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Sex differences in the mediating role of brain-derived neurotrophic factor between inflammation and memory in cirrhotic patients with minimal hepatic encephalopathy

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ABSTRACT

Minimal hepatic encephalopathy (MHE) affects attention, visuo-motor coordination, and visual perception, with mixed evidence on its impact on memory. Brain-derived neurotrophic factor (BDNF) is associated with memory dysfunction, and plays a crucial role in modulating neuroplasticity. This study investigates the mediating role of BDNF in the relationship between pro-inflammatory cytokines (IL-6, IL-15, IL-18), and declarative memory performance, and the moderating effects of sex. Sixty-eight cirrhotic patients and 22 healthy volunteers performed the Psychometric Hepatic Encephalopathy Score for MHE diagnosis and logical memory subtest (Wechsler Memory Scale-III). Moderated mediation analysis using bias-corrected bootstrapping and multiple regression was performed. Results showed that increased levels of IL-18 and IL-15 were significantly associated with lower BDNF levels ($p = 0.03$ and $p = 0.02$ respectively). However, no direct effect was observed between IL-18 and IL-15 and memory. The conditional effects of BDNF on memory were significant only for women with and without MHE, and lower BDNF levels were associated with lower memory performance (without MHE: $p = 0.002$; MHE: $p = 0.001$). Moreover, BDNF mediated indirectly the relationship between pro-inflammatory cytokines and memory. IL-18 and IL-15 impacted memory through reduced BDNF levels only in women with and without MHE, whereas IL-6 showed no significant effect on BDNF or memory across groups. These findings underscore the important role of BDNF in memory in cirrhotic patients, especially women with MHE, by mediating the IL-18 and IL-15 effects. The study highlights the role of IL-18 and IL-15 cytokines in neuroplasticity-related memory decline, positioning BDNF as a key biomarker for inflammation-associated cognitive impairment in this population.

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1. Introduction

Cognitive impairment is a frequent complication among patients with liver cirrhosis, particularly in those suffering from minimal hepatic encephalopathy (MHE), a subclinical stage of hepatic encephalopathy (HE) (Vilstrup et al., 2014). MHE is often characterized by deficits in attention, visuomotor coordination, and visual perception (Felipo et al., 2014; McCrea et al., 1996; Weissenborn et al., 2001), though its impact on memory remains less clear (Vilstrup et al., 2014). Studies investigating memory in MHE patients have produced conflicting findings (García-García et al., 2018; Tarter et al., 1987; Thomas et al., 1998; Weissenborn et al., 2003; Weissenborn et al., 2005), leaving uncertainty regarding the presence of memory impairment.

In the context neurodegenerative disorders, blood-based biomarkers have emerged as minimally invasive tools for assessing cognitive impairment. Particularly in patients with MHE, increased ammonia levels and inflammation play significant roles in neurological dysfunction (Felipo, 2013; Shawcross et al., 2004, 2007; Felipo et al., 2012a,b; Montoliu et al., 2009).

Several studies support the interplay between hyperammonemia and inflammation in the neurological alterations observed in MHE (Felipo et al., 2012a,b; Shawcross et al., 2004), with elevated serum levels of IL-6 and IL-18 correlating with its presence (Montoliu et al., 2009). While these cytokines do not freely cross the blood-brain barrier (BBB), peripheral inflammation can compromise BBB integrity, increasing its permeability and allowing pro-inflammatory mediators to access the central nervous system (Nikolopoulos et al., 2023; Takechi et al., 2017). In addition, these cytokines interact with endothelial cells, activate neuroimmune signaling, and promote leukocyte infiltration, collectively exacerbating neuroinflammation and could be associated to cognitive deficits (Huang et al., 2021).

IL-6 and IL-18 are significantly elevated in cirrhotic patients, where they impact neuronal plasticity and contribute to neuroinflammatory processes that impair cognitive function (Mangas-Losada et al., 2017; Montoliu et al., 2009; Yadav et al., 2016). Additionally, IL-15 has been involved in inflammation-driven neuroplasticity alterations, although its role in cirrhosis-related cognitive impairment remains less understood (Pan et al., 2013). Given their involvement in neuroinflammatory pathways, these cytokines are likely to play a crucial role in the progression of cognitive disturbances associated with cirrhosis. In particular, studies in MHE patients have demonstrated that elevated inflammatory markers correlate with deficits in learning and long-term memory, with García-García et al. (2018) highlighting an inverse relationship between interleukin serum levels and memory performance.

Moreover, Brain-derived neurotrophic factor (BDNF) is a key regulator of neuroplasticity and has been extensively linked to memory dysfunction, affecting both short- and long-term memory (Alonso et al., 2002; Yamada and Nabeshima, 2003). BDNF supports neuronal health and counteracts the effects of pro-inflammatory cytokines (Charlton et al., 2023; Yu et al., 2022; Xu et al., 2017), which are central to conditions like MHE. Verbal declarative memory, highly dependent on medial temporal lobe integrity, is particularly vulnerable to neuroinflammation and synaptic dysfunction (Lima Giacobbo et al., 2019). Efficient cognitive function relies on neuroplasticity-driven synaptic modifications at both dendritic and axonal levels, which can be disrupted by inflammatory processes (Lima Giacobbo et al., 2019).

BDNF can have a large diversity of sources including neurons, immune system cells such as monocytes or lymphocytes, the thymus, the liver, and platelets (Brigadski and Leβmann, 2020; Bouhaddou et al., 2024). Patients with liver cirrhosis exhibit immune system alterations, lymphopenia and thrombocytopenia, which could affect their peripheral BDNF levels and, consequently, its effects on the central nervous system. In patients with liver cirrhosis, BDNF functions not only as a neurotrophic marker but also as a molecule associated with the severity of inflammation and fibrosis (Xu et al., 2017). Reduced serum concentrations of BDNF in cirrhotic patients reflect the degree of liver inflammation and disease progression (Stawicka et al., 2021). Additionally, significantly lower BDNF levels have been observed in patients with liver cirrhosis and those with MHE. The relationship between BDNF and cognitive function is complex, involving interactions with various signaling pathways and regulatory mechanisms. Systemic inflammation in patients with liver cirrhosis can reduce BDNF levels, impairing cognitive function. Moreover, hyperammonemia directly impacts BDNF content in the brain, further contributing to memory deficits (Galland et al., 2017; Lima Giacobbo et al., 2019).

Differences in neuroplasticity and inflammatory responses between men and women have garnered attention, highlighting sex-specific variations in BDNF signaling and functions. Studies have shown that these differences affect not only susceptibility to inflammatory conditions but also synaptic plasticity, essential for learning and memory (Chan and Ye, 2017). Women, in particular, exhibit elevated BDNF levels in regions such as the prefrontal cortex and hippocampus, critical for cognitive processing, which may be linked to an increased vulnerability to inflammatory markers (Hayley et al., 2015). Conversely, men tend to display distinct patterns of cognitive decline, potentially related to differences in BDNF receptor phosphorylation and specific inflammatory responses (Hill & van den Buuse, 2011; Spencer-Segal et al., 2011). Given the influence of these sex-specific variations on neuroprotection and deterioration processes, it is crucial to investigate how inflammatory processes and BDNF interact to influence memory performance in men and women with liver cirrhosis.

