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The Predictive Potential of Intestinal Microbiota for the Chemotherapy Efficacy of Patients with Cachexia in Advanced Colorectal Cancer

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Abstract

Objective: The study aims to explore the predictive potential of intestinal microbiota characteristics for the chemotherapy efficacy of patients with cachexia in the middle and advanced stages of colorectal cancer. **Methods:** A total of 96 patients were enrolled. According to the inclusion and exclusion criteria, 40 patients were ultimately included in the study. The blood and fecal samples were collected from patients before chemotherapy and 6 months after treatment for routine blood tests and 16S rRNA-based intestinal microbiota sequencing. At the same time, the nutritional risk screening (NRS 2002) score and body composition analysis indicators were dynamically monitored and recorded. After completing the standardized chemotherapy, a review of abdominal CT was conducted at the 6th month of follow-up. According to the CT results, the patients were divided into three groups using the Response Evaluation Criteria in Solid Tumors (RECIST 1.1 version): 22 cases in the remission group (RG), 8 cases in the stable group (SDG), and 10 cases in the progression group (PDG). **Results:** Before and after treatment, there were no statistically significant differences in NRS 2002 scores, KPS scores, BMI, grip strength, serum indicators, and body composition index among the groups ($P > 0.05$). Before treatment, the ACE index, Chao1 index, Shannon index, and Simpson index of intestinal microbiota diversity of the RG group were higher than those of the PDG group ($P < 0.05$). After treatment, the above diversity

indices are still higher in the RG group than those in the PDG group ($P < 0.01$). The LEfSe analysis showed that before treatment, the dominant bacteria in the RG group were butyrate-producing beneficial bacteria such as *Faecalibacterium*. While those in the SDG group were amino acid metabolism-related genera, such as *Acidaminococcus*. And those in the PDG group were *Lactobacillaceae* and *Clostridium*. After treatment, the dominant bacteria in the RG group were from the family *Carnobacteriaceae*, whereas those in the SDG group were *Desulfovibrio*. And those in the PDG group were *Streptococcaceae* and other pro-inflammatory bacteria. **Conclusion:** The intestinal microbiota composition of patients with cachexia at baseline could be a potential biomarker for predicting the efficacy of chemotherapy.

Keywords

Advanced Colorectal Cancer, Cachexia, Intestinal Microbiota, Chemotherapy Efficacy, Predictive Value

1. Introduction

Currently, colorectal cancer is one of the most common malignant tumors of the digestive tract. It ranks third in terms of incidence and second in terms of mortality worldwide. According to statistics, in 2022, there were 517,000 new cases and 240,000 deaths in China. Among them, patients in the middle and advanced stages accounted for an extremely high proportion [1]-[3]. Malnutrition is a common complication in middle and advanced-stage colorectal cancer, with an incidence rate of 40% to 80%. It mainly involves weight loss, muscle atrophy, and metabolic disorders, which can reduce the quality of life of patients, weaken the tolerance to chemotherapy, affect prognosis, and limit the survival benefits of patients [4] [5]. Chemotherapy is the main treatment method for colorectal cancer. It remains the main treatment approach for patients with malnutrition in the middle and advanced stages of colorectal cancer. However, there are significant individual differences in clinical efficacy. Some patients experience disease progression after treatment, and chemotherapy may further disrupt intestinal homeostasis, exacerbating dysbiosis and malnutrition symptoms [6]. Recent studies have confirmed that the intestinal microbiota, as the "second genome" of the human body, can affect the occurrence and development of colorectal cancer and the efficacy of chemotherapy through mechanisms such as immune regulation, metabolic regulation, and drug biotransformation. Abnormal composition and function of the intestinal microbiota have been proven to be closely related to the treatment response of patients with colorectal cancer [7] [8].

At present, there have been reports that the individual associations between the intestinal microbiota and the chemotherapy efficacy of colorectal cancer or cachexia [9] [10]. However, the specific association between the intestinal microbiota characteristics of patients with advanced colorectal cancer cachexia and the

efficacy of chemotherapy has not yet been clarified. Also, no specific microbiota markers that can effectively predict the chemotherapy efficacy of such patients have been identified. Therefore, it is impossible to provide precise references for the formulation of individualized chemotherapy regimens in clinical practice.

This study focuses on the special clinical group of cachexia in advanced colorectal cancer. It directly links the characteristics of the intestinal microbiota with the efficacy of chemotherapy. This can provide new ideas and an experimental basis for clinical prediction of chemotherapy efficacy, optimization of treatment strategies, and improvement of patient prognosis.

2. Objects and Methods

2.1. Objects

From July 2024 to August 2025, a total of 96 patients diagnosed with advanced colorectal cancer were enrolled. Based on the inclusion and exclusion criteria, 56 patients were excluded, which included 18 cases in the terminal stage of wasting syndrome, 12 cases with severe organ dysfunction, 23 cases who had used antibiotics within the past 3 months, 1 case unable to take oral medication, and 2 cases with other tumors. Ultimately, 40 patients were included in the study. All patients completed 6 months of standardized chemotherapy and efficacy assessment, and provided fecal and blood samples both before and 6 months after chemotherapy for the detection of intestinal flora and blood indicators. There was no loss or exclusion of samples.

Inclusion criteria: 1) Confirmed diagnosis of colorectal cancer through pathological examination, with clinical stage of II - IV (mid-late stage) [11]; 2) Meets the diagnostic criteria for cancer cachexia and is diagnosed as the cachexia stage [12]; 3) According to the NCCN guidelines, chemotherapy is required, and the patient can tolerate the treatment [13]; 4) More than 21 days have elapsed since the last surgery, radiotherapy, chemotherapy, targeted therapy, immunotherapy, etc.; 5) There is a risk of malnutrition (NRS2002) with a score of ≥3, and enteral nutrition support treatment is required [14]; 6) The patient's physical condition score on the KPS scale is ≥70 points, and they are capable of taking care of themselves in daily life [15]; 7) Age and Gender: Over 18 years old, gender not restricted; 8) Expected survival period: ≥6 months; 9) No use of antibiotics in the past 3 months; 10) At least one measurable lesion meeting the RECIST 1.1 criteria was required.

Exclusion criteria: 1) According to the diagnostic criteria for cancer cachexia, those diagnosed as the refractory stage of cachexia (expected lifespan < 3 months); 2) Comorbidities: patients with severe dysfunction of the heart, lung, liver, or kidney; 3) Concurrently having other tumors or having participated in other clinical trials. This study was approved by the Ethical Committee of Youjiang Medical University for Nationalities. The ethical review number: 2024070202. All patients were informed and signed the informed consent form.

This study included patients with stage II - IV colorectal cancer, all of whom

satisfied the criteria for advanced stages and required standard first-line chemotherapy. The treatment principles and efficacy evaluation criteria remained consistent. The predominant pathological type was adenocarcinoma, with a minimal proportion of squamous cell carcinoma. The overall pathological composition was uniform.

