ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
In press.

Effects of Serum S100A12 and Diamine Oxidase Levels on Gut Microbiota Dysbiosis and Immune Function in Patients with Colon Cancer

Jixiang Liu[1], Shan Liu[2], Jinghang Cong[2], Xuesong Zhao[2], Liwei Zhang[3], and Wei Li[2]

[1] Department of Health Insurance Affairs, The First Affiliated Hospital of Qiqihar Medical University, Qiqihar, China
[2] Department of Clinical Laboratory, The Second Affiliated Hospital of Qiqihar Medical University, Qiqihar, China
[3] Department of Gastroenterology, The Second Affiliated Hospital of Qiqihar Medical University, Qiqihar, China

Received: 21 October 2025; Received in revised form: 4 January 2026; Accepted: 31 January 2026

ABSTRACT

Colon cancer, a leading cause of death, demands early detection. We evaluate serum S100 calcium-binding protein A12 (S100A12) and diamine oxidase (DAO) effects on gut dysbiosis/immunity and their combined diagnostic value.

This retrospective study included 105 colon cancer patients (study group), 90 benign lesions, and 105 matched healthy controls. Serum S100A12 and DAO were measured by ELISA (enzyme-linked immunosorbent assay). Gut flora (*Escherichia coli*, *Enterococcus faecalis*, *Bifidobacterium*, *Lactobacillus*) were cultured; T cell subsets ($CD4^+$, $CD8^+$, Treg) by flow cytometry. Patients were stratified by median levels into high/low groups. Correlations and diagnostic efficacy were assessed using Pearson test and ROC (receiver operating characteristic) analysis.

Baseline data were comparable among three groups. Fecal flora (*E. coli*, *E. faecalis*, *Bifidobacterium*, *Lactobacillus*) and T cells ($CD4^+$, $CD8^+$, regulatory T cells) differed significantly. Serum S100A12 and DAO were elevated in colon cancer versus benign/control groups. High/low subgroups showed disparities in flora and T cells. S100A12 and DAO positively correlated with *E. coli*/*E. faecalis*, $CD8^+$ T, and Treg, but negatively with *Bifidobacterium*, *Lactobacillus*, and $CD4^+$ T. The cancer group had reduced $CD4^+$ and $CD4^+/CD8^+$ ratio, and elevated $CD8^+$ and Treg. Combined S100A12+DAO detection outperformed single markers

Elevated serum S100A12 and DAO in colon cancer are associated with gut microbiota dysbiosis and immune dysregulation. Their combination shows promise as a potential biomarker for early diagnosis and disease evaluation.

Keywords: Amine oxidase (Copper-Containing); Colonic neoplasms; Gastrointestinal microbiome; Immunity; S100A12 protein

INTRODUCTION

Colon cancer (CRC) remains a leading cause of cancer-related mortality worldwide. With the change in dietary structure and the acceleration of the aging process of the population, China has experienced a notable rise in CRC incidence over time, and the prevention and treatment situation is severe. Clinically, patients with early-stage CRC can achieve a good prognosis through surgery. Owing to nonspecific early

Corresponding Author: Wei Li, PhD;
Department of Clinical Laboratory, The Second Affiliated Hospital of Qiqihar Medical University, Qiqihar, China. Tel: (+86 138) 0361 1464, Fax: (+86 045) 2273 6280, Email: Liwei584@hotmail.com

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

symptoms, diagnosis is frequently delayed until intermediate or advanced disease stages, causing patients to miss the best treatment opportunity and experience an unsatisfactory 5-year survival rate.[1,2] Therefore, the development of convenient, effective biomarkers is critical for advancing CRC diagnosis and care.

In recent years, the role of the human microbiome, especially the gut microbiota, in health and disease has received unprecedented attention.[3] The intestine is a vast and complex micro-ecosystem, home to trillions of microorganisms. It maintains a dynamic balance with the host and jointly participates in key physiological processes such as nutritional metabolism, barrier maintenance, and immune regulation. A large amount of evidence indicates that once this balance is disrupted, it is called intestinal flora dysbiosis, which is closely related to the occurrence, development, and metastasis of CRC, transforming it from a simple "resident" to an active "participant" in the carcinogenic process.[4,5] Research has confirmed a distinct gut microbiota composition in CRC patients compared to that of healthy individuals, usually manifested as a decrease in microbial diversity and abnormal changes in the proportion of specific microbiota. For instance, opportunistic pathogenic bacteria such as *Fusobacterium nucleatum* accumulate, while beneficial bacteria like *Bifidobacterium* and *Lactobacillus* decrease. This disorder of the microbiota structure not only leads to the accumulation of bacterial metabolic products, directly damaging the host DNA, but also disrupts the intestinal mucosal barrier function, activates a chronic inflammatory state, and thereby promotes tumor occurrence.[6–8]