Furthermore, as BDNF plays a pivotal role in synaptic plasticity and mitigates the harmful effects of neuroinflammation (Charlton et al., 2023; Xu et al., 2017; Yu et al., 2022), it has emerged as a promising therapeutic target for cognitive impairment.

Therefore, this study aims to explore the mediating role of BDNF in the relationship between inflammatory biomarkers and memory function in cirrhotic patients, with a focus on how these relationships are moderated by sex. Hence, the specific objectives of this study are: 1) To analyze the association between pro-inflammatory cytokines and BDNF levels and memory performance. 2) To investigate whether BDNF mediates the relationship between pro-inflammatory cytokines and memory. 3) To analyze the role of sex in these relationships.

This study will provide a deeper understanding of the inflammatory mechanisms underlying memory impairment in patients with liver cirrhosis and the influence of sex on these processes. The results will clarify the relationship between peripheral inflammation, BDNF levels, and their effects on memory, improving the research and development of sex-specific therapeutic targets to treat memory decline in cirrhotic patients, especially those with MHE, our findings could help guide the development of personalized therapeutic approaches for MHE patients, ultimately improving clinical outcomes and quality of life.

2. Material and methods

2.1. Participants

A total of 68 patients with liver cirrhosis were consecutively recruited from the outpatient clinics at Clínico and Arnau de Vilanova hospitals in Valencia, Spain. The diagnosis of liver cirrhosis was confirmed through clinical, biochemical, and ultrasonographic evaluations. Inclusion criteria included chronic liver cirrhosis, the ability to stand and walk unaided, stable medication regimen, and being over 18 years of age. Exclusion criteria comprised the presence or history of overt HE, recent alcohol consumption within the past six months, and a history of substance abuse or dependence other than alcohol.

Additionally, a control group of 22 healthy volunteers was included after excluding liver disease via clinical, analytical, and serological tests. Participants showed no signs of fever or recent infection. All participants underwent the Psychometric Hepatic Encephalopathy Score (PHES), a battery of five psychometric tests used for diagnosing MHE (Ferenci et al., 2002; Weissenborn et al., 2001). The PHES score, adjusted for age

and educational level, was calculated using Spanish normality tables (http://www.redeh.org/TEST_phes.htm). Cirrhotic patients were classified as having MHE if their score was ≤−4 points. Based on the PHES score, the patients were divided into 25 individuals with MHE (MHE) (19 men and 6 women) and 43 without MHE (NMHE) (30 men and 13 women). The 22 healthy volunteers (11 men and 11 women) served as controls, and they completed the PHES battery to rule out cognitive impairment. Psychometric tests, and blood collection were carried out on the same day.

All participants provided written informed consent before inclusion in the study. The study protocols were approved by the Scientific and Research Ethics Committees of Hospital Clínico Universitario and Arnau de Vilanova Hospital in Valencia, Spain [approval code: 2018/210 (March 2, 2018) and 2023/130 (March 1, 2024)] and adhered to the ethical guidelines of the Helsinki Declaration.

2.2. Blood collection and measurements

2.2.1. Blood collection

Blood samples were collected at 8:30 a.m., following an overnight fast, to avoid diurnal variations in cytokine levels. For plasma isolation, venous blood was taken in BD Vacutainer tubes with EDTA, and centrifuged at 1500×*g* for 10 min. The supernatant was collected and stored frozen at – 80 °C until analysis.

2.2.2. Quantification of cytokines and BDNF

BDNF, IL-6, IL-18, IL-15 and CCL20 were measured in plasma samples using Enzyme-Linked ImmunoSorbent Assay (ELISA) with Commercial DuoSet® ELISA Kits (R&D Systems; Minneapolis, Minnesota, United States). The IL-17A levels were measured with the IL-17A 2.0 Advantage Assay using SIMOA™ HD-X equipment (Quanterix Corp., Billerica, MA, USA), with intra- and inter-assay coefficients of variations (CV) of 4.3 % and 5.9 % respectively, and a detection limit of 0.002 pg/mL. For the BDNF and cytokines determinations, intra-assay CV ranged from 3 to 10 %, and inter-assay CV, from 9 to 25 %. Lower curve concentrations were 23.4, 9.4, 15.6, 11.7, and 15.6 pg/mL for BDNF, IL-6, IL-15, IL18, and CCL20, respectively. Lower concentration detected for each cytokine was 23.4, 0.05, 0.8, 7, and 0.7 pg/mL for BDNF, IL-6, IL-15, IL18, and CCL20, respectively. All procedures were carried out according to the manufacturer's protocol.

2.2.3. Verbal declarative memory

To test verbal memory, we used the Logical Memory subtest from the Wechsler Memory Scale-III (WMS-III) (Wechsler, 1997). In this task, participants are read two brief stories (A and B), with story B presented twice. After hearing each story, participants are asked to reproduce it as accurately as possible. After 30 min, they are asked to recall what they remembered. Participants then take a recognition test consisting of 15 true or false questions for each story. The recorded scores include immediate recall (the sum of the scores for recalling story A and the two presentations of story B, for both thematic units and themes).

2.3. Statistical analysis

Continuous variables were reported as mean and standard error of the mean (SEM) or median and interquartile range. The Kolmogorov-Smirnov test was used to assess the normality. Comparisons were performed using one-way analysis of variance (ANOVA), followed by post-hoc Bonferroni multiple comparisons test for parametric variables. For variables which did not follow normal distribution, such as BDNF and cytokine levels, group differences were assessed using Kruskal-Wallis test followed by Dunn's multiple comparisons test. Categorical data were analyzed by Chi-squared test.

We conducted a moderated mediation analysis (Preacher et al., 2007), using Hayes' PROCESS macro (model 18) in SPSS (version 26; IBM Corporation, Armonk, NY, USA) to explore the role of BDNF as a mediator in the relationship between inflammation and memory performance, with the moderating effects of both group (control, without MHE, with MHE) and sex (male, female). In this analysis, the outcome variable (Y) was memory performance, the independent variable (X) was each cytokine independently (IL-18, IL15, IL-6), and the mediating variable (M) was BDNF levels, we included Group (W) and sex (Z) as moderators. Standardized values were used to perform the moderated mediation analysis. Bootstrap data resampling procedures establish confidence intervals (CIs) to test the statistical significance of an indirect effect (Shrout and Bolger, 2002). CIs are considered statistically significant when they do not include zero. The analysis was based on 10,000 bootstrap iterations, and the CI was set at 95 %, as recommended by Mallinckrodt et al. (Mallinckrodt et al., 2006). The level of significance was set at 0.05.

