Neuropsychiatric Disease and Treatment

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Potential Inflammatory Biomarkers and Differential Gut Microbiota in Cognitive Impairment After Ischemic Stroke

Lishuo Wu[1,2,*], Yajie Jiang[3,*], Wei Xu[2], Liwen Wang[2], Liping Li[2], Chunli Zhang[1], Keyu Huang[1], Yi Yang[2], Jian Dai[2], Fengkun Zhou[1]

[1]Department of Neurology, The First People's Hospital of Nanning, Nanning, Guangxi, People's Republic of China; [2]Department of Psychological and Sleep Rehabilitation,Jiangbin Hospital of Guangxi Zhuang Autonomous Region, Nanning, Guangxi, People's Republic of China; [3]Engineering Research Center of Innovative Traditional Chinese, Zhuang and Yao Materia Medica, Ministry of Education, Guangxi University of Chinese Medicine, Nanning, Guangxi, People's Republic of China

*These authors contributed equally to this work

Correspondence: Fengkun Zhou, Department of Neurology, The First People's Hospital of Nanning, Nanning, Guangxi, People's Republic of China, Email zfk1224@163.com; Jian Dai, Department of Psychological and Sleep Rehabilitation,Jiangbin Hospital of Guangxi Zhuang Autonomous Region, Nanning, Guangxi, People's Republic of China, Email fjptdj@163.com

Objective: Ischemic stroke, one of the main public health problems worldwide, causes a variety of physiological dysfunction, including cognitive impairment. Although studies have been focused on posted-stroke cognitive impairment (PSCI), its pathological mechanism remains unclear.

Methods: Here, we enrolled 66 participants stratified into three groups: healthy controls (HC, n=15), post-stroke patients without cognitive impairment (PSWCI, n=15), and PSCI patients (n=36). We analyzed clinical parameters and changes of several cytokines and gut microbiota profiles.

Results: We found that compared with healthy control (HC) group, levels of low-density lipoprotein (LDL) cholesterol, total cholesterol (TC), homocysteine (Hcy), CRP and IL-6 were significantly increased in PSWCI and PSCI patients. Of them, levels of Hcy and IL-6 in PSCI group were significantly higher than that in PSWCI. ROC curve analysis identified Hcy and IL-6 as potential diagnostic biomarkers for PSCI. Furthermore, 16S rRNA sequencing of gut microbiota shows that the abundance of *blaut, bifidobacterium* and *macromonas* increased, while the abundance of *bacteroides* and *bifidobacterium brevis* decreased significantly.

Conclusion: These findings suggest that elevated Hcy and IL-6 levels may serve as risk factors for PSCI, with gut microbiota dysregulation potentially contributing to its pathogenesis.

Keywords: post-stroke cognitive impairment, IL-6, homocysteine, diagnostic efficacy, gut microbiota

Introduction

With the growing incidence of stroke, the number of patients suffering from posted-stroke cognitive impairment (PSCI) is continuously increasing. Studies have shown that the probability of dementia in stroke patients was increased by 4–12 times.[1] In Europe, 24%–39% of the stroke patients exhibit cognitive impairment within three months,[2] while in Asia, this proportion is as high as 69.8%.[3] These individuals experience cognitive deficits in memory, attention, executive function, comprehension, and judgment, significantly impacting their daily lives. Although drugs for PSCI are available, they have side effects such as prolonged medication periods, poor compliance, and adverse reactions. Therefore, it is an urgent scientific and clinical problem to explore the pathological mechanism of PSCI and develop newly effective prevention.

Previous studies have confirmed that inflammatory factors,[4] immune cells, metabolites[5,6] and gut microbiota[7,8] play vital roles in PSCI. Among these, alterations in gut microbiota have garnered significant attention due to their regulatory effects on the nervous, metabolic and immune system through the intestinal-brain axis.[9,10] Gut microbiota consists of

Received: 13 December 2024
Accepted: 18 March 2025
Published: 28 April 2025

thousands of bacteria, fungi and viruses and the bacterial genes are even about 150 times than that of human.[11] These microorganisms coexist with human and involve in processes of energy absorption, material metabolism and immune response, called the "second genome" of human.[12] In recent years, gut microbiota and its metabolites in both physiological and pathological processes have been widely concerned.[13] Studies have shown that dysregulation in abundance of *Chlamydia pneumoniae, Helicobacter pylori* and *Porphyromonas gingivalis* increase the level of C-reactive protein (CRP)[14] and potentially lead to atherosclerosis[15] and ischemic stroke. Additionally, stress-induced bacterial translocation and inflammatory responses have been reported to affect the prognosis of ischemic stroke.[16] Notably, *Clostridium butylicum* has been found to protect against brain injury and cognitive dysfunction in cerebral ischemia/reperfusion of diabetic mice through enhancing the diversity of gut microbiota.[17] Nevertheless, the roles of gut microbiota in ischemic PSCI remain to be elucidated.