Chronic inflammation has been recognized as the seventh hallmark of tumors and plays a core role in the initiation and progression of CRC.[9] The fact that patients with inflammatory bowel disease are at high risk of CRC strongly proves that chronic inflammation is an important driver of CRC.[10] In the tumor microenvironment, various immune cells and inflammatory mediators form a complex regulatory network. Among them, the balance of helper T cells is of vital importance.[11,12] $CD4^+$ T cells are the command centers of adaptive immunity, while $CD8^+$ T cells are the main effector cells, responsible for recognizing and killing tumor cells.[13] Regulatory T cells (Tregs), as a subpopulation of $CD4^+$ T cells, play an immunosuppressive role. By inhibiting the function of effector T cells, they help tumors achieve immune escape. During the process of CRC, it is often observed that the infiltration of $CD8^+$ T cells and Treg cells increases, while the number of $CD4^+$ T cells and the ratio of $CD4^+/CD8^+$ decrease. This indicates a tilt towards an immunosuppressive state, which is conducive to tumor progression and metastasis.[14] S100 calcium-binding protein A12 (S100A12) is precisely a key molecule that links inflammation with immunity. As a member of the S100 protein family, S100A12 is mainly expressed and secreted by neutrophils and is an important damage-related molecular pattern molecule. It activates signaling pathways such as nuclear factor-κB (NF-κB) by binding to the receptor of advanced glycation end products, thereby amplifying the inflammatory response and recruiting more immune cells to the inflammatory site.[15,16] S100A12 has been widely studied in acute and chronic inflammatory diseases such as pneumonia, sepsis, and rheumatoid arthritis, and has been confirmed to be a sensitive marker of disease activity.[17,18] Accumulating evidence indicates that S100A12 is overexpressed in multiple solid tumors, including gastric and hepatocellular carcinomas, and promotes the malignant behavior of tumor cells through mechanisms such as regulating the cell cycle and epithelial-mesenchymal transition.[19] However, systematic investigation into S100A12's role in CRC remains limited, especially in its interaction with the gut microbiota and systemic immune function. A complete intestinal mucosal barrier is the first physical and immune line of defense that isolates a large number of microorganisms and toxins in the intestinal tract from the internal environment of the host.[20] Diamine oxidase (DAO) is a cytoplasmic enzyme mainly present in the apical cells of intestinal mucosal villi, responsible for catalyzing the metabolism of polyamines within cells. Its activity in serum is widely regarded as a sensitive and specific indicator reflecting the integrity of intestinal mucosa and the renewal rate of epithelial cells.[21] When the intestinal mucosa is damaged due to ischemia, hypoxia, infection, or inflammation and other reasons, DAO will be released in large quantities from the necrotic intestinal epithelial cells into the blood, thereby elevating serum DAO levels. In the context of CRC, the invasive growth of tumors and the related local inflammatory response undoubtedly cause damage to the intestinal barrier. The increase in DAO levels not only indicates damage to the intestinal mucosa but may also be involved in the vicious cycle of disordered microbiota-immune axis.[22,23] Although DAO is widely used in intestinal

diseases, its role in the dysbiosis and immune function changes of CRC remains unexplored in depth.

Based on the above research background and existing research gaps, this study adopted a retrospective cohort study design to systematically explore the effects of serum S100A12 and DAO expression levels on intestinal flora imbalance and immune function in CRC, investigating the diagnostic value of their combination. To examine gut microbiota dysfunction related to barrier integrity and immunity, we focused on two key functional categories: conditionally pathogenic bacteria (*E. coli* and *E. faecalis*), linked to inflammation and barrier disruption,[24] and beneficial bacteria (*Bifidobacterium* and *Lactobacillus*), known for maintaining epithelial homeostasis and immune regulation through short-chain fatty acid production.[25] This pathogenic-beneficial imbalance is a recognized dysbiosis signature in colorectal cancer. We put forward the following hypotheses: CRC patients exhibited a significant elevation in serum S100A12 and DAO compared to both comparison groups. High expression correlated with both gut dysbiosis (notably elevated *E. coli*/*E. faecalis*, reduced *Bifidobacterium*/*Lactobacillus*) and impaired immunity (specifically reduced $CD4^+$ T cells and $CD4^+/CD8^+$ ratio). These increases in $CD8^+$ T and Treg cell levels were also significantly correlated with high expression. The combined detection of the two has a better diagnostic value for CRC than the individual detection. Through ELISA, fecal culture, flow cytometry, and correlation and receiver operating characteristic (ROC) curve analysis, the inter-group differences, association relationships, and combined diagnostic efficacy of the above indicators were verified. Ultimately, this study provided noninvasive auxiliary indicators for the early diagnosis of CRC, helping to improve patient prognosis and save medical resources.

MATERIALS AND METHODS

Study Design

This study adopted a retrospective cohort research design. Patients with CRC or benign lesions from our Digestive Surgery Department, alongside healthy individuals from our Examination Center (February 2024 to August 2025), were enrolled and categorized into three groups by diagnosis: the electronic medical record system yielded 105 CRC, 92 patients with benign colorectal lesions, and 110 healthy control cases, including the healthy physical examination population (control group), patients with benign colon lesions (benign colon lesion group), and patients with CRC (study group). Final exclusion comprised five controls (ineligibility) and two benign cases (lost to follow-up). The study design flowchart is shown in Figure 1.

Ethical Explanation

The study was approved by the Institutional Review Board of the Second Affiliated Hospital of Qiqihar Medical University. The principles of the Declaration of Helsinki were strictly followed and all research procedures were in accordance with international ethical standards. All participants provided written informed consent; in emergency situations, their legal representatives or guardians were permitted to provide consent on their behalf. All data from the study were anonymized to ensure the privacy and confidentiality of the participants.

Inclusion Criteria

Control group: (1) Age 40- 74 years. (2) Normal physical examination results (blood routine, liver and kidney function, tumor markers, and colonoscopy). (3) No history of intestinal infection in the past 3 months, and no antibiotics or immunomodulators. (4) No history of malignant tumors, chronic inflammatory diseases (such as rheumatoid arthritis), or metabolic diseases (such as diabetes). (5) Complete clinical data and voluntary signing of informed consent forms.