A post hoc power analysis was conducted using G*Power software (version 3.1.9.7 for Windows) to calculate statistical power. Based on a moderate effect size ($f^2 = 0.15$) and significance level ($\alpha = 0.05$), with a total sample size of $n = 90$, the power analysis yielded $1-\beta = 0.867$.

Additionally, we performed analyses to investigate the potential mediating role of BDNF in the relationship between other pro-inflammatory biomarkers, including IL-17A and CCL20, and memory performance. These analyses followed the same moderated mediation model (Model 18, PROCESS) used for IL-18, IL-15 and IL-6. Detailed results for these additional biomarkers, which did not show significant effects, can be found in the Supplementary Data.

3. Results

3.1. Demographic, clinical, and neuropsychological results

Table 1 shows the sociodemographic, clinical, and neuropsychological results of the study groups, showing no significant differences in age. Declarative memory scores were significantly lower in MHE patients compared to both NMHE and controls. Biochemical analyses revealed that BDNF plasma levels were significantly reduced in NMHE and MHE patients relative to controls. IL-18 and CCL20 levels were significantly elevated in both patient groups compared to controls, while IL-6 showed a significant increase only in NMHE versus controls. IL17A showed significant higher levels in plasma from MHE patients compared to controls. In contrast, IL-15 levels did not reach statistical significance (Table 1).

3.2. Moderated mediation analysis of IL-18 on declarative memory through BDNF considering the influence of group and sex

We tested a moderated mediation model to examine whether BDNF, acting as a mediator, plays a role in the relationship between IL-18 and memory performance, with group (Controls, NMHE, MHE) and sex (men/women) as moderators. The analysis indicated that higher levels of IL-18 were significantly associated with lower BDNF levels (path *a*: B = −0.228, SE = 0.104, $p = 0.031$, BootLLCI = −0.434, BootULCI = −0.021). This negative relationship suggests that increased IL-18, a pro-inflammatory cytokine, may contribute to reduced BDNF levels, which are crucial for neuroplasticity and cognitive functioning. However, no direct effect was found between IL-18 and memory (path *c*': B = −1.123, SE = 1.310, $p = 0.394$, BootLLCI = −3.729, BootULCI = 1.483) (Fig. 1).

3.2.1. Conditional effects of BDNF on declarative memory by group and sex in IL-18 model

When examining the effect of BDNF on memory in model including IL18, the interaction between BDNF and group (i.e., Control, NMHE, MHE) was statistically significant ($p = 0.008$), indicating that impact of BDNF on memory performance differed depending on the patient group. However, the interaction between BDNF and sex was not significant ($p = 0.539$), indicating that the influence of BDNF on memory was not consistently different between men and women across all groups.

Table 1
Main demographic, clinical and neuropsychological characteristics, and plasma cytokines measured in all participants.

	Controls (n = 22)	NMHE (n = 43)	MHE (n = 25)	Global *p* value
Sex (m/w)	11/11	30/13	19/6	0.141
Age (years)	63 ± 2	62 ± 1	63 ± 2	0.851
Etiology of cirrhosis				
Alcohol		19	11	
HBV/HCV		0/11	1/4	
HCV + alcohol		0	1	
MASH/MASH + alcohol		10/2	6/1	
Other		1	1	
Child-Pugh (A/B/C)		35/7/1	18/5/2	0.483
MELD score		9 ± 0.4	10 ± 0.9	0.144
Declarative memory (total score)	26.1 ± 1.6	22.6 ± 1.1	16.6 ± 1.5***/ αα	<0.001
Plasma cytokines (pg/mL)				
BDNF	1363 (452–3245)	291** (208–531)	254*** (137–613)	<0.001
IL-18	132 (90–281)	281** (207–474)	449*** (231–645)	<0.001
IL-15	5.9 (2–12.6)	6.6 (5.6–21.16)	8.8 (4.7–14.7)	0.223
IL-6	1.34 (0.76–1.98)	2.53* (1.55–6.30)	2.62 (1.73–3.42)	0.012
IL-17A	0.140 (0.06–0.235)	0.220 (0.110–0.695)	0.245* (0.167–1.035)	0.04
CCL-20	7.1 (4.9–12.4)	24*** (13–34)	17* (5–43)	0.009

Values are expressed as mean ± standard error or median (interquartile range). Abbreviations: NMHE and MHE, patients without and with minimal hepatic encephalopathy according to PHES score; m, men; w, women; HBV, hepatitis B virus; HCV, hepatitis C virus; MASH, metabolic dysfunction-associated steatohepatitis; MELD, model for end-stage liver disease. Child Pugh and MELD scores measure the severity of chronic liver disease, the higher the score represent the more severe liver disease. Differences between groups were analyzed using one-way ANOVA followed by post-hoc Bonferroni test for parametric variables. *P* values are shown after using Bonferroni correction for multiple comparisons. For variables which did not follow normal distribution, Kruskal-Wallis test followed by Dunn's multiple comparisons test were used to assess group differences. Proportions between groups for categorical variables were analyzed with Chi-squared test. Significant differences compared to control group are indicated by asterisks (*); significant differences between NMHE and MHE patients are indicated by α: *p < 0.05; **/αα p < 0.01; ***p < 0.001.

Nonetheless, the three-way interaction among BDNF, group, and sex was significant ($p = 0.019$), suggesting that the role of BDNF in memory may vary based on both group status and sex.

Specifically, the positive effect of BDNF on memory was statistically significant only for women in both the NMHE and MHE groups. For women without MHE, lower BDNF levels were associated with worse memory performance (path *b*: B = 10.265, SE = 3.202, $p = 0.002$, BootLLCI = 3.895, BootULCI = 16.635). Similarly, in women with MHE, lower BDNF was linked to worse memory outcomes (path *b*: B = 21.653, SE = 6.334, $p = 0.001$, BootLLCI = 9.039, BootULCI = 34.268). In contrast, this positive association between BDNF and memory was not significant for women in the Control group or for men in any of the groups, as all *p*-values were greater than 0.140 (Fig. 2A).

3.2.2. Conditional indirect effect of IL-18 on memory through BDNF by group and sex

Results of the conditional indirect effect of IL-18 on memory through BDNF across the three groups (Controls, NMHE, and MHE) and sexes show a significant indirect effect of IL-18 on memory through BDNF for women without MHE (B = −2.338, BootSE = 1.953, BootLLCI = −7.549, BootULCI = −0.210) and with MHE (B = −4.931, BootSE = 3.903, BootLLCI = −15.149, BootULCI = −0.610). These findings suggest that higher levels of IL-18 are associated with lower memory performance through decreased BDNF levels in these groups of women. In contrast, the indirect effects were not significant for women controls or for men in any of the groups (Table 2).