2.2. Grouping

Patients with advanced colorectal cancer cachexia who met the inclusion and exclusion criteria were given chemotherapy according to the 2023 NCCN colorectal cancer guidelines (oxaliplatin + leucovorin + 5-fluorouracil, mFOLFOX6 regimen; or oxaliplatin + capecitabine, XELOX regimen; or irinotecan + leucovorin + 5-fluorouracil, FOLFIRI regimen) [16]. From the start of the first chemotherapy cycle to the end of the last chemotherapy cycle, a total of 6 months (including 6 - 8 chemotherapy cycles) were covered. After completing the standardized chemotherapy, a review of abdominal CT was conducted at the 6th month of follow-up. Based on the imaging results, the patients were classified into 3 groups according to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1 version) [17]. The chemotherapy regimens for the three patient groups are as follows: The remission group included 14 cases of the mFOLFOX6 regimen, 5 cases of the XELOX regimen, and 3 cases of the FOLFIRI regimen; the stable group comprised 4 cases of the mFOLFOX6 regimen, 1 case of the XELOX regimen, and 3 cases of the FOLFIRI regimen; the progression group consisted of 5 cases of the mFOLFOX6 regimen, 3 cases of the XELOX regimen, and 2 cases of the FOLFIRI regimen. For patients in stages II - IV included in this study, baseline confirmation was achieved through full abdominal CT, which identified measurable lesions (target lesions) that satisfied the RECIST 1.1 criteria. For non-metastatic patients in stages II and III, the primary lesion was designated as the target lesion, whereas for stage IV patients, the target lesion comprised the primary lesion ± metastatic lesion. The measurement methods and evaluation criteria have been thoroughly standardized.

2.3. Observation Target

Before chemotherapy and at the end of the last chemotherapy (after 6 months of treatment), the following contents should be recorded: nutritional risk screening score sheet (NRS2002), Karnofsky performance status (KPS) score, body mass index (BMI), grip strength (in kg), body composition analysis, and abdominal CT imaging examination. Venous blood was drawn in the morning to test relevant indicators: hemoglobin (Hb), total protein (TP), albumin (ALB), prealbumin (PA), and C-reactive protein (CRP). Fecal samples were collected to test the intestinal microbiota.

2.4. Fecal 16S rRNA Sequencing Detection

During sample collection, instruct the patient to urinate first. Place clean and hygienic toilet paper in the toilet to prevent urine contamination. Use a disposable

sterile stool sampling box to collect 200 - 500 mg of fresh fecal mid-section internal samples. The sampling should be completed within 2 hours of the marked time. After marking, store the samples at −80˚C in the refrigerator within 6 hours. During the sample preparation stage, weigh 200mg of the mixed fecal sample, add PBS solution, shake it, then centrifuge at 10,000 rpm for 3 minutes and discard the supernatant. Use the E.Z.N.A™ Mag-Bind soil DNA kit to extract the fecal genomic DNA. Target the V3-V4 variable region of the 16S rRNA gene, and perform PCR amplification using the forward primer：5'-CTACGGGCGCAG-3' and the reverse primer：5'-GACTACHVGGTATCATCC-3'. Quantify the library concentration using the Qubit 3.0 fluorescence quantitative instrument and sequence the two-round amplification products. Data analysis is conducted using the RDP, Silva, and NCBI 16S databases for comparison, and analyses of intestinal microbiota diversity and composition are carried out. The fecal 16S rRNA sequencing detection is performed [18]. This experiment was supported by Shenggong Biotechnology Engineering (Shanghai) Co., Ltd.

2.5. Quality Control

1) Sample quality: The fecal samples were refrigerated within 2 hours after collection to prevent bacterial contamination; the blood biochemistry, grip strength, and body composition tests were all conducted by the same laboratory and the same operator to ensure uniform testing methods.

2) Efficacy assessment: The CT examinations were evaluated by two experienced radiologists in a double-blind manner. In case of disagreement, consensus was reached through consultation to reduce assessment errors.

3) Statistical methods: Statistical analysis was performed using Prism software, and the data analysis results were verified by the supervisor.

2.6. Statistical Approach

Statistical analysis was performed using GraphPad Prism (version 9.5) software [19]. For the measurement data, the Shapiro-Wilk test is used to determine whether the data conform to a normal distribution [20]. The measurement data that follow a normal distribution are expressed as mean ± standard deviation (SD). The measurement data that do not follow a normal distribution are expressed by the median (M) and interquartile range (IQR). The comparisons among multiple groups were conducted using one-way analysis of variance or Kruskal-Wallis test, and pairwise comparisons between groups were performed using the Least Significant Difference (LSD) test or Dunnett T3 test [21]. The comparison within this group before and after treatment is performed using the paired sample t-test or the Wilcoxon signed-rank test [22]. For the count data, they are expressed as frequencies and percentages (n, %), and the chi-square test is used. For intestinal microbiota analysis, the Benjamini-Hochberg (BH) method was employed to correct for multiple comparisons. The Adonis (PERMANOVA) test was used to assess the beta diversity. The LEfSe analysis incorporated built-in corrections and LDA threshold con-

trols to mitigate false positives. The application of the receiver operating characteristic curve (ROC) was used to assess the predictive performance of various baseline alpha diversity indices for chemotherapy remission by computing the area under the curve (AUC) and corresponding P-value. When the P-value is less than 0.05, the difference is statistically significant.

3. Result

3.1. Patient General Information

From July 2024 to August 2025, a total of 40 patients with advanced colorectal cancer cachexia who met the criteria were included. After 6 months of treatment, there were 22 cases in the response group (complete response + partial response, RG), 8 cases in the stable group (SDG), and 10 cases in the progression group (PDG). The general clinical data, including gender, age, BMI, lesion location (colon, rectum), pathological type (adenocarcinoma, squamous cell carcinoma), and TNM stage (II, III, IV), showed no statistically significant differences among the groups (P > 0.05) (Table 1).

Table 1. Comparison of general clinical data of patients in each group [n (%), (mean ± SD).

Project [n (%), (Mean ± SD)]	RG (n = 22)	SDG (n = 8)	PDG (n = 10)	H/F/x^2	p
Gender				1.901	0.387
Man	12 (55)	3 (38)	3 (30)		
Female	10 (45)	5 (62)	7 (70)		
Age (Years)	59.59 ± 9.50	58.75 ± 8.05	59.30 ± 5.17	0.020	0.980
BMI (kg/m^2)	23.43 ± 1.71	22.21 ± 0.90	22.42 ± 1.77	2.363	0.108
Diseased Region				1.434	0.488
Colon	20 (90)	6 (75)	9 (90)		
Rectum	2 (10)	2 (25)	1 (10)		
Pathological Type				0.410	0.815
Glandular Cancer	18 (82)	7 (88)	9 (90)		
Squamous Carcinoma	4 (18)	1 (12)	1 (10)		
TNM Staging				4.008	0.135
Phase II	3	0	2		
Phase III	12	4	7		
Phase IV	7	4	1		

Note: BMI: Body Mass Index.

3.2. Changes in Nutrition-Related Indicators before and after Treatment for Each Group of Patients

Before treatment, there was no statistically significant difference in the nutritional risk screening (NRS 2002) score, BMI (body mass index), HG (grip strength), KPS score, and CRP (C-reactive protein) among the groups (P > 0.05) (Figure 1). After

treatment, there was no statistically significant difference in the nutritional risk screening (NRS 2002) score, BMI, HG, KPS score, and CRP among the groups ($P > 0.05$) (Figure 2). However, there was no statistically significant difference in the paired comparison of the above nutrition-related indicators before and after treatment within the same group ($P > 0.05$) (Figure 1 and Figure 2).