Benign colon lesion group: (1) Age 40 -74 years. (2) Confirmed as benign colonic lesions through colonoscopy and pathological examination (such as colonic polyps, chronic colitis, colonic diverticulitis) excluding precancerous lesions or malignant tendencies.[26] (3) No history of antibiotics, immunosuppressants, or intestinal microecological regulators within the past month. (4) Complete clinical data and voluntary signing of informed consent forms.

Study group: (1) Age 40- 74 years.(2) Postoperative pathological examination, clearly diagnosed as CRC (referring to the pathological diagnosis criteria of the "Chinese CRC Diagnosis and Treatment Specifications (2023 Edition)"),[26] with no preoperative chemotherapy, radiotherapy, immunotherapy, or intestinal flora regulation treatment (such as probiotics, antibiotics) .(3) Complete clinical data (including demographic characteristics, history of underlying diseases, and laboratory test data). (4) Voluntarily participation in this study and signed the informed consent form.

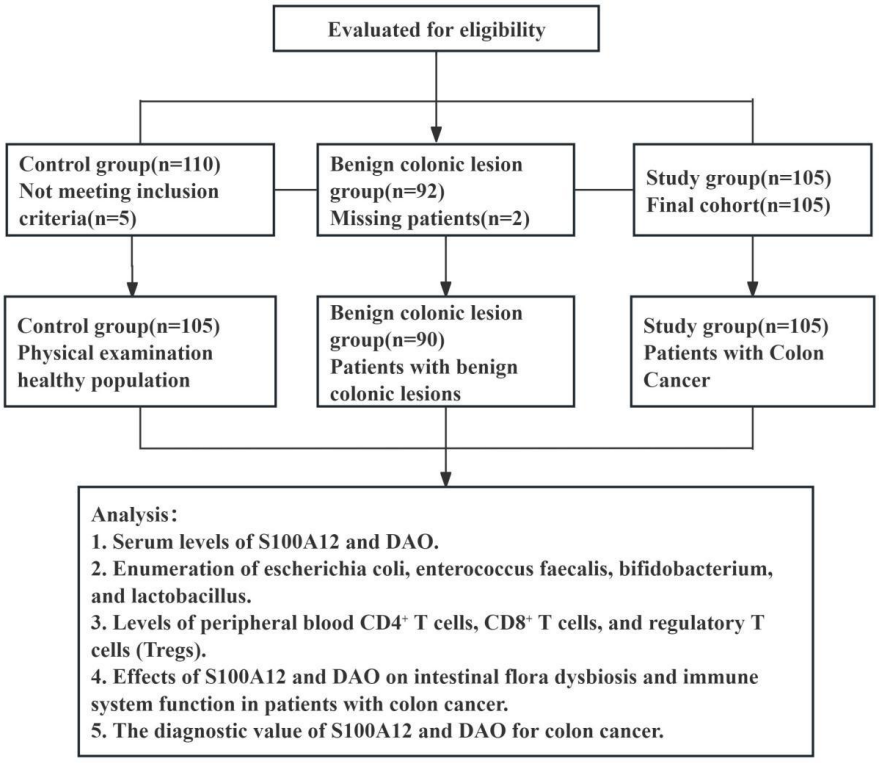

Figure 1. Design Flow Chart.

Exclusion Criteria

(1) Combined with malignant tumors in other parts of the body (such as gastric cancer, lung cancer) or metastatic tumors. (2) History of acute infection (such as pneumonia, urinary tract infection), severe trauma, or surgery within the past 1 month. (3) Presence of autoimmune diseases (such as systemic lupus erythematosus), severe liver and kidney dysfunction (Child-Pugh grade C), or blood system diseases (such as leukemia). (4) Long-term use of glucocorticoids (such as prednisone), immunosuppressants (such as cyclosporine), or broad-spectrum antibiotics (course of treatment≥7 days) within the past 3 months.[27] (5) Pregnant or lactating women; (6) Key observation index data are missing in medical records.

Sample Collection

Serum sample: All the research subjects had 5 mL of peripheral venous blood collected on an empty stomach in the early morning of the next day after enrollment. The blood was placed in anticoagulant-free blood collection tubes and left to stand at room temperature for 30 minutes. Then, the blood was centrifuged at 3000 *g* for 15 minutes (with a centrifugation radius of 10 cm). The serum was separated and aliquoted into EP tubes for storage in a −80°C ultra-low temperature refrigerator. The levels of S100A12 and DAO are awaiting batch testing.[28]

Fecal samples: Within 24 hours after enrollment, 1 g of fresh morning stool of the research subjects was collected and placed in a sterile fecal collection tube. Immediately, was add 9 mL of sterile normal saline, shaken thoroughly to mix well, and then a 10-fold series dilution was performed (10^{-1} to 10^{-6}). The samples were then inoculated and cultured to count the intestinal flora.[29]

Peripheral blood immune cell samples: Two mL of fasting peripheral venous blood were collected and placed in an EDTA anticoagulant blood collection tube. The tube was gently inverted and mixed well. To avoid coagulation or hemolysis, samples were shipped within 2 hours for flow cytometry-based T-cell subset ($CD4^+$, $CD8^+$, Treg) analysis.[30]

Observation Indicators

Documented baseline data covered demographics (age, gender, BMI), clinical features (colonic symptoms), underlying diseases and lifestyle habits (history of hypertension, history of diabetes, smoking history, drinking history, and intestinal medication history in the past month), as well as routine laboratory indicators (white blood cell count, hemoglobin, liver function, and kidney function indicators).