3.3. Moderated mediation analysis of IL-15 on declarative memory through BDNF considering the influence of group and sex

We also tested a moderated mediation model to examine whether BDNF, acting as a mediator, plays a role in the relationship between IL-15 and memory performance, with group and sex as moderators. The analysis revealed that IL-15 was negatively associated with BDNF levels (path *a*: B = −0.271, SE = 0.115, $p = 0.022$, BootLLCI = −0.501, BootULCI = −0.041). This significant association suggests that higher levels of IL-15 correlate with lower BDNF levels. However, no direct effect of IL-15 on memory was detected (path *c'*: B = 1.496, SE = 0.828, $p = 0.076$, BootLLCI = −0.158, BootULCI = 3.150).

3.3.1. Conditional effects of BDNF on declarative memory by group and sex in IL-15 model

On regard of the conditional effects of BDNF on declarative memory as a function of group and sex in model including IL-15, we observed that the interaction between BDNF and group was positive and significant ($p = 0.004$). However, the interaction between BDNF and sex was not significant ($p = 0.947$). The three-way interaction between BDNF, group, and sex was significant ($p = 0.016$) suggesting that the role of BDNF in memory may vary based on both group status and sex.

The conditional effects analysis revealed that the effect of BDNF on memory was statistically significant only for women in both the NMHE and MHE groups; for women without MHE lower BDNF levels were associated with worse memory performance (path *b*: B = 11.941, SE = 3.283, $p = 0.001$, BootLLCI = 5.383, BootULCI = 18.499), the same for women with MHE (path *b*: B = 24.150, SE = 6.392, $p = 0.000$, BootLLCI = 11.378, BootULCI = 36.916), but it was not significant for control women or for men in any of the groups (all $p > 0.214$) (Fig. 2B).

3.3.2. Conditional indirect effect of IL-15 on memory through BDNF by group and sex

Table 3 presents the conditional indirect effects of IL-15 on memory, mediated by BDNF, across groups and sex. Significant indirect effects were observed for women in both the NMHE (B = −3.237, BootSE = 2.679, BootLLCI = −10.468, BootULCI = −0.601) and MHE groups (B = −6.545, BootSE = 5.271, BootLLCI = −20.657, BootULCI = −1.388). These findings indicate that in woman cirrhotic patients with and without MHE, higher IL-15 levels are linked to poorer memory performance through their negative impact on BDNF. No significant indirect effects were found for women in the Control group or for men in any of the groups.

3.4. Moderated mediation analysis of IL-6 on declarative memory through BDNF considering the influence of group and sex

Results of the moderated mediation analysis of IL-6 on declarative memory through BDNF showed that the relationship between IL-6 and BDNF was not significant (path *a*: B = −0.137, SE = 0.147, $p = 0.354$, BootLLCI = −0.431, BootULCI = 0.156), suggesting that, unlike other inflammatory markers studied, IL-6 does not appear to influence BDNF levels. Additionally, the effect of IL-6 on memory was not significant (path *c'*: B = −0.889, SE = 1.018, $p = 0.387$, BootLLCI = −2.931, BootULCI = 1.153). Regarding the conditional effects of BDNF on declarative memory as a function of group and sex in model including IL-6, we observed that the interaction between BDNF and group was significant (p = 0.003). However, the interaction between BDNF and sex was not significant ($p = 0.536$). The three-way interaction between BDNF, group, and sex was also significant ($p = 0.032$), indicating that the relationship between BDNF and memory varies depending on both

