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ORIGINAL RESEARCH

A Prospective Observational Study on the Effects of *XingQi HuoXue* Decoction on Gut Microbiota and Clinical Outcomes in Sepsis Patients with Acute Gastrointestinal Injury

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Background: Therapeutic options for sepsis-associated acute gastrointestinal injury (AGI) remain limited. In this study, we explored the potential of a decoction called *XingQiHuoXue* (XQHX), which was formulated by us, in patients with septic AGI.
Materials and Methods: It was a non-randomized observational study. Forty-nine patients diagnosed with septic AGI classified as qi stagnation and blood stasis syndrome (QSBSS), a condition characterized by unsmooth flow of qi and blood, were enrolled. The patients were divided into the treatment group (routine treatment and the XQHX) and the control group (routine treatment). Clinical characteristics encompassed demographics, underlying diseases, disease severity and prognostic outcomes. Gut microbiota data were derived from characterization of pre- and post-treatment fecal specimens collected from 15 patients enrolled. The clinical and gut microbiota characteristics were compared between the two groups.
Results: Among the 49 patients, 30 showed improvement, including 19 patients from the treatment group and 11 from the control group. The difference in the improvement rate between the groups was significant ($P < 0.05$). With regard to changes in the microbiome composition, the treatment group showed a significant decrease in the abundance of *Bacteroides*, and *Enterobacteriaceae/g-* ($q = 0.04$ and $q = 0.006$), accompanied by a significant increase in the abundance of *Enterococcus* ($q = 0.01$). These changes were not found in the control group. The abundance of *Bacteroides* and *Enterobacteriaceae/g-* was positively correlated with the Acute Physiology and Chronic Health Evaluation II and Sequential Organ Failure Assessment score ($R = 0.778$ and 0.499), while the abundance of *Enterococcus* showed a negative correlation with the score ($R = -0.811$ and -0.656).
Conclusion: Our preliminary findings suggest that XQHX may improve the prognosis of patients with septic AGI and QSBSS. The milder disease presentation and better prognosis could be associated with alterations in the gut microbiota induced by XQHX.
Keywords: sepsis, acute gastrointestinal injury, traditional Chinese medicine, gut microbiota

Introduction

Sepsis is a common occurrence in the intensive care unit (ICU) that is characterized by recurrent episodes of high-grade inflammation and high mortality rates.[1,2] It has been reported that the mortality rate of patients with sepsis in the ICU is as high as 25.8%.[3] The gastrointestinal (GI) system has a number of sophisticated and autonomous functions in the body, including digestion, absorption, excretion, and protection.[4] However, the GI is vulnerable to sepsis. In fact, about 60% of patients with sepsis develop acute gastrointestinal injury (AGI) and experience nausea, vomiting, or gastrointestinal bleeding during hospitalization.[5] AGI in patients with sepsis is associated with a high mortality rate.[6] Therefore, its timely treatment is vital to survival. The treatment of AGI in patients with sepsis is limited. Therapeutic approaches are

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often selected based on the symptoms. Measures such as using metoclopramide and erythromycin to enhance gastric motility, avoiding gastrointestinal motility suppressants, reducing or discontinuing enteral nutrition formulations, and implementing intensified systemic fluid and electrolyte management are commonly utilized in clinical practice.[7] The measures approaches alleviate clinical symptoms but may not slow disease progression. Consequently, proactive exploration of novel treatment strategies remains imperative.

In sepsis patients, varying degrees disruptions of gut microbiota are detected. Antibiotics are invaluable in the treatment of sepsis. However, treatment with broad-spectrum antibiotics has been found to aggravate the disorder of gut microbiota.[8] Disorders often manifest as a decrease in diversity and an imbalance in the proportion of bacterial communities, such as Firmicutes/Bacteroidetes (F/B),[9,10] Butyric acid producing bacteria such as *lactobacilli, fecal bacilli*, and *Bifidobacterium* decrease, while opportunistic pathogenic bacteria such as *Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacterium, and Bacteroides fragilis* increase.[8] The disorders aggravate the occurrence of AGI and deteriorated gastrointestinal motility.[11] One of the unique targets of treatment in traditional Chinese medicine (TCM) is the regulation of intestinal function and gut microbiota.[12] For example, Magnoliae Officinalis Cortex, a common Chinese medicinal herb, demonstrates therapeutic effects including anti-emetic, anti-diarrheal, anti-ulcerative, and functional digestive disorder alleviation. Besides, it can protect the intestinal barrier by upregulating intestinal epithelial tight junction proteins (eg, ZO-1) and regulate gut microbiota such as enhances butyrate-producing bacterial abundance when septic AGI occurs.[13] These effects have also been found with TCM formulas such as *Shenling Baizhu powder*[14] and *Gegen Qinlian decoction*.[15] Based on these studied effects of TCM treatments, it might be promising to investigate their effects in patients who have sepsis associated with AGI.

The features of AGI associated with sepsis are described as “gastric fullness” and “abdominal distension” in TCM, and these conditions are considered to be commonly related to qi stagnation and blood stasis (QSBSS).[16] QSBSS describes the condition of unsmooth flow of qi and blood, manifesting with symptoms such as abdominal pain, abdominal distension, and constipation.[17] We have created a decoction called *XingQi HuoXue* (XQHX) based on the principles of TCM and the famous prescription called *Taohong Siwu* (THSW),[18] which is one of the most widely prescribed qi-promoting and blood-activating decoctions in practice. And from the perspective of modern medicine, pharmacological studies confirm that THSW decoction, the foundation of XQHX, can alleviate the blood stagnation and promote angiogenesis,[18,19] aligning with its traditional applications. It is known that invigorating qi and promoting blood circulation early on can improve intestinal ischemia and hypoxia and, thereby, protect the intestinal environment and improve clinical outcomes.[20] Furthermore, we incorporated additional herbs such as Rhei Radix et Rhizoma,[21] Magnoliae Officinalis Cortex[22] into XQHX, which exhibit efficacy in alleviating gastrointestinal symptoms (eg, abdominal distension, constipation, nausea, and vomiting). XQHX holds therapeutic potential for septic AGI patients classified with QSBSS; however, the clinical performance of the formulation remains inadequately investigated.

Therefore, we conducted a prospective observational study in patients with septic AGI and QSBSS, aiming to investigate the effects of XQHX on the prognosis and gut microbiota of these patients. Additionally, we sought to explore the potential relationship between changes in disease severity and alterations in the gut microbiota. We hope to pave a new way for the management of septic AGI.

Materials and Methods

Study Design and Patient Selection

We designed a prospective observational study. A cohort of 49 subjects was recruited for this study. All the patients were admitted to the ICU of Zhejiang Hospital of Traditional Chinese Medicine between September 2021 and March 2023 and met the inclusion and exclusion criteria (Figure 1).

The inclusion criteria are as follows:

1. The patients were suffering life-threatening organ dysfunction due to a dysregulated host response to infection, which we defined define them as sepsis.[23,24]

2. The patients had all developed AGI, which was diagnosed based on the AGI guidelines proposed by the European Society of Intensive Care Medicine (ESICM) in 2012.[7] Briefly, AGI score I: the function of the digestive tract is

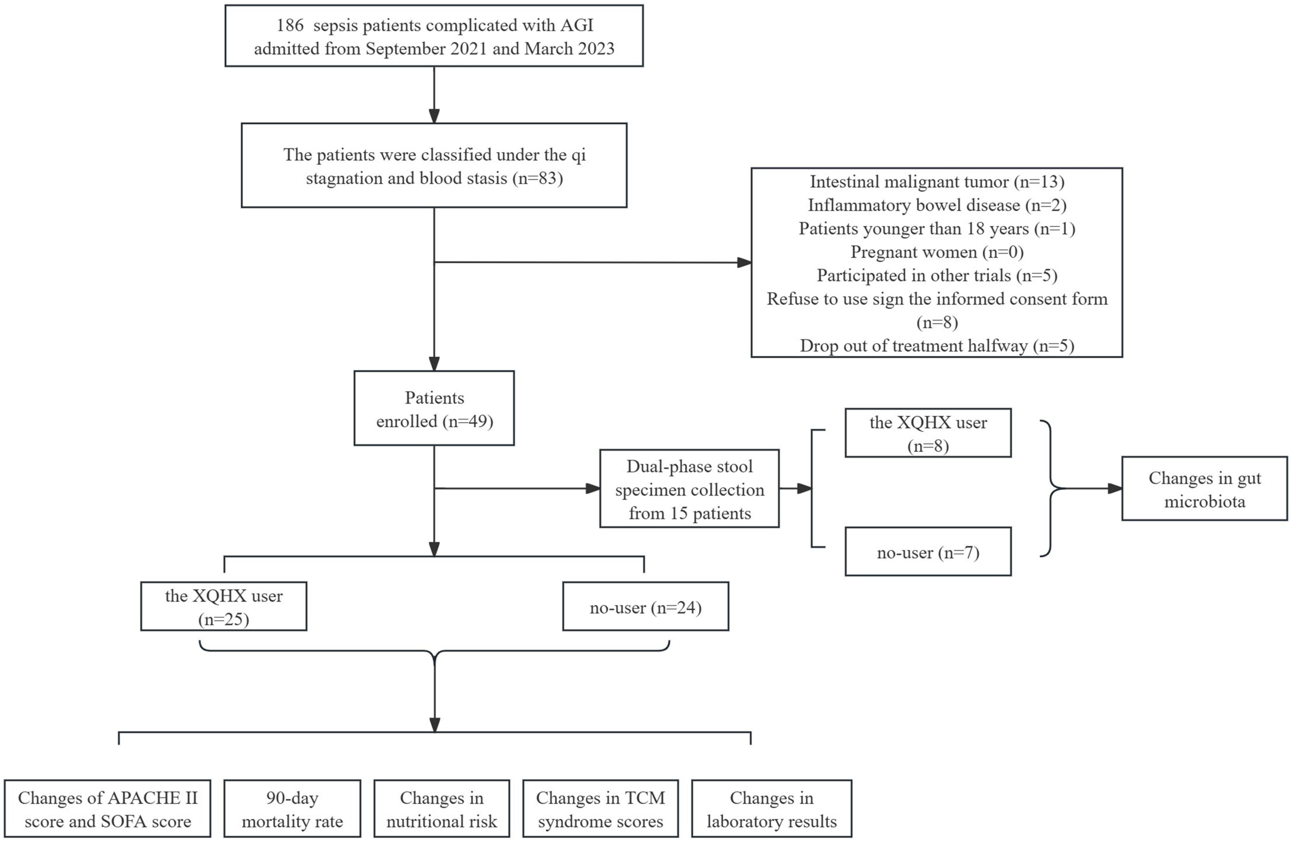

Figure 1 Flow diagram of the research.

temporarily damaged and can recover on its own; AGI score II: the function of the digestive tract cannot recover on its own, but can be improved after medication or other interventions; AGI score III: the function of the digestive tract cannot be recovered through conventional treatment; AGI score IV: the gastrointestinal function failure that can directly endanger life and affect multiple organs.

3. The patients were classified under the QSBSS type based on “Expert consensus on gastrointestinal dysfunction secondary to sepsis with integrating Traditional Chinese Medicine and Western medicine”[23] and evaluation from professional Chinese medicine practitioners. QSBSS was diagnosed according to the following symptoms:

(1) Main symptoms: Abdominal pain, abdominal distension and constipation

(2) Secondary symptoms: Poor appetite, nausea, vomiting, dim complexion, even squamous and dry skin.

(3) Tongue image: Pale purple and dark tongue

(4) Pulse condition: Thread and sluggish pulse

Patients with one of the main symptoms or two of the secondary symptoms and tongue and pulse support were diagnosed. TCM syndrome scoring method is shown in (Table S1).

The exclusion criteria are as follows: patients younger than 18 years of age, pregnant women, those who refuse to who refuse to sign the informed consent form, those who were allergic to TCM, those with primary gastrointestinal damage at the time of ICU admission, such as intestinal malignant tumor, inflammatory bowel disease and other bowel diseases, those who enrolled in other trials and drop out studies halfway.

Treatment Protocol and Drug Selection

All the patients received the standard treatment for sepsis, with the regime tailored to each patient according to the cause.[23] Patients were divided into a control group and a treatment group based on whether XQHX therapy was added. XQHX, modified formulation derived from THSW, consists of 13 types of herbs: *Prunus persica* (L). Batsch in *Beytr.*

Entw. Gewächsreich: 30 (1801) [Rosaceae; Persicae Semen], *Rheum officinale* Baill. in *Adansonia 10: 246 (1872)* [Polygonaceae; Rhei Radix et Rhizoma], *Neolitsea cassia* (L). Kosterm. in *J. Sci. Res. (Jakarta) 1: 85 (1952)* [Lauraceae; Cinnamomi Ramulus], *Carthamus tinctorius* L. *in Sp. Pl.: 830 (1753)* [Asteraceae; Carthami Flos], *Curcuma phaeocaulis* Valeton in *Bull. Jard. Bot. Buitenzorg, sér. 2, 27: 69 (1918)* [Zingiberaceae; Curcumae Rhizoma], *Angelica sinensis* (Oliv). Diels in *Bot. Jahrb. Syst. 29: 500 (1900)* [Apiaceae; Angelicae Sinensis Radix], *Rehmannia glutinosa* (Gaertn). Libosch. ex DC in *Prodr. 9: 275 (1845)* [Orobanchaceae; Rehmanniae Radix], *Ligusticum chuanxiong* Hort. in *Acta Pharm. Sin. 12(10): 670 (1957)* [Apiaceae; Chuanxiong Rhizoma], *Salvia miltiorrhiza* Bunge in *Enum. Pl. China Bor.: 50 (1833)* [Lamiaceae; Salviae Miltiorrhizae Radix et Rhizoma], *Paeonia lactiflora* Pall. in *Reise Russ. Reich. 3: 286 (1776)* [Paeoniaceae; Paeoniae Radix Rubra], *Citrus aurantium* L. in *Sp. Pl. 2: 782. (1753)* [Rutaceae; Aurantii Fructus], *Magnolia officinalis* Rehder & E.H.Wilson in *C.S.Sargent, Pl. Wilson. 1: 391 (1913)* [Magnoliaceae; Magnoliae Officinalis Cortex], *Areca catechu* L. in *Sp. Pl.: 1189 (1753)* [Arecaceae; Arecae Pericarpium]. All species be validated taxonomically (eg, http://mpns.kew.org/mpns-portal/ and https://ydz.chp.org.cn/#/main). The decoction was prepared according to the adult prescription raw drug dosage of 174 g:[19,25] Persicae Semen (10g), Rhei Radix et Rhizoma (10g), Mirabilite (10g), Cinnamomi Ramulus (6g), Carthami Flos (6g), Curcumae Rhizoma (15g), Angelicae Sinensis Radix (12g), Drying Rehmanniae Radix (10g), Chuanxiong Rhizoma (10g), Salviae Miltiorrhizae Radix et Rhizoma (15g), Paeoniae Radix Rubra (10g), Aurantii Fructus (15g), Magnoliae Officinalis Cortex (15g), Arecae Pericarpium (30g). All the Chinese medicinal materials were purchased and stored by the First Affiliated Hospital of Zhejiang Chinese Medical University and identified by Professor Caihua Sun (The First Affiliated Hospital of Zhejiang Chinese Medical University, China). The voucher specimens (Table S2) were preserved at the First Affiliated Hospital of Zhejiang Chinese Medical University. According to the TCM decoction method:[26] all herbs (except Rhei Radix et Rhizoma and Mirabilite) were mixed and boiled for 1.5 h. Add the Rhei Radix et Rhizoma in the last 5 minutes. The Mirabilite should be added into the TCM decoction directly at the last time. All the process was repeated twice. Patients were evaluated two weeks after the initiation of the treatment protocol.[27] Researchers did not participate in the development of clinical treatment protocols.

Data Collection and Follow-up

Demographic and clinical data were collected at the time of patient enrollment, including comorbidities, laboratory test results, use of vasopressors, mechanical ventilation status, continuous renal replacement therapy (CRRT), and other relevant treatment information. This data collection was repeated two weeks after the completion of treatment. All information was retrieved from patients' medical records and laboratory information systems (Table 1). Researchers followed up with patients and recorded their prognostic outcomes.

For all enrolled patients, nutritional risk screening (NRS 2002) scores, Traditional Chinese Medicine (TCM) syndrome scores, Acute Physiology and Chronic Health Evaluation II (APACHE II) scores, and Sequential Organ

Table 1 Demographic and Clinical Characteristics of Acute Gastrointestinal Function Injury in 49 Patients with Sepsis

	All Patients (n=49)	Treatment Group (n=25)	Control Group (n=24)	P-value
Sex (male), n (%)	33(67.30%)	17(68%)	16(66.70%)	1.000
Age (years), (mean ± standard deviations)	75.27±14.83	74.96±15.94	75.58±13.93	0.885
NRS2002 value, median (IQR)	6(5~7)	6(5~7)	6(5~6.75)	0.654
APACHEII value, median (IQR)	22(19~27)	24(19.50~27)	21(18.25~27.50)	0.214
SOFA value, median (IQR)	14(11~15)	14(11~16.5)	12.5(11~14)	0.164
AGI				
I~II grade, n (%)	22(44.90%)	11(44%)	11(45.80%)	1.000
III~IV grade, n (%)	27(55.10%)	14(56%)	13(54.20%)	1.000

(*Continued*)

Table 1 (Continued).

	All Patients (n=49)	Treatment Group (n=25)	Control Group (n=24)	P-value
Infection sites (n, %)				
Lungs	26(53.10%)	11(44%)	15(62.50%)	0.256
Bloodstream	11(22.40%)	7(28%)	4(16.70%)	0.496
Abdominal cavity	12(24.50%)	7(28%)	5(20.80%)	0.742
Underlying disease, n (%)				
Hypertension	32(65.30%)	17(68%)	15(62.50%)	0.796
Diabetes	24(49%)	13(52%)	11(45.80%)	0.778
Cardiovascular diseases	15(30.60%)	7(28%)	8(33.30%)	0.762
Respiratory diseases	23(46.90%)	13(52%)	10(41.70%)	0.571
Kidney diseases	8(16.30%)	4(16%)	4(16.70%)	1.000
Hepatopathy	2(4.10%)	0(0%)	2(8.30%)	0.235
Cerebrovascular diseases	17(34.70%)	10(40%)	7(29.20%)	0.551
Antibiotic therapy, n (%)				
Broad-spectrum agents	49(100%)	25(100%)	24(100%)	1.000
Narrow-spectrum agents	19(38.78%)	9(36%)	10(41.67%)	0.773
Laboratory findings, median (IQR)				
WBC (*10^9/L)	13.11(10.40~17.71)	12.7(8.50~16.30)	14.4(11.53~18.98)	0.396
N(%)	86.2(81.30~91.20)	85.4(78.10~91.90)	88(84.10~91.15)	0.509
PLT (*10^9/L)	132(85~208)	132(101~208)	132.5(33.25~211)	0.706
CPR (mg/L)	166.36(110.79~193.74)	166.36(131.70~205.45)	161.35(106.01~187.29)	0.246
PCT (ug/mL)	2.34(1.12~6.12)	2.39(0.68~12.34)	2.274(1.25~5.70)	0.880
ALT (U/L)	28(16~46)	30(14~67)	26(17.50~33.75)	0.806
AST (U/L)	18(11~34)	16(10~35)	21.5(12.25~27.25)	0.755
TB (mg/L)	17.10(7.30~27.40)	16.50(6.80~27.40)	17.35(9.83~27.33)	0.304
DB (mg/L)	10.10(4~15.10)	8.50(3.90~13.30)	10.35(4.60~18.78)	0.317
LDH (U/L)	315(233~450)	266(222~384)	326.50(253.50~501)	0.180
CK (U/L)	69(30~181)	67(30~181)	75.50(28.75~192.25)	0.865
Cr (U/L)	68(57~154)	74(61~201)	63(45~115.75)	0.089
Alb (g/L)	28(25.80~30.20)	28(24~29.50)	27.90(25.83~32.88)	0.203
BUN (mmol/l)	8.90(6.6~17.20)	8.90(7.30~20.50)	9(5.93~17.05)	0.491
cTnI (ug/l)	0.02 (0.01~0.11)	0.03(0.01~0.07)	0.04(0.01~0.15)	0.345
BNP (ng/l)	151.70(55~422.40)	146.90(53.50~422.40)	188.25(58.78~439.18)	0.748
Prognosis				
Improvement	30(61.20%)	19(76%)	11(45.80%)	0.042*
Death	19(38.80%)	6(24%)	13(54.20%)	0.042*

Notes: The Fisher's exact test was used to compare categorical data. Continuous variables were reported as means ± standard deviations, and statistical comparisons were made using the independent *t*-test. Non-normally distributed variables were expressed as the median and interquartile range (IQR), and comparisons were made using the Mann–Whitney *U*-test, Significant differences between treatment and control groups are indicated by asterisks (*: P < 0.05).

Abbreviations: WBC, white blood cell; N%, Neutrophil %; CRP, C-reactive protein; PCT, procalcitonin; TB, total bilirubin; DB, direct bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; Cr, Creatinine; Alb, Albumin; Broad-spectrum agents, The agents which antibacterial activity of both Gram-positive bacteria and Gram-negative bacteria; Narrow-spectrum agents, The agents which antibacterial activity only one of Gram-positive or Gram-negative bacteria.

Failure Assessment (SOFA) scores were assessed on the day of enrollment and two weeks after the completion of treatment. These assessments were used for disease evaluation.

Researchers prospectively collected the clinical information and completed the Case Report Forms (CRFs).

Sample Collection and Processing

We randomly selected 15 patients for fecal collection, including 8 patients from the treatment group and 7 patients from the control group. Fecal samples from the patients were collected at admission and after the 2-week treatment period. Two samples were obtained from each of the 15 patients. Fecal samples were processed in the laboratory within 4h after collection and stored at −80°C until analysis.

DNA Extraction and PCR Amplification

Considering the chances of live virus being present in feces, all the fecal samples were inactivated at 56°C for 30 min before DNA extraction. Microbial DNA was extracted from 200 mg of feces per sample using the E.Z.N.A.®soil DNA kit (Omega Bio-Tek, Norcross, GA, US) with a bead-beating step according to the manufacturer's protocol. The V4-V5 region of the bacteria 16S ribosomal RNA gene were amplified by PCR (95°C for 2 min, followed by 25 cycles at 95°C for 30s, 55°C for 30s, and 72°C for 30s and a final extension at 72°C for 5 min). The primers were 341F 5′-CCTAYGGGRBGCASCAG-3′ and 806R 5′-GGACTACNNGGGTATCTAAT-3′, where barcode is an eight-base sequence unique to each sample.[28] PCR reactions were carried out in triplicate 20μL mixture containing 4μL of 5 × FastPfu Buffer, 2μL of 2.5mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10ng of template DNA. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, US) according to the manufacturer's instructions.

Library Construction and Sequencing

Purified PCR products were quantified by Qubit®3.0 (Life Invitrogen) and every twenty-four amplicons whose barcodes were different were mixed equally. The pooled DNA product was used to construct Illumina Pair-End library following Illumina's genomic DNA library preparation procedure. Then, the amplicon library was paired-end sequenced (2 × 250) on an Illumina platform (Shanghai BIOZERON Biotech. Co., Ltd) according to the standard protocols.

Bioinformatics Analysis

Microbiome sequencing data analysis was performed using QIIME 2 (https://qiime2.org/).[29] The Cutadapt plugin trimmed primer sequences from both ends of reads and filtered sequences <200 bp. Denoising analysis was conducted via the DADA2 plugin: paired-end sequences were merged, low-quality reads and chimeras were removed, generating a feature table and representative sequences. The feature-table plugin subsequently filtered singletons and performed rarefaction on the feature table Finally, representative sequences were taxonomically annotated using a Naive Bayes classifier against the SILVA database (https://www.arb-silva.de/).

Processed data were imported into R, where the microeco package (https://github.com/ChiLiubio/microeco) was employed to perform: 1) microbial community composition analysis, 2) alpha and beta diversity analyses, and 3) differential abundance analysis. Alpha diversity was assessed using the Chao 1 index and Shannon index. Beta diversity was estimated by the Bray-Curtis distance and was visualized by principal coordinate analysis. Differential abundance analysis results underwent false discovery rate (FDR) correction, with adjusted values reported as q-values throughout the manuscript.

Outcomes

Primary Outcomes

The primary outcomes of the study include changes in disease severity, as measured by variations in the APACHE II score and SOFA score, as well as changes in gut microbiota.

Secondary Outcomes

The secondary outcomes include:

90-day mortality rate: The proportion of patients who die within 90 days of enrollment.

Improvement in condition: Defined as successful weaning from mechanical ventilation, discontinuation of continuous renal replacement therapy (CRRT), cessation of vasopressor treatment, discharge from the intensive care unit (ICU), or hospital discharge.

Changes in nutritional risk: Measured using NRS 2002 score.

Changes in TCM syndrome scores.

Changes in laboratory results: Including white blood cell count, percentage of neutrophils, C-reactive protein levels, and procalcitonin levels.

Statistical Analysis

SPSS23.0 was used for statistical analysis of data. The Fisher's exact test and Chi-square test was used to compare categorical data. Continuous variables were reported as means ± standard deviations, and statistical comparisons were made using the independent *t*-test. Non-normally distributed variables were expressed as the median and interquartile range (IQR), and comparisons were made using the Mann–Whitney *U*-test. Spearman rank correlation analysis was used to determine the correlation between the abundance of gut microbiota and the APACHE II and SOFA score. P-value or q-value (false discovery rate adjusted) less than 0.05 were considered statistically significant.

Results

Clinical Characteristics, Treatments and Outcomes

Among the 49 patients included in this study (Figure 1), 24 patients received the standard treatment for sepsis alone and were assigned to the control group, which comprised 16 (66.70%) were males. Twenty-five patients were administered XQHX in addition to standard therapy and were assigned to the treatment group, which comprised 17 (68.00%) males. The patients were mainly middle-aged or elderly, with the median age being 76 years. There was no significant difference with regard to sex or age between the two groups ($p > 0.05$). We used the NRS2002 score, the APACHE II score and the SOFA score to evaluate nutritional status and disease severity, respectively. There was no significant difference in either score between the two groups ($p > 0.05$). AGI was graded according to the AGI guidelines proposed by the European Society of Intensive Care Medicine (ESICM) in 2012. Twenty-two patients had grade I to II AGI, including 11 in the treatment group and 11 in the control group; 27 had grade III to IV AGI, including 14 in the treatment group and 13 in the control group. There was no significant difference in the ratio of the number of patients with grade I to II AGI to the number of patients with grade III to IV AGI between the two groups ($p > 0.05$), and there were no significant differences in inflammation grade, heart function, liver function, and renal function either (Table 1).

All the patients who were enrolled in the study received broad-spectrum antibiotics treatment, as per the standard protocol for sepsis treatment, with β-lactams and tetracyclines being the major classes of broad-spectrum antibiotics administered. β-lactams were the most frequently used: 32 cases (65.31%) were treated with β-lactam antibiotics alone. Tetracycline was not used in patient alone. However, 10 patients (20.41%) were treated with β-lactam combined with tetracycline (Table 2). The treatment of other antibiotics is shown in Table 2. There was no significant difference in the antibiotic regimens between the treatment and control groups (Table 1). In the treatment group, 13 patients (81.30%) showed improvement, but only 6 patients (40.00%) in the control group showed improvement. The difference in the improvement rate was significant between the two groups ($P = 0.042$) (Table 1).

Changes in Laboratory Indicators and Disease Severity Before and After Treatment

In the treatment group, there was a significant decrease in white blood cell (WBC) count, neutrophil percentage (N%), hypersensitive C-reactive protein (CRP) level, and procalcitonin (PCT) level after 14 days of treatment ($P < 0.05$ for all) (Figure 2). In the control group, we only observed a significant reduction in the WBC count ($P = 0.035$), and no significant changes were observed in the other indicators ($P > 0.05$ for all) (Figure 2). There were no significant different in WBC, N%, CRP or PCT before treatment in the two groups ($P > 0.05$), while after treatment, the level of CRP and PCT was significant lower in the treatment group (Figure 2). A decrease in the severity of disease was observed in both

Table 2 Antibiotic Regimen in 49 Patients with Acute Gastrointestinal Injury in Sepsis

	All Patients (n=49)	Treatment Group (n=25)	Control Group (n=24)
Broad-spectrum agents, n (%)			
β-lactams	32(65.31%)	17(68.00%)	15 (62.50%)
Aminoglycosides	1(2.04%)	0(0.00%)	1(4.17%)
β-lactams+ Tetracyclines	10(20.41%)	5 (20.00%)	5(20.83%)
β-lactams+ Tetracyclines +Fosfomycin	3(6.12%)	2(8.00%)	1(4.17%)
β-lactams+4-quinolones	1(2.04%)	0(0.00%)	1(4.17%)
Aminoglycosides+4-quinolones	1(2.04%)	0(0.00%)	1(4.17%)
Aminoglycosides+ Tetracyclines	1(2.04%)	1(4.00%)	0(0.00%)
Narrow-spectrum agents, n (%)			
Polypeptide	10(20.41%)	4 (16.00%)	6 (25.00%)
Linezolid	7(14.29%)	3(12.00%)	4 (16.67%)
Daptomycin	1(2.04%)	0(0.00%)	1 (4.17%)
Teicoplanin	1(2.04%)	1(4.00%)	0 (0.00%)

Abbreviations: Broad-spectrum agents, The agents which antibacterial activity of both Gram-positive bacteria and Gram-negative bacteria; Narrow-spectrum agents, The agents which antibacterial activity only one of Gram-positive or Gram-negative bacteria.

groups after treatment, but this change was more prominent in the treatment group. The scores of APACHEII and SOFA were decreased significantly in the treatment group, while were not in the other group (Figure 3).

Changes in Gut Microbiota Diversity Before and After Treatment

According to the Shannon diversity index and Chao diversity index, there was no significant difference in microbial diversity between the two groups before and after treatment. However, we observed a trend toward decreased gut microbiota diversity during sepsis, the change was not statistically significant (Figure 4). Principal coordinate analysis of Bray-Curtis distances indicated no significant differences in the fecal microbiota composition between the two groups before and after treatment. However, significant differences were observed within each group before and after treatment (Figure 4).

Differences in Gut Microbiota Composition Before and After Treatment

Before treatment, there was no significant difference in the microbial composition at the phylum, class, order, family, or genus between two groups. As expected, Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria were the four most dominant phyla. After treatment, we found that the abundance of Firmicutes in the treatment group ($q = 0.002$) and the control group ($q < 0.001$) increased significantly, while that of Proteobacteria decreased significantly ($q < 0.05$). In addition, the abundance of Bacteroidetes was significantly decreased in the treatment group ($q = 0.002$), but no significant change was observed in the control group (Figure 5A). Further, we found that in the treatment group, the ratio of Firmicutes to Bacteroidetes increased significantly after treatment ($p = 0.005$) (Figure 6).

Within Firmicutes, the relative abundance of *Enterococcaceae* increased significantly in both the treatment group ($q = 0.01$) and the control group ($q = 0.03$), while the relative abundance of *Lactobacillaceae* did not change significantly in either group. In the treatment group, the abundance of *Enterobacteriaceae* and *Bacteroidaceae* decreased significantly after treatment ($q = 0.04$ and $q = 0.006$, respectively), while there were no obvious changes in the control group (Figure 5D).

Further analysis of differences at the genus level showed that the gut microbiota of the two groups was mainly composed of *Enterococcus, Enterobacteriaceae/g-, Bacteroides, Bifidobacterium, Lactobacillus*, and *Parabacteroides* before treatment. After treatment, enrichment of *Enterococcus* was observed in both groups. The abundance of *Parabacteroides* and *Bacteroides* was significantly decreased in the treatment group ($q < 0.001$ and $q = 0.003$,

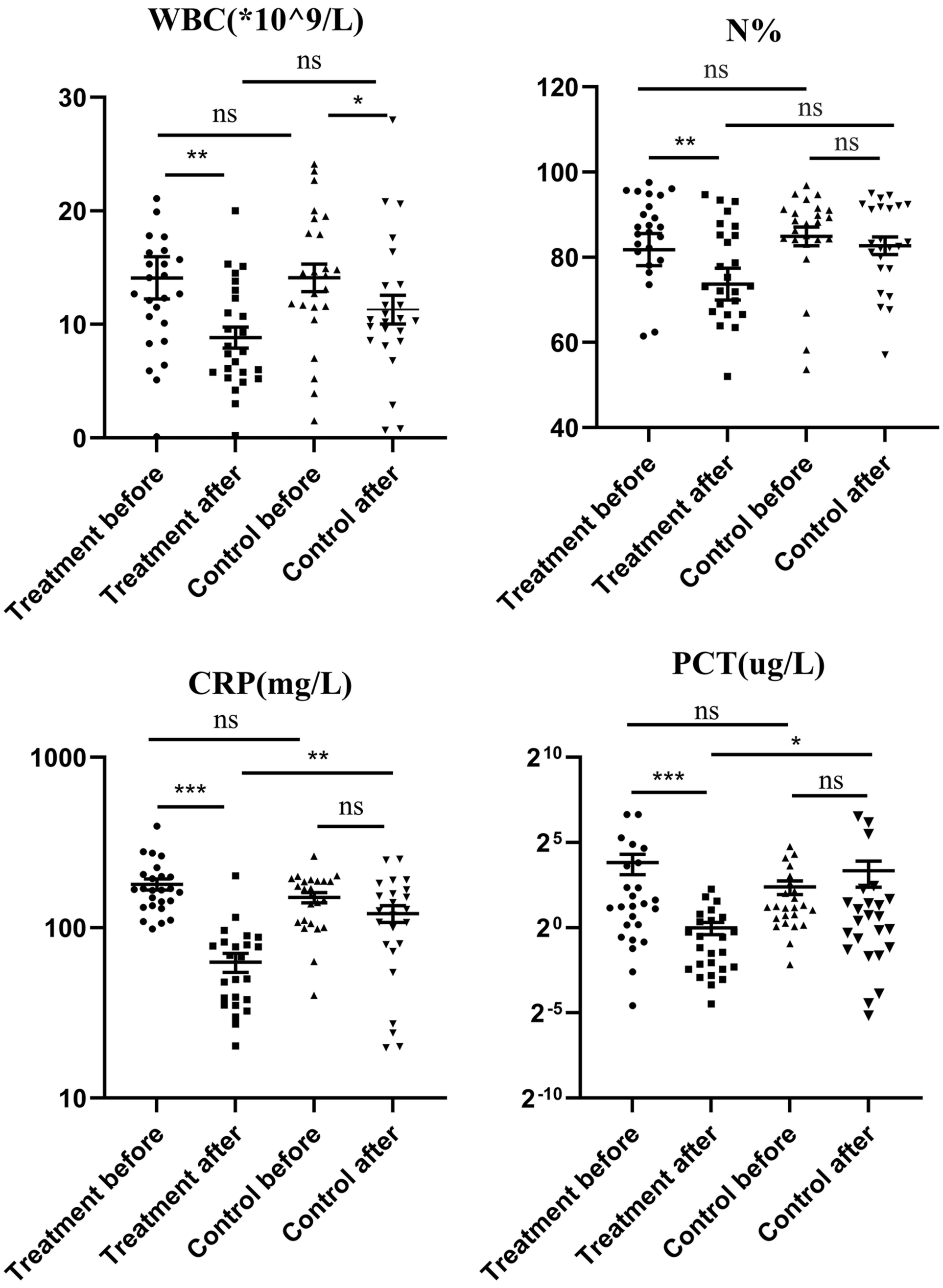

Figure 2 Comparison of the laboratory indicators between treatment group (n=25) and control group (n=24). The treatment group showed a significant decrease in inflammatory markers before and after treatment. P values 0.05–0.01, 0.01–0.001, <0.001 were considered statistically significant and marked as *, **, ***.
Abbreviations: WBC, white blood cell; N%, Neutrophil %; CRP, C-reactive protein; PCT, procalcitonin; ns: non-significant.

respectively), but this was not observed in the control group. The abundance of *Enterobacteriaceae/g-* also decreased significantly in the treatment group (q = 0.002), although this was not found in the control group (Figure 5E).

In addition, we found that in the treatment group, the abundance of *Bifidobacterium* increased after treatment, but it decreased in the control group. However, these changes were not significant (q > 0.05) (Figure 5E).

Correlation Between Gut Microbe Abundance and Disease Severity

The severity of sepsis associated with AGI was evaluated with the APACHE II score and SOFA score, which was based on the clinical data collected. Gut microbiota composition was mainly characterized at the genus level. The results

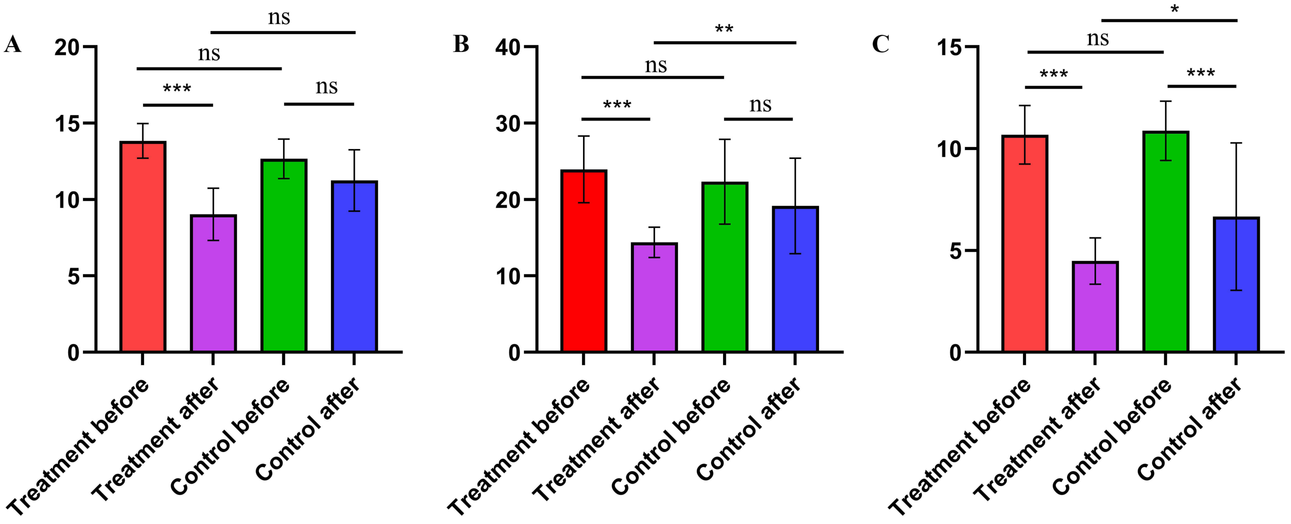

Figure 3 Differences in disease severity between the treatment group (n=25) and control group (n=24) before and after treatment. Compare the score of Sequential Organ Failure Assessment (SOFA), Acute Physiology and Chronic Health Evaluation-II (APACHEII), and Traditional Chinese medicine (TCM) syndrome in different groups. (**A**) SOFA, (**B**) APACHEII, (**C**) TCM syndrome. P values 0.05–0.01, 0.01–0.001, <0.001 were considered statistically significant and marked as *, **, ***.
Abbreviation: ns, non-significant.

showed that there was a strong negative correlation between the abundance of *Enterococcus* and the APACHE II score and SOFA score (R = −0.811 and −0.656). Additionally, the abundance of an unclassified genus of *Enterobacteriaceae* and *Bacteroides* was positively correlated with the APACHE II score (R = 0.778) and SOFA score (R = 0.499) (Figure 7).

Discussion

The present study assessed the effects of a TCM concoction, XQHX, formulated by our group, on patients with AGI associated with sepsis. Overall, the findings indicated that treatment with XQHX regulated the composition of gut microbiota in patients classified under the QSBSS according to TCM. And it seems to reduce the disease severity and improve the prognosis of these patients.

Sepsis is a critical disease that commonly occurs in the clinical setting and can involve multiple organs.[1] It is often associated with ischemia of the GI tract that results from a low perfusion rate and redistribution of blood.[5] It is also characterized by damage, and even necrosis, of intestinal mucosal cells and loss of normal function. As per TCM theory, this condition is regarded as a state of QSBSS[17], necessitating early intervention with qi-promoting and blood-activating therapies. Accordingly, the XQHX concoction prepared by us is based on the classic decoction called THSW, which has a well-established safety profile supported by extensive clinical experience.[25] It contains Aurantii Fructus, Magnoliae Officinalis Cortex, and Arecae Pericarpium for qi invigoration, and Angelicae Sinensis Radix, Drying Rehmanniae Radix, Chuanxiong Rhizoma, and Paeoniae Radix Rubra for improving blood circulation. As mentioned above, XQHX exhibits significant therapeutic potential for septic AGI, as explained by TCM theory and modern medicine. This is consistent with the findings of our study, in which patients who received early adjunctive treatment with the XQHX demonstrated lower APACHE II and SOFA scores, reduced inflammatory levels, and more favorable clinical outcomes.

Furthermore, we propose that XQHX decoction modulates intestinal microecology, offering insights into its therapeutic effects through gut microbiota regulation. Gut microbiota is an important factor associated with the condition of patients.[30,31] In patients with AGI associated with sepsis, the gut microbiome often exhibits a highly disturbed state characterized by a reduction in diversity and the abundance of harmful bacterial species.[8] TCM is widely recognized for its unique ability to modulate gut microbiota and restore microbial homeostasis, thereby playing a therapeutic role in the management of diverse diseases.[32,33] XQHX discussed contains several medicinal herbs with modulate gut microbiota, such as Rhei Radix et Rhizoma, Salviae Miltiorrhizae Radix et Rhizoma, Chuanxiong Rhizoma and Paeoniae Radix Rubra. For example, the compound emodin extracted from Rhei Radix et Rhizoma has been found to have a killing effect on *Bacteroides*,[34] and Paeoniae Radix Rubra also has an inhibitory effect on the growth of *Bacteroides*.[35] In our study,

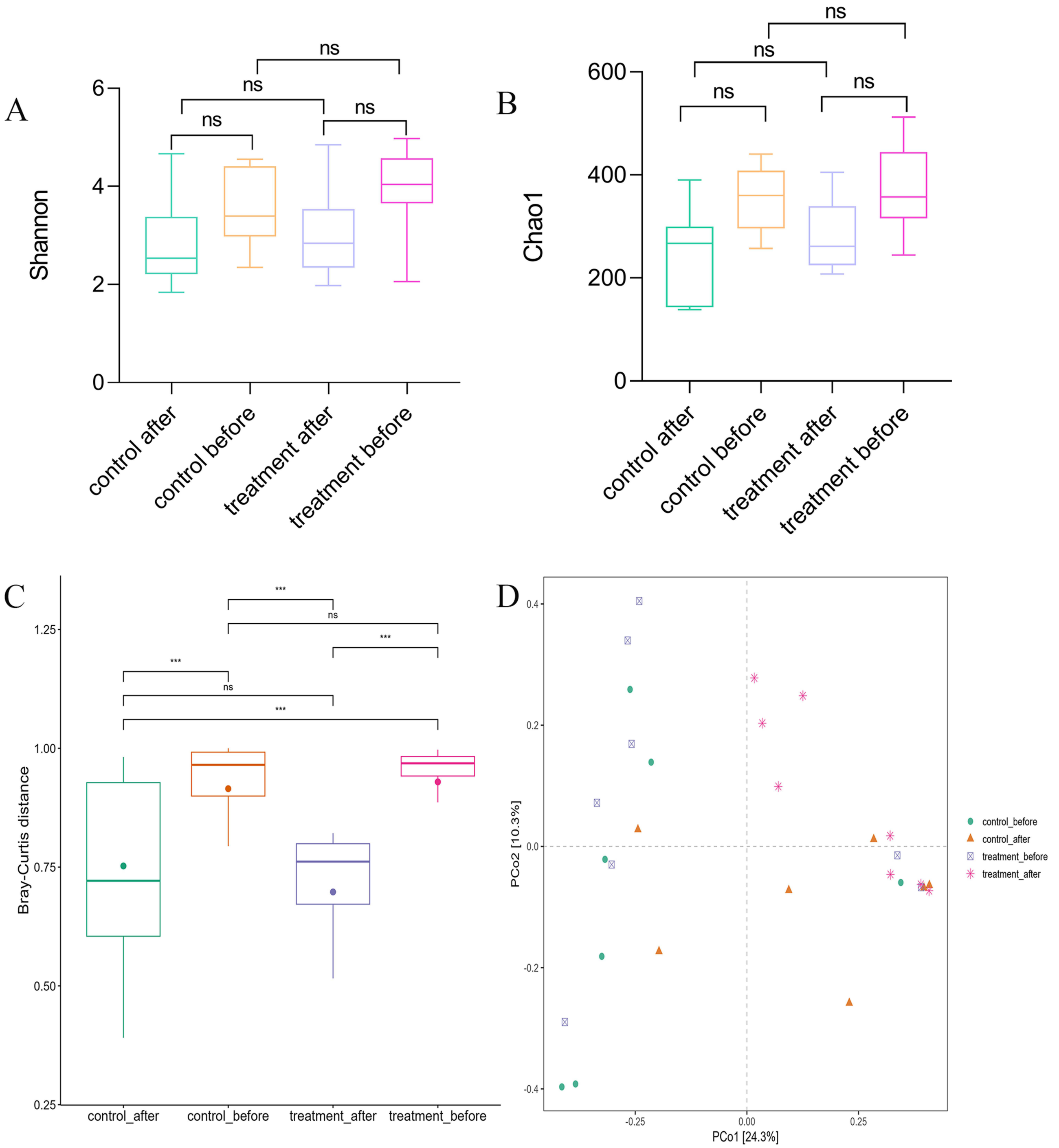

Figure 4 Changes in gut microbial diversity and community structures in the treatment group (n=8) and control group (n=7) before and after treatment. There were no significant differences in the alpha diversity of the gut microbiota among the groups based on the Shannon index (**A**) and Chao index (**B**). Beta diversity results were assessed by principal coordinate analysis (PCoA) of Bray-Curtis distances (**C** and **D**). P <0.05 was considered statistically significant and P-value<0.001 was marked as ***.
Abbreviations: ns, non-significant.

the treatment group showed a significant increase in the Firmicutes/Bacteroidetes (F/B) ratio after 2 weeks (Figure 6). The F/B ratio is widely recognized as a critical indicator of gut health[36]. Ojima et al reported that the ratio <1:10 may indicate elevated risk of poor prognosis, potentially associated with an increase in intestinal inflammation and weakening of intestinal barrier function.[10] Consequently, the elevated F/B ratio observed in our treatment group, absent in controls, may reflect the therapeutic efficacy of XQHX treatment.

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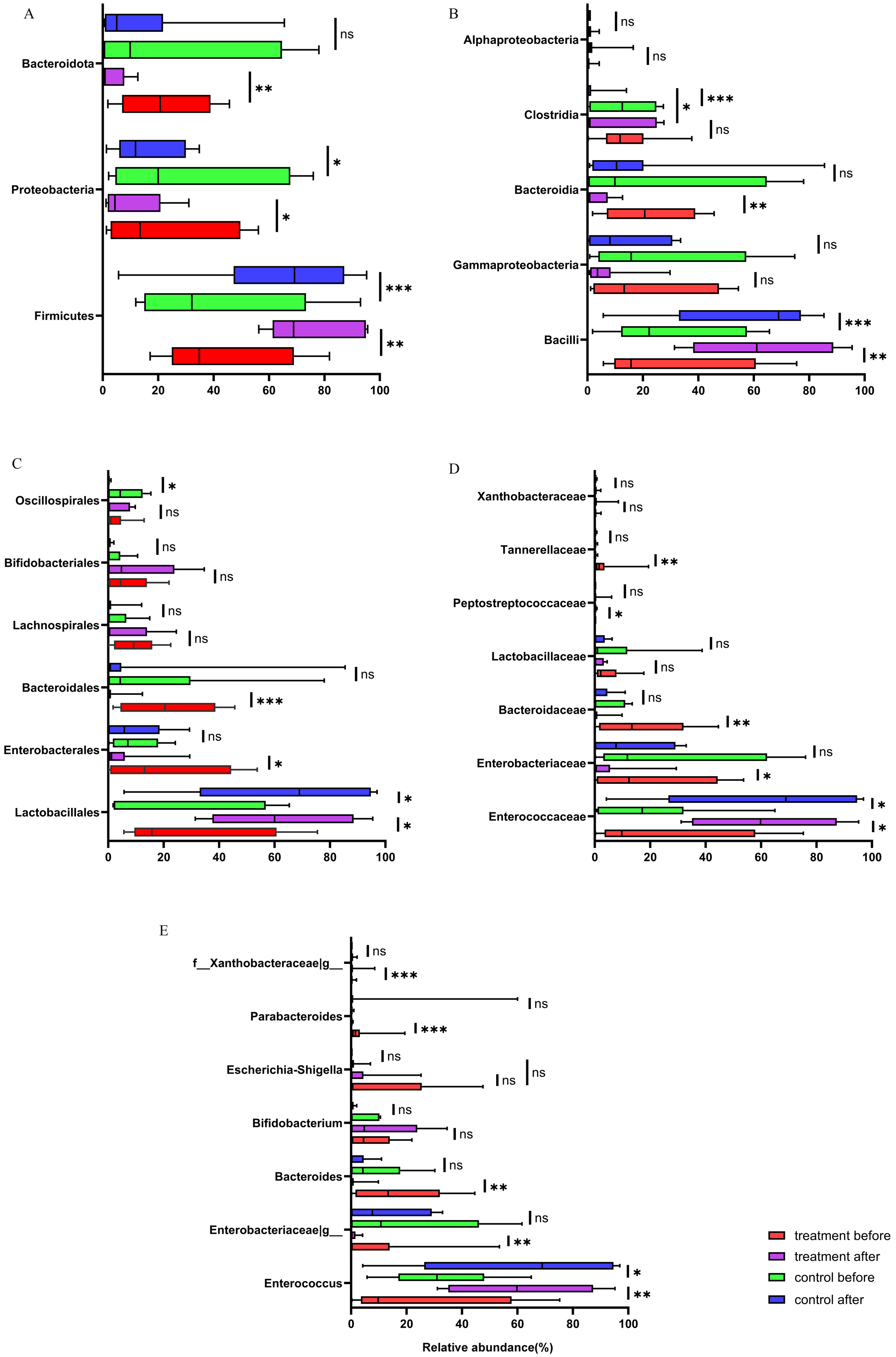

Figure 5 Taxonomic differences in the gut microbiota between the treatment group (n=8) and control group (n=7) before and after treatment. Comparison of the relative abundance at the phylum (**A**) class (**B**) order (**C**) family (**D**) and genus (**E**) levels across the groups. Each box represents an interquartile range (first and third quartiles) of taxa abundance, and the black line corresponds to median abundance. q-values 0.05–0.01, 0.01–0.001, <0.001 were considered statistically significant and marked as *, **, ***.
Abbreviations: ns, non-significant.

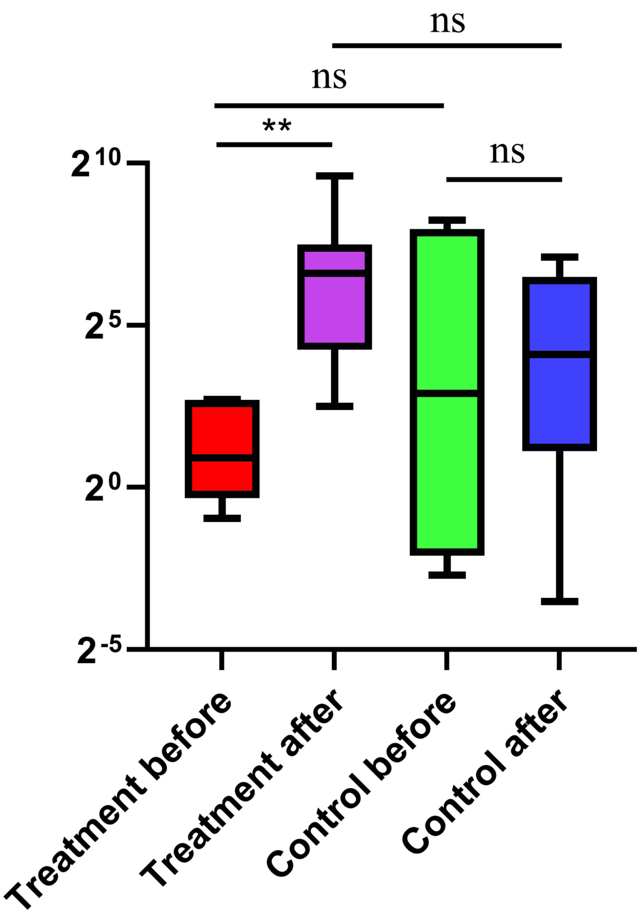

Figure 6 Changes in the ratio of Firmicutes to Bacteroidota between the treatment group (n=8) and control group (n=7) before and after treatment. In the treatment group, the ratio of Firmicutes to Bacteroidota increased significantly. P <0.05 was considered statistically significant and P value between 0.01 and 0.001 was marked as **.

During sepsis, the gut microbiota exhibits substantial proliferation of hypervirulent bacterial strains, which has been pathophysiologically implicated in both disease progression severity and the development of secondary infections[8] *Enterobacteriaceae/g-* and *Bacteroides* are high-virulence pathogenic microorganisms in the intestine that are associated with meningitis, abdominal infection, pneumonia, bloodstream infection, and so on.[37–39] They have synergistic effects, so the risk of enterogenic infection increases greatly when both these bacteria thrive.[39] In addition, it has been reported that *Parabacteroides* can metabolize succinic acid, which can promote the level of reactive oxygen species and the pro-inflammatory cytokines TNFα and IL-1β and, thus, increase intestinal inflammation.[40] Similarly, *Enterococcus* is a common pathogenic genus that is often associated with urinary tract infections, abdominal and pelvic infections, endocarditis, and other infections.[41] However, *Enterococcus* species can produce short-chain fatty acids,[42] which are important for maintaining the normal immune barrier of the gut. Moreover, *Enterococcus* species have genes that encode antimicrobial substances, such as bacteriocins, which can inhibit the growth of other pathogenic microorganisms.[43] We found that in the treatment group, the abundance of *Parabacteroides, Bacteroides*, and *Enterobacteriaceae/g-* decreased significantly, while *Enterococcus* abundance showed a significant increase (Figure 5). According to the study by Liu et al, in ICU patients, the aforementioned alterations in gut microbiota were closely associated with a reduced risk of septic shock and improved hyperlactatemia.[39] Similar findings were observed in US ICUs.[44] As noted above, *Parabacteroides, Bacteroides, and Enterobacteriaceae/g-* are highly virulent bacteria in the gut. *Enterococcus* contributes to pathogenicity while also maintaining intestinal barrier integrity and suppressing other pathogenic bacteria.[41–43] The properties of these microbiota may be linked to the observation, though the speculation awaits validation through further research. We further analyzed the correlation between these microbial shifts and disease severity. We found that abundance of *Enterobacteriaceae/g-* and *Bacter*oides was positively correlated with the APACHE II score (R = 0.778) and SOFA score (R = 0.499), while abundance of *Enterococcus* showed a negative correlation with the APACHE II score and SOFA score (R = −0.811 and −0.656) (Figure 7). This appears to seem to confirm our hypothesis that the modulation of gut microbiota by XQHX plays a significant role in reducing disease severity and improving clinical outcomes.

This study has several limitations. First, as a small-sample observational study, it lacked randomization and blinding. Controlling for confounding biases was challenging. Limiting causal inference regarding the relationship between the use of XQHX, clinical improvement, and alterations in the gut microbiota. Thus, our results require validation in larger-sample randomized clinical trials. Secondly, attrition occurred during the study. Only 15 patients completed fecal sample collection both pre- and post-treatment. This reduced the statistical power of the gut microbiota analysis. For example, while an upward trend in the probiotic (*Bifidobacterium)* was observed in the treatment group post-therapy, it did not reach statistical significance. The lack of significant validation may be attributable to the small number of fecal specimens collected. Third, patient assessment occurred only at 14 days post-XQHX treatment. The short follow-up

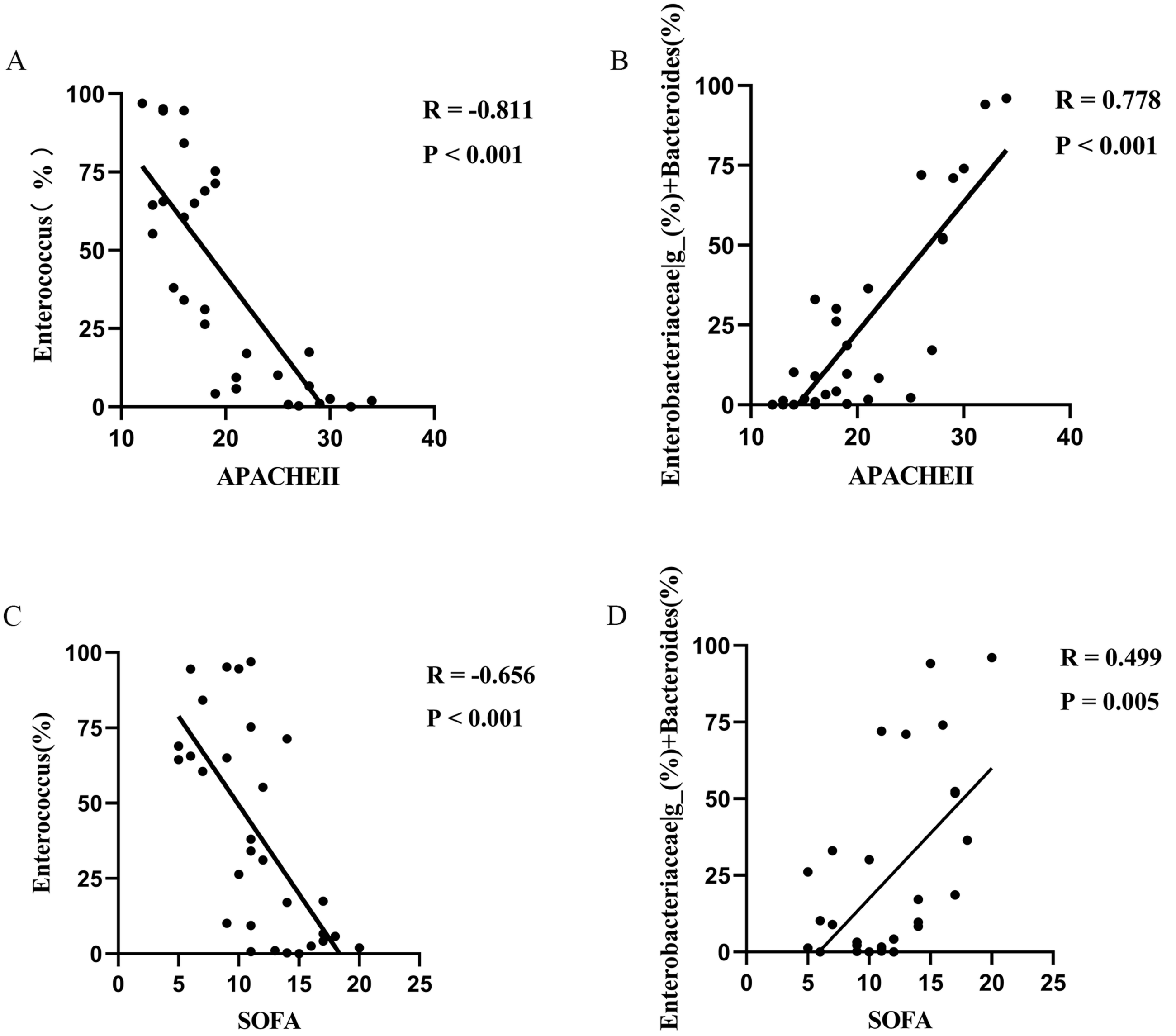

Figure 7 The associations between Acute Physiology and Chronic Health Evaluation-II (APACHEII) score, Sequential Organ Failure Assessment (SOFA) score and bacterial levels. The abundance of gut microbiota measured from sepsis patients with Acute Gastrointestinal Injury. The associations were analyzed using Spearman rank correlation analysis. (**A**) *Enterococcus* and APACHEII score, (**B**) *Enterobacteriaceae|g-* + *Bacteroides* and APACHEII score, (**C**) *Enterococcus* and SOFA score, (**D**) *Enterobacteriaceae|g-* + *Bacteroides* and SOFA score The abundance of *Bacteroides and Enterobacteriaceae|g-* were positively correlated with the APACHE-II score and SOFA score, while the *enterococcus* level showed a negative correlation. Data were derived from pre- and post-treatment measurements in 8 patients from the treatment group and 7 patients from the control group, with a total of 30 data points analyzed for correlation.

period limited our observation of long-term outcomes and precluded evaluation of any potential positive effect of XQHX on gut microbiota reconstruction. For instance, we observed a declining trend in gut microbiota diversity post-sepsis treatment, though statistically insignificant. And according to studies, it takes approximately 4–8 weeks or longer timeframe for microbiota diversity restoration.[45,46] Therefore, we intend to incorporate additional follow-up time points in future studies to better characterize XQHX's long-term efficacy. Finally, although XQHX evolved from the classical formula (THSW), its composition was augmented with additional components (eg, Rhei Radix et Rhizoma, Magnoliae Officinalis Cortex, and Aurantii Fructus, etc). This increased complexity necessitates further investigation into precise mechanisms of action. We consider it imperative to conduct clinical and pharmacological studies with expanded sample sizes.

Despite these limitations, our findings provide crucial real-world evidence suggesting a beneficial role for the TCM formulation XQHX in treating patients with septic AGI classified as QSBSS according to TCM theory. This study strikes a balance between the encouraging real-world performance of TCM and the necessity for rigorous evidence generation, laying a foundation for larger-scale randomized controlled clinical trials.

Conclusion

Our preliminary findings suggest that patients with septic AGI classified as QSBSS according to TCM theory exhibited accelerated recovery and improved clinical outcomes following adjunctive treatment with the XQHX. These improved outcomes may be associated with alterations in the gut microbiota induced by XQHX. The small sample size and observational nature of this study limit definitive conclusions. Future randomized controlled trials are warranted to validate these findings, which serve as an important foundation for subsequent research. We will further investigate the efficacy of XQHX, elucidate its underlying mechanisms of action, and explore its potential to expand therapeutic options for septic AGI.

Abbreviations

AGI, Acute gastrointestinal injury; APACHE II, Acute Physiology and Chronic Health Evaluation II; ESICM, European Society of Intensive Care Medicine; SOFA, Sequential Organ Failure Assessment; TCM, Traditional Chinese medicines; XQHX, *XingQiHuoXue*; QSBSS, Qi stagnation and blood stasis syndrome; THSW, *Taohong Siwu*; WBC, white blood cell; N%, Neutrophil %; CRP, C-reactive protein; PCT, Procalcitonin; TB, Total bilirubin; DB, Direct bilirubin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; CK, Creatine kinase; LDH, Lactate dehydrogenase; Cr, Creatinine; Alb, Albumin.

Data Sharing Statement

The data analyzed in this study can be made available by the corresponding author on reasonable request, and all sequence data were deposited in the NCBI Sequence Read Archive (SRA) database (PRJNA1065323).

Ethics Approval

This study was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Zhejiang Chinese Medical University (No. 2020-KL-101-03), following the Declaration of Helsinki.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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