562
abstraction	-0.356	0.006*	-0.341	0.008*	-0.220	0.094
Delayed recall	-0.515	0.0001**	-0.364	0.005	-0.447	0.0001**
orienteering	-0.492	0.0001**	-0.376	0.003*	-0.437	0.001*

PV, Periventricular; DWM, Deep White Matter. *P < 0.05, **P < 0.001.

Table 4. Correlation analysis between serum NfL, TNF-α, IL-1β and neurocognitive univariate scores in the alcohol-dependent group (P < 0.05)

	NfL		TNF-α		IL-1β	
	r	p	r	p	r	p
Visuospatial and executive functions	-0.410	0.001*	-0.309	0.017*	-0.302	0.02*
naming	-0.303	0.02*	-0.113	0.394	-0.178	0.177
attention	-0.354	0.006*	-0.1.57	0.234	-0.267	0.041*
calculation	-0.298	0.022*	-0.226	0.086	-0.182	0.168
language	-0.253	0.054	-0.228	0.082	0.025	0.852
abstraction	-0.374	0.003*	-0.136	0.304	-0.065	0.624
Delayed recall	-0.487	0.0001**	-0.264	0.043*	-0.123	0.355
orientation	-0.407	0.001*	-0.212	0.107	-0.125	0.346

NfL, Neurofilament Light; TNF-α, Tumour Necrosis Factor Alpha; IL-1β, Interleukin-1β. *P < 0.05, **P < 0.001.

Receiver operating characteristic (ROC) curves of serum NfL, TNF-α and IL-1β to predict alcohol-related cognitive impairment

ROC curves were drawn based on the MoCA score (testers scoring ≥ 26 were considered to have normal cognitive function) and serum NfL levels to assess the prediction value for cognitive impairment in alcohol-dependent patients: the area under the curve (AUC) was 0.815, with a 95% confidence interval of 0.741-0.889 (P < 0.0001), and the serum NfL diagnostic threshold for alcohol-related cognitive impairment was 10.0 ng/L, corresponding to a sensitivity of 68.6% and a specificity of 100%. It is indicated that serum NfL has a high diagnostic value for alcohol-related cognitive impairment (**Figure 4**).

ROC curves were drawn based on the MoCA score and serum TNF-α levels to assess the prediction value for cognitive impairment in alcohol-dependent patients: the AUC was 0.695 with a 95% confidence interval of 0.593-0.796 (P < 0.001), and the threshold of serum TNF-α for diagnosing alcohol-related cognitive impairment was 4.1 nmol/L, corresponding to a sensitivity of 47.7% and the specificity was 90.6%. It is indicated that serum TNF-α has a high diagnostic value for alcohol-related cognitive impairment (**Figure 4**).

ROC curves were drawn based on the MoCA score and serum IL-1β levels to assess the prediction value for cognitive impairment in alcohol-dependent patients: the AUC was 0.725 with a 95% confidence interval of 0.630-0.820 (P < 0.0001), and the threshold for serum IL-1β to diagnose alcohol-related cognitive impairment was 5.66 nmol/L, corresponding to a sensitivity of 66.3% and a specificity of 75%. It is indicated that serum IL-1β has a high diagnostic value for alcohol-related cognitive impairment (**Figure 4**).

Discussion

In recent years, as social standards and living conditions have improved, the number of alco-

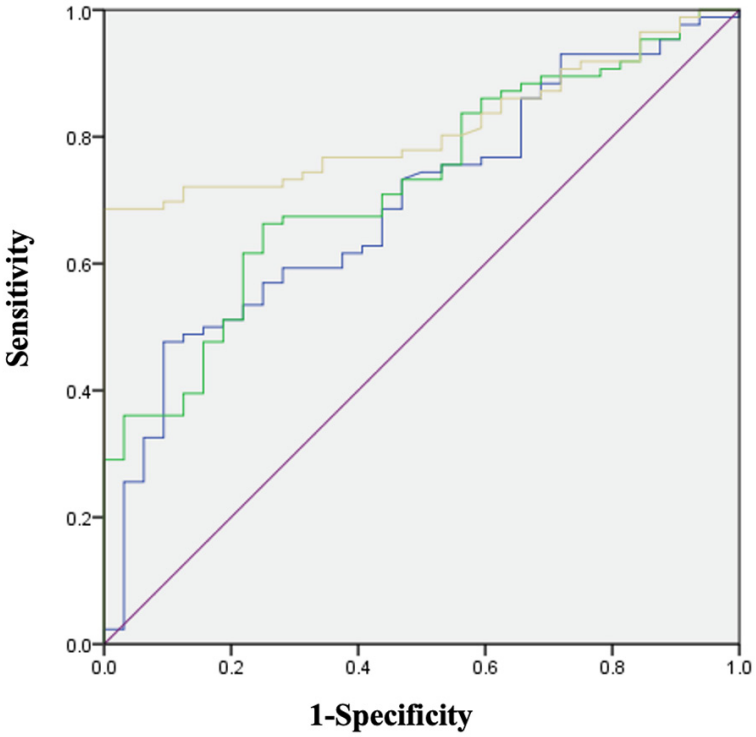


Figure 4. ROC curves of serum NfL, TNF-α and IL-1β for predicting alcohol-related cognitive impairment. The areas under the curve for NfL, TNF-α and IL-1β levels were 0.815, 0.695 and 0.725, respectively. ROC, Receiver Operating Characteristic; NfL, Neurofilament Light; TNF-α, Tumour Necrosis Factor Alpha; IL-1β, Interleukin-1β.

hol-dependent people has increased year by year, along with a variety of negative effects such as physical illnesses, psychiatric abnormalities and neuropsychological changes occurring as a result of long-term heavy drinking. Excessive alcohol consumption is known to increase the risk of alcohol-related structural brain damage and cognitive decline, manifested by irreversible structural brain damage, cognitive impairment and psychiatric symptoms [13].

Previous studies have shown that the white matter of the brain is one of the main targets of alcohol toxicity in the CNS [14]. Dysregulation of the central stress response system and inflammatory mechanisms caused by chronic alcohol consumption have also been suggested as potential causes of CNS damage [15]. Specifically, these factors can lead to various types of neuronal and glial cell damage and cause demyelination and axonal damage, which can manifest on imaging as WMLs. McEvoy et al. [16] showed that alcohol intake was associated with WMLs in a "U" shape, with moderate alcohol consumption having a protective effect on white matter compared to non-drinkers, while excessive alcohol intake could damage white matter. In our study, the Fazekas, PV and DWM scores were higher in the alcohol-dependent group than in the non-alcohol-dependent group, suggesting that alcohol-dependent patients develop cerebral WMLs, similar to the findings of McEvoy et al. Prospective studies have shown that alcohol-dependent patients are at more than twice the risk of developing severe cognitive impairment later in life compared to control patients [17, 18]. Our study used the MMSE, MOCA, HAMD and HAMA to assess the cognitive and psychological status of alcohol-dependent patients. The results showed that both MMSE and MoCA scores were lower in the alcohol-dependent group than in the non-alcohol-dependent group, suggesting that cognitive impairment was already present in the enrolled alcohol-dependent patients, in line with the tendency of previous studies [17, 18]. According to the correlation analysis of Fazekas score with MMSE and MOCA scores in this study, Fazekas score was negatively correlated with both MMSE and MOCA scores. Further correlations between individual Fazekas scores and MOCA scores suggested that cognitive impairment was mainly in visuospatial and executive functions, attention, calculation, abstraction, delayed recall and orientation, and that the correlation between WMLs and cognitive function was probably higher in DWM than in PV. Clarke et al. [19] found that cognitive impairment in alcohol-dependent patients was closely related to white matter atrophy in the brain, affecting visuospatial and executive functions, memory and attention, especially executive dysfunction. Zahr et al. [20] suggested that alcohol-dependent patients would develop selective cognitive and motor dysfunction in cognitive

judgment, cognitive control, decision making, inhibition, working memory, affective and reward processing, visuospatial functions, processing speed, and gait and static postural stability. Our findings suggested that levels of cognitive function decline with increasing severity of cerebral WMLs primarily affected visuospatial and executive functions, attention, calculation, abstraction, delayed recall and orientation, which is consistent with previous research. Analysis of possible mechanisms: The inflammatory response plays a key role in the development of alcohol-induced WMLs. Ethanol exposure increases levels of pro-inflammatory factors in brain tissue, triggering an inflammatory response. The subsequent neuroinflammation has a cascading effect, activating microglia, which contributes to white matter pathology in the form of volume reduction and loss of oligodendrocyte precursor cells, with local white matter damage in turn sending cytokine-mediated signals back to the periphery, and the increase in inflammatory factor levels becomes more pronounced as the severity of WMLs increases.

Our study also found that the HAMA score in the alcohol-dependent group was higher than that in the control group, suggesting that patients in the alcohol-dependent group had anxiety problems. The disruption of the morphological structure and function of the brain as a result of alcohol can lead to serious psychiatric and psychological disorders. Clinical studies have shown that there is a significant two-way relationship between anxiety disorders and excessive alcohol consumption. People with anxiety disorders were 2.6 times more likely to be at risk of alcohol dependence than those without, and more than one third of alcohol-dependent people had at least one type of anxiety disorder. Chronic heavy alcohol consumption leads to neurological changes, which in turn lead to negative emotions such as anxiety and exacerbate the disease development [21]. In the present study, based on the HAMA scores, we obtained similar results that patients in the alcohol-dependent group had anxiety problems.

Alcoholic cognitive impairment is a highly complex chronic brain disease for which the pathogenesis and early screening biological markers are not yet fully understood, and early identification and intervention are important for improving clinical outcomes. The current evidence [22] suggests a pro-inflammatory response during long-term chronic drinking and alcohol withdrawal. The central immune system responds in a protective or dynamic equilibrium to small amounts of short-term drinking, but long-term drinking induces a destructive pro-inflammatory state, which is reflected in elevated serum pro-inflammatory factors [6]. TNF-α has a wide range of biologically active functions that affect normal cell growth and differentiation, immune function and induction of stress responses through the activation of different downstream signalling via two receptors on the cell surface, TNFR1 and TNFR2. TNF-α has been identified as a major regulator of the inflammatory response compared to other pro-inflammatory factors and is known to be involved in the pathogenesis of inflammatory and autoimmune diseases pathogenesis [23]. IL-1β plays an important role in the CNS in regulating the inflammatory process. It is the initiator of many cytokines, cell adhesion molecules and inflammatory mediators, mainly by binding to its specific receptor IL-1 receptor 1, releasing pro-inflammatory cytokines, prostaglandins and other toxic mediators (e.g., reactive oxygen species), amplifying the inflammatory response and participating in the process of neuroinflammatory damage, chronic neurodegeneration and aging [24]. TNF-α, in conjunction with IL-6, disrupts the microvascular endothelium and permeability of the blood-brain barrier, stimulating pro-inflammatory effects on vascular endothelial cells and causing an increase in serum pro-inflammatory factors, leading to damage to the intima, degeneration of myelin sheaths and reduced neurological function, resulting in neurodegenerative changes. As in this experiment, the serum concentrations of TNF-α and IL-1β factors were increased in the alcohol-dependent group compared with those in the non-alcohol-dependent group. It is indicated that long-term heavy alcohol consumption leads to increased concentration of TNF-α and IL-1β factors in the body, which confirms the immune system-induced neuroinflammatory responses in alcohol-dependent patients.

NfL is a neuronal cytoskeletal protein and a marker of neural tissue damage in neuroinflammatory and neurodegenerative diseases [25]. Under normal conditions, only small amount of

NfL is released into the extracellular fluid, whereas in the presence of CNS damage, NfL is released from the axons into the cerebrospinal fluid and crosses the blood-brain barrier, raising serum NfL level [26]. Our results showed that serum NfL level was higher in the alcohol-dependent group than in the non-alcohol-dependent group. The present study further confirmed that serum NfL, TNF-α and IL-1β levels were negatively correlated with both MMSE and MoCA scores, and that serum NfL, TNF-α and IL-1β levels were also negatively correlated with neurocognitive univariate scores. Similarly, the results of ROC curve analysis suggested that serum NfL, TNF-α and IL-1β could all be used as predictive markers for alcoholic cognitive impairment. However, serum NfL is a more reliable biomarker than TNF-α and IL-1β, and has a higher diagnostic value for the development of cognitive impairment in alcohol-dependent patients. The reason [27, 28] may be that long-term heavy alcohol intake leads to cerebral WMLs, which decrease the reserve capacity of the brain and further cause neuroinflammation, neuronal loss, cortical layer thinning and neurodegenerative lesions that affect cognitive function. In the process, NfL is released into the blood, causing an increase in serum NfL level. In this study, serum NfL level was measured by ELISA. Although the sensitivity of ELISA is low, the specificity of serum NfL for diagnosing alcohol-related cognitive impairment was found to be as high as 100% in ROC curve analysis, which may be due to the fact that most of the alcohol-dependent patients enrolled had a history of heavy drinking for decades, and the sample size was small, leading to possible bias in the test results.

In summary, patients with alcohol dependence develop cerebral WMLs and cognitive impairment, and as the severity of cerebral WML increases, cognitive function is further impaired, mainly in the aspects of visuospatial and executive functions, attention, calculation, abstraction, delayed recall and orientation. NfL, TNF-α and IL-1β may be used as biomarkers to assess cognitive impairment in alcohol-dependent patients. The present study is a single-centre study, and the changes in serum NfL, TNF-α and IL-1β levels were not monitored dynamically. The role of serum NfL, TNF-α and IL-1β in predicting cognitive impairment in alcohol-dependent patients needs to be further confirmed by expanding the sample size and conducting multi-centre studies.

Disclosure of conflict of interest

None.

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