The S100A12 kits were purchased respectively from R&D Systems (USA), and the DAO kit from Shanghai Enzyme-Linked Biotechnology Co., LTD. (China). All procedures were performed strictly according to the manufacturers' instructions. The detection instrument is the ELx808 microplate reader from Bio-Tek of the United States. The absorbance (OD value) is read at a wavelength of 450 nm to detect the concentrations of serum S100A12 (unit: ng/mL) and DAO (unit: U/L).

Culture and quantification of gut microbiota: A 1-g aliquot of fresh fecal homogenate was spread onto selective agars: MacConkey Agar for *Escherichia coli*, Azide Bile Esculin Agar for *Enterococcus faecalis*, and modified MRS Agar (supplemented with 0.05% L-cysteine) for *Bifidobacterium* and *Lactobacillus* (all media from Qingdao Haibo Biotechnology, China). Plates were incubated anaerobically at 35°C for 72 hours in an anaerobic chamber (YQX-II; Shanghai Yuejin Medical Instrument, China) with a gas mixture (85% N_2, 10% CO_2, 5% H_2). Each batch included negative (sterile saline) and positive (ATCC reference strains) controls. Colonies were counted independently by two experienced researchers, and results were averaged and expressed as $\log_{10}$ colony-forming units (CFU) per gram of wet feces.

A BD FACSCanto II flow cytometer was used with the corresponding fluorescence-labeled antibodies CD4-FITC, CD8-PE, CD25-APC, Foxp3-PE-Cy7 antibodies (all purchased from BD Biosciences in the United States) to detect the percentages (%) of $CD4^+$ T cells, $CD8^+$ T cells, and Tregs in peripheral blood, and the $CD4^+/CD8^+$ ratio was calculated. The gating strategy was as follows: Lymphocytes were first gated based on forward scatter (FSC) and side scatter (SSC) profiles. From this population, $CD4^+$ T cells were selected. Regulatory T cells (Tregs) were then identified as the $CD25^+Foxp3^+$ subset within $CD4^+$ T cells.

Sample Size Calculation

The sample size was calculated based on the primary outcome measure of this study (the correlation between serum S100A12 and the number of bifidobacteria), referring to the research results of Li et al.[31] This study included 116 patients with advanced CRC. Pearson correlation analysis confirmed that the serum S100A12 level demonstrated a significant inverse correlation with fecal *bifidobacterium* abundance ($r=-0.62$, $p<0.05$). Using G*Power 3.1 software, with the test level α set at 0.05 (two-sided) and the power $(1-\beta)=0.90$, and substituting the correlation coefficient $r=-0.62$, it was calculated that the minimum sample size required for the CRC group was 38 cases. To ensure sufficient robustness of the research results in subgroup analyses (such as the high/low expression group of serum S100A12 and the high/low expression group of DAO), and considering the possible case dropout during the follow-up process, it was ultimately determined that 105 cases would be included in the CRC group. Control and benign group sizes were determined by the cancer group's size and the feasibility of recruiting concurrent cases: The benign lesion and control groups, with sample sizes of 90 and 105 respectively, satisfied all statistical power criteria. Our sample size (105 cases, 195 controls) is adequate for diagnostic accuracy assessment. Per empirical guidelines, >100 cases are recommended to reliably estimate an AUC exceeding 0.8. The control group size further ensures robust specificity estimation, supporting the validity of our ROC analyses.

Statistical Analysis

Data were analyzed with SPSS 26.0. Normally distributed metrics (mean ± SD) were compared using *t* test/ANOVA, while nonparametric data [median (IQR)] employed the Mann-Whitney *U* test. Categorical variables were presented as n (%) and analyzed using χ^2 or Fisher exact test (for expected frequencies<5). Associations between serum S100A12/DAO and microbiota/immune indices were assessed using Pearson or Spearman correlation, based on data distribution. Independent associations were verified by multiple linear regression, adjusting for age, sex, BMI, CEA, and CA19-9, with standardized coefficients (β) reported. Diagnostic performance was evaluated using ROC curves (AUC, sensitivity, specificity). Differences between AUCs were compared via Delong test. A binary logistic regression model (disease status as outcome, S100A12 and DAO as predictors) was used to generate a combined probability score for joint ROC analysis. All statistical tests were two-sided, and $p<0.05$ was considered statistically significant.

RESULTS

Comparison of Baseline Data

The study comprised 105 healthy controls, 90 benign colon lesion patients, and 105 CRC cases. As shown in Table 1 demographic characteristics (including age, sex, BMI), underlying diseases and living habits (including history of hypertension, diabetes, smoking, and drinking), and routine laboratory parameters (including hematological and biochemical profiles) showed no statistically significant differences across all groups (all p>0.05). This result indicates that the three groups of subjects were well balanced and highly comparable on the vast majority of potential confounders. In terms of clinical characteristics, the distribution of colonic symptoms differed markedly across the three groups (p<0.001). Specifically, most of the individuals (93.3%) in the healthy control group were asymptomatic, while the two patient groups mainly had clinical symptoms such as abdominal pain/bloating, hematochezia/melena. This distribution was completely consistent with the different clinical health status of the three groups, and also indirectly verified the rationality and correctness of the grouping. The conventional CRC biomarkers, CEA and CA19-9, exhibited distinct levels across the study cohorts (p<0.001). This is consistent with the disease characteristics of CRC and further confirms the validity of the study-group assignment. Therefore, the three groups were well-balanced and comparable in key baseline data, with the exception of clinical symptoms and specific tumor markers.

Table 1. Comparison of baseline data of the three groups of research subjects (mean ± SD).

