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The role of probiotics in coronavirus disease-19 infection in Wuhan: A retrospective study of 311 severe patients

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ABSTRACT

Purpose: Researches revealed that probiotics maybe a potential strategy for COVID-19, whereas there is a lack of related evidence. This study aims to analyze the role of probiotics on severe COVID-19 patients.
Methods: In the current retrospective single-center study, we collected data of 311 consecutive severe patients with confirmed COVID-19 in Wuhan Union Hospital from Feb 3rd to Feb 20th, 2020. Epidemiological, clinical and medication characteristics were compared and analyzed between patients with or without probiotics.
Results: In total, 93 of the 123 patients (75.61%) who were treated with probiotics survived to hospital discharge with the median inpatient day of 32 days and mean virus clearance time of 23 days, which were significantly longer than those of patients without probiotics. There were no bias in laboratory parameters, except for IL-6 and ESR, which were significantly higher in patients treated probiotics. We tracked the dynamic changes of 8 selected laboratory parameters (IL-6, CRP, total T lymphocytes, NK cells, B lymphocyte, CD4 + T cells, CD8 + T cells and CD4/CD8 ratio) and found that probiotics could not reduce the increased IL-6 levels but possessed the ability to moderate the immunity and decreased the incidence of secondary infection in COVID-19 patients.
Conclusions: Probiotics could be an effective strategy for the treatment of COVID-19 patients to reduce the secondary infection and moderated the immunity.

1. Introduction

Because it is highly contagious and person-to-person transmission occurs through direct contact, droplets or fomites, COVID-19 has spread all over the world rapidly since December 2019, when the novel coronavirus (2019-nCoV) was isolated [1,2]. As once the epicenter of COVID-19, there were 50,340 patients confirmed COVID-19 in Wuhan, approximately 7–10% of which were severe/critical cases [3] and transferred to the designated hospital for treatment.

At present, there is no proven regimen other than conventional medicine. Antiviruses (arbidol, alpha-interferon, ribavirin, lopinavir/ritonavir and resochin), anti-inflammatory agents (corticosteroids) and symptomatic supportive treatments (immunopotentiators, intestinal probiotics) are recommended for the treatment of severe COVID-19 patients in the Chinese management guidelines for COVID-19 (version 7.0) [4].

Probiotics, which are usually used to treat diarrhea, are live microorganisms or components of dead bacteria which could regulate the immunity through the route of the gut to exert the resistance against antibiotics, xenobiotics and pathogenicity or toxicity factors [5]. Interestingly, recent research found that probiotics could be used as coadjuvants in treating metabolic disorders such as type 2 diabetes, obesity and metabolic syndrome [6]. At present, although the lesions are primarily located in the lung, later studies, expanded the distribution of COVID-19 to the GI system [7], which might explain the presence of diarrhea as one of the symptoms of COVID-19. Dysbiosis of the human gut microbiome is linked to respiratory tract infections (RTIs) through the gut-lung axis. Previous studies showed that various strains of *Lactobacillus* spp. and *Bifidobacterium* spp. through oral or intranasal administration have shown suppression of infection symptoms against

Abbreviations: CREA, creatinine; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; T-BIL, total bilirubin; HGB, hemoglobin; PLT, platelets; WBC, white blood cell; NE, neutrophil; EO, eosinophilic granulocyte; CRP, c-reactive protein; PCT, procalcitonin; IL-6, interleukin 6; FIB, Fibrinogen; ESR, erythrocyte sedimentation rate.

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viral infections [8,9]. Other research found that SARS-CoV could bind angiotensin-converting enzyme 2 (ACE2) [10], which is expressed in endothelial cells of the vasculature, epithelia of the lungs and intestine [11]. ACE2 exerted protective effects in infection induced acute lung injury mediated through activation of the reninangiotensin system (RAS), and the expression of ACE2 was increased in patients treated with ACE inhibitors [12]. Meanwhile, several probiotics, particularly probiotic lactic acid bacteria, have been reported to be able to produce peptides with ACE inhibitory effects [13]. Thus, probiotics might improve RTIs in COVID-19 patients through the ACE2 pathway.