Here, we recruited 66 participants, including 15 healthy controls (HC), 15 posted-stroke without cognitive impairment (PSWCI) and 36 posted-stroke cognitive impairment (PSCI). We collected and analyzed clinical data, serum factors, and gut microbiota profiles from these participants. Our study provides evidence of these factors in diagnostic efficiency of PSCI and also offers insight into the alterations into gut microbiota associated with ischemic PSCI, laying the theoretical basis foundation for the diagnosis and treatment of ischemic PSCI.

Materials and Methods

Participants Recruitment

We recruited 66 participants who underwent physical examination or were hospitalized in the Department of Neurology of the First People's Hospital of Nanning (Guangxi, China) from July 2020 to December 2023. The cohort comprised 15 cases of healthy controls (HC), 15 participants of PSWCI and 36 participants of PSCI. This study was approved by the Ethics Committee of Nanning First People's Hospital and informed consent was obtained from all these participants in accordance with the guidelines outlined in the Declaration of Helsinki. The diagnosis of PSCI was based on the criteria for the diagnosis of various cerebrovascular diseases formulated at the Fourth National Conference on Cerebrovascular Diseases of the Chinese Medical Association. Multiple cerebral infarction was confirmed by craniocranial computed tomography (CT) or magnetic resonance imaging (MRI), consistent with the diagnostic criteria for PSCI in China. The evaluation of Mini-Mental State Examination (MMSE, permission for use was obtained from Par Inc) and Montreal Cognitive Assessment (MoCA) was conducted with at least two psychiatrists within 1 to 3 months. *Inclusion and exclusion criteria: Inclusion criteria*: (1) Diagnosis of ischemic stroke confirmed by cranial CT or MRI, in line with the standard of diagnostic essentials of various cerebrovascular diseases formulated at the fourth National Academic Conference on Cerebrovascular Diseases of Chinese Medical Association and Chinese ischemic stroke subclassification (CISS) classification. (2) The total score of modified Hachinski ischemic scale (HIS) is ≥7; (3) Age 30–80; (4) No aphasia symptom; *Exclusion criteria*: (1) Family history of mental illness. (2) Severe neurological deficits, such as hemiplegia, aphasia, audio-visual impairment or combined with conscious disorder; (3) Other brain organic diseases induced cognitive impairment, including brain trauma, epilepsy, Parkinson's disease, etc. (4) Other physical diseases induced cognitive impairment, including malignant tumor, severe organ dysfunction, etc., (5) Usage of antibiotics, probiotics or prebiotic preparations within one month; (6) Chronic diarrhea and constipation.

Methods

Clinical Data Collection

The general clinical information of all participants such as age, sex, height, weight, blood pressure, low density lipoprotein (LDL), total cholesterol (TC), triglyceride (TG), homocysteine (Hcy) and uric acid (UA) was collected. These data were analyzed to investigate potential indicators associated with PSCI.

Preparation of Fecal Samples

Fresh fecal samples (3–5g) were collected into a sterile frozen tube (QIAGEN) in the morning using a clean sterile spoon and then screw it tightly to create an anaerobic environment; the samples are transported to the laboratory using dry ice

and stored in a −80°C refrigerator until further processing. Fecal collection procedure was completed on the same day of physical examination. Following sample collection, 16S rRNA sequencing was then performed.

Detection of Serum Factors

The quantification of serum IL-6 and CRP using a 5mL of blood sample was performed at the laboratory of Nanning First People's Hospital. After centrifugation (3000 rpm, 10 min, 4°C), the serum was harvested, and IL-6 concentration was determined by enzyme-linked immunosorbent assay (ELISA) assay. IL-6 detection kit is from Roche Technology Co., Ltd. CRP levels were determined by chemiluminescence method (Roche Technology Co., Ltd). Steps of these experiment were performed according to the manufacturer's instructions.

Determination of LDL, TC, TG, Hcy and UA Serum factors, including LDL, TC, TG, Hcy and UA, was quantitatively analyzed using a fully automated biochemical analyzer (cobas 800, Roche, USA) at the clinical laboratory of Nanning First People's Hospital. All measurements were performed in strict accordance with the manufacturer's protocols provided in the reagent kit instructions.

Statistics

Statistical analyses were performed using the GraphPad Prism 9.0 software (San Diego, CA, USA). The normal distribution data were expressed as (x ±s). Based on the characteristics of the data, analysis of variance or Kruskal–Wallis test was used among groups, and T test or Mann–Whitney U-test was used for comparison between two groups. $P < 0.05$ was considered statistically significant.