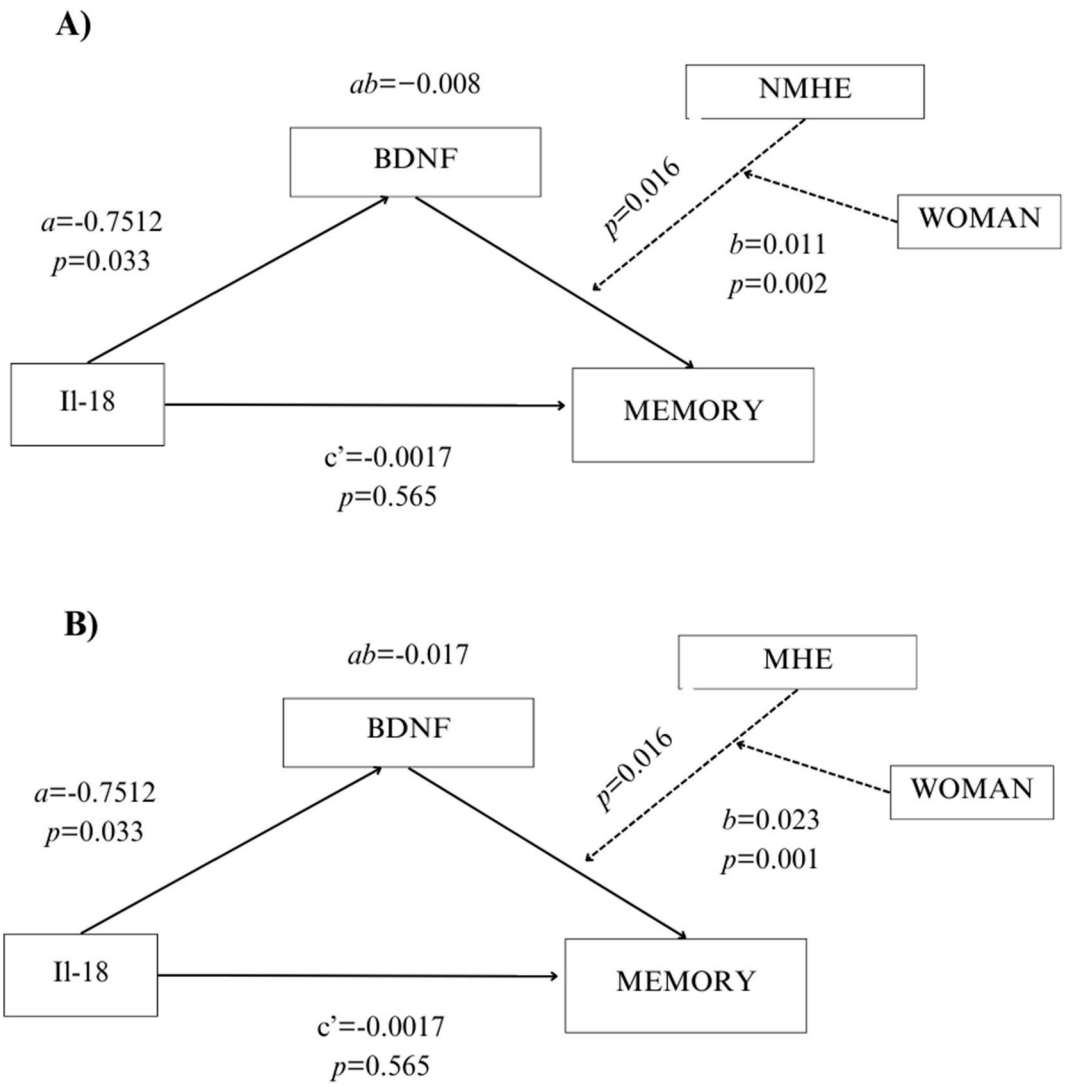

Fig. 1. Moderation and mediation analysis with group and sex, using bias-corrected bootstrapping in conjunction with multiple regression analysis. Numbers on the lines show B and p-values. Solid lines indicate direct effects; dashed lines indicate moderations. A) Results are reported for the woman without MHE. B) Results are reported for the woman with MHE. IL-18 was negatively related with BDNF (path *a*: B = −0.228, SE = 0.104, $p = 0.031$). The three-way interaction between BDNF, group, and sex was significant ($p = 0.019$). Thus, the relationship between BDNF and memory was significant only for women without MHE (B = 10.265, SE = 3.202, $p = 0.002$) and woman with MHE (B = 21.653, $p = 0.001$). The conditional direct effect of the relationship between IL-18 and memory was not significant (path *c*': B = −1.123, SE = 1.310, $p = 0.394$, BootLLCI = −3.729, BootULCI = 1.483). There was an indirect effect of ILl-18 on memory through BDNF only in women without MHE (path ab: B = −2.338, BootSE = 1.953, BootLLCI = −7.549, BootULCI = −0.210) and with MHE (path ab: B = −4.931, BootSE = 3.903, BootLLCI = −15.149, BootULCI = −0.610).

group and sex. Therefore, the conditional effects analysis revealed that the effect of BDNF on memory was significant only for women without MHE (path *b:* B = 11.177, SE = 3.748, $p = 0.004$, BootLLCI = 3.659, BootULCI = 18.694) and women with MHE (path *b:* B = 23.433, SE = 7.481, $p = 0.003$, BootLLCI = 8.428, BootULCI = 38.438). However, this effect was not significant for control women or for men in any of the groups (all $p > 0.387$) (Fig. 2C).

Analysis of the conditional indirect effect of IL-6 on memory through BDNF across the three groups and sexes showed no significant indirect effects of IL-6 on memory through BDNF for any group or sex. These results suggest that IL-6 does not significantly influence memory performance through BDNF in any of the analyzed groups or sexes (Table 4).

3.5. Additional analysis

Additionally, we performed analyses to investigate the potential mediating role of BDNF in the relationship between other pro-inflammatory biomarkers, including IL-17A and CCL20, and memory performance. These analyses followed the same moderated mediation model (Model 18, PROCESS) used for IL-18 and IL-15. However, no significant direct or indirect effects were found for these biomarkers. The detailed results of these additional analyses are presented in the Supplementary Tables 1 and 2

4. Discussion

The present study explored the mediating role of BDNF in the relationship between pro-inflammatory cytokines, specifically IL-18, IL-15, and IL-6, and declarative memory function in patients with liver cirrhosis, with a particular emphasis on how these effects are moderated by sex. The following discussion is structured into four sections to comprehensively address these findings: (1) Direct effects of interleukins and BDNF, (2) Conditional Effects of BDNF on Declarative Memory (3) Moderating effects of sex, and (4) Indirect effects of interleukins on memory through BDNF mediation.

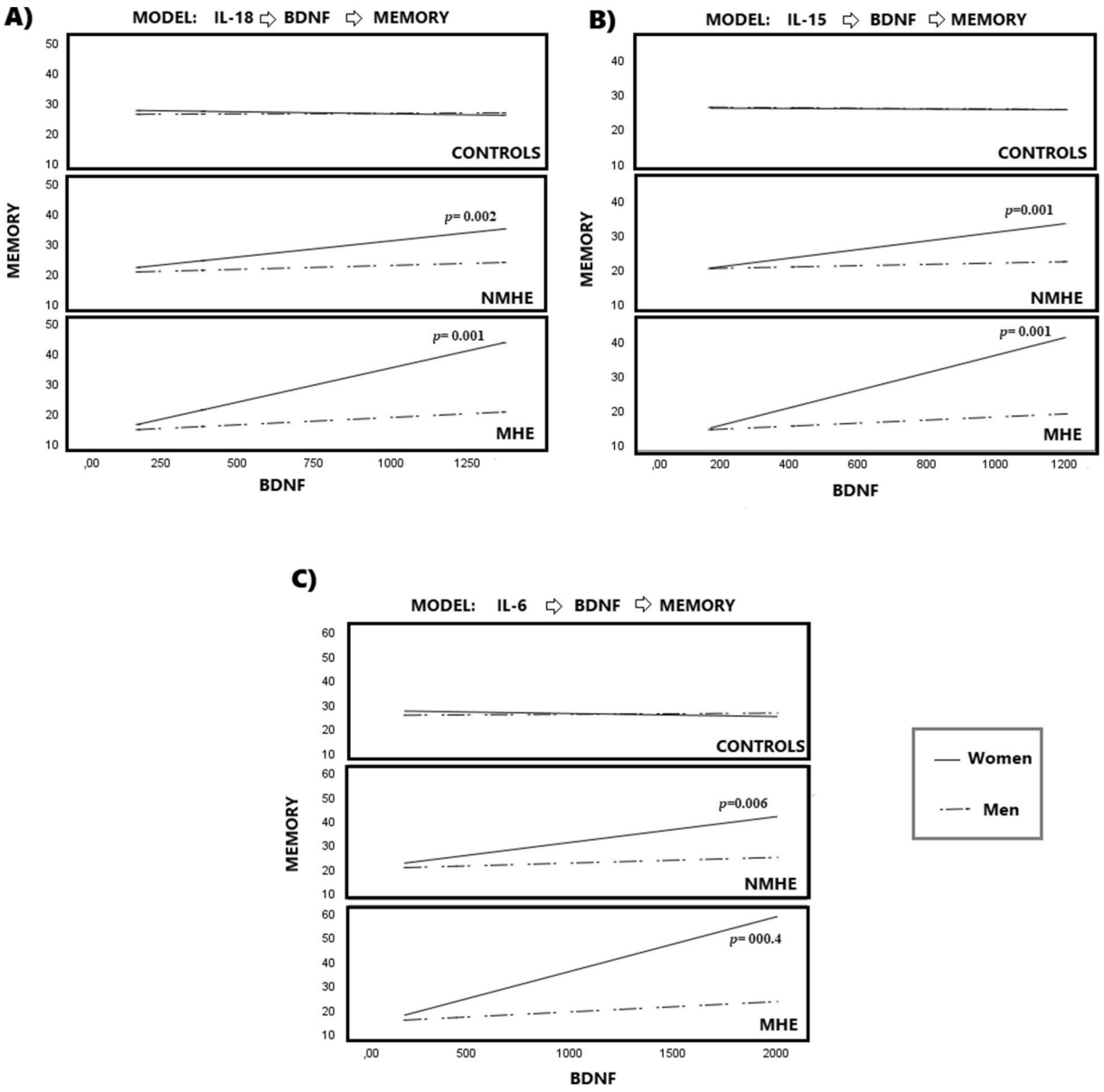

Fig. 2. Conditional effects of BDNF on declarative memory as a function of group (Control, NMHE or MHE) and sex in all the models tested. A) Results are reported for the model IL-18 (X), BDNF (M), Memory (Y), with group (Controls, NMHE, MHE) and sex (men/women) as moderators. B) Results are reported for the model IL-15 (X), BDNF (M), Memory (Y), with group (Controls, NMHE, MHE) and sex (men/women) as moderators. C) Results are reported for the model IL-6 (X), BDNF (M), Memory (Y), with group (Controls, NMHE, MHE) and sex (men/women) as moderators. The values shown represent raw values and not log-transformed values. Abbreviations: NMHE and MHE, patients without and with minimal hepatic encephalopathy, respectively.