Moreover, the health effect of probiotics are benefit for the treatment of COVID-19, such as the ability to support intestinal integrity and maintain intestinal permeability, competition with pathogens for nutrients and attachment sites, and the modulation of immune cell activity against invading pathogens [14] the regulation of balancing proinflammatory and anti-inflammatory cytokines [15], which are grossly imbalanced in COVID-19 patients who suffer cytokine storms [16]. Patients with high concentrations of TNF-α, and IL-6 showed severe consequences of COVID-19. Probiotics have an immune regulation effect through local immunity (by maintaining gut wellbeing and gut wall integrity) and systemic immunity (by enhancing specific and nonspecific immune system) [17]. Probiotics improved the levels of NK cells, T and B lymphocytes, type I interferons and antigen-presenting cells (APCs) in the lung immune system [18]. Another study suggested that probiotics modulate the expression of interleukin-10 (IL-10) and decrease the expression of inflammatory cytokines, such as TNF-α, IL-1 and IL-8 [19]. And many studies have found that probiotics possessed a therapeutic role in viral respiratory infections [20]. Probiotic *bifidobacteria* could decrease the duration of respiratory, symptoms caused by the common cold coronavirus and the days with fever [21]. And clinical trial indicated that patients treated with probiotics containing *Lactobacillus rhamnosus* GG, live *Bacillus subtilis*, and *Enterococcus faecalis* showed significantly less ventilator-associated pneumonia compared to those without probiotics [22]. However, the therapeutic strength of probiotics in COVID-19 patients is still uncertain due to limitation of available clinical data [5].

Immunonutrition, which includes feeding various pharmaconutrients, such as arginine, vitamins and probiotics, was used to modify inflammatory or immune responses. Many studies have shown that immunonutrients promote patient recovery by inhibiting inflammation or immune responses [23]. In fact, the gene expression and maturation and differentiation of immune cells are regulated by some nutrients and their metabolites [24]. Hence, immunonutrition, including functional food components, prebiotics and probiotics, may be a potential treatment for COVID-19 patients via microbiota modulation and immunoregulation [25]. However, all these assumptions were based on previous studies, and more clinical evidence is needed to support the use of probiotics in COVID-19 patients. Although many clinical trials on COVID-19 are ongoing, few studies the effect of intestinal probiotics on COVID-19.

Here, to confirm and further explore the possible mechanisms of probiotics in COVID-19 patients, we performed a single-center retrospective analysis at Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. We collected the clinical characteristics, medication and outcomes of 311 severe COVID-19 patients and analyzed the effect of probiotics on immunity and inflammation. We hope this study may provide evidence for the treatment of probiotics in COVID-19.

2. Methods

2.1. Ethics statement

This study was approved by the Medical Ethical Committees of Wuhan Union Hospital (2020-0104). The requirement for written informed consent was waived because there was no intervention for treatment and potential risk to patients and the data were analyzed anonymously.

2.2. Study design and participants

This retrospective single-center study included severely ill adult inpatients (≥18 years old), who were admitted to Wuhan Union Hospital, the designated hospital for severe patients, from February 3 to February 20, 2020. All patients were diagnosed as COVID-19 positive according to WHO interim guidance, and the treatment was in line with the Chinese management guideline for COVID-19 (version 7.0). The clinical characteristics were followed up to March 15, 2020. Regarding complications of medication regimens, we excluded patients who died.

2.3. Medication

Probiotics were recommended to maintain intestinal microecological balance and prevent secondary bacterial infection in Chinese management guidelines for COVID-19 (version 7.0). According to whether probiotics were used, patients were divided into probiotic group and nonprobiotic group. Probiotics treatment included oral Combined *Bifidobacterium, Lactobacillus, Enterococcus* and *Bacillus* tablets (*Bifidobacterium infantis* [26], *Lactobacillus acidophilus* [27], *Dung enterococcus, Bacillus cereus* [28]) 1.5 g tid; Live combined *Bifidobacterium* and *Lactobacillus* tablets (*Bifidobacterium longum* [29], *Lactobacillus bulgaricus* [30], *Streptococcus thermophiles* [31]) 2 g tid; Live combined *Bacillus Subtilis* and *Enterococcus Faecium Enteric*-coated Capsules (*Enterococcus faecium* [32], *Bacillus subtilis* [33]) 0.5 g tid. It was up to the attending physician to decide whether to give probiotics based on the patient's condition and the mean duration of probiotics was 12.94 days.