Indicators	Control Group (n=105)	Benign Colonic Lesion Group (n=90)	Study Group (n=105)	Statistic (χ^2/F)	p
Demographics					
Age, y	55.8 ± 8.6	56.1 ± 8.9	55.7 ± 8.7	0.15	0.859
Gender (Male/Female)	55/50	48/42	56/49	0.58	0.748
BMI, kg/m^2	23.5 ± 2.8	24.0 ± 3.2	23.9 ± 2.9	0.785	0.457
Clinical Characteristics					
Colonic Symptoms, n (%)				105.325	<0.001
Asymptomatic	98 (93.3%)	15 (16.7%)	10 (9.5%)		
Abdominal pain/discomfort	5 (4.8%)	45 (50.0%)	50 (47.6%)		
Bloody stool/black stool	2 (1.9%)	20 (22.2%)	35 (33.3%)		
Altered bowel habits	0 (0.0%)	10 (11.1%)	10 (9.5%)		
Comorbidities & Habits					
History of Hypertension, n (%)	24 (22.9%)	23 (25.6%)	25 (23.8%)	0.41	0.815
History of Diabetes, n (%)	18 (17.1%)	16 (17.8%)	19 (18.1%)	0.12	0.942
Smoking History, n (%)	23 (21.9%)	16 (17.8%)	21 (20.0%)	0.85	0.654
Drinking History, n (%)	20 (19.0%)	17 (18.9%)	21 (20.0%)	0.08	0.961
History of Intestinal Medication, n (%)	6 (5.7%)	6 (6.7%)	7 (6.7%)	0.35	0.841
Laboratory Indicators					
White Blood Cell, ×10^9/L	6.6 ± 1.3	6.7 ± 1.2	6.8 ± 1.4	0.47	0.625
Hemoglobin, g/L	134.8 ± 12.3	133.9 ± 11.8	133.2 ± 12.5	0.72	0.487
ALT, U/L	25.5 ± 8.4	26.1 ± 8.7	25.8 ± 8.9	0.23	0.793
AST, U/L	24.3 ± 7.6	24.8 ± 7.9	24.5 ± 8.1	0.15	0.859
Serum Creatinine, μmol/L	78.8 ± 12.1	79.5 ± 11.6	79.2 ± 12.3	0.11	0.896
CEA, ng/mL	1.5 ± 0.8	1.7 ± 0.9	5.9 ± 3.1	132.85	<0.001
CA19-9, U/mL	18.8 ± 7.4	20.1 ± 8.2	46.2 ± 20.8	79.36	<0.001

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; CA19-9: carbohydrate antigen 19-9; CEA: carcinoembryonic antigen.

The Serum Levels of S100A12 and DAO, Intestinal Flora and Immune Indexes Were Compared Among the Three Groups

The comparisons of serum markers, intestinal flora and key indicators of immune function among the control group, the benign colon lesion group and the study group are shown in Table 2. There were statistically significant differences in all observed indicators among the three groups of research subjects (p<0.001). Serum S100A12 and DAO showed a gradient elevation across groups, with the study group > benign group > controls (all p<0.05), suggesting that these two serum markers may be related to the severity of colon diseases, especially with marked overexpression in the CRC cohort. The number of opportunistic pathogenic bacteria (*E. coli*, *E. faecalis*) was the highest in the study group, while the number of beneficial bacteria (*Bifidobacterium*, *Lactobacillus*) was the lowest. The differences between any two groups were statistically significant (all p<0.05). This result indicates substantial microbial imbalance in CRC patients, manifesting as microecological disruption. CRC patients exhibited marked shifts in T-cell immunity, including suppressed $CD4^+$ levels and amplified $CD8^+$/Treg responses compared to other groups (all p<0.05). These changes collectively suggest the typical characteristics of CRC patients, such as elevated serum S100A12 and DAO levels, intestinal flora imbalance, and suppressed immune function.

The Influence of High and Low Expression of S100A12 and DAO on the Microbiota and Immunity in the CRC Group

In the CRC group, based on the median expression levels of serum S100A12 and DAO, the patients were further divided into high and low expression subgroups for comparison. The results are shown in Table 3. An inverse relationship was observed between biomarker levels and gut flora: pathogen enrichment and probiotic reduction in high-expression groups (all p<0.001). In terms of immune function, both the S100A12 and DAO high-expression groups presented a more significant immunosuppressive state, specifically manifested as reciprocal alterations: decreased $CD4^+$ parameters with concurrently increased $CD8^+$ and Treg percentages (all p<0.001). The findings indicate that among CRC patients, higher serum S100A12 or DAO concentrations correlate with more severe intestinal microbial dysbiosis and immune impairment.

Table 2. Comparison of serum S100A12 and DAO levels, intestinal flora and immune function indicators among the three groups of research subjects (mean ± SD).

Indicators	Control Group (n=105)	Benign Colonic Lesion Group (n=90)	Study Group (n=105)	*F*	*p*
Serum Markers					
S100A12, μg/L	98.45 ± 28.63	172.36 ± 38.92[a]	285.61 ± 58.74[a,b]	398.120	<0.001
DAO, ng/L	9.84 ± 0.72	26.33 ± 1.45[a]	36.28 ± 2.15[a,b]	376.450	<0.001
Intestinal Flora, log_{10} CFU/g					
E coli	6.12 ± 0.45	6.58 ± 0.51[a]	7.85 ± 0.62[a,b]	45.217	<0.001
E faecalis	5.89 ± 0.52	6.34 ± 0.48[a]	7.41 ± 0.59[a,b]	38.945	<0.001
Bifidobacterium	8.41 ± 0.38	7.92 ± 0.42[a]	6.88 ± 0.51[a,b]	52.331	<0.001
Lactobacillus	8.25 ± 0.41	7.80 ± 0.39[a]	6.95 ± 0.47[a,b]	49.876	<0.001
Immune Function Indicators, %					
$CD4^+$ T cells	42.35 ± 5.12	39.81 ± 4.76[a]	31.26 ± 4.35[a,b]	65.412	<0.001
$CD8^+$ T cells	25.64 ± 3.88	27.95 ± 3.95[a]	35.82 ± 4.21[a,b]	58.963	<0.001
$CD4^+/CD8^+$ ratio	1.65 ± 0.25	1.43 ± 0.22[a]	0.87 ± 0.18[a,b]	71.258	<0.001
Treg cells	5.12 ± 1.05	6.33 ± 1.21[a]	9.45 ± 1.54[a,b]	47.815	<0.001

[a]p<0.05, compared with the Control Group.