Table 2
Indices of conditional indirect effect of IL-18 on memory through BDNF across the three groups (Controls, NMHE, and MHE) and sexes.

Group	Sex	Effect	BootSE	BootLLCI	BootULCI
Control	women	0.2558	0.4092	−0.2674	0.9752
Control	Men	−0.0066	0.4119	−0.9003	0.7276
NMHE	**women**	**−2.3375**	**1.9527**	**−7.5493**	**−0.2094**
NMHE	Men	−0.550	0.386	−1.4595	0.0275
MHE	**women**	**−4.9307**	**3.903**	**−15.149**	**−0.6102**
MHE	Men	−1.0934	0.7726	−2.9348	0.0162

Abbreviations: LLCI, Lower limit confidence interval; ULCI, Upper limit confidence interval; SE, standard error; NMHE, patients without minimal hepatic encephalopathy; MHE, patients with minimal hepatic encephalopathy (based on PHES score). The confidence intervals (CIs) were calculated using bootstrap resampling procedures. An indirect effect is considered statistically significant if the CI does not include zero, indicating a meaningful mediation effect.

Table 3
Indices of conditional indirect effect of IL-15 on memory through BDNF across the three groups (Controls, NMHE, and MHE) and sexes.

Group	Sex	Effect	BootSE	BootLLCI	BootULCI
Control	women	0.0716	0.4201	−0.7689	0.9224
Control	Men	0.1088	0.5467	−1.3127	0.8717
NMHE	**women**	−3.2368	2.6794	−10.4679	−0.6012
NMHE	Men	−0.5225	0.5316	−1.7525	0.3776
MHE	**women**	−6.5452	5.2711	−20.6573	−1.3878
MHE	Men	−1.1537	1.0159	−3.3931	0.595

Abbreviations: LLCI, Lower limit confidence interval; ULCI, Upper limit confidence interval; SE, standard error; NMHE, patients without minimal hepatic encephalopathy; MHE, patients with minimal hepatic encephalopathy (based on PHES score). The confidence intervals (CIs) were calculated using bootstrap resampling procedures. An indirect effect is considered statistically significant if the CI does not include zero, indicating a meaningful mediation effect.

Table 4
Indices of conditional indirect effect of IL-6 on memory through BDNF across the three groups (Controls, NMHE, and MHE) and sexes.

Group	Sex	Effect	BootSE	BootLLCI	BootULCI
Control	women	0.1481	0.4451	−0.4109	1.149
Control	Men	−0.0076	0.4496	−0.9397	0.8745
NMHE	women	−1.5334	3.3691	−11.3862	0.7944
NMHE	Men	−0.2709	0.5633	−1.6661	0.4556
MHE	women	−3.215	6.8174	−23.2404	1.6789
MHE	Men	−0.5343	1.0965	−3.3867	0.7816

Abbreviations: LLCI, Lower limit confidence interval; ULCI, Upper limit confidence interval; SE, standard error; NMHE, patients without minimal hepatic encephalopathy; MHE, patients with minimal hepatic encephalopathy (based on PHES score). The confidence intervals (CIs) were calculated using bootstrap resampling procedures. An indirect effect is considered statistically significant if the CI does not include zero, indicating a meaningful mediation effect.

4.1. Associations between interleukins and BDNF levels

In our study, a significant relationship was observed between the pro-inflammatory cytokines IL-15 and IL-18 and reduced BDNF levels in patients with liver cirrhosis, suggesting that increased systemic inflammation may negatively impact neuroplasticity. These findings align with previous studies where cytokines such as IL-2, IL-4, TNF-α, and IL-6 showed inverse associations with BDNF in patients with cognitive impairment (Guan and Fang, 2006; Yap et al., 2021). However, unlike prior research, our study did not find a significant association between IL-6 and BDNF levels, even though IL-6 has shown notable interactions in other cognitive impairment contexts; this may indicate that the role of IL-6 in regulating BDNF is condition-specific and may not be universal across all neurocognitive inflammatory states, such as those in patients with liver cirrhosis (Guan and Fang, 2006). The IL-6 levels vary due to several factors as severity of liver cirrhosis (Rey and Effendi-Ys, 2021) and are influenced by conditions such as hyperglycemia, hyperlipidemia, and diabetes (Gunes et al., 2023), as well as by age in the presence of chronic diseases (Franceschi et al., 2000, Ershler and Keller, 2000), which can also affect memory. Although the study cohort is age-matched, there is still some variability in this factor, as well as in disease severity, leading to individual differences in IL-6 levels. This variability complicates the exact identification of the relationship between IL-6, BDNF, and memory. Future analyses should aim to clarify this relationship by controlling for all potential confounding factors that may influence IL-6 levels.

Collectively, these results indicate that elevated levels of IL-15 and IL-18 could directly contribute to neuroinflammation and reduction of BDNF levels, thereby compromising neuroplasticity and potentially worsening cognitive outcomes in MHE. This mechanism supports the broader literature suggesting that targeting specific cytokines could mitigate BDNF decline and protect against inflammation-induced cognitive impairment (Dadkhah et al., 2024; Ibrahim et al., 2024).

4.2. Conditional effects of group and sex on BDNF role in memory

The significant interaction between BDNF and group in both models indicates that the relationship between BDNF and memory varies across different groups. BDNF, a protein prevalent in most areas of the brain and in the blood, is known to decrease in patients with liver cirrhosis (Stawicka et al., 2021). Our findings indicate that reduced BDNF levels are linked to impaired immediate memory performance, especially among women with cirrhosis and MHE. These results are consistent with other studies that have reported significantly lower serum BDNF levels in patients with overt HE and MHE. These studies highlight BDNF as a potential predictive marker in the context of MHE. (Bedewy et al., 2024; Stawicka et al., 2021).

Findings in this study underscore the critical role of BDNF in modulating memory in the presence of inflammation and other factors related to liver cirrhosis. These results also indicate that BDNF is a crucial biomarker for memory function in patients with liver cirrhosis. BDNF not only promotes nervous tissue health and counteracts the effects of pro-inflammatory cytokines but also its reduction is associated with the severity of liver inflammation and fibrosis (Xu et al., 2017; Stawicka et al., 2021). These results are consistent with previous studies showing that decreased BDNF is related to cognitive impairment in conditions of hyperammonemia and neuroinflammation (Galland et al., 2017; Lima Giacobbo et al., 2019).

Moreover, reduced BDNF levels have been observed in patients with chronic diseases, which are often associated with cognitive impairment, particularly in direct memory. Decreased BDNF levels are also linked with conditions such as dementia, autism, multiple sclerosis, Parkinson's disease, Alzheimer's, and Huntington's disease (Azman and Zakaria, 2022; Bathina and Das, 2015). Additionally, a study by Lim et al. (2016) showed that the presence of the Val66Met polymorphism associated with BDNF is responsible for synaptic excitation and the integrity of neurons, leading to moderate memory loss, amyloid-beta accumulation, and hippocampal atrophy in Alzheimer's disease.

In patients with MHE, reduced BDNF levels might result from hyperammonemia, chronic inflammation, oxidative stress, or impaired liver function affecting the brain-liver axis. BDNF expression is known to mitigate both stress and inflammation (Mondelli et al., 2011). Thus, cirrhosis associated cognitive changes in the brain can contribute to decreased BDNF levels. This underscores the importance of BDNF as both a diagnostic marker and a potential therapeutic target for cognitive impairments related to liver cirrhosis and other neurodegenerative diseases.

4.3. Moderating effects of sex

The present findings reveal a significant interaction between sex and BDNF levels in relation to memory performance, with female patients, particularly those with MHE, exhibiting a marked sensitivity to BDNF fluctuations. This sensitivity could be mediated by estrogen, which has been shown to upregulate BDNF expression and signaling, thereby promoting neuroplasticity and supporting cognitive function (Sohrabji and Lewis, 2006; Scharfman and MacLusky, 2006). The positive regulation of estrogens on BDNF within the hippocampus and other memory critical brain regions supports synaptic plasticity, potentially underlying sex-specific cognitive advantages observed in females (Weinstein et al., 2014).

A substantial body of in vivo and in vitro research has demonstrated that estrogen exerts significant neuroprotective effects through multiple mechanisms. It plays a crucial role in reducing inflammation and oxidative stress while also enhancing neurogenesis and angiogenesis, contributing to overall brain health (Liu et al., 2009; Herson et al., 2009). Beyond these fundamental functions, estrogen also regulates key molecular and cellular processes in the brain, particularly by increasing the expression of BDNF mRNA and protein in regions vital for memory, such as CA1 and CA3 (Scharfman and MacLusky, 2006). This estrogen-BDNF interaction is believed to enhance synaptic plasticity, reinforcing memory resilience in females and providing a possible explanation for the cognitive benefits associated with female sex. Additionally, research suggests that elevated serum BDNF levels are correlated with a lower risk of dementia in women, an effect likely mediated by estrogen influence on BDNF regulation (Weinstein et al., 2014; Sohrabji and Lewis, 2006). Findings from ovariectomized animal models further support this notion, as estrogen replacement therapy has been shown to boost BDNF expression in the hippocampus and cortex, suggesting a protective mechanism against age-related cognitive decline (Sohrabji and Lewis, 2006).

In postmenopausal women, estrogen's neuroprotective function is likely shaped more by the indirect effects of reduced estrogenic signaling rather than by its direct hormonal influence. The decline in estrogen levels, along with the lack of other essential neuroprotective factors,

may increase neuronal susceptibility to metabolic stressors, such as ammonia-induced astrocyte dysfunction (Brown et al., 2008; Dhandapani and Brann, 2002). This imbalance in cerebral homeostasis is thought to play a role in the greater prevalence of neuropsychiatric impairments in women compared to men, emphasizing the vital role of estrogen in preserving brain function beyond the reproductive years.

Further evidence of sex-specific differences in BDNF function is provided by Chan and Ye (2017), who report that cognitive processes related to BDNF are more significantly modulated in females than in males, with female susceptibility to BDNF deficient conditions, such as depression, suggesting a crucial role of sex hormones in BDNF activity. Specifically, the estrogen interaction with TrkB receptors abundantly expressed in brain regions associated with memory enhances estrogen neurotrophic effects, promoting neuronal survival and cognitive processing (Hill & van den Buuse, 2011; Spencer-Segal et al., 2011). This neuroprotective impact of estrogen on BDNF signaling may partly account for the observed cognitive advantages in females, which tend to decline with age or estrogen deficiency, underscoring the critical role of BDNF in female cognitive health.

4.4. Indirect associations between interleukins and memory via BDNF mediation

Firstly, no direct effect of IL-15 and IL-18 on memory was found. These two cytokines are linked to the pathophysiology of MHE and other neuroinflammatory disorders (Mangas-Losada et al., 2017; Montoliu et al., 2009; Broux et al., 2015; Alboni et al., 2010). Despite their relevance in MHE, their direct impact on memory may be modulated or influenced by other factors, such as neuroinflammatory mediators, metabolic changes, or oxidative stress, for this reason we found no direct relationship. Patients with MHE show oxidative damage (Gimenez-Garzó et al., 2015). Moreover, some studies suggest that oxidative stress levels can modulate the effects of peripheral factors on the central nervous system in HE and that oxidative stress varies by sex (Macedo de Oliveira et al., 2022). Therefore, sex should also be considered as a factor in how peripheral blood components influence the central nervous system.

Furthermore, the results of the indirect effects of interleukins on memory through BDNF underscore a sex-specific pathway through which inflammatory cytokines, particularly IL-15 and IL-18, impact memory performance through BDNF in women. The indirect relationship between elevated cytokine levels and reduced BDNF in female patients highlights a potentially heightened vulnerability to inflammation-driven cognitive decline in women, especially those with MHE. This phenomenon is partially attributable to the influence of estrogens, which not only regulate BDNF but also interact with inflammatory pathways that impact neuroplasticity and cognitive function (Sohrabji and Lewis, 2006).

It is worth noting that additional analyses conducted for IL-17 and CCL20 did not yield significant results, further suggesting that the effects observed for IL-18 and IL-15 may be specific to these particular biomarkers in the context of inflammation and memory decline in cirrhosis.

In women, estrogen modulates immune responses and is associated with both protective and detrimental effects in the brain, depending on the inflammatory context. This response might exacerbate the impact of cytokines like IL-15 and IL-18 on BDNF reduction, thus heightening susceptibility to memory impairment (Weinstein et al., 2014). Studies show that estrogen regulation of BDNF is highly sensitive to systemic inflammation; in inflammatory environments, estrogen-mediated BDNF expression can be disrupted, diminishing its neuroprotective benefits and leaving women particularly vulnerable to cognitive deficits linked with reduced neurotrophic support (Sohrabji and Lewis, 2006).

Additionally, inflammatory cytokines, when chronically elevated, can impair estrogen regulatory effects on BDNF, potentially by influencing estrogen receptor signaling pathways. This interference disrupts BDNF role in supporting synaptic plasticity, which is vital for memory processes. As a result, high levels of IL-15 and IL-18 may more profoundly affect memory in women due to this dual impact on inflammation and neurotrophic support (Weinstein et al., 2014). Moreover, as estrogen levels naturally decline with age, especially post-menopause, the protective effects on BDNF diminish, which may explain why older women or those with lower estrogen levels are at increased risk for memory impairments when inflammation is present (Sohrabji and Lewis, 2006). The indirect pathway observed in this study, whereby IL-15 and IL-18 impact memory through BDNF reduction, suggests a feedback loop where inflammation and reduced BDNF exacerbate each other, particularly in women.

Sex-specific differences in the relationship between BDNF and memory suggest that biological factors significantly influence how inflammation impacts cognition. While estrogen supports BDNF regulation and cognitive resilience, its decline in postmenopausal women may contribute to increased neural vulnerability. However, other mechanisms could play a role. Women may be more susceptible to cognitive impairments due to heightened sensitivity to inflammation, motor-cognitive interactions, and genetic or epigenetic factors influencing BDNF expression. This increased susceptibility may stem from biological factors that also make women more prone to the detrimental cognitive and behavioral effects of chronic inflammation (Lasselin et al., 2018). Beyond hormonal effects, sex differences in immune regulation have a genetic basis, as key immune-related genes on the X chromosome provide women with greater immune diversity, potentially shaping neuroinflammatory responses (Klein and Flanagan, 2016; Casimir et al., 2013). These factors highlight the importance of considering sex differences when evaluating the neurological effects of cirrhosis.

In addition to estrogen, testosterone may also play a role in modulating BDNF and cognitive function in men. Testosterone has been linked to neuroprotection and synaptic plasticity, with evidence suggesting that testosterone enhanced antioxidant defense system, BDNF levels and cell proliferation, additional it have seen that treatment only with testosterone significantly weakened oxidative stress (Fanaei et al., 2014). Given that testosterone levels decline with age (Golan et al., 2015), this could differentially impact BDNF expression and memory function in older male participants. Future studies should investigate how sex-specific hormonal differences, beyond estrogen, interact with inflammation and BDNF pathways to influence cognition in cirrhotic patients.

Targeting the IL-18 and IL-15 pathways presents a promising strategy for alleviating neuroinflammation-driven cognitive decline in cirrhotic patients. Experimental research has demonstrated that inhibiting IL-18 can mitigate neuroinflammatory damage and enhance cognitive function in other neurological disorders (Xie et al., 2017) suggesting its potential applicability in MHE. Similarly, IL-15 modulation has been explored for its major role of IL15 signaling in cerebral function (Pan et al., 2013), highlighting the need for further investigation into its therapeutic relevance in cirrhosis-related cognitive impairment. Additionally, strategies to enhance BDNF expression, such as physical exercise, dietary interventions, or pharmacological agents like BDNF mimetics (See Choi et al., 2018; Romero Garavito et al., 2025), could complement cytokine-targeting therapies, potentially improving cognitive resilience in MHE patients. Future research should explore these combined approaches to develop more effective neuroprotective strategies for this vulnerable population that could be especially beneficial for female patients experiencing inflammation-driven cognitive decline.

This study underscores the critical role of BDNF in modulating memory in the presence of inflammation and other factors related to liver cirrhosis, positioning BDNF as a potential therapeutic target for cognitive impairment in MHE. However, the study is not without limitations. The cross-sectional nature of this study limits causal inference, as it only captures associations rather than establishing temporal relationships between inflammation, BDNF, and memory performance. Longitudinal studies are needed to confirm these pathways and assess

the progressive impact of neuroinflammation on cognitive function. Additionally, while plasma BDNF levels have been used as a proxy for central nervous system activity, they may not fully reflect brain-derived BDNF dynamics due to contributions from peripheral sources such as platelets and immune cells. This limitation should be considered when interpreting our findings, and future research should aim to integrate cerebrospinal fluid (CSF) or neuroimaging markers to obtain a more precise measure of BDNF role in neuroplasticity and memory function. Future studies should incorporate longitudinal designs and larger, more diverse samples to validate these sex-specific pathways and assess potential therapeutic strategies to counteract the inflammatory effects on BDNF and memory, particularly in vulnerable populations. Expanding the evaluation of memory subdomains could further elucidate differential cognitive vulnerabilities in this group.

5. Conclusion

In conclusion, this study highlights the critical role of BDNF as a mediator in the relationship between pro-inflammatory cytokines and memory function in cirrhotic patients, with distinct sex-specific effects that underscore women's heightened vulnerability, especially in the presence of MHE. The findings suggest that BDNF levels, influenced by cytokines such as IL-15 and IL-18, may serve as a key mechanism through which inflammation exacerbates cognitive decline, particularly in female patients. Given the observed impact of IL-18 and IL-15 on BDNF levels, interventions aimed at modulating these cytokines or enhancing BDNF signaling could hold therapeutic promise. Future research should prioritize translational approaches, including clinical trials evaluating anti-inflammatory therapies, BDNF-enhancing strategies, and biomarker-driven interventions to mitigate neuroinflammation and preserve cognitive function in MHE patients. Expanding our understanding of these pathways could pave the way for precision medicine approaches tailored to neuroinflammatory-driven cognitive impairment in liver disease. Additionally, evaluating the effectiveness of BDNF in comparison to other biomarkers for diagnosing MHE and assessing cognitive function in liver disease could establish its relative utility. Further validation through well-designed clinical trials is essential to determine whether targeting BDNF and related inflammatory pathways can effectively translate into therapeutic benefits for patients with cirrhosis-related cognitive impairment. Incorporating BDNF as a diagnostic tool into current protocols could improve early detection and management of cognitive impairment in liver disease, ultimately benefiting patient outcomes.

CRediT authorship contribution statement

Daniela Batallas: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Juan José Gallego:** Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Franc Casanova-Ferrer:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis. **Adriá López-Gramaje:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis. **Pablo Rivas-Diaz:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Javier Megías:** Writing – review & editing, Resources, Investigation. **Desamparados Escudero-García:** Writing – review & editing, Supervision, Resources, Investigation. **Lucía Durbán:** Writing – review & editing, Resources, Investigation. **Salvador Benlloch:** Writing – review & editing, Supervision, Resources, Investigation. **Amparo Urios:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Vanesa Hidalgo:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Alicia Salvador:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Carmina Montoliu:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Ethics approval and consent to participate

Study protocols were approved by the Scientific and Research Ethics Committees of Hospital Clinico Universitario and Arnau de Vilanova Hospital of Valencia, Valencia, Spain [approval code: 2018/210 (March 2, 2018) and 2023/130 (March 1, 2024)] and were in accordance with the ethical guidelines of the Helsinki Declaration. Written informed consent was obtained from all individual participants included in the study.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2025.100998.

Data availability

Data will be made available on request.

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