Additional medicines included the following. Chloroquine phosphate, 500 mg bid, the course of treatment should not exceed 10 days. Arbidol, 200 mg tid was administered, and the course of treatment should not exceed 10 days. Lopinavir/ritonavir, 200 mg/50 mg/grain, 2 grain a time, bid, the course of treatment should not exceed 10 days. Ribavirin[b] was recommended for use in combination with interferon alpha inhalation or lopinavir/ritonavir, 500 mg bid, and the course of treatment should not exceed 10 days. Interferon alpha inhalation, 5 million U bid. Antibiotics included quinolone, cephalosporin and carbapenems. Lianhua qingwen capsule, 4 grain a time, tid. Traditional Chinese medicine decoction, bid. The immune enhancer included thymopetin for injection (1 mg, qd), and thymalfasin for injection (1.6 mg, biw). Sedative hypnotic therapy included estazolam tablets (1 mg, qn). Corticosteroids (Solu Medrol) were given not more than 1–2 mg/kg/day (duration not more than 5 days). The utilization rates of these medications are showed in Table 3.

2.4. Procedures

The epidemiological and clinical characteristics, medication, and laboratory parameters of confirmed severe cases of COVID-19 were collected from electronic medical records by a standardized case report form. All data were checked by two experienced individuals independently. The illness severity of COVID-19 was defined according to the Chinese management guideline for COVID-19 (version 7.0) [4].

The method used for laboratory confirmation of COVID-19 is to perform real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) assay tests using throat swab specimens that were obtained from upper respiratory tracts every other day after clinical remission of symptoms, including fever, cough, and dyspnea. These laboratory confirmations were performed at Wuhan Union Hospital following the standard protocol. Routine blood examinations, coagulation profiles, serum biochemical tests, plasma levels of inflammatory factors (including interleukin-6 (IL-6)), C-reactive protein, and procalcitonin were collected as long as these tests were performed. The frequency of tests was determined by the treating physician according to the

conditions of the patients. Medication regimen were divided into antiviral drugs, antimicrobial drugs, traditional Chinese medicine, immunopotentiators, and probiotics.

The criteria for discharge were absence of fever for at least 3 days, substantial improvement in both lungs on chest CT, clinical remission of respiratory symptoms, and two throat-swab samples negative for the COVID-19 test obtained at least 24 h apart [4].

2.5. Statistical analysis

Categorical variables are described as frequency rates and percentages. Proportions for categorical variables were compared using the $\chi 2$ test, although Fisher's exact test was used when the data were limited. Means for continuous variables were compared using independent group t tests when the data were normally distributed and described using the mean ± standard deviation; otherwise, the Mann-Whitney test was used and were described using mean, median, and interquartile range (IQR) values. Logistic regression was used to select independent risk factors that affect outcomes. All statistical analyses were performed using SPSS version 20.0 software. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Outcome

The current study described 311 severe patients with COVID-19 who admitted in Wuhan Union hospital from Feb 3, 2020 to Feb 15, 2020. Outcomes were assessed as discharged, remaining in hospital till Mar 15, 2020. Fig. 1 illustrated the outcome of patients confirmed with COVID-19. In total, 93 of the 123 patients (75.61%) who were treated with probiotics survived to hospital discharge with the median inpatient day of 32 days and mean virus clearance time of 23 days, and 147 of the 188 (78.19%) patients without the treatment of probiotics discharged with the median inpatient day of 20 days and mean virus clearance time of 17 days. Significant differences existed across the treatment of probiotics with regards to inpatient days ($P < 0.001$) and virus clearance time ($P < 0.001$).

4. Demographic and epidemiologic characteristics

Table 1 shows the epidemiologic characteristics of 311 severe patients infected with COVID-19. Of 311 patients, the mean time from illness onset to hospital admission ($P = 0.323$) was 13 days. A total of 184 patients (59.16%, $P = 0.712$) had comorbid diseases, such as chronic bronchitis (4.18%, $P = 0.069$), hypertension (35.05%, $P = 0.482$), diabetes (19.29%, $P = 0.497$), heart disease (15.43%, $P = 0.110$), renal failure (3.22%, $P = 0.199$), liver failure (2.57%, $P = 0.394$) and tumors (7.72%, $P = 0.499$). On admission, 254 patients had fever (81.67%, $P = 0.182$) as the initial symptom, and 226 patients had cough (72.67%, $P = 0.719$). Other symptoms were cough phlegm (36.98%, $P = 0.042$), shortness of breath (45.33%, $P = 0.774$) and diarrhea (21.54%, $P = 0.323$). Among them, only cough phlegm ($P < 0.05$) showed a significant difference between patients with or without probiotics.

Table 1
Demographic and Epidemiologic Features of severe COVID-19 Patients on admission.

	No. (%)			P Value
	Total(n = 311)	Nonprobioctics (n = 188)	Probiotics (n = 123)	
Age	60.10 ± 12.37	60.20 ± 12.67	62.019 ± 10.88	0.196
Sex (Male)	150 (48.23%)	83(44.15%)	67(54.47%)	0.075
Comorbidities	184 (59.16%)	114 (60.64%)	72 (58.54%)	0.712
Chronic bronchitis	13(4.18%)	11(5.85%)	2(1.63%)	0.069
Hypertension	109 (35.05%)	63(33.51%)	46(37.40%)	0.482
Diabetes	60 (19.29%)	34(18.09%)	26(21.14%)	0.497
Heart disease	48 (15.43%)	34(18.09%)	14(11.38%)	0.110
Renal failure	10(3.22%)	8(4.26%)	2(1.63%)	0.199
Liver failure	8(2.57%)	6(3.19%)	2(1.63%)	0.394
Tumor	24(7.72%)	13(6.91%)	11(8.94%)	0.499
Time from onset of illness to inpatients	13.00 ± 6.13	12.3 ± 5.88	13.1 ± 6.41	0.323
Fever	254 (81.67%)	158 (84.04%)	96(78.05%)	0.182
Cough	226 (72.67%)	138(73.40%)	88(71.54%)	0.719
Cough phlegm	115 (36.98%)	78(41.49%)	37(30.08%)	0.042*
Shortness of breath	141 (45.33%)	84(44.68%)	57(46.34%)	0.774
Diarrhea	67 (21.54%)	37(19.68%)	30(24.39%)	0.323

Data are presented as mean ± SD, n (%).
* Significant at $P < 0.05$.

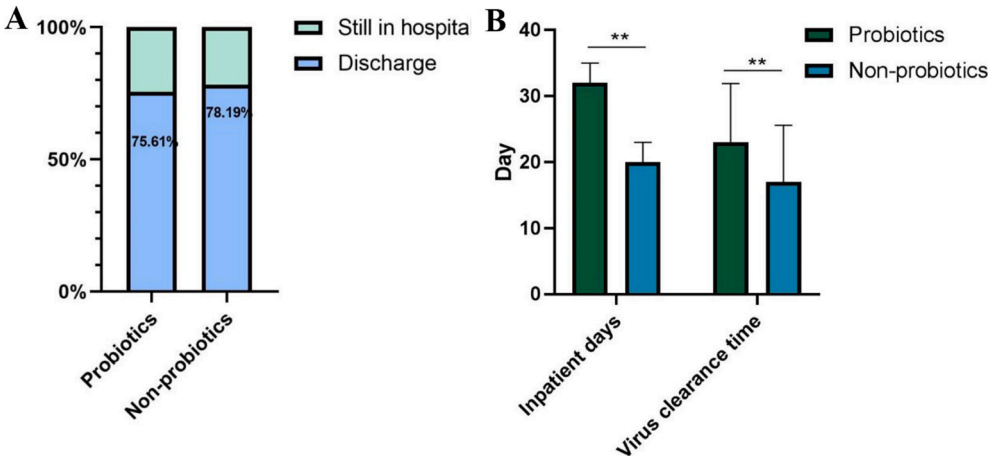

Fig 1. The outcomes between patients treated with or without probiotics. (A), the discharge rate of patients in different probiotics treated groups; (B), inpatient days and virus clearance time of patients in different probiotics treated groups.

4.1. Laboratory findings

Table 2 shows the laboratory findings of severe COVID-19 patients on admission.

We included 6 blood biochemistry parameters (CREA (P = 0.088), BUN (P = 0.183), AST (P = 0.692), ALT (P = 0.0.595), LDH (P = 0.122) and T-BIL (P = 0.847), 6 routine blood parameters (lymphocytes (P = 0.635), HGB (P = 0.254), PLT (P = 0.159), WBC (P = 0.858), NE (P = 0.923) and EO (P = 0.820)), 4 inflammatory-related biomarkers (CRP (P = 0.077), PCT (P = 0.476), ESR (P = 0.049) and IL-6 (P = 0.001)), 2 coagulation function biomarkers (D-Dimer (P = 0.697) and FIB (P = 0.476)), and 6 immune-related biomarkers (Total T lymphocyte (P = 0.965), CD4 (P = 0.750), CD8 (P = 0.669), B lymphocyte (P = 0.462), NK cell (P = 0.280) and CD4/CD8 ratio (P = 0.651)). Among these biochemistry parameters, only IL-6 (P = 0.001) and ESR (P = 0.049) were at significantly higher levels in patients treated with probiotics.

Table 2
Laboratory findings of severe COVID-19 patients on admission.

	Normal range	Median (IQR)		P Value
		Nonprobioctics (n = 188)	Probiotics(n = 123)	
Blood biochemistry				
CREA, μmol/L	57–111	63.8(53.8–76.4)	68.0 (53.8–72.1)	0.088
BUN, mmol/L	2.9–8.2	4.0(1.0–4.5)	4.0(3.4–5.6)	0.183
AST, U/L	8–40	41.0(35.5–51.0)	23.0 (15.0–32.0)	0.692
ALT, U/L	5–40	69.0(53.0–91.0)	19.0 (13.0–41.5)	0.595
LDH, U/L	109–245	194.0 (166.5–249.5)	211.0 (188.0–239.5)	0.122
T-BIL, umol/L	3–20	12.5 ± 7.19	12.4 ± 5.72	0.847
Blood routine				
Lymphocytes, G/L	1.1–3.2	1.1 ± 0.49	1.0 ± 0.46	0.635
HGB, g/L	40–50	122.8 ± 21.47	125.4 ± 16.30	0.254
PLT, G/L	125–350	248.8 ± 92.4	233.4 ± 92.69	0.159
WBC, G/L	3.5–9.5	5.9 ± 2.43	5.9 ± 2.43	0.858
NE, G/L	1.8–6.3	4.4 ± 2.42	4.3 ± 2.35	0.923
EO, G/L	0.01–0.52	0.07 ± 0.13	0.07 ± 0.30	0.820
Infection-related biomarkers				
CRP, mg/L	0–8	2.5(1.3–2.7)	11.7(2.7–73.2)	0.077
PCT, ng/ml	≤0.05	0.05(0.03–0.05)	0.05 (0.03–0.12)	0.476
ESR, mm/h	0–20	49.0 ± 30.59	63.4 ± 36.56	0.049*
IL-6	0.1–5	4.1(3.6–5.4)	8.1(5.2–12.6)	0.001**
Coagulation function				
D-Dimer, mg/L	0–0.5	1.3(0.7–1.4)	0.4(0.3–1.0)	0.697
FIB, G/L	2.0–4.0	4.4 ± 1.19	4.3 ± 1.31	0.476
Immunity				
Total T lymphocyte	58.17–84.22	71.8 ± 9.88	71.9 ± 8.53	0.965
CD4	25.34–51.37	42.8 ± 8.66	43.4 ± 9.01	0.750
CD8	14.23–38.95	24.95 ± 9.79	26.01 ± 8.6	0.669
B lymphocyte	4.1–18.31	13.77 ± 9.75	12.44 ± 8.33	0.462
NK cell	3.33–30.47	8.5 ± 5.24	9.8 ± 6.11	0.280
CD4/CD8 ratio	0.41–2.72	2.18 ± 1.23	2.07 ± 1.36	0.651

Data are presented as medians (interquartile ranges, IQR), n (%) and mean ± SD; *P < 0.05, **P < 0.01.

CREA, creatinine; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; T-BIL, total bilirubin; HGB, hemoglobin; PLT, platelets; WBC, white blood cell; NE, neutrophil; EO, eosinophilic granulocyte; CRP, c-reactive protein; PCT, procalcitonin; IL-6, interleukin 6; FIB, Fibrinogen.

4.2. Treatment analysis

Patients confirmed with COVID-19 were treated with supportive care and empiric medication. As Table 3 shows, almost all patients received one or more antiviral treatments, such as chloroquine (13.18%, P = 0.156), arbidol (97.11%, P = 0.699), lopinavir and ritonavir (26.05%, P = 0.001), ribavirin (31.83%, P = 0.012) and interferon (27.01%, P = 0.001). The use of lopinavir/ritonavir (P = 0.001), ribavirin[b] (P = 0.012) and interferon alpha inhalation (P = 0.001) was significantly higher in patients treated with probiotics. Other treatments, including antibiotics (72.03%, P = 0.879), Lianhua qingwen capsules (51.77%, P = 0.992), traditional Chinese medicine decoctions (95.82%, P = 0.935), immune enhancement (77.81%, P = 0.231), sedative hypnotic therapy (17.04%, P = 0.543) and corticosteroids (21.86%, P = 0.756) were also used in COVID-19 patients and showed no significant differences between patients treated with probiotics and those treated without probiotics.

4.3. Effect of probiotics on immune and inflammation

To determine the potential effects of probiotics on immunity and inflammation, we tracked the dynamic changes in 8 selected laboratory parameters (IL-6, CRP, total T lymphocytes, NK cells, B lymphocytes, CD4 + T cells, CD8 + T cells and CD4/CD8 ratio) during the course of COVID-19. T1 refers to the first test after hospital admission, T2 refers to the midpoint test during the whole hospital stay, and T3 refers to the last test before discharge. As Fig. 2A shows, patients treated with probiotics had higher multiple antibiotic ratios (P = 0.001) and longer antibiotic durations (P = 0.001). However, there were partial superpositions between the curve of CRP for patients who were treated with or without probiotics (Fig. 2C). For IL-6 (Fig. 2B), the curve separated clearly (P greater than 0.05) and showed an increasing trend in probiotic-treated patients. As Fig. 2D-2I shows, immune parameters between patients with probiotics and without probiotics revealed opposite trends. Total T lymphocytes, NK cells and B lymphocytes were upregulated in probiotic-

Table 3
Treatment analysis of severe COVID-19 patients.

	No. (%)			*P* Value
	Total(n = 311)	Non-probioctics(n = 188)	Probiotics(n = 123)	
Chloroquine phosphate	41 (13.18%)	29(15.43%)	12(9.76%)	0.156
Arbidol	302 (97.11%)	182(96.81%)	120 (97.56%)	0.699
Lopinavir/ritonavir	81 (26.05%)	31(16.49%)	50(40.65%)	0.001**
Ribavirin[b]	99 (31.83%)	50(26.60%)	49(39.84%)	0.012*
Interferon alfa inhalation	84 (27.01%)	36(19.15%)	48(29.27%)	0.001**
Antibiotic	224 (72.03%)	136(72.34%)	88(71.54%)	0.879
Traditional Chinese medicine				
Lianhua qingwen capsule	161 (51.77%)	98(52.13%)	63(51.22%)	0.992
Traditional Chinese medicine decoction	298 (95.82%)	180(95.74%)	118 (62.77%)	0.935
Immune enhancer	242 (77.81%)	142(75.53%)	100 (81.30%)	0.231
Sedative hypnotic therapy	53 (17.04%)	30(15.96%)	23(18.70%)	0.543
Corticosteroid	68 (21.86%)	40(21.28%)	28(22.76%)	0.756

Data are presented as n (%).
* P < 0.05.
** P < 0.01.

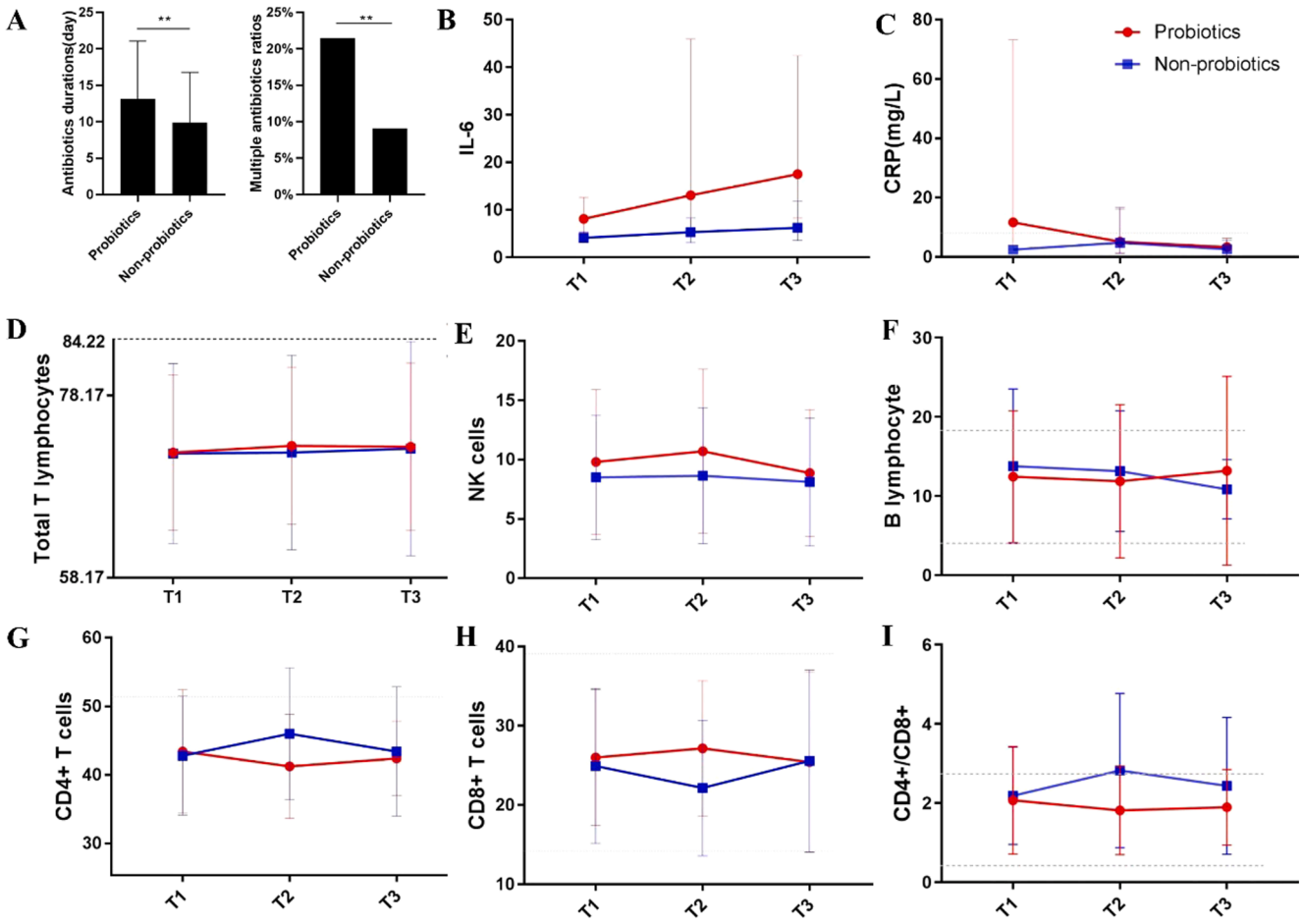

Fig 2. The dynamic changes in selected inflammatory and immunological parameters during the course of COVID-19. (A), the antibiotic treatment of patients in different probiotics treated groups; (B), the dynamic change of IL-6 in patients in different probiotics treated groups; (C), the dynamic change of CRP level in patients in different probiotics treated groups; (D), the dynamic change of total T lymphocytes in patients in different probiotics treated groups; (E), the dynamic change of NK cells in patients in different probiotics treated groups; (F), the dynamic change of B lymphocytes in patients in different probiotics treated groups; (G), the dynamic change of CD4 + T cells in patients in different probiotics treated groups; (H), the dynamic change of CD8 + T cells in patients in different probiotics treated groups; (I), the dynamic change of CD4/CD8 ratio in patients in different probiotics treated groups.

treated patients. The CD4+/CD8 + ratio in patients without probiotics increased beyond the normal range, whereas this parameter in probiotic-treated patients remained within the normal range.

5. Discussion

At present, there are no approved specific antiviral agents targeting the novel coronavirus [34], and supportive therapies are the main treatments. Many researchers believe probiotics may be a potential therapy for COVID-19 [35–37]. However, to our knowledge, there is a lack of research evidence about the effect of probiotics on COVID-19. In the current study, we conducted a single center retrospective analysis of 311 severe COVID-19 patients, providing clinical evidence that probiotics could enhance immune function and reduce secondary infection in severe COVID-19 patients.

The epidemiological data for COVID-19 showed no probiotic bias except for the significant reduction in cough phlegm in probiotic-treated patients. However, there are no studies of cough phlegm in COVID-19 outcomes. Regarding clinical characteristics, probiotic-treated patients showed a significantly higher level of IL-6 on admission (Table 2). Many studies have found that increased IL-6 levels are in turn significantly associated with adverse clinical outcomes, and inhibition of IL-6 may be a target for therapeutics for the management of dysregulated host responses in patients with COVID-19 [38,39]. Therefore, to some extent, this study showed that probiotics could not attenuate the increased IL-6 levels, which maybe the cause of the significantly longer inpatient day of probiotic-treated patients (Fig. 1). At present, a clinical trial of lopinavir/ritonavir has shown that no benefit was observed with lopinavir-ritonavir treatment beyond standard care in severe COVID-19 patients [40]. In our study, patients treated with probiotics showed significantly higher rates of in lopinavir-ritonavir, ribavirin[b] and interferon alfa inhalation (Table 3). However, the virus clearance time was significantly longer in probiotic-treated patients (Fig. 1). These results indicated that lopinavir-ritonavir, ribavirin[b] and interferon alpha may not have beneficial effects in severe COVID-19 patients, which is consistent with previous studies. Although probiotics might have the ability to protect against SARS-CoV through ACE2, the effect of probiotic-treatment on SARS-CoV was limited in this study.

Lymphocytopenia was observed in many COVID-19 patients [26,41]. Lymphocyte subsets were regarded as immunological biomarkers for host immune system. Studies have found that the total number of B cells, T cells and NK cells were significantly decreased in patients with COVID-19 and much lower in severe cases [42]. Lower levels of CD4 + T cells and CD8 + T cells also found in severe COVID-19 patients and these characteristics were important for predicting the state of the illness changes from mild to severe and the disease outcomes [43]. Consistent with previous study that probiotics supplementation increased the number of neutrophils and CD4 and CD8 T lymphocytes [44], our studies found that patients treated with probiotics have higher levels of total T cells, CD8 + T cells compared to patients who not used probiotics

(Fig. 2), which indicated that probiotics could moderate the reduction immunity in severe COVID-19 patients.

During treatment in the hospital, antibiotics were used to treat and prevent infection in the early days of the outbreak, which may lead to the intestinal dysbacteriosis, bacterial resistance and secondary infection. Probiotics supplementation were found to significantly remodel the microbiome of an individual recovering from antibiotic therapy [45] and, therefore, were recommended for the prevention of secondary infection. In our study, patients treated with probiotics showed significantly longer antibiotic durations and higher multiple antibiotic ratios (Fig. 2A), which meant a higher rate of secondary infection in these patients. We analyzed dynamic changes of CRP level, which is a parameter of infection [46]. The results showed that the CRP level of probiotics treated patients in T2 and T3 were almost the same as those of patients without probiotics, which indicated that the secondary infection in probiotic-treated patients was not higher than that in patients without probiotics. Moreover, recent studies have reported a consistent association between CRP and disease severity and outcomes [47,48]. Higher levels of CRP were observed in severe patients compared to non-severe patients and more likely to get bad outcomes. Lu et al. [49] developed a simple death risk index, which consisted of age and CRP, to predict the short-term mortality of COVID-19 patients. Therefore, CRP is recognized as a certain inflammatory parameter to predict the progression and outcome of COVID-19. In our study, CRP was higher in probiotic-treated patients at T1 and reduced to the level of T2, which was close to the level of non-probiotic-treated patients at T2. Finally, the CRP levels decreased with the same trend as nonprobiotic-treated patients. All these results indicated that probiotics could reduce the inflammatory factor CRP and then prevent the progression of COVID-19.

This study has some limitations. First, this is a single-center retrospective study, and large-scale research is needed to provide high-quality evidence. Second, fatal cases of COVID-19 were excluded, and selection bias might have occurred. Third, pathological findings were not available. Therefore, additional studies are needed to investigate the outcomes of severe patients confirmed with COVID-19.

In summary, we reported the probiotic-specific differences in the epidemiology, medication and dynamic changes in inflammatory and immunological parameters in severe COVID-19 patients and provided evidence that probiotics could reduce secondary infection and moderate the immunity in severe COVID-19 cases, which is consistent with the findings of other studies. Further studies on the mechanism of probiotics on COVID-19 are warranted. It is our hope that these findings may serve as a guide to the clinical therapy of COVID-19.

CRediT authorship contribution statement

Qiang Li: Data curation, Writing - original draft, Visualization. **Fang Cheng:** Data curation, Formal analysis, Writing - review & editing. **Qiling Xu:** Data curation, Software, Validation. **Yuyong Su:** Data curation, Investigation. **Xuefeng Cai:** Data curation, Supervision. **Fang Zeng:** Conceptualization, Methodology. **Yu Zhang:** Conceptualization, Funding acquisition.

Declaration of Competing Interest

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

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Data availability

The datasets are available from the corresponding author on reasonable request.

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