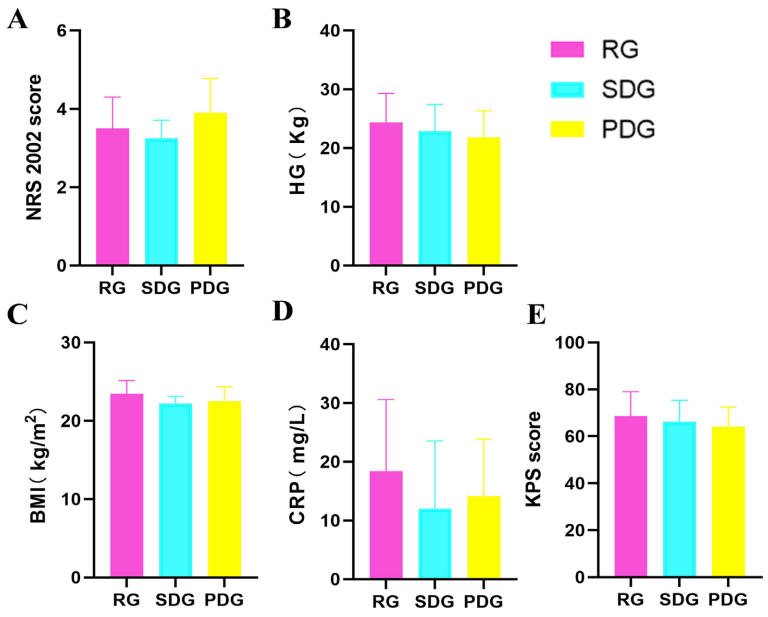

Figure 1. Nutrition-related indicators of patients in each group before treatment. (A) Nutritional risk screening score; (B) Grip strength; (C) Body mass index; (D) C-reactive protein; (E) KPS score. RG: Remission group; SDG: Stable group; PDG: Progressive group.

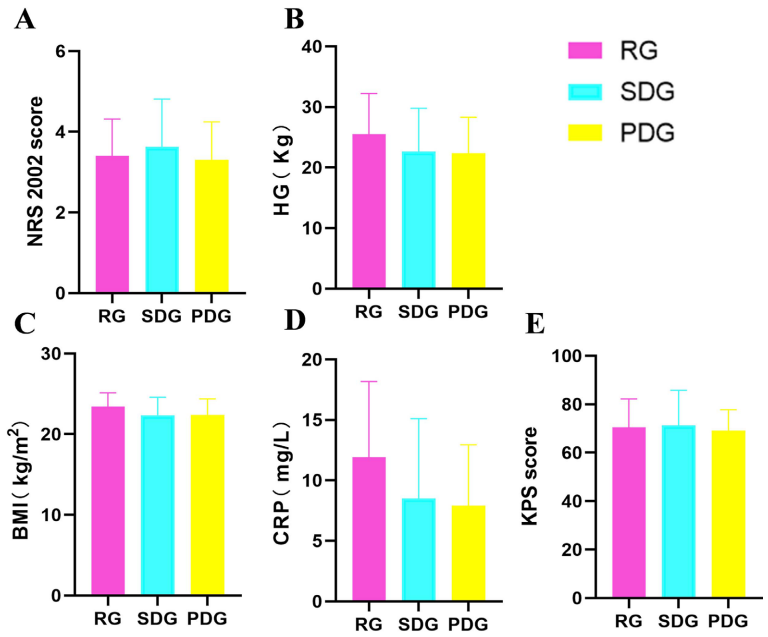

Figure 2. Nutrition-related indicators of patients in each group after treatment. (A) Nutritional risk screening score; (B) Grip strength; (C) Body mass index; (D) C-reactive protein; (E) KPS score; RG: Remission group; SDG: Stable group; PDG: Progressive group.

3.3. Changes in Nutrition-Related Blood Indicators before and after Treatment in Each Group of Patients

Before treatment, there was no statistically significant difference in serum total protein (TP), albumin (ALB), prealbumin (PA), and hemoglobin (HB) among all groups ($P > 0.05$) (Figure 3). After treatment, there was no statistically significant difference in serum total protein (TP), albumin (ALB), prealbumin (PA), and hemoglobin (HB) among all groups ($P > 0.05$) (Figure 4). However, there was no statistically significant difference in the paired comparison of the above blood indicators before and after treatment within the same group ($P > 0.05$) (Figure 3 and Figure 4).

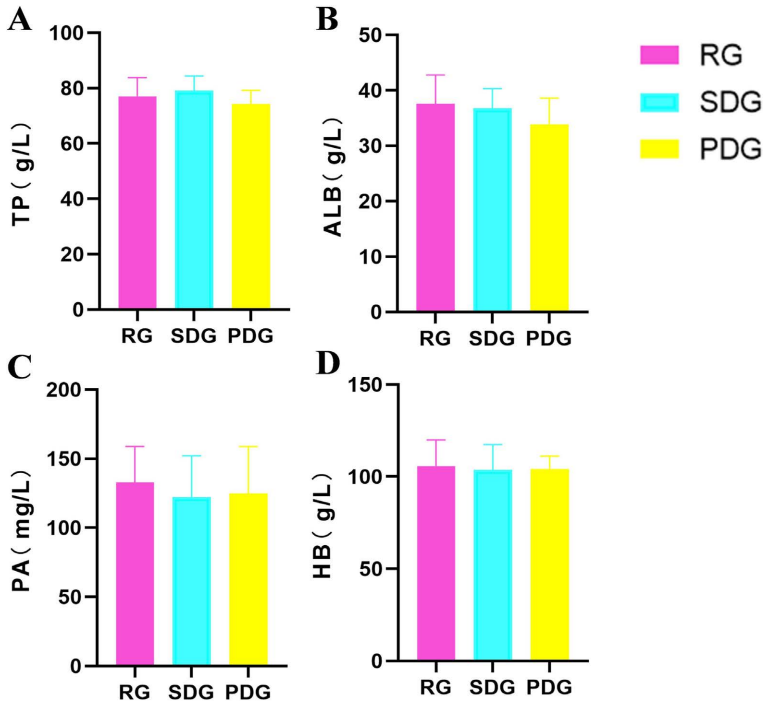

Figure 3. Nutrition-related blood indicators of patients in each group before treatment. (A) Total protein; (B) Albumin; (C) Prealbumin; (D) Hemoglobin; RG: Remission group; SDG: Stable group; PDG: Progressive group.

3.4. Changes in Body Composition of Patients in Each Group before and after Treatment

Before treatment, there was no statistically significant difference in fat mass index (FMI), fat-free mass index (FFMI), skeletal muscle mass index (SMI), and extracellular water ratio (ECW/TBW) among the groups ($P > 0.05$) (Figure 5). After treatment, there was no statistically significant difference in fat mass index (FMI), fat-free mass index (FFMI), skeletal muscle mass index (SMI), and extracellular water ratio (ECW/TBW) among the groups ($P > 0.05$) (Figure 6). However, there was no statistically significant difference in the paired comparison of the above body composition indicators before and after treatment within the same group ($P > 0.05$) (Figure 5 and Figure 6).