Results

Participant Characteristics and Cognitive Ability Assessment

We recruited a total of 66 participants, including 15 healthy controls (HC), 15 ischemic PSWCI and 36 ischemic PSCI patients. Demographic and clinical characteristics, including gender, age, weight and blood pressure, were systematically collected for all participants. As presented in Table 1, the three groups demonstrated comparable baseline characteristics, with no statistically significant differences observed in sex distribution, age, or body weight. Cognitive function was evaluated using two standardized neuropsychological assessments: MMSE and MoCA. The assessment results revealed significant cognitive impairment in PSCI patients compared to HC, while no significant change when compared to PSWCI (see Figure 1A and B).

Detection of Several Serum Factors

We further analyzed the level changes of serum factors, including LDL, TC, TG, Hcy, CRP and IL-6 in three groups. As shown in Figure 2, compared with HC group, levels of LDL, TC, Hcy, CRP and IL-6 in stroke patients are significantly increased, except UA and TG. Notably, the contents of Hcy and IL-6 in patients with PSCI are much higher than that of the PSWCI, suggesting a potential pathophysiological role of these two serum factors in the development of cognitive dysfunction following stroke.

Table 1 Participant Characteristics

	HC (n=15)	PSWCI (n=15)	PSCI (n=36)	Significant Difference
Age	60.5±7.3	61.2±9.9	62.1±8.1	N.S.
Weight	60.7±5.6	60.1±6.9	60.3±8.0	N.S.
Sex	M(7) F(8)	M(6) F(9)	M(19) F(17)	—
MMSE	29.8±1.8	28.3±3.9	21.8±3.5 *	*p<0.01
HAMA	1.2±0.2	1.1±0.2	5.4±0.8 *	*p<0.01

Note: *indicates p value <0.01.
Abbreviations: M indicates male participant; F indicates female participant. N.S. indicates no significance.

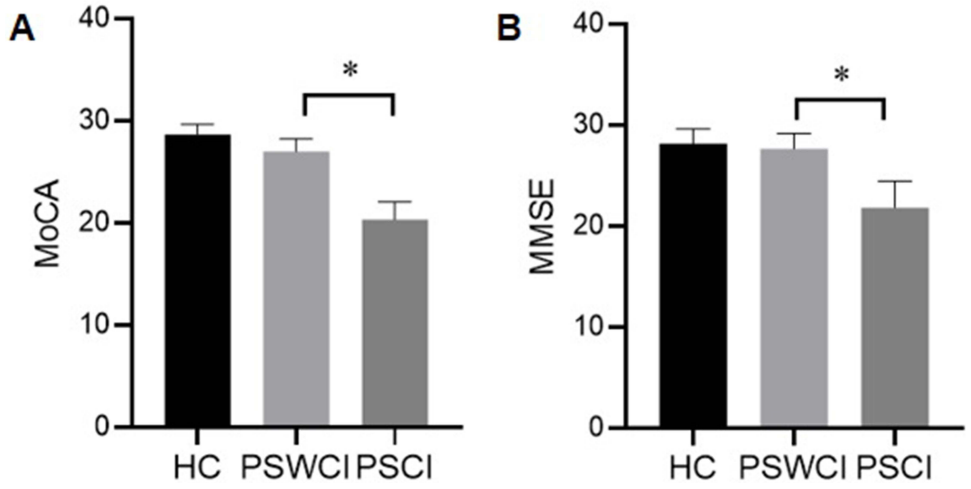

Figure 1 Evaluation of MoCA (**A**) and MMSE (**B**) of all participants. (* indicates p <0.05).

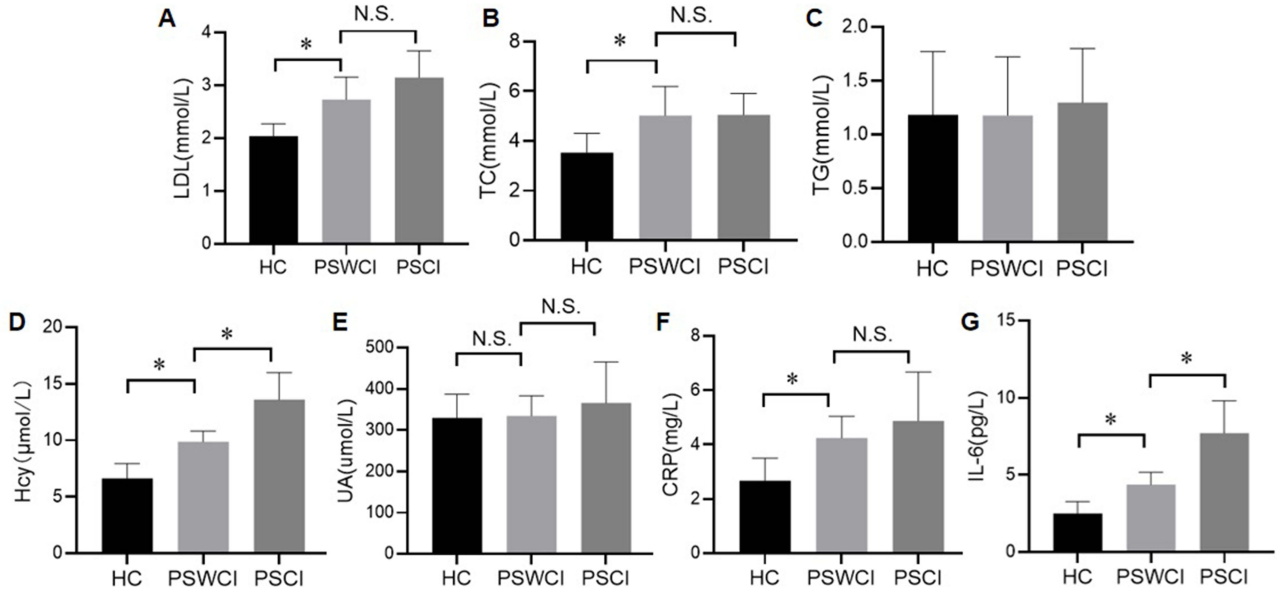

Figure 2 Levels of serum factors of all participants were detected. LDL (**A**), TC (**B**), TG (**C**), Hcy (**D**) and UA (**E**) was evaluated with the biochemical analyzer. CRP was detected by chemiluminescence method (**F**) and IL-6 was determined by ELISA assay (**G**).(* indicates p <0.05, NS means no significance).

Potential Biomarkers in Cognitive Impairment of the PSCI

To investigate whether serum Hcy and IL-6 levels could be served as the biological biomarkers of cognitive impairment in PSCI, we evaluated the diagnostic efficiency of these factors by receiver operating characteristic (ROC) curve. As depicted in Figure 3A and B below, the area under the curve (AUC) of Hcy is 0.947 (P value < 0.0001, 95% CI 0.8826 to 1), with a cutoff value 10.9 umol/L demonstrating a sensitivity of 87.88% and specificity of 100%, respectively. Similarly, the AUC of IL-6 is 0.9604 (P value < 0.0001, 95% CI 0.9100 to 1), with a cutoff value 6.145 pg/L showing a sensitivity of 81.25% and specificity of 100%, respectively. These results indicate that both Hcy and IL-6 may serve as promising biomarkers for the diagnosis of PSCI.

Abundance Changes of Gut Microbiota Between PSWCI and PSCI Groups

Given the pivotal roles of gut microbiota in the brain physiological processes, we conducted DNA extraction from fecal samples to investigate alterations in gut microbiota abundance through 16S rRNA sequencing. We extracted DNA from

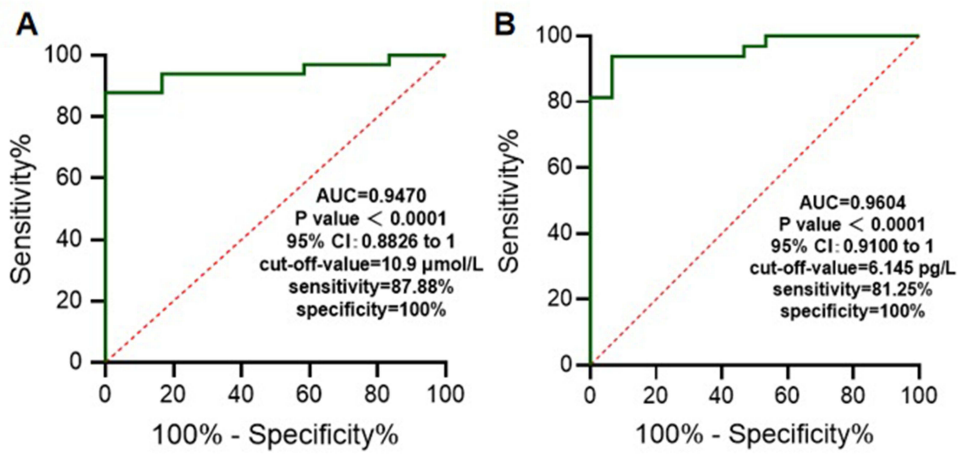

Figure 3 Diagnostic efficiency evaluation of Hcy (**A**) and IL-6 (**B**) using ROC curve.