[b]p<0.05, compared with the Benign Colonic Lesion Group.

[c]DAO: diamine oxidase; S100A12: S100 calcium-binding protein A12.

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

Table 3. Comparison of microbiota and immune indicators between the high and low expression subgroups of S100A12 and DAO within the CRC group (mean ± SD).

Indicators	S100A12 Low-Expression Group (n=53)	S100A12 High-Expression Group (n=52)	*t*	*p*	DAO Low-Expression Group (n=52)	DAO High-Expression Group (n=53)	*t*	*p*
Intestinal Flora, log_{10} CFU/g								
E coli	7.52 ± 0.55	8.18 ± 0.58	6.012	<0.001	7.48 ± 0.53	8.21 ± 0.59	6.874	<0.001
E faecalis	7.15 ± 0.54	7.67 ± 0.56	4.856	<0.001	7.18 ± 0.55	7.64 ± 0.58	4.235	<0.001
Bifidobacterium	7.15 ± 0.48	6.61 ± 0.49	5.678	<0.001	7.12 ± 0.47	6.64 ± 0.50	5.145	<0.001
Lactobacillus	7.18 ± 0.44	6.72 ± 0.46	5.234	<0.001	7.15 ± 0.45	6.75 ± 0.47	4.567	<0.001
Immune Function Indicators, %								
$CD4^+$ T cells	33.25 ± 4.12	29.25 ± 4.08	5.045	<0.001	33.01 ± 4.05	29.50 ± 4.20	4.412	<0.001
$CD8^+$ T cells	33.85 ± 4.05	37.82 ± 4.10	4.987	<0.001	34.12 ± 4.00	37.51 ± 4.15	4.256	<0.001
$CD4^+/CD8^+$ ratio	0.98 ± 0.17	0.77 ± 0.16	6.412	<0.001	0.97 ± 0.16	0.79 ± 0.17	5.678	<0.001
Treg cells	8.52 ± 1.42	10.40 ± 1.48	6.745	<0.001	8.61 ± 1.40	10.28 ± 1.52	5.987	<0.001

CFU: colony-forming units; CRC: colon cancer; DAO: diamine oxidase; S100A12: S100 calcium-binding protein A12.

Correlation Analysis

Correlations of serum S100A12 and DAO with gut microbiota and immune parameters are presented in Table 4. Both markers showed significant positive associations with conditionally opportunistic pathogens (*E. coli*, *E. faecalis*; all $p<0.001$) but negative associations with beneficial bacteria (*Bifidobacterium*, *Lactobacillus*; all $p<0.001$). Immune profiling revealed negative correlations with $CD4^+$ T cells and positive correlations with $CD8^+$ T cells and regulatory T cells (Tregs; all $p<0.001$). The $CD4^+/CD8^+$ ratio was also inversely related to both markers (all $p<0.001$). Although correlation coefficients were moderate ($r \approx 0.5–0.7$), their strong statistical significance suggests biological relevance. Multiple linear regression adjusted for age, sex, BMI, CEA, and CA19-9 confirmed these as independent associations (Table 4). For example, S100A12 independently predicted Treg levels (standardized $\beta=0.52$, $p<0.001$) and *Bifidobacterium* abundance ($\beta=-0.45$, $p<0.001$), with DAO showing a similar pattern. These findings indicate that S100A12 and DAO influence gut dysbiosis and immune dysregulation independently of conventional demographic and tumor burden indicators. Key relationships are illustrated in scatter plots (Figure 2). Both S100A12 and DAO were positively associated with Treg percentage (Figure 2A, C) and negatively associated with *Bifidobacterium* abundance (Figure 2B, D), with all trends remaining highly significant ($p<0.001$).

Analysis of Diagnostic Efficacy

To evaluate the clinical potential of the novel markers, we systematically compared the diagnostic performance of serum S100A12 and DAO with conventional tumor markers CEA and CA19-9 for colorectal cancer (Table 5). The AUCs for S100A12 and DAO alone were 0.823 and 0.801, respectively, while those for CEA and CA19-9 were 0.852 and 0.821. Notably, the combined detection of S100A12 and DAO achieved an AUC of 0.894, which was significantly higher than that of any single marker, including CEA ($p=0.032$) and CA19-9 ($p<0.001$) alone. The combined S100A12+DAO AUC was also higher than that of CEA+CA19-9 combined (AUC=0.875), although this difference did not reach statistical significance ($p=0.086$). Based on the Youden index, preliminary cutoff values for S100A12 and DAO were 212.5 μg/L and 31.6 ng/L, respectively. These results indicate that S100A12 and DAO alone have good diagnostic value, and their combination provides superior performance to conventional tumor markers.

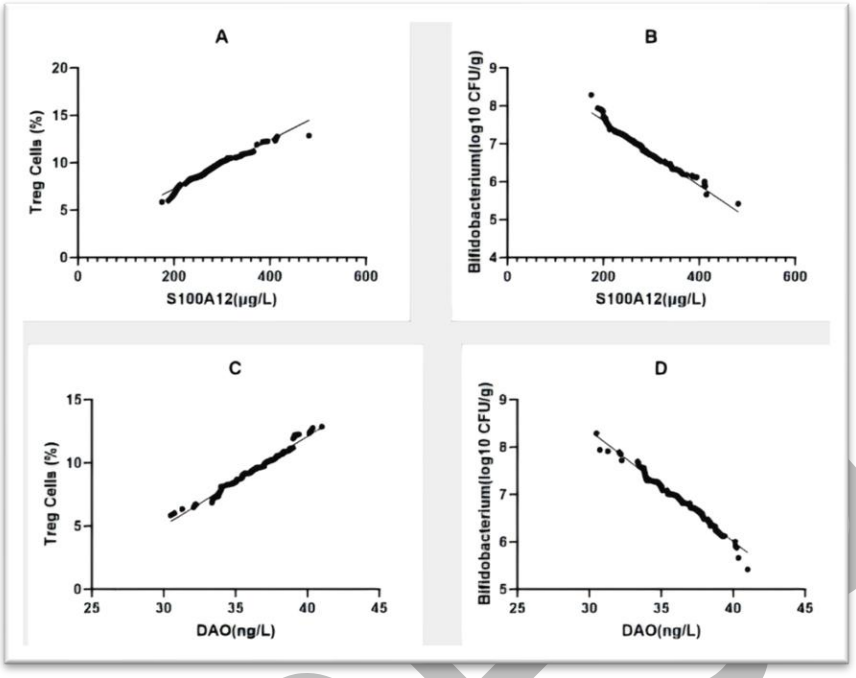

Figure 2. Correlation analysis of serum S100A12 and DAO with key immune and gut microbiota parameters. (A) Positive correlation between S100A12 and Treg percentage. (B) Negative correlation between S100A12 and *Bifidobacterium* abundance (log_{10} CFU/g). (C) Positive correlation between DAO and Treg percentage. (D) Negative correlation between DAO and *Bifidobacterium* abundance.

Table 4. Correlation and multiple regression analysis of S100A12 and DAO with immune and microbial parameters.

Parameter	Analysis Type	S100A12 Effect size (*r* / β)	DAO Effect size (*r* / β)	*p*
Immune Function				
$CD4^+$ T cells, %	Simple correlation (*r*)	−0.623	−0.589	<0.001
	Multiple regression (β)	−0.48	−0.43	<0.001
$CD8^+$ T cells, %	Simple correlation (*r*)	0.585	0.548	<0.001
	Multiple regression (β)	0.42	0.39	<0.001
$CD4^+/CD8^+$ ratio	Simple correlation (*r*)	−0.61	−0.58	<0.001
	Multiple regression (β)	−0.41	−0.38	<0.001
Treg cells, %	Simple correlation (*r*)	0.647	0.601	<0.001
	Multiple regression (β)	0.52	0.47	<0.001
Gut Microbiota				
E. coli, log CFU/g	Simple correlation (*r*)	0.612	0.587	<0.001
	Multiple regression (β)	0.46	0.44	<0.001
E. faecalis, log CFU/g	Simple correlation (*r*)	0.534	0.521	<0.001
	Multiple regression (β)	0.4	0.38	<0.001
Bifidobacterium, log CFU/g	Simple correlation (*r*)	−0.598	−0.563	<0.001
	Multiple regression (β)	−0.45	−0.41	<0.001
Lactobacillus, log CFU/g	Simple correlation (*r*)	−0.556	−0.538	<0.001
	Multiple regression (β)	−0.4	−0.39	<0.001

[a]BMI: body mass index; CA19-9: carbohydrate antigen 19-9; CEA: carcinoembryonic antigen; CFU: colony-forming units; DAO: diamine oxidase; S100A12: S100 calcium-binding protein A12. [b]Multiple regression analysis was adjusted for age, sex, BMI, CEA, and CA19-9.

Table 5. Diagnostic efficacy of serum S100A12 and DAO for CRC.

Test Indicator	AUC	95% CI	Sensitivity (%)	Specificity (%)	*p*
S100A12	0.823	0.774–0.872	78.1	75.6	<0.001
DAO	0.801	0.750–0.852	75.2	73.3	<0.001
CEA	0.852	0.806–0.898	80	77.1	<0.001
CA19-9	0.821	0.772–0.870	76.2	74.3	<0.001
S100A12 + DAO	0.894	0.857–0.931	86.7	82.4	<0.001
CEA + CA19-9	0.875	0.836–0.914	84.8	80	<0.001

AUC: area under the curve; CA19-9: carbohydrate antigen 19-9; CEA: carcinoembryonic antigen; CI: confidence interval; CRC: colon cancer; DAO: diamine oxidase; S100A12: S100 calcium-binding protein A12.

DISCUSSION

This study systematically explored the expression levels of serum S100A12 and DAO in patients with CRC and their clinical significance. This study confirms a significant elevation of serum S100A12 and DAO in individuals with CRC, and these markers are closely related to specific structural changes in the intestinal flora (an increase in opportunistic pathogenic bacteria and a decrease in beneficial bacteria) and systemic immunosuppressive status (a reduction in $CD4^+$ T cells and an increase in $CD8^+$ T cells and Treg cells). More importantly, the combined detection of S100A12 and DAO demonstrated superior diagnostic efficacy compared to a single indicator, providing a new potential biomarker combination for the early diagnosis and disease assessment of CRC.

Specifically, this study revealed progressively elevated serum S100A12 and DAO levels from controls to benign lesions to CRC, demonstrating a clear ascending trend, and they increased in a stepwise manner with the aggravation of the degree of intestinal flora imbalance. This phenomenon reveals the core position of both in the pathophysiological process of CRC. As an S100 protein family member, S100A12 shows overexpression in CRC represents a direct manifestation of tumor-associated chronic inflammation. Qiao et al[32] confirmed through immunohistochemistry that the serum S100A12 level in patients with CRC (293.76 ± 62.54 μg/L) was notably higher than in healthy individuals (105.41 ± 30.39 μg/L). Li et al[31] also reported a significant increase in serum S100A12 in patients with CRC, and its correlation pattern with intestinal flora imbalance was highly consistent with ours. In addition, the cancer-promoting effect of S100A12 in digestive tract tumors such as gastric cancer and hepatocellular carcinoma has also been reported many times. Shu et al[33] included 82 patients with CRC and 14 healthy controls in their study. Through ELISA detection, significantly high expression of S100 protein family members such as S100A8/A9 was found, and they were regarded as potential diagnostic biomarkers. This provides strong evidence for the findings of our research group regarding the high expression of S100A12 in CRC from independent studies.

The observed positive correlation of S100A12 level with $CD8^+$ T cells and Treg cells and negative correlation with $CD4^+$ T cells can be explained by molecular mechanisms. As a damage-associated molecular pattern molecule, S100A12 activates NF-κB and MAPK signaling pathways by binding to its receptor RAGE, thereby driving the massive release of proinflammatory cytokines (such as IL-6, TNF-α, IL-1β). This chronic inflammatory environment is a key driving force for inducing the differentiation of naive $CD4^+$ T cells into Treg cells. Studies have confirmed that activation of the NF-κB pathway can upregulate the expression of Foxp3, which is a key transcription factor and functional marker of Treg cells. For example, Luo and Li's review clearly showed that NF-κB signaling, especially its c-Rel subunit, plays a key role in Treg lineage profiling by directly binding to the regulatory region of Foxp3 gene and driving its transcription.[34] Therefore, S100A12-mediated inflammatory signaling may be an important bridge connecting neutrophil-derived inflammation and adaptive immunosuppression (Treg expansion). In addition, the present study observed an increased proportion of $CD8^+$ T cells in the peripheral blood of colon cancer patients. In tumor

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

immunology, an increased number of $CD8^+$ T cells alone is not equivalent to effective antitumor immunity. On the contrary, under chronic antigen stimulation and an immunosuppressive microenvironment, $CD8^+$ T cells often show "functional exhaustion" or "dysregulation", characterized by high expression of inhibitory receptors such as PD-1, TIM-3, LAG-3, and impaired cytotoxic function (such as secretion of granzyme B and perforin).[35] Although these depletion markers were not examined in this study, the observed pattern of concurrent elevation of $CD8^+$ T cells with S100A12 (a proinflammatory marker) and Treg (immunosuppressive cells) strongly suggests that these $CD8^+$ T cells are more likely to be dysfunctional than fully activated effector cells. This interpretation is consistent with the overall pattern of immunosuppression revealed by the decreased $CD4^+/CD8^+$ ratio. The significant reduction of the $CD4^+/CD8^+$ ratio is not only a sensitive indicator of systemic immune imbalance, but also closely related to the progression and poor prognosis of colorectal cancer.

DAO, as an enzyme mainly existing in the villous epithelial cells of the small intestinal mucosa, has a serum elevation that is established as the gold standard for assessing intestinal barrier integrity. Although DAO has traditionally been considered a sensitive indicator of the integrity of the small intestinal mucosa, its significant increase in colon cancer patients in this study may be indicative of broader gastrointestinal barrier dysfunction resulting from systemic inflammation and disruption of the gut-system axis. Colon cancer-related local inflammation, dysbiosis and systemic reactions may jointly lead to the increase of intestinal barrier permeability and the release of DAO into the blood. Testa et al[36] showed that the expression level of DAO in colorectal cancer tissues was as high as 80.0%, suggesting that DAO may be related to the occurrence of colorectal tumors. The study by Snezhkina et al[37] also pointed out that high DAO expression was associated with the reduction of intestinal health-related flora as well as impaired intestinal barrier function.

The imbalance of the intestinal flora plays a key driving role in this pathological process. S100A12 and DAO exhibited synchronous dynamics with the progressive disruption of microbiota structure. On the one hand, the excessive proliferation of pathogenic bacteria can directly damage the intestinal epithelium and produce toxins that exacerbate inflammation. On the other hand, the reduction of beneficial bacteria means that the protective and anti-inflammatory effects of short-chain fatty acids (such as butyrate) produced by these bacteria on the intestinal mucosa are lost. The dysbiotic microbiota profile observed in this study—characterized by enrichment of potential pathogens and depletion of commensal beneficial bacteria—could act in concert with S100A12 to foster an immune milieu that favors Treg expansion. Specifically, overgrowth of opportunistic pathobionts like *E. coli* and *E. faecalis* elevates circulating levels of microbial products such as lipopolysaccharide (LPS), which engages the TLR4/MyD88/NF-κB cascade, amplifying inflammatory signaling initiated by the S100A12/RAGE axis. Concurrently, reduced abundance of health-associated genera including *Bifidobacterium* and *Lactobacillus* leads to diminished production of microbial metabolites, notably the short-chain fatty acid butyrate. Butyrate functions as a histone deacetylase inhibitor and facilitates Treg differentiation by epigenetