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ORIGINAL ARTICLE

Gut Microbiota Characteristics in Severe Acute Pancreatitis and their Association with Hypertriglyceridemia and miR-29b

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ABSTRACT

Background: **This study aimed to characterize the gut microbiota structure in patients with severe acute pancreastitis (SAP) and analyze correlations with serum miR-29b, blood lipids, and inflammatory factors.**
Methods: **A total of 77 adult SAP patients admitted to the Department of Critical Care Medicine, Chongqing University Three Gorges Hospital between March 2024 and January 2025 were enrolled. Based on triglyceride levels, patients were categorized into hypertriglyceridemic SAP (HTG-SAP, *n* = 33) and non-hypertriglyceridemic SAP (non-HTG-SAP, *n* = 44) groups. Gut microbiota composition and diversity were assessed using 16S rRNA gene sequencing. Quantitative real-time polymerase chain reaction (RT-qPCR) was used to determine serum miR-29b-3p expression. Correlations between gut microbiota characteristics and serum miR-29b, blood lipids, and inflammatory factors were analyzed.**
Results: **Gut microbiota α-diversity analysis showed that the Chao1 and phylogenetic diversity (PD) indices in the HTG-SAP group were significantly lower than those in the non-HTG-SAP group ($p < 0.05$). At the genus level, the HTG-SAP group exhibited increased relative abundances of *Anaerococcus* and *Finegoldia* but a decreased abundance of *Enterococcus*. Serum miR-29b-3p expression was significantly elevated in the HTG-SAP group ($p = 0.002$). Correlation analysis indicated that the gut microbiota diversity indexes were significantly negatively correlated with serum miR-29b-3p expression in SAP patients (Chao1 index: $r = -0.237$, $p = 0.038$; PD index: $r = -0.266$, $p = 0.019$). The relative abundances of *Anaerococcus*, *Finegoldia*, and *Peptoniphilus* showed positive correlations with miR-29b-3p expression ($r = 0.369$, $p = 0.001$; $r = 0.303$, $p = 0.007$; $r = 0.307$, $p = 0.007$, respectively), while *Enterococcus* abundance was negatively correlated ($r = -0.343$, $p = 0.002$), with serum miR-29b-3p expression. *Anaerococcus* abundance correlated positively with plasma cholesterol (Chol) levels ($r = 0.281$, $p = 0.013$). Furthermore, serum miR-29b-3p expression was positively correlated with both plasma Chol and triglyceride (TG) levels ($r = 0.347$, $p = 0.002$; $r = 0.276$, $p = 0.015$).**
Conclusions: **The gut microbiota characteristics differ among SAP patients with different etiologies. Gut microbiota dysbiosis in HTG-SAP patients is associated with high expression of miR-29b and hyperlipidemia, suggesting that these factors may be linked to the pathogenesis of this SAP subtype.**
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KEYWORDS

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INTRODUCTION

Acute pancreatitis (AP) is a common digestive system disease characterized by local pancreatic injury and systemic inflammatory response, with a global incidence of approximately 34 per 100,000 annually [1,2]. While the condition of most AP patients is self-limiting, about 20% may progress to severe acute pancreatitis (SAP), accompanied by persistent organ failure and pancreatic necrosis, with a mortality rate as high as 20% - 40% [3]. Gallstones, alcohol, hyperlipidemia, endoscopic retrograde cholangiopancreatography, and pancreatic injury induce inappropriate activation and release of trypsinogen, which underlies AP incidence [4].

An estimated 25 - 50% of the global population presents with serum triglyceride (TG) levels ≥ 150 mg/dL, which is associated with an increased risk of pancreatitis [5]. Hypertriglyceridemia (HTG) is the third most common cause of AP globally and the second leading cause in some regions of China [6]. Hypertriglyceridemic acute pancreatitis (HTG-AP) has an acute onset, rapid progression, a higher likelihood of developing into SAP, and a higher incidence of complications [7]. Its pathogenesis is complex, involving dyslipidemia, systemic inflammatory response, and intestinal barrier dysfunction, among other processes. However, the primary mechanism of HTG-AP is thought to involve pancreatic lipases, which decompose triglycerides into free fatty acids, causing lipotoxicity and inflammatory responses that underlie disease severity [8].

The role of the "gut-pancreas axis" in disease progression is increasingly recognized. As the core component of the gut microecology, the gut microbiota plays a crucial role in maintaining intestinal immune homeostasis and barrier function by regulating the renewal of intestinal mucosal epithelial cells, the release of intestinal antimicrobial peptides, and intestinal permeability [9, 10]. The gut microbiota is also involved in the pathogenesis of AP. Abnormal increases in intestinal pathogens can breach an already impaired intestinal barrier, exacerbating systemic or local inflammation and triggering secondary infections, thereby causing a "second hit" to AP patients [11]. Studies have shown that AP patients have significant gut microbiota dysbiosis, manifested as decreased diversity and altered microbial structure, and these changes are correlated with disease severity [12, 13]. The composition of the gut microbiota in AP patients may also vary depending on the etiology, with specific microbial changes associated with gallstones, alcohol, and hyperlipidemia [14]. Notably, HTG-AP patients exhibit unique microbial characteristics, such as reduced diversity, enrichment of conditional pathogens, and reduction of beneficial bacteria, which may be one reason for their poorer clinical prognosis and greater propensity to progress to SAP [15]. Some studies have shown that HTG-regulated gut microbiota may promote glycerophospholipid metabolism and increase lysophosphatidylcholine content through a Toll-like receptor 4-dependent mechanism, thereby aggravating pancreatic injury in AP [16]. However, the role and specific mechanism of gut microbiota in the process of HTG aggravation of AP are still unclear.

MicroRNAs (miRNAs), a class of highly conserved non-coding RNAs, post-transcriptionally regulate pathophysiological processes such as cell proliferation, apoptosis, inflammatory response, and metabolism [17, 18]. The miR-29 family is known to play an important role in the regulation of lipid metabolism, affecting lipid synthesis and metabolism through different pathways [19,20]. Nevertheless, its interaction with the gut microbiota in HTG-SAP remains unclear.

To further characterize the gut-pancreas axis in HTG-SAP, including the potential roles of microbiota and the miR-29 family member miR-29b, we systematically analyzed the gut microbiota structural characteristics of patients with HTG-SAP and non-HTG-SAP. We further explored the correlations between gut microbiota and serum miR-29b expression levels, blood lipids, and inflammatory factors. These findings reveal the potential pathogenic mechanisms of HTG-SAP from the cross-perspective of microecology and molecular regulation, thus providing a new theoretical basis for its early intervention.

MATERIALS AND METHODS

Study subjects

SAP patients with different etiologies admitted to the Department of Critical Care Medicine at Chongqing University Three Gorges Hospital between March 2024 and January 2025 were included. The inclusion criteria were as follows: 1) Age ≥ 18 years; 2) Met the diagnostic criteria for SAP according to the revised Atlanta classification (2012) [21]; 3) Time from onset to admission to the intensive care unit (ICU) ≤ 72 hours; 4) Informed consent obtained from the patient or their family members. The exclusion criteria were as follows: 1) Combined with chronic pancreatitis, immunosuppressive diseases, cancer, gastroenteritis, inflammatory bowel disease, or irritable bowel syndrome; 2) Severe heart, liver, or renal failure. SAP patients were divided into HTG-SAP and non-HTG-SAP groups based on whether their triglyceride level was > 1000 mg/dL.

Ethics

This study complied with international medical ethics standards and was approved by the Chongqing University Three Gorges Hospital Ethics Committee. Informed consent was obtained from the subjects or their legally authorized representative.

Observation indicators

The following laboratory results within 24 hours of ICU admission were recorded for SAP patients: white blood cell (WBC) count, neutrophils, albumin (ALB), total bilirubin (TBil), direct bilirubin (DBil), alanine aminotransferase (ALT), aspartate aminotransferase (AST),

serum creatinine (Crea), cholesterol (Chol), triglycerides (TG), serum amylase (AMY), serum lipase (LIP), C-reactive protein (CRP), interleukin-6 (IL-6), and procalcitonin (PCT).

Gut microbiota 16S rRNA high-throughput gene sequencing

Fecal samples were collected using sterile anal swabs from SAP patients within 24 hours of ICU admission and stored at -80°C until testing. Genomic DNA (gDNA) was purified from samples using the Zymo Research BIOMICS DNA Microprep Kit. The gDNA integrity was verified by 0.8% agarose gel electrophoresis, followed by the Tecan F200 (PicoGreen dye) detection. The following specific primers with index sequences were synthesized to amplify the V4 region of the bacterial 16S rDNA: 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Three replicates were evaluated for each sample, and each PCR reaction was terminated during the linear amplification phase. After PCR, the products from the same sample were pooled and detected by electrophoresis. The target DNA fragments were recovered by gel extraction using a gel recovery kit and eluted with TE buffer. Based on preliminary electrophoresis results, the recovered PCR products were quantified and mixed in equimolar amounts. Libraries were then constructed using the NEBNext Ultra II DNA Library Prep Kit for Illumina (BioLabs). Sequencing was performed using the PE250 method on the ILLUMINA platform.

Bioinformatics analyses

Paired-end sequences were merged using Fast Length Adjustment of Short reads (FLASH). Sabre was used to demultiplex the raw reads into sample-specific sequences based on barcodes and to trim barcode sequences. Strict quality control was performed in Quantitative Insights Into Microbial Ecology 2 (QIIME 2) to filter out sequences with an average quality score < 30, length < 200 bp, or ambiguous bases (N > 0). Based on the Deblur algorithm in QIIME 2, sequence denoising and chimera removal were conducted to generate an amplicon sequence variant (ASV) feature table and representative sequences. Finally, a classifier based on the naive Bayes algorithm was used to construct a taxonomic classification dataset based on the SILVA database, and this dataset was subsequently used to perform taxonomic annotation of the ASV representative sequences.

Serum miR-29b-3p detection

Peripheral venous blood (2 - 5 mL) was collected from SAP patients within 24 hours of ICU admission. The blood was rested at room temperature for 30 minutes and then centrifuged at 3,000 rpm for 10 minutes. Separated serum was aliquoted and stored at -80°C for testing. Total RNA was extracted from the serum using the miRcute Serum/Plasma miRNA Extraction Kit (Centrifugal Plate Type) from Tiangen (Beijing). Reverse transcription was performed using the miRcute Enhanced miRNA cDNA First Strand Synthesis Kit. RT-qPCR was performed using the miRcute enhanced miRNA fluorescence quantification detection kit, along with commercially available specific primers for miR-29b-3p (Catalog No. CD201-0028, Tm: 60°C) and the internal reference U6 (Catalog No. CD201-0145, Tm: 60°C) purchased from Tiangen Biotech. The thermal cycling conditions were set as follows: an initial denaturation at 95°C for 15 minutes; followed by 5 cycles of denaturation at 94°C for 20 seconds, annealing at 64°C for 30 seconds, and extension at 72°C for 34 seconds; and a subsequent 45 cycles of denaturation at 94°C for 20 seconds and combined annealing/extension at 60°C for 34 seconds.

Statistical analysis

Data analysis was performed using SPSS software (version 26.0). Categorical data were expressed as numbers or percentages, and comparisons between groups were performed using the χ^2 test. Measurement data were tested for normality. If normally distributed, the data were expressed as mean ± standard deviation ($\bar{x} \pm s$), and comparisons between groups were performed using the independent samples *t*-test. If not normally distributed, they were expressed as median (interquartile range) [*M* (*P25*, *P75*)], and comparisons between groups were performed using the Mann-Whitney *U* test. Linear discriminant analysis effect size (LEfSe) analysis was performed to identify differentially abundant taxa between groups. Taxa with a linear discriminant analysis (LDA) score > 2.0 and a p-value < 0.05 were considered significantly different. The relative expression of the target gene miR-29b in serum was calculated using the ΔΔCt method, with the non-HTG-SAP group serving as the control group, where ΔCt = Ct (target gene) - Ct (reference gene), and ΔΔCt = ΔCt (case group) - ΔCt (control group). The correlation between two variables was analyzed using Spearman's correlation analysis. A p-value < 0.05 was considered statistically significant.

RESULTS

Comparison of laboratory indicators in non-HTG-SAP and HTG-SAP patients

To evaluate the gut-pancreas axis of HTG-SAP patients, we enrolled 77 patients, including 44 in the non-HTG-SAP group and 33 in the HTG-SAP group. There were no significant differences in gender, history of hypertension, or history of biliary diseases between the two groups. However, compared with the non-HTG-SAP group, patients in the HTG-SAP group were younger (40.2 ± 13.7 years vs. 54.6 ± 16.5 years, p < 0.001), and the proportions of patients with a history of hyperlipidemia (30.3% vs. 0%) and AP (39.4% vs. 15.9%, p = 0.020) were significantly higher. A comparison of laboratory indicators confirmed that plasma Chol and TG

Table 1. Comparison of different indicators between the HTG-SAP and non-HTG-SAP groups.

Indicators	non-HTG-SAP (n = 44)	HTG-SAP (n = 33)	$t/Z/\chi^2$	p
Male [n (%)]	23 (52.3)	20 (60.6)	0.531	0.466
Age [years, $\bar{x} \pm s$]	54.6 ± 16.5	40.2 ± 13.7	-4.07	< 0.001
Hypertension [n (%)]	12 (27.3)	8 (24.2)	0.090	0.764
Hyperlipidemia [n (%)]	0 (0.0)	10 (30.3)	12.759	< 0.001
Pancreatitis [n (%)]	7 (15.9)	13 (39.4)	5.409	0.020
Biliary tract disease [n (%)]	7 (15.9)	1 (3)	2.119	0.146
WBC [× 10^9/L, *M* (*P25*, *P75*)]	13.1 (9.4, 16.9)	8.4 (6.8, 14.9)	-1.595	0.111
Neutrophils [%, *M* (*P25*, *P75*)]	88.1 (85.5, 91.4)	85.7 (82.4, 89)	-2.255	0.024
ALB [g/L, $\bar{x} \pm s$]	32.9 ± 6.1	35.0 ± 5.7	1.560	0.124
TBil [μmol/L, *M* (*P25*, *P75*)]	20.1 (11.8, 27.2)	17.0 (11.0, 23.3)	-1.148	0.251
DBil [μmol/L, *M* (*P25*, *P75*)]	8.8 (4.7, 16.4)	5.3 (2.9, 9.5)	-2.424	0.015
ALT [U/L, *M* (*P25*, *P75*)]	20.2 (12.5, 64.4)	16.4 (11.3, 29.7)	-1.498	0.134
AST [U/L, *M* (*P25*, *P75*)]	32.0 (20.2, 88.9)	25.2 (16.6, 53.4)	-1.637	0.102
Chol [mmol/L, *M* (*P25*, *P75*)]	3.5 (2.8, 4.6)	9.0 (5.6, 11.3)	-5.749	< 0.001
TG [mmol/L, *M* (*P25*, *P75*)]	1.7 (1.0, 3.1)	19.6 (12.8, 44.0)	-7.267	< 0.001
Crea [μmol/L, *M* (*P25*, *P75*)]	78.0 (51.3, 133.0)	69.0 (48.5, 107.5)	-0.952	0.341
AMY [U/L, *M* (*P25*, *P75*)]	231.0 (62.8, 608.3)	194.0 (78.0, 500.5)	-0.484	0.629
LIP [U/L, *M* (*P25*, *P75*)]	201.0 (111.5, 817.5)	520.0 (141.0, 1109.5)	-1.523	0.128
CRP [mg/L, $\bar{x} \pm s$]	181.3 ± 97.2	210.1 ±133.0	1.060	0.296
IL-6 [ng/L, *M* (*P25*, *P75*)]	142.9 (43.2, 259.6)	147.7 (48.5, 377.8)	-0.422	0.673
PCT [ng/mL, *M* (*P25*, *P75*)]	2.3 (0.6, 5.9)	1.4 (0.3, 5.4)	-0.896	0.370
miR-29b-3p [$2^{-\Delta\Delta CT}$, *M* (*P25, P75*)]	0.9 (0.3, 3.2)	4.4 (1.1, 10.4)	-3.129	0.002

non-HTG-SAP: non-hypertriglyceridemic severe acute pancreatitis, HTG-SAP: hypertriglyceridemic severe acute pancreatitis, WBC: white blood cell, ALB: albumin, TBil: total bilirubin, DBil: direct bilirubin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, Crea: serum creatinine, Chol: cholesterol, TG: triglyceride, AMY: serum amylase, LIP: serum lipase, CRP: C-reactive protein, IL-6: interleukin-6, PCT: procalcitonin, miR: MicroRNA.

Table 2. Correlation analysis between gut microbiota characteristics and miR-29b, inflammatory factors, and blood lipids in HTG-SAP versus non-HTG-SAP patients.

Microbiota features	Correlation coefficient (r)					
	miR-29b-3p	Chol	TG	CRP	IL-6	PCT
Chao1 index	-0.237 *	-0.183	-0.103	-0.069	-0.035	0.048
PD index	-0.266 *	-0.150	-0.084	-0.054	-0.042	0.055
Anaerococcus	0.369 **	0.281 *	0.181	-0.115	-0.126	0.054
Finegoldia	0.303 **	0.196	0.106	-0.152	-0.190	0.045
Peptoniphilus	0.307 **	0.201	0.097	-0.058	-0.201	0.003
Enterococcus	-0.343 **	-0.075	-0.101	0.001	0.164	-0.102

***Anaerococcus*, *Finegoldia*, and *Peptoniphilus* are genera in the class *Clostridia* in the phylum *Firmicutes*. *Enterococcus* is a genus under the class *Bacilli* in the phylum *Firmicutes*. * p < 0.05, ** p < 0.01.**

Table 3. Correlation analysis between miR-29b and inflammatory factors and blood lipids in in HTG-SAP versus non-HTG-SAP patients.

miRNAs	Correlation coefficient (*r*)				
	Chol	TG	CRP	IL-6	PCT
miR-29b-3p	0.347 **	0.276 *	0.105	-0.246	0.006

*** p < 0.05, ** p < 0.01.**

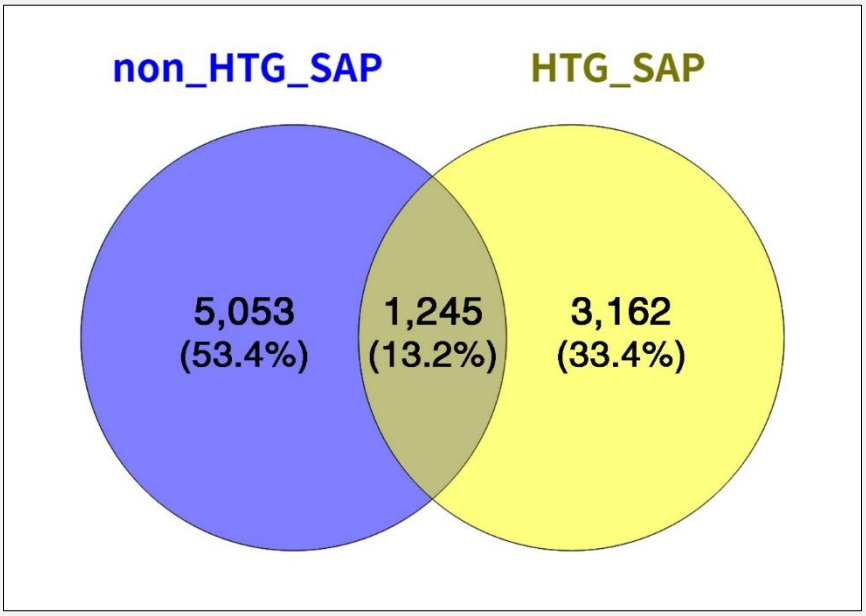

Figure 1. Comparison of ASVs in the non-HTG-SAP and HTG-SAP groups.

Venn diagram of ASVs in the gut microbiota of the two patient groups.
non_HTG_SAP: non-hypertriglyceridemic severe acute pancreatitis, HTG_SAP: hypertriglyceridemic severe acute pancreatitis, ASVs: operational taxonomic units.

levels were significantly higher in the HTG-SAP group than in the non-HTG-SAP group (both $p < 0.001$). In contrast, the neutrophil percentage and DBil in the HTG-SAP group were lower than those in the non-HTG-SAP group ($p = 0.024$ and $p = 0.015$, respectively). There were no statistically significant differences in WBC, ALB, TBil, ALT, AST, Crea, AMY, LIP, CRP, IL-6, or PCT between the two groups (all $p > 0.05$). Interestingly, the relative expression level of serum miR-29b-3p in the HTG-SAP group was significantly higher than that in the non-HTG-SAP group ($p = 0.002$), and this elevation remained statistically significant after adjusting for age as a potential confounder via multiple linear regression analysis ($p = 0.003$). Given the established role of the miR-29 family in regulating lipid metabolism [19,20], these results are consistent with the possibility that miR-29b regulates the elevated lipid levels in HTG-SAP (Table 1).

Comparison of gut microbiota between the non-HTG-SAP and HTG-SAP groups

To evaluate differences in the gut microbiota composition between HTG-SAP and non-HTG-SAP patients, we collected rectal swabs from 77 patients and performed high-throughput rRNA sequencing. The sequences were then denoised and filtered for chimeras to generate amplicon sequence variants (ASVs). As shown in Figure 1, the HTG-SAP group had 3,162 unique

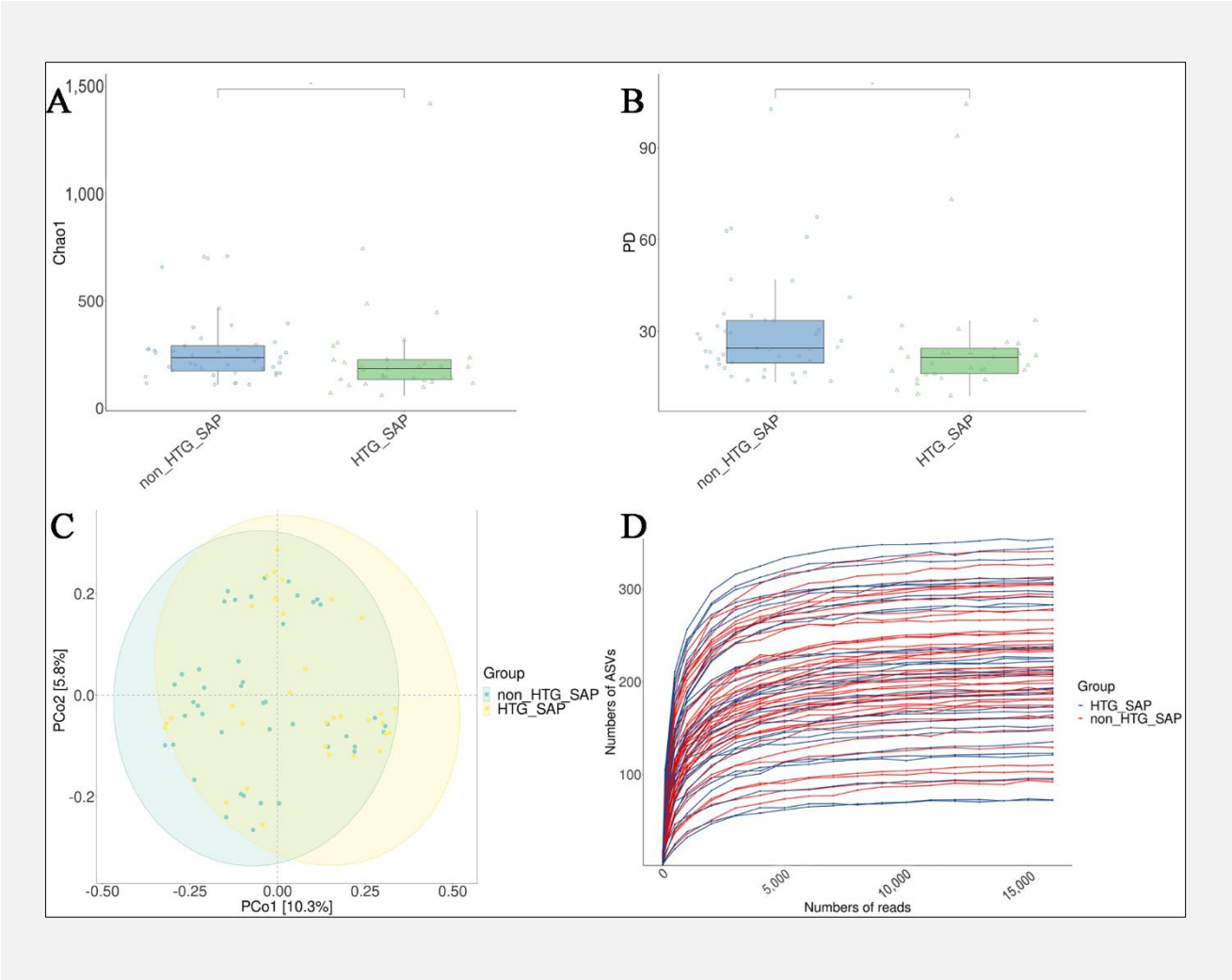

Figure 2. Comparison of gut microbiota α-diversity between the non-HTG-SAP and HTG-SAP groups.

A: Comparison of the Chao1 index, B: Comparison of the phylogenetic diversity (PD) index. * $p < 0.05$. C: Beta diversity analysis based on PCoA. The abscissa and ordinate represent the contribution rate of the first principal component (10.3%) and the second principal component (5.8%) to the sample difference. Each symbol represents the gut microbiota of a sample. D: Rarefaction curves of the gut microbiota in the two patient groups.

ASVs, the non-HTG-SAP group had 5,053 unique ASVs, and 1,245 ASVs were shared between the two groups.

Next, we evaluated the gut microbiota α-diversity. The Chao1 index and phylogenetic diversity (PD) index of the gut microbiota in the HTG-SAP group were significantly lower than those in the non-HTG-SAP group (both $p < 0.05$), suggesting reduced gut microbiota diversity in the HTG-SAP group (Figure 2A and B). Furthermore, to evaluate the overall structural divergence of the gut microbiota between the two groups, beta diversity was assessed using principal coordinate analysis (PCoA) based on unweighted UniFrac distances. As shown in Figure C, although the PCoA plot displayed considerable overlap between the HTG-SAP and non-HTG-SAP groups, statistical evaluation using permutational multivariate analysis of variance (PERMANOVA) confirmed a significant shift in the overall microbial community structure ($R2 = 0.020$, $p = 0.009$). The rarefaction curves plateaued with increasing sequencing depth, indicating sufficient sequencing depth was achieved (Figure 2D).

At the phylum level, *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* were the dominant phyla in both groups, accounting for 35.62%, 36.15%, and 17.03% in the HTG-SAP group; and 35.46%, 30.88%, and 17.65% in the non-HTG-SAP group, respectively. The relative abundance of *Firmicutes* was statistically higher in the HTG-SAP group than in the non-HTG-SAP group (Figure 3A). At the class level, the relative abundance of

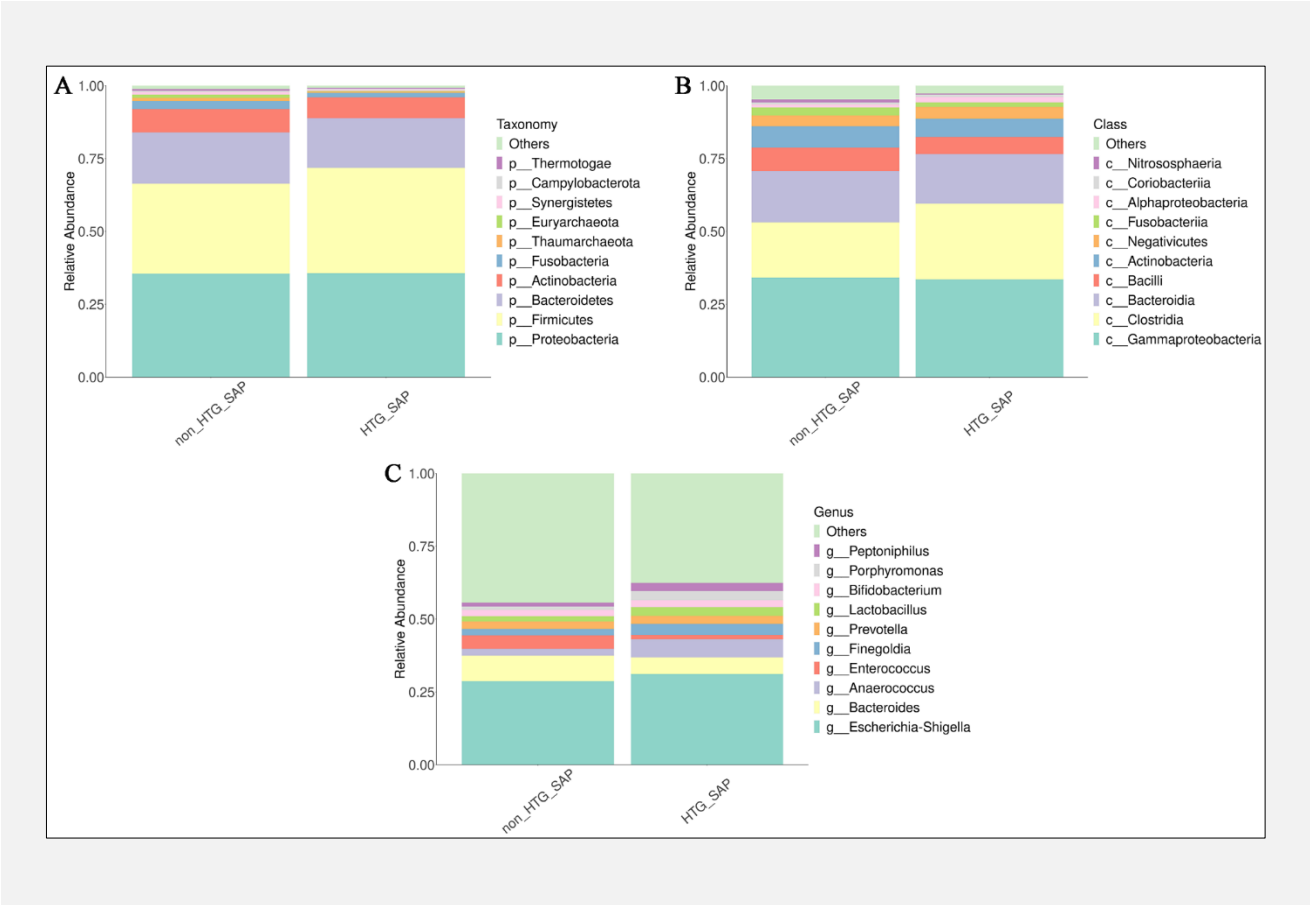

Figure 3. Comparison of gut microbiota in the non-HTG-SAP and HTG-SAP groups.

A: Comparison at the phylum level, B: Comparison at the class level, C: Comparison at the genus level.

Clostridia (under *Firmicutes*) was higher, while that of *Bacilli* (under *Firmicutes*) was lower in the HTG-SAP group (Figure 3B). At the genus level, the relative abundances of *Escherichia-Shigella* (under *Proteobacteria*), *Anaerococcus*, and *Finegoldia* (both under *Clostridia*, *Firmicutes*) were significantly higher, while the relative abundances of *Bacteroides* (under *Bacteroidetes*) and *Enterococcus* (under *Bacilli*, *Firmicutes*) were significantly lower in the HTG-SAP group (Figure 3C). LEfSe analysis results showed that several taxa, including *Anaerococcus*, *Peptoniphilus*, and *Megamonas* (under *Firmicutes*), were significantly enriched in the HTG-SAP group ($p < 0.05$), while *Campylobacter* (under *Campylobacterota*) was significantly enriched in the non-HTG-SAP group ($p < 0.05$) (Figure 4A). The cladogram of differentially abundant taxa in the fecal samples of the two groups is shown in FigureB. Collectively, these results indicate distinct microecological differences in the microbiota structure of non-HTG-SAP and HTG-SAP patients.

Correlation analysis between gut microbiota, miR-29b, inflammatory factors, and blood lipids in SAP patients

Given our above evidence showing differences in the miR-29b serum levels, laboratory indicators (Chol, TG), and gut microbiota composition in non-HTG-SAP and HTG-SAP patients, we sought to evaluate correlations between these differences. Correlation analysis demonstrated that the Chao1 and PD indices of the gut microbiota were significantly negatively correlated with the serum miR-29b-3p expression in SAP patients ($r = -0.237$, $p = 0.038$; $r = -0.266$, $p = 0.019$). Furthermore, the relative abundances of *Anaerococcus*, *Finegoldia*, and *Peptoniphilus* were positively correlated with serum miR-29b-3p expression ($r = 0.369$, $p = 0.001$; $r = 0.303$, $p = 0.007$; $r = 0.307$, $p = 0.007$, respectively), while the relative abundance of *Enterococcus* was negatively correlated ($r = -0.343$, $p = 0.002$). Additionally, the relative abundance of *Anaerococcus* was positively correlated with plasma Chol levels ($r = 0.281$, $p = 0.013$). No correlations were observed with TG, CRP, IL-6 or PCT levels (Table). Next, we evaluated the correlations between serum miR-29b-3p and other plasma compo-

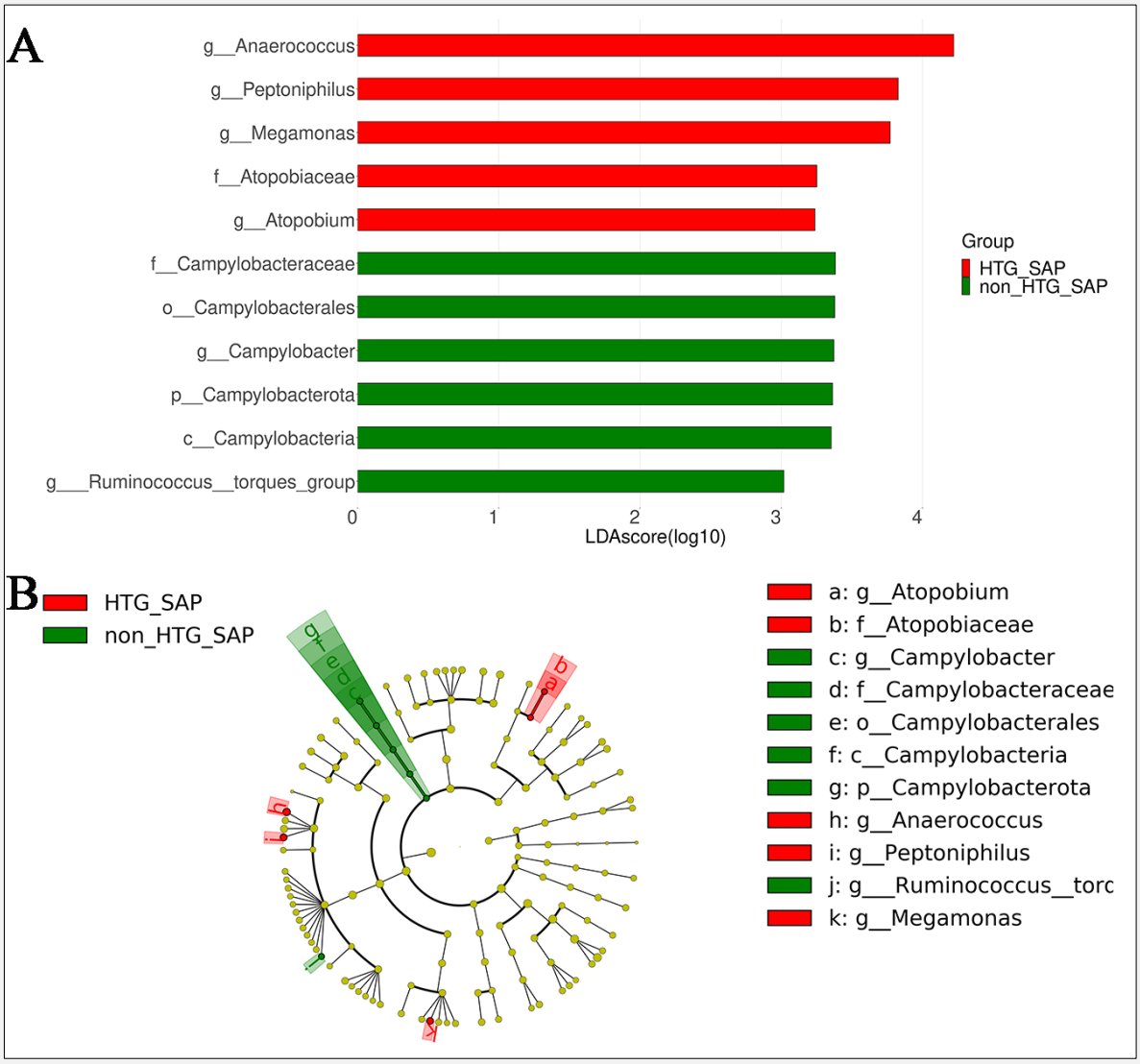

Figure 4. Comparison of differentially abundant gut microbiota species in the non-HTG-SAP and HTG-SAP groups.

A: LEfSe analysis of species, B: Cladogram of species.

nents. Serum miR-29b-3p was positively correlated with plasma Chol and TG levels (r = 0.347, p = 0.002; r = 0.276, p = 0.015) (Table 2). These findings are consistent with the potential role of miR-29b and microbiota in HTG-SAP patient pathology.

DISCUSSION

This study systematically analyzed the structural characteristics of the gut microbiota in SAP patients with different etiologies using 16S rRNA high-throughput sequencing technology, focusing on the important subtype, HTG-SAP. The results showed that compared with the non-HTG-SAP group, HTG-SAP patients had significant gut microbiota dysbiosis, characterized by reduced diversity, enrichment of conditional pathogens, and reduction of beneficial bacteria. Importantly, the gut microbiota structural characteristics in SAP were closely related to the expression of serum miR-29b-3p and blood lipid levels, suggesting that these factors may play a synergistic role in the pathogenesis of HTG-SAP. In this study, we demonstrated characteristic gut microbiota structural dysbiosis in HTG-SAP patients. The gut

microbiota contains 100 trillion microbial cells and produces a variety of metabolites, including short-chain fatty acids, vitamins, anti-inflammatory and antioxidant substances, neurotoxins, carcinogens, and immunotoxins [22]. In AP, changes in the gut microbiota are associated with the risk and severity of AP [23-25], with evidence for an increased abundance of *Bacteroidetes* and *Proteobacteria*, and a decreased abundance of *Firmicutes* and *Actinobacteria* [12]. Additional studies suggest that a decreased abundance of *Ruminococcaceae* and *Enterococcus* is associated with SAP classification [26], while an increased abundance of *Escherichia/Shigella* is associated with disease severity, HTG status, and patient prognosis [15]. The later studies suggest broad differences in the gut microbiota according to disease classification; however, our study extends previous work by specifically focusing on severe acute pancreatitis and by integrating gut microbiota profiling with serum miR-29b-3p expression and lipid-related correlation analyses in HTG-SAP and non-HTG-SAP patients. Our results demonstrated that compared with the non-HTG-SAP group, HTG-SAP patients had significantly lower Chao1 and PD indices, indicating impaired richness and phylogenetic diversity of their gut microecology and destruction of microecosystem stability. This is consistent with prior studies showing that the HTG status and SAP classification each separately contribute to reducing bacterial diversity [15,26]. In terms of microbiota structure, we observed a significant increase in the relative abundance of genera belonging to the *Peptoniphilaceae* family of *Firmicutes*, such as *Anaerococcus*, *Finegoldia*, and *Peptoniphilus*, in the gut of HTG-SAP patients. These genera are mostly conditional pathogens that can destroy the intestinal epithelial barrier by producing toxins and metabolites, and their over-proliferation is associated with various inflammatory diseases [27]. A previous study reported similar enrichment of conditional pathogens in SAP patients [28]. In our study, we also found that the abundance of *Enterococcus* (also containing conditional pathogenic strains) was significantly reduced in the HTG-SAP group compared to the non-HTG-SAP group. *Enterococcus* is known to be decreased in the guts of SAP versus AP patients and has potential probiotic properties [26,29]. Therefore, the ecological imbalance pattern of "increased pathogens and decreased beneficial bacteria" observed in this study provides a microecological basis for intestinal barrier dysfunction and bacterial/endotoxin translocation in HTG-SAP, which may correlate with the deterioration of the disease condition.

Our study also confirmed that the expression of miR-29b-3p in the serum of HTG-SAP versus non-HTG-SAP patients was significantly up-regulated. The miR-29 family (including miR-29a, miR-29b, and miR-29c) is known to play key roles in apoptosis, proliferation, and differentiation [30]. Increasing evidence indicates that the miR-29 family plays an important role in the regulation of lipid metabolism, potentially providing a link between lipid metabolism disorders and AP. Ru et al. [19] demonstrated that miR-29 targets the SREBP cleavage-activating protein/sterol regulatory element-binding protein 1 pathway (SCAP/SREBP-1), forming a negative feedback loop, thereby regulating the expression of lipid synthesis-related genes. Kurtz et al. [20] also reported that miR-29 can inhibit the activation of key lipid metabolism genes mediated by forkhead box A2 (FOXA2). Consistent with the latter studies, we determined that the expression level of serum miR-29b-3p in SAP patients was positively correlated with TG levels and Chol levels. These results suggest that miR-29b-3p may be involved in the occurrence and development of HTG-SAP by affecting lipid metabolism, though the potential roles of SCAP/SREBP-1 and FOXA2 in mediating this process warrant further investigation.

Importantly, we revealed for the first time that the serum miR-29b-3p level in SAP patients was negatively correlated with the Chao1 and PD indexes, reflecting lower gut microbiota diversity. In addition, the miR-29b-3p level was positively correlated with the relative abundance of intestinal conditional pathogens (*Anaerococcus*, *Finegoldia*, and *Peptoniphilus*) and negatively correlated with the abundance of probiotic *Enterococcus*. These data suggest that the high expression of miR-29b-3p is closely related to gut microecological imbalance, thus providing a new perspective for understanding HTG-SAP pathogenesis.

Based on these findings, a hypothetical conceptual framework is proposed: in HTG-SAP, elevated miR-29b-3p, lipid disorders, and gut dysbiosis occur concurrently. This microecological imbalance is associated with impaired mucosal barrier integrity and systemic inflammation. Together, these correlated elements form a "metabolic disorder-microbiota dysbiosis–inflammation amplification" cycle that parallels HTG-SAP progression, although any causal or mechanistic relationships require further experimental validation.

This study has several limitations. First, this was a single-center cross-sectional study with a sample size determined by clinical availability rather than formal power calculations. Furthermore, no healthy control group or mild AP group was included. Although this design allowed a more specific focus on etiology-associated differences within SAP, it also limited the ability to determine whether the observed microbial patterns were specific to HTG-SAP or instead reflected gut microbiota alterations commonly associated with SAP itself. Moreover, detailed information on clinical exposures that may influence gut microbiota composition, such as antibiotic use, nutritional support, proton pump inhibitors, and other medications, was not available. Second, although serum miR-29b-3p expression was measured, other miR-29 family members, such as miR-29a and miR-29c, were not evaluated. Therefore, the present study could not assess the role of the entire miR-29 family in HTG-SAP. Third, the HTG-SAP patients were younger than patients without HTG-SAP, reflecting the epidemiological characteristics of this subtype. Al-

though multivariate regression confirmed that miR-29b-3p elevation remained significant after age adjustment ($p = 0.003$), age cannot be entirely excluded as a contributing factor to the observed microbiota shifts. Future age-matched cohort studies or animal models are warranted to further clarify the mechanisms. In addition, given the exploratory nature of the correlation analyses, p-values were not adjusted for multiple testing, which may increase the risk of false-positive findings.

CONCLUSION

In summary, this study confirms that HTG-SAP patients have characteristic gut microbiota structural dysbiosis, manifested as reduced diversity, enrichment of conditional pathogens (*Anaerococcus*, *Finegoldia*, and *Peptoniphilus*), and reduction of the potentially beneficial *Enterococcus*. Notably, we found that this dysbiosis pattern is associated with increased serum miR-29b-3p levels. Based on these concurrent phenomena, we propose a model of a "metabolism-microbiota-inflammation" cycle, wherein miR-29b-3p upregulation, lipid disorders, and microecological imbalance are closely interlinked features of HTG-SAP. While this study provides a new perspective on the gut-pancreas axis mechanism in SAP, further research is needed to validate this mechanism and explore its therapeutic potential.

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Data Availability Statement:
The data presented in this study are available from the corresponding author upon reasonable request. The data are not publicly available due to ethical and privacy considerations, including the potential risk of compromising patient confidentiality.

Ethics Approval and Consent to Participate:
This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Chongqing University Three Gorges Hospital Ethics Committee (approval number: 2022 Scientific Research No. (174), and Application for Ethics Extension [Approval Number: 2022 Scientific Research No. (174)-1)]. Informed consent was obtained from the participants or their legally authorized representatives.

Declaration of Generative AI in Scientific Writing:
The authors declare that no generative AI or AI-assisted technologies were used in the writing process, data analysis, or creation of figures for this manuscript.

Declaration of Interest:
The authors declare that they have no conflicts of interest in this work.

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