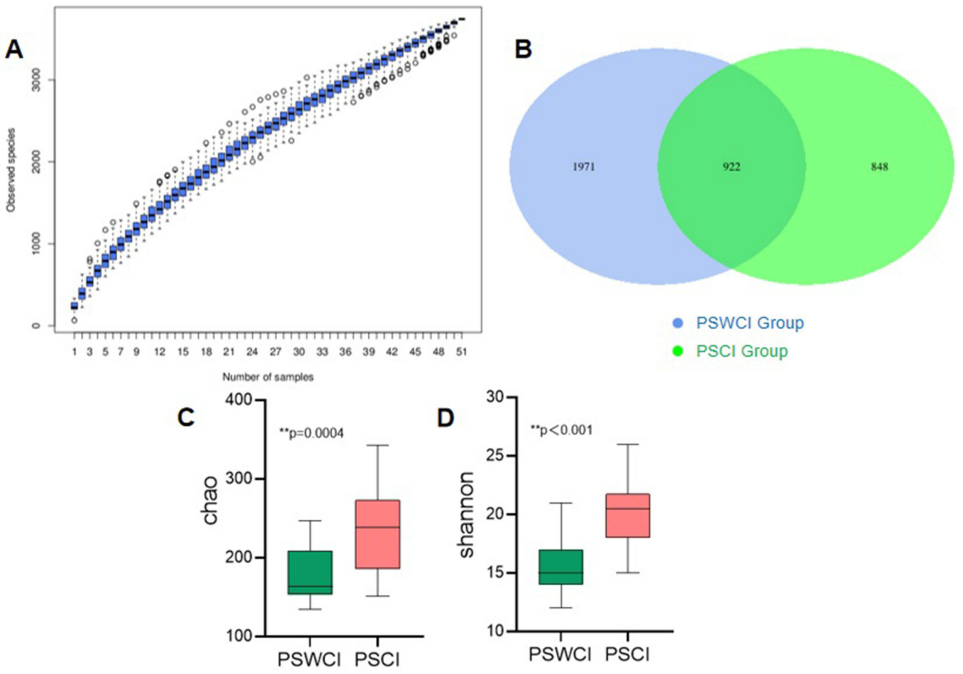

Figure 4 Fecal microbiome analysis with 16S rRNA sequencing. Relationship between the number of bacterium and the number of samples (**A**). Venn diagram (**B**). Fecal microbiome diversity between PSWCI and PSCI groups was elucidated using the Chao 1 index (**C**) and Shannon index (**D**).

fecal samples to investigate abundance alterations in gut microbiota abundance by 16S rRNA sequencing. As illustrated in Figure 4A, the species accumulation curve demonstrates that our study has achieved sufficient sampling depth for reliable analysis. Wayne diagram shows 922 shared bacteria genera between PSWCI and PSCI groups (see Figure 4B). Chao1 and Shannon index analyses indicate statistically significant differences in microbial abundance and diversity between these two groups (see Figure 4C and D). As depicted in Figure 5, we observed a marked increase in the relative abundance of *Blaut, Bifidobacterium* and *Macromonas*, while *Bacteroides* and *Bifidobacterium brevis* showed significant reduction in their abundance profiles.

Discussion

Ischemic stroke, characterized by its high incidence, high mortality and high disability rate, is one of the major public health problems in the world. In China alone, approximately 2.4 million new cases of stroke are reported annually, and

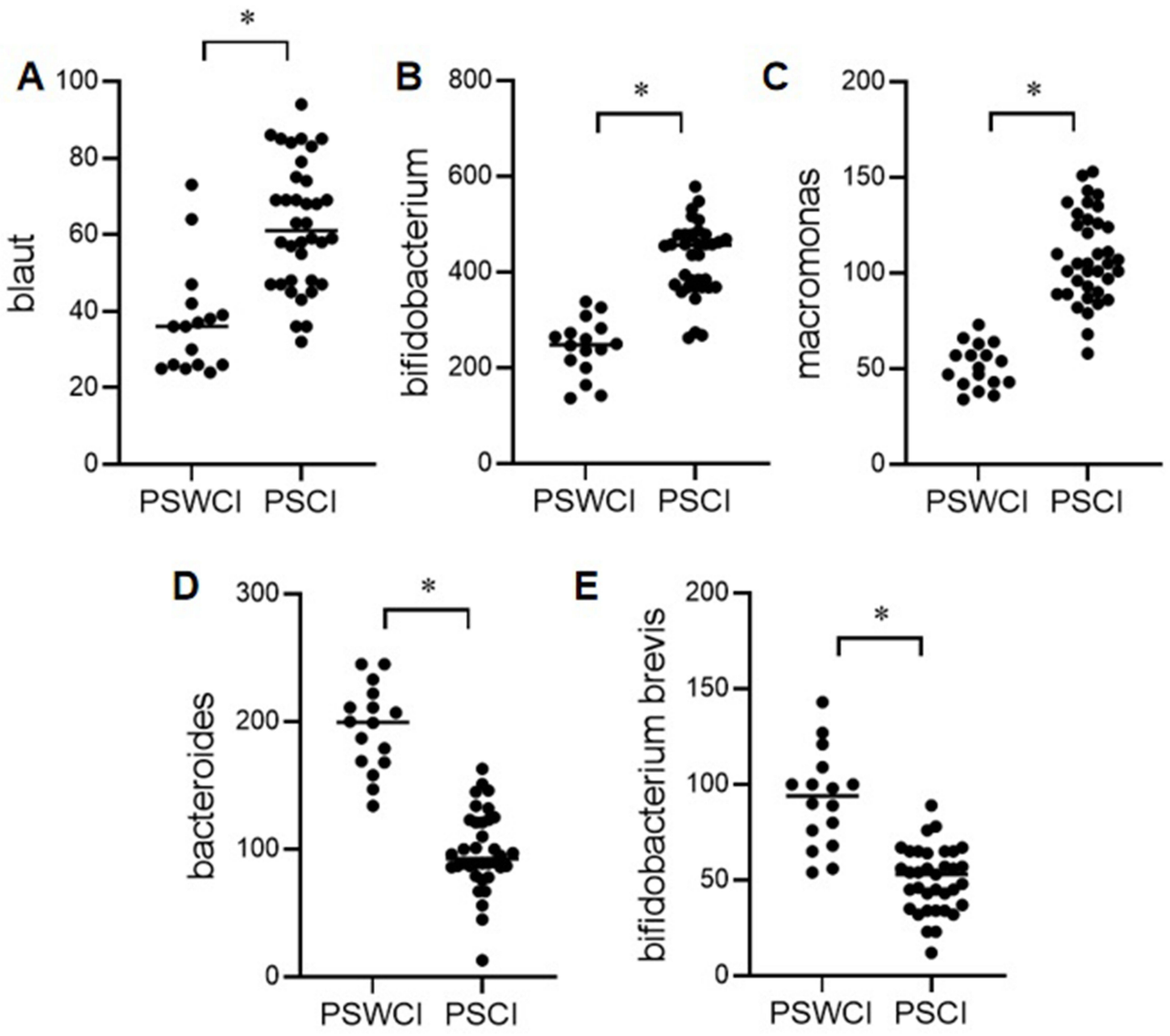

Figure 5 Abundance alterations of gut microbial composition. (* indicates p <0.05). Abundance changes of *Blaut* (**A**), *Bifidobacterium* (**B**), *Macromonas* (**C**), *Bacteroides* (**D**) and *Bifidobacterium brevis* (**E**) between PSWCI and PSCI groups.

around 70% of these patients experience different degrees of cognitive impairment in the early stages. Consequently, elucidating the pathological mechanisms and identifying biomarkers for PSCI hold substantial scientific and clinical importance, underscoring the urgent need for the development of early intervention strategies.

Currently, there is no standardized treatment for PSCI. The primary pharmacological interventions for cognitive impairment typically include cholinesterase inhibitor, memantine and nimodipine. Studies have shown that the levels of IL-6, CRP and IFN-γ in peripheral blood are related to cognitive dysfunction.[18,19] In our study, we investigated the alterations of several serum factors and found that compared with the HC group, levels of LDL, TC, Hcy, CRP and IL-6 are significantly higher in PSWCI and PSCI patients, consistent with the findings of Benn et al.[20] Notably, levels of Hcy and IL-CRP in PSCI group were even much higher than that of PSWCI. Further analysis using the ROC curve showed that Hcy and IL-6 possess strong diagnostic potential for PSCI. These results indicate that Hcy and IL-6 might serve as valuable biomarkers of cognitive impairment in PSCI patients.

The dysregulation of gut microbiota, characterized by alterations in both abundance and diversity, has been increasingly implicated in various diseases.[21] An increasing evidences suggest that gut microbiota rebalancing holds great medical value for the prevention and treatment of cognitive impairment.[22] For example, treatment with the probiotic

Clostridium butyricum alleviates the cerebral ischemia-reperfusion injury and exert neuroprotective effects.[23] Many scholars also reported that probiotics may be a promising therapeutic strategy for neurological dysfunction.[24–26] It was found that the abundance of *Ruminococcus* and *Blaut* in patients with acute ischemic stroke increased significantly, while the abundance of *Bacteroides, Purpura* and *Fusobacterium* decreased significantly.[27] Nan Xu et al found that the characteristics of gut microbiota in patients with acute ischemic stroke are mainly elevated levels of *Bifidobacterium, Macromonas, Brucella, Haldeman* and *Clostridiu*m.[28] Another study demonstrated an increase in the abundance of short-chain fatty acid-producing bacterium *Odoribacter* and *Aikman* in patients with ischemic stroke.[29] Moreover, *Lactobacillus helveticus* supplementation has been shown to reverse anxiety and depressive behaviors while alleviating cognitive impairment.[30] In line with these findings, our study also revealed significant differences in the abundance and diversity of gut microbiota between PSWCI and PSCI participants. Specifically, we observed an increase in the abundance of *Blaut, Bifidobacterium* and *Macromonas*, alongside a significant decrease in the abundance of *Bacteroides* and *Bifidobacterium brevis*. These results suggest that these specific gut microbiota may play a role in the pathogenesis of cognitive impairment, further underscoring the potential of microbiota-targeted therapies in cognitive health.

Studies on biomarkers were believed to be a promising avenue for advancing the treatment of PSCI, although their clinical application remains limited. To address this gap, our study focused on analyzing alterations in serum factors, lipid metabolites and gut microbiota. Our results offer novel insights into the diagnostic potential of certain factors for PSCI and highlight significant changes in gut microbiota among patients with ischemic PSCI. However, several limitations should be acknowledged. First, as participants in our study are limited, a large number of high-quality clinical trials are needed to confirm the results. Second, our research exclusively included patients with ischemic stroke, underscoring the need for future studies to encompass other stroke subtypes. Lastly, the study did not account for the potential influences of lymphocytes,[31] platelets,[32] diabetes mellitus on PSCI, which warrant further investigation.

Acknowledgments

This work was supported by the fundings from Health Committee of Guangxi Zhuang Autonomous Region (Z20201417), Nanning Qingxiu District Science and Technology Plan Project (2019041) and Guangxi Medical and Health Key Cultivation Discipline Construction Project.

An unauthorized version of the Chinese MMSE was used by the study team without permission, however this has now been rectified with PAR. The MMSE is a copyrighted instrument and may not be used or reproduced in whole or in part, in any form or language, or by any means without written permission of PAR (www.parinc.com). We also thank Par. Inc for providing us with MMSE Test Forms.

Disclosure

The authors declare that they have no competing interests in this work.

References

1. Rost NS, Brodtmann A, Pase MP, et al. Post-stroke cognitive impairment and dementia. *Circulation Research*. 2022;130:1252–1271. doi:10.1161/CIRCRESAHA.122.319951
2. Sun J-H, Tan L, Yu J-T. Post-stroke cognitive impairment: epidemiology, mechanisms and managem ent, Annals of translational medicine. 2:80.
3. Yu K-H, Cho S-J, Oh MS, et al. Korean-vascular cognitive impairment harmonization standards study gro, cognitive impairment evaluated with vascular cognitive impairment harm onization standards in a multicenter prospective stroke cohort in kore a. *Stroke*. 2013;44:786–788. doi:10.1161/STROKEAHA.112.668343
4. Kulesh A, Drobakha V, Kuklina E, Nekrasova I, Shestakov V. cytokine response, tract-specific fractional anisotropy, and brain mor phometry in post-stroke cognitive impairment. *J Stroke Cerebrovasc Dis*. 2018;27:1752–1759. doi:10.1016/j.jstrokecerebrovasdis.2018.02.004
5. Xiao X, Yeoh BS, Vijay-Kumar M. Lipocalin 2: an emerging player in iron homeostasis and inflammation. *Annu Rev Nutr*. 2017;37:103–130. doi:10.1146/annurev-nutr-071816-064559
6. Kim J-H, Ko P-W, Lee H-W, et al. Astrocyte-derived lipocalin-2 mediates hippocampal damage and cognitiv e deficits in experimental models of vascular dementia. *Glia*. 2017;65:1471–1490. doi:10.1002/glia.23174
7. Benakis C, Brea D, Caballero S, et al. Commensal microbiota affects ischemic stroke outcome by regulating int estinal γδ T cells. *Nature Med*. 2016;22:516–523. doi:10.1038/nm.4068
8. Xu K, Gao X, Xia G, et al. Rapid gut dysbiosis induced by stroke exacerbates brain infarction in turn. *Gut*. 2021;70:1486–1494. doi:10.1136/gutjnl-2020-323263

9. Teng Z, Dong Y, Zhang D, An J, Lv P. Cerebral small vessel disease and post-stroke cognitive impairment. *Int J Neurosci*. 2017;127:824–830. doi:10.1080/00207454.2016.1261291
10. Angoorani P, Ejtahed H-S, Siadat SD, Sharifi F, Larijani B. Is there any link between cognitive impairment and gut microbiota? *A S Ystematic Review Gerontology*. 68:1201–1213.
11. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces sha microbial diversity in the human intestine. Cell, 124. 837–848.
12. Gasmi A, Mujawdiya PK, Pivina L, et al. Relationship between gut microbiota, gut hyperpermeability and obesity. *Curr Med Chem*. 2021;28:827–839. doi:10.2174/0929867327666200721160313
13. Silva YP, Bernardi A, Frozza RL. The role of short-chain fatty acids from gut microbiota in gut-brain C ommunication. *Front Endocrinol*. 2020;11:25. doi:10.3389/fendo.2020.00025
14. Nadareishvili ZG, Koziol DE, Szekely B, et al. Increased CD8 + T cells associated with chlamydia pneumoniae in symptomatic carotid plaque. *Stroke*. 2001;32:1966–1972. doi:10.1161/hs0901.095633
15. Hayashi C, Viereck J, Hua N, et al. Porphyromonas gingivalis accelerates inflammatory atherosclerosis in t he innominate artery of ApoE deficient mice. *Atherosclerosis*. 2011;215:52–59. doi:10.1016/j.atherosclerosis.2010.12.009
16. Ponferrada A, Caso JR, Alou L, et al. The role of PPARgamma on restoration of colonic homeostasis after expe rimental stress-induced inflammation and dysfunction. *Gastroenterology*. 2007;132:1791–1803. doi:10.1053/j.gastro.2007.02.032
17. Caso JR, Hurtado O, Pereira MP, et al. Colonic bacterial translocation as a possible factor in stress-worseni ng experimental stroke outcome. *Am J Physiol Regul Integr Comp Physiol*. 2009;296:R979–985. doi:10.1152/ajpregu.90825.2008
18. Rothenburg LS, Herrmann N, Swardfager W, et al. The relationship between inflammatory markers and post stroke cognitiv e impairment. *J Geriatr Psychiatry Neurol*. 2010;23:199–205. doi:10.1177/0891988710373598
19. Alexandrova ML, Danovska MP. Cognitive impairment one year after ischemic stroke: predictors and dynamics of significant determinants. *Turk J Med Sci*. 2016;46:1366–1373. doi:10.3906/sag-1403-29
20. Benn M, Nordestgaard BG, Frikke-Schmidt R, Tybjærg-Hansen A. Low LDL cholesterol, *PCSK9* and *HMGCR* genetic variation, and risk of Alzheimer's disease and Parkinson's disease. *MENDELIAN Ran Domisation Study BMJ*. 357:j1648.
21. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol*. 2021;19:55–71. doi:10.1038/s41579-020-0433-9
22. Ettinger S. Diet, gut microbiome, and cognitive decline. *Curr Nutr Rep*. 2022;11:643–652. doi:10.1007/s13668-022-00435-y
23. Sun J, Wang F, Ling Z, et al. Clostridium butyricum attenuates cerebral ischemia/reperfusion injury in diabetic mice via modulation of gut microbiota. *Brain Res*. 180–188.
24. Koszewiczz M, Jaroch J, Brzecka A, Ejma M, Aliev G. Dysbiosis is one of the risk factor for stroke and cognitive impairment and potential target for treatment. *Pharmacol Res*. 2020;164:105277. doi:10.1016/j.phrs.2020.105277
25. Li K, Wei S, Hu L. Protection of fecal microbiota transplantation in a mouse model of mul tiple sclerosis. *Mediators Inflammation*. 2020;2020:2058272. doi:10.1155/2020/2058272
26. Akhoundzadeh K, Vakili A, Shadnoush M, Sadeghzadeh J. Effects of the oral ingestion of probiotics on brain damage in a trans ient model of focal cerebral ischemia in mice. *Iran J Med Sci*. 2018;43:32–40. doi:10.4161/gmic.29232
27. Soldán M, Argalášová Ľ, Hadvinová L, Galileo B, Babjaková J. The effect of dietary types on gut microbiota composition and development of non-communicable diseases: a narrative review. *Nutrients*. 2024;16:3134. doi:10.3390/nu16183134
28. Xu N, Kan P, Yao X, et al. Astragaloside IV reversed the autophagy and oxidative stress induced b y the intestinal microbiota of AIS in mice. *J Microbiol*. 2018;56:838–846. doi:10.1007/s12275-018-8327-5
29. Li N, Wang X, Sun C, et al. Change of intestinal microbiota in cerebral ischemic stroke patients. *BMC Microbiol*.
30. Liang S, Wang T, Hu X, et al. Administration of lactobacillus helveticus NS8 improves behavioral, co gnitive, and biochemical aberrations caused by chronic restraint stres s. *Neuroscience*. 2015;310:561–577. doi:10.1016/j.neuroscience.2015.09.033
31. Ren H, Liu X, Wang L, Gao Y. Lymphocyte-to-monocyte ratio: a novel predictor of the prognosis of ac ute ischemic stroke. *J Stroke Cerebrovasc Dis*. 2017;26:2595–2602. doi:10.1016/j.jstrokecerebrovasdis.2017.06.019
32. Xu M, Chen L, Hu Y, et al. The HALP (hemoglobin, albumin, lymphocyte, and platelet) score is asso ciated with early-onset post-stroke cognitive impairment. *Neurol Sci*. 2023;44:237–245. doi:10.1007/s10072-022-06414-z