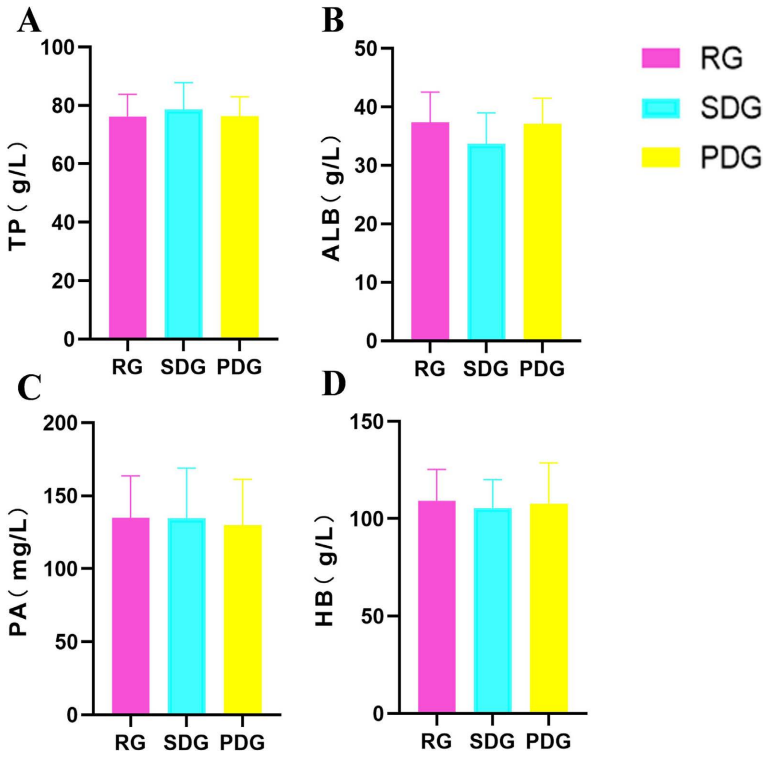

Figure 4. Nutrition-related blood indicators of patients in each group after treatment. (A) Total protein; (B) Albumin; (C) Prealbumin; (D) Hemoglobin; RG: Remission group; SDG: Stable group; PDG: Progressive group.

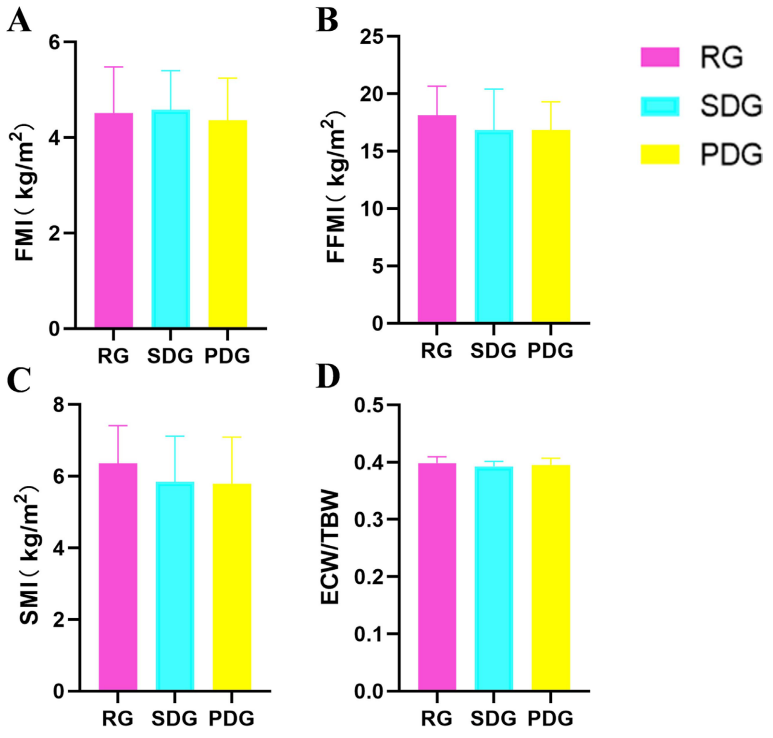

Figure 5. Body composition of patients in each group before treatment. (A) Fat mass index; (B) Lean body mass index; (C) Skeletal muscle mass index; (D) Extracellular water ratio; RG: Remission group; SDG: Stable group; PDG: Progressive group.

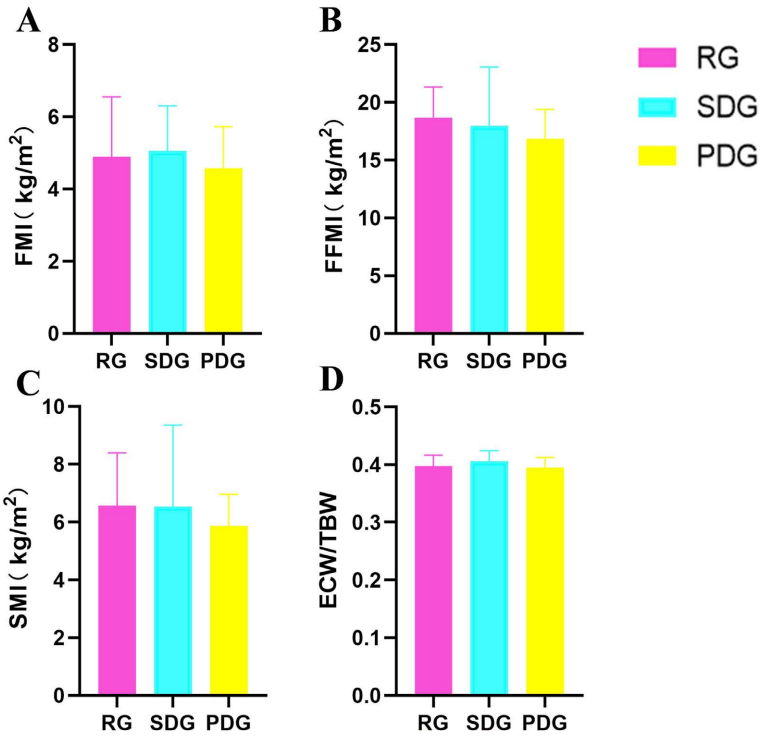

Figure 6. Body composition of patients in each group after treatment. (A) Fat mass index; (B) Lean body mass index; (C) Skeletal muscle mass index; (D) Extracellular water ratio; RG: Remission group; SDG: Stable group; PDG: Progressive group.

3.5. Changes in the Intestinal Microbiota of Patients in Each Group before and after Treatment

Before treatment, the ACE index and Chao1 index of the intestinal microbiota in the RG group were significantly higher than those in the SDG group and the PDG group ($P < 0.05$), while the Shannon index and Simpson index were significantly higher in the RG group than in the PDG group ($P < 0.01$) (**Figure 7**). After treatment, the ACE index, Chao1 index, and Shannon index of the intestinal microbiota in the RG group were significantly higher than those in the SDG group and the PDG group ($P < 0.01$), and the Simpson index was significantly higher in the RG group than in the PDG group ($P < 0.001$) (**Figure 8**).

Principal coordinate analysis (PCoA) based on Bray-Curtis distance shows [23]. Before treatment, the samples of each group were highly overlapping, and there was no statistically significant difference in the overall structure of the intestinal microbiota among the groups ($P > 0.05$) (**Figure 9(A)**). After treatment, the samples of each group still did not form distinct, separate clusters ($P > 0.05$) (**Figure 9**).

The Venn diagram analysis shows that before treatment, the total number of core bacterial species common to all groups was 112, and the number of unique species was 6 in the RG group, 40 in the SDG group, and 60 in the PDG group. After treatment, the total number of core bacterial species common to all groups was 113, and the number of unique species was 5 in the RG group, 59 in the SDG group, and 41 in the PDG group (**Figure 10**).

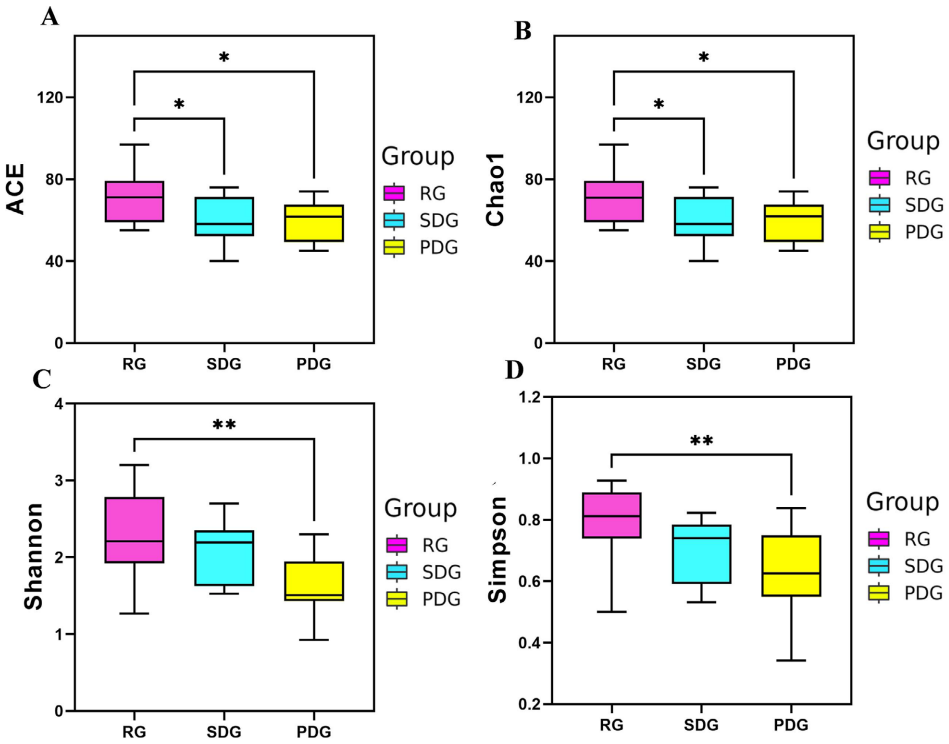

Figure 7. Alpha diversity of intestinal microbiota before treatment in each group of patients. (A) ACE index; (B) Chao1 index; (C) Shannon index; (D) Simpson index; RG: Remission group; SDG: Stable group; PDG: Progression group. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

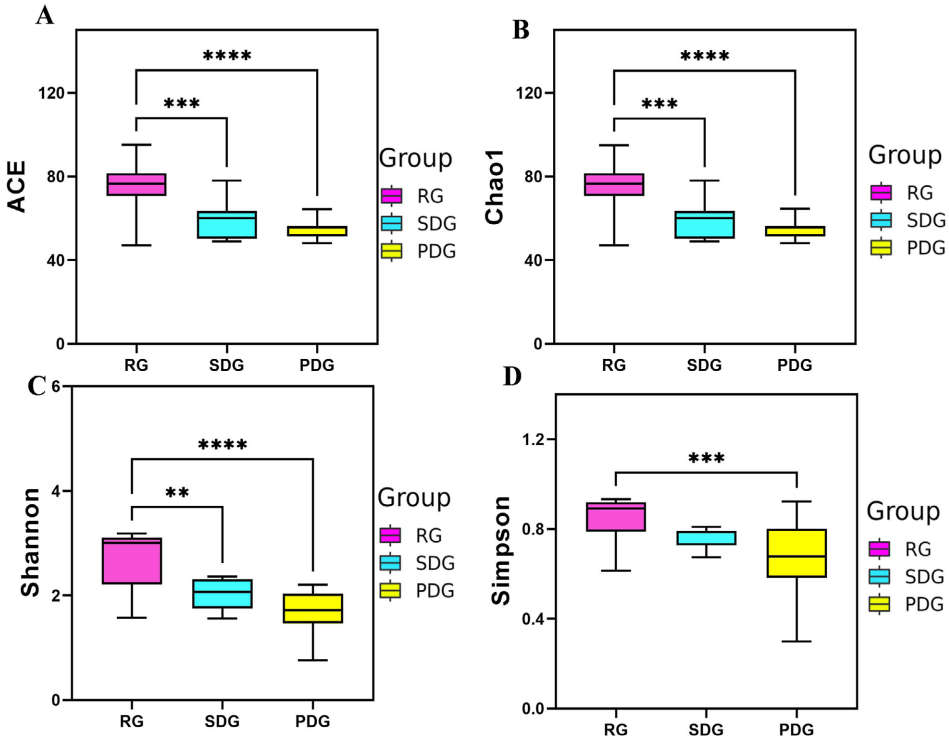

Figure 8. Alpha diversity of intestinal microbiota in each group of patients after treatment. (A) ACE index; (B) Chao1 index; (C) Shannon index; (D) Simpson index. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; RG: Remission group; SDG: Stable group; PDG: Progression group.

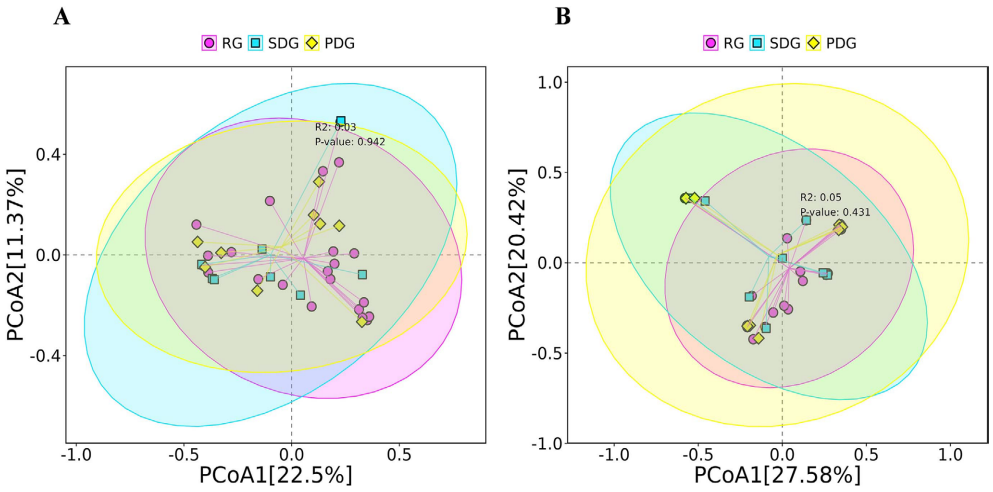

Figure 9. Beta diversity of intestinal microbiota in each group of patients before and after treatment. (A) PCoA analysis of intestinal microbiota in each group of patients before treatment; (B) PCoA analysis of intestinal microbiota in each group of patients after treatment; RG: Remission group; SDG: Stable group; PDG: Progression group.

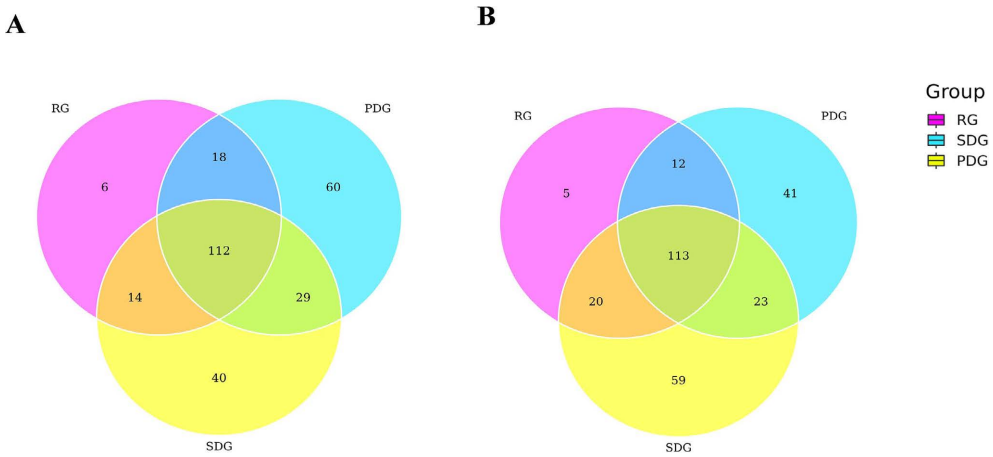

Figure 10. Analysis of core microbiota species before and after treatment in each group of patients. (A) Core microbiota species of each group of patients before treatment; (B) Core microbiota species of each group of patients after treatment; RG: Remission group; SDG: Stable group; PDG: Progressive group.

At the phylum level, before and after treatment, Firmicutes, Proteobacteria, Actinobacteriota, Verrucomicrobiota, and Bacteroidota accounted for more than 90% of each group (**Figure 11**). At the genus level, before and after treatment, *Escherichia-Shigella*, *Bifidobacterium*, *Blautia*, *Enterococcus*, *Streptococcus*, *Lactobacillus*, *Klebsiella*, *Akkermansia*, *Subdoligranulum*, and *Faecalibacterium* accounted for more than 75% of each group (**Figure 12**).

The LEfSe analysis revealed, before treatment, the top three dominant bacterial groups in the RG group were Faecalibacterium, Burkholderiales, and Erysipelotrichaceae-UCG-003; in the SDG group, the top three were Acidaminococcales, Acidaminococcaceae, and *Phascolarctobacterium*; and in the progressive group, the top three were *Lactoacillaceae*, *Lactoacillus*, and *Clostridiales* (**Figure 13**) [24].

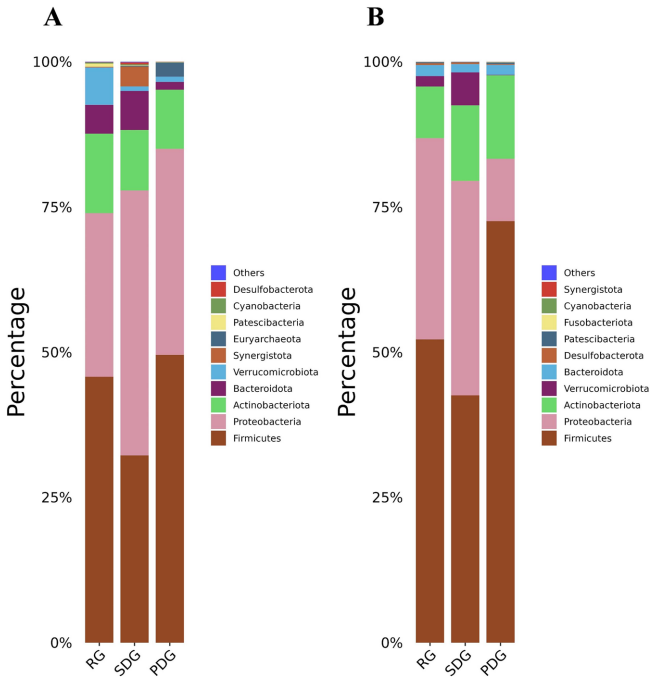

Figure 11. Analysis of the composition of intestinal microbiota in patients of each group at the phylum level before and after treatment. (A) Intestinal microbiota of patients in each group at the phylum level before treatment; (B) Intestinal microbiota of patients in each group at the phylum level after treatment; RG: Remission group; SDG: Stable group; PDG: Progressive group.

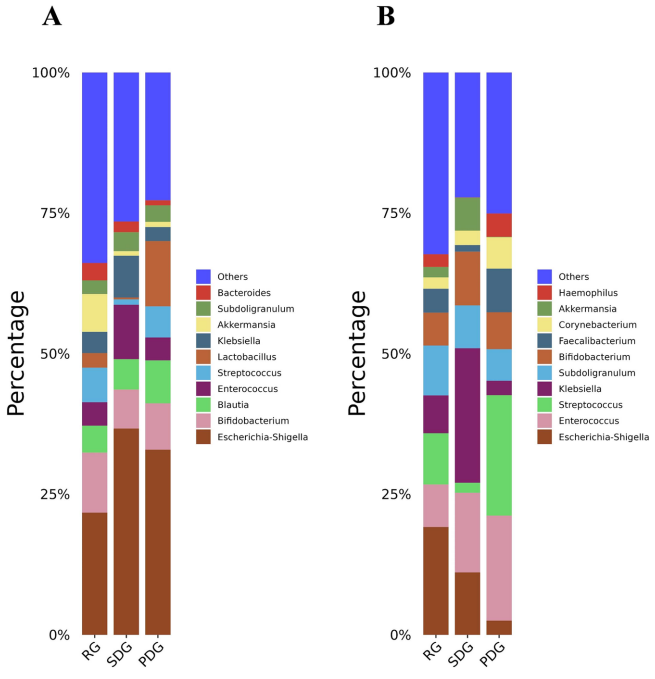

Figure 12. Analysis of the composition of intestinal microbiota in patients of each group at the genus level before and after treatment. (A) Intestinal microbiota of patients in each group at the genus level before treatment; (B) Intestinal microbiota of patients in each group at the genus level after treatment; RG: Remission group; SDG: Stable group; PDG: Progressive group.

After treatment, the dominant bacterial group in the RG group was *Carnobacteriaceae*, while in the SDG group it was *Desulfovibrio*, *Unassigned-Desulfovibrio*; in the PDG group, it was *Streptococcaceae*, *Lactobacillus*, *Unassigned-Lactobacillus*, *Streptococcus salivarius*, and *Actinomycetales* (Figure 14).

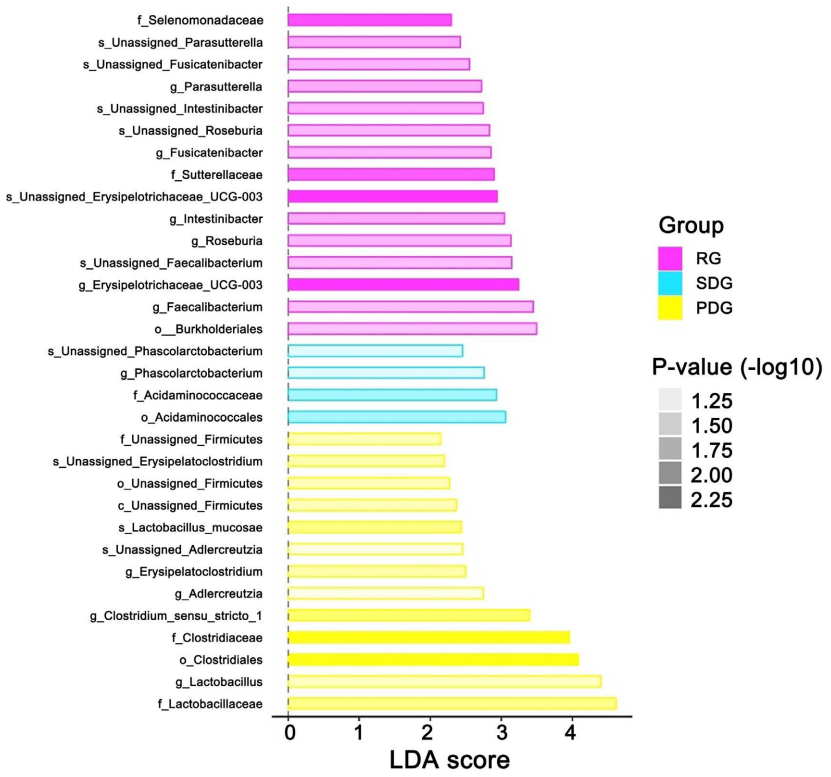

Figure 13. Analysis of species differences in the intestinal microbiota of patients in each group before treatment. RG: Remission group; SDG: Stable group; PDG: Progressive group.

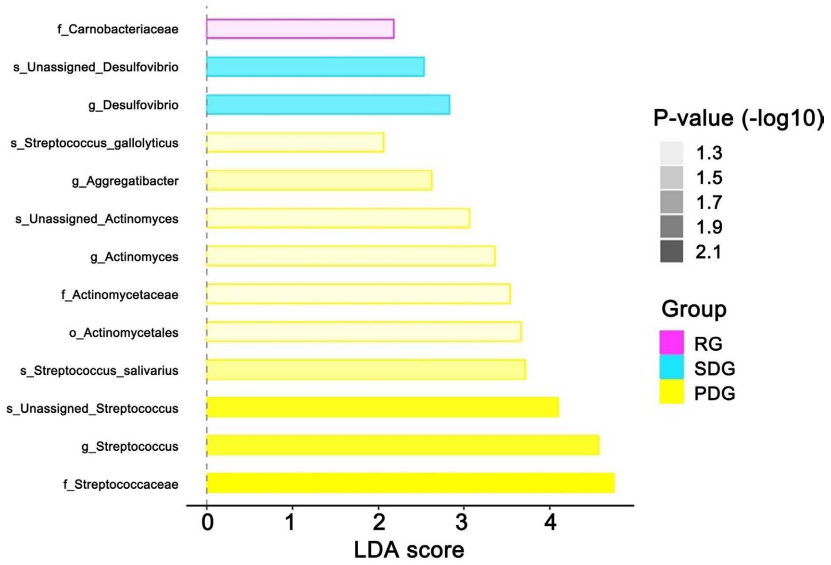

Figure 14. Analysis of species differences in the intestinal microbiota of patients in each group after treatment. RG: Remission group; SDG: Stable group; PDG: Progressive group.

3.6. ROC Prediction Analysis of Baseline Gut Microbiota Alpha Diversity

To assess the potential of intestinal microbiota indicators in predicting the baseline efficacy of chemotherapy, ROC prediction analysis revealed that the AUC values for the ACE, Chao1, Shannon, and Simpson indices of the baseline patients' intestinal microbiota alpha diversity exceeded 0.7, with all results achieving statistical significance ($P < 0.05$) (Figure 15).

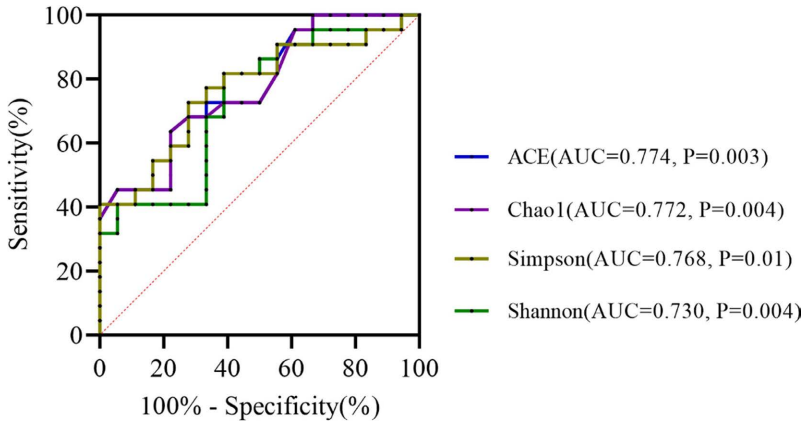

Figure 15. ROC prediction analysis of baseline gut microbiota alpha diversity. RG: Remission group; SDG: Stable group; PDG: Progressive group.

4. Discussion

Based on the NCCN Clinical Practice Guidelines for Colorectal Cancer, the CSCO Guidelines for the Diagnosis and Treatment of Colorectal Cancer, and pertinent phase III clinical trials, following standard first-line chemotherapy for advanced colorectal cancer, partial response predominates as the primary effective outcome, comprising approximately 43% - 55%, with complete response being notably rare, at only 2% - 5%. In alignment with these findings, this investigation identified 17 instances of partial response and merely 5 cases of complete response, indicating a significantly higher frequency of partial response over complete response. Given the preliminary nature of this study with a limited sample size, it amalgamated partial response and complete response into a unified response category, in adherence to established research protocols both domestically and internationally, to uphold the statistical robustness and clinical validity of the analysis.

Previous studies have suggested that patients with advanced colorectal cancer cachexia often experience progressive deterioration of nutritional status and loss of skeletal muscle mass during chemotherapy. This is manifested by significant decreases in BMI, serum albumin, and skeletal muscle mass index, which is closely related to tumor consumption, adverse effects of chemotherapy, and intestinal dysfunction [25]-[27]. The results of this study show that there were no statistically significant differences in the nutritional risk screening (NRS 2002) scores, KPS scores, BMI, grip strength (HG), C-reactive protein (CRP), serum total protein (TP), albumin (ALB), prealbumin (PA), hemoglobin (HB), fat mass index

(FMI), fat-free mass index (FFMI), skeletal muscle mass index (SMI), and extracellular water ratio (ECW/TBW) among all groups of patients before and after treatment. This is different from the conclusions of some previous studies. Before treatment, there was no statistically significant difference in the above indicators among the groups, suggesting that at the time of enrollment, patients in each group were all in the advanced stage of colorectal cancer with cachexia. And their nutritional status and body composition levels were similar, indicating the balance and comparability of the study baseline. After the treatment, there was still no significant difference in the above indicators among the different groups. This might be related to the long-term chemotherapy regimen of 6 months adopted in this study. The continuous tumor consumption and the toxic effects of chemotherapy jointly prevented significant recovery of the nutritional status and muscle mass in all groups of patients. On the other hand, all the patients included in this study received standardized nutritional support intervention during the perichemotherapy period. This, to some extent, slowed down the process of nutritional deterioration and narrowed the differences between the groups. Furthermore, the sample size of this study is relatively small, which may result in insufficient statistical power and prevent the detection of small but clinically significant differences between groups. This is a common limitation in clinical research.

Most previous studies have shown that higher alpha diversity of the intestinal microbiota is associated with better chemotherapy response and prognosis in cancer patients. Decreased diversity often indicates microbiota imbalance and adverse outcomes [28]-[30]. This study shows that before treatment, the ACE index and Chao1 index of the alpha diversity of the intestinal microbiota in the RG group were significantly higher than those in the SDG group and the PDG group. While the Shannon index and Simpson index were significantly higher than those in the PDG group. After treatment, the above indicators in the RG group were still significantly higher than those in the other two groups, which was consistent with the results of previous studies. The mechanism might be as follows: A highly diverse microbiota can enhance chemotherapy tolerance and sensitivity through generating short-chain fatty acids, strengthening the intestinal mucosal barrier, and regulating the immune microenvironment [31] [32]. The baseline high bacterial diversity in the mitigation group constitutes a "beneficial reserve". Under chemotherapy stress, it is easier to maintain homeostasis and promote tumor remission. In contrast, the diversity in the PDG group is low, and the imbalance of intestinal bacteria is prone to triggering inflammation and weakening the therapeutic effect, leading to disease progression. Furthermore, the ROC results of the baseline gut microbiota alpha diversity indicate that it has predictive potential for the chemotherapy efficacy of patients.

The PCoA analysis based on the Bray-Curtis distance showed that, before and after the treatment, the samples of each group were highly overlapping, and there was no statistically significant difference in the overall structure among the groups [23]. Suggesting that the intervention did not significantly reshape the community

structure. The Venn diagram analysis shows that the number of core species in the central group remained stable at 112 to 113 before and after treatment, suggesting that the core species of the intestinal microbiota in patients with cachexia have high stability. However, the number of species unique to each group changed significantly. The SDG group increased from 40 to 59, the progressive group decreased from 60 to 41, and the RG group slightly decreased. Combined with the result of no difference in the overall structure, it can be inferred that the intervention mainly regulates the distribution of local species rather than changing the overall community framework.

At the genus level, the proportions of the five dominant phyla (Bacteroidetes, Firmicutes, etc.) exceeded 90%, which was consistent with the core phylum-level structure of patients with colorectal cancer [33]. At the genus level, the core genus groups such as *Escherichia-Shigella*, *Bifidobacterium*, etc., accounted for over 75%, suggesting that the core functional groups of the microbiota have not changed in the state of cachexia. The LEfSe analysis revealed that before treatment, the dominant bacteria in the RG group were genera such as *Faecalibacterium* that produce butyric acid, which have anti-inflammatory and barrier-protecting effects and are closely related to chemotherapy remission. The dominant bacteria in the SDG group were genera such as *Acidaminococcus* that are involved in amino acid metabolism, which may maintain disease stability. The dominant bacteria in the PDG group were genera such as *Lactobacillus* and *Clostridiales*, which contain opportunistic pathogens, and their enrichment suggests dysbiosis and inflammation, and is related to progression. After treatment, the dominant bacteria in the RG group were genera such as *Coprococcus*, which are related to immune homeostasis. The dominant bacteria in the SDG group were genera such as *Desulfurococcus*, whose production of hydrogen sulfide can damage intestinal mucosa, suggesting potential inflammation. The dominant bacteria in the PDG group were genera such as *Streptococcus*, which are pro-inflammatory and are related to poor prognosis. Previous studies have suggested that the differential microbiota can affect the efficacy of chemotherapy by regulating inflammation and metabolite production, but the mechanism remains unclear. This study indicates that a high baseline alpha diversity and the enrichment of beneficial bacteria such as *Faecalibacterium* species are key features for predicting a good response to chemotherapy, while low alpha diversity and the enrichment of pro-inflammatory bacteria suggest poor efficacy.

Even though the ROC predictive analysis of baseline intestinal microbiota alpha diversity indicates predictive potential, beta diversity did not demonstrate clear separation between groups, suggesting that the differences in the overall structure of the microbiota are relatively limited. The above results may be attributed to the small sample size, insufficient statistical power, and high biological variation among individuals within each group. Consequently, the findings of this study primarily suggest that gut microbiota diversity has certain predictive potential for chemotherapy efficacy, but a predictive biomarker suitable for direct clinical ap-

plication has yet to be established. Future research should aim to expand the sample size, conduct multi-center prospective studies, and integrate metagenomic, metabolomic, and other technologies to further validate the stability and reliability of microbiota markers, thereby elucidating their clinical application value.

5. Conclusion

This study demonstrated that the baseline alpha diversity of the intestinal microbiota, along with the characteristics of differential bacteria in patients with advanced colorectal cancer cachexia, possesses predictive potential for the efficacy of chemotherapy: Higher alpha diversity and enrichment of beneficial bacteria indicated better chemotherapy remission outcomes, while lower diversity and enrichment of pro-inflammatory bacteria might be associated with disease progression. This provides a theoretical basis for clinically precise prognosis stratification and targeted bacterial intervention, and also lays a foundation for subsequent research on the mechanism of the association between the microbiota and chemotherapy efficacy.

6. Limitation and Further Study

This study has the following limitations: First, it is a single-center, small-sample exploratory study. The subgroup sample sizes are limited, resulting in restricted statistical power. Therefore, the findings may be subject to certain biases. Second, the patients received various first-line chemotherapy regimens, leading to treatment heterogeneity. This may affect the research results. Additionally, dietary habits and the use of other medications may affect the data regarding the intestinal microbiota and nutritional status. Finally, 16S rRNA sequencing technology provides only information on microbiota composition, without yielding direct functional metabolic data. Further multi-center, large-sample size studies, with dietary data collection, metagenomics, or metabolomics application, are necessary.

Authors' Contributions

Shi, W.: Design of the work, Acquisition, Analysis, Interpretation of data, Draft the manuscript; Zhu, X.T. and Ruan, J.J.: Sample collection, Experimental operation; Yi, S.G.: Interpretation of data, Revise the manuscript; Wang, J.H.: Conception, Design of the work, Interpretation of data, Revise the manuscript, Final approval of the manuscript to be published, Funding Acquisition; Huang, L.L.: Conception, Design of the work, Interpretation of data, Revise the manuscript, Final approval of the manuscript to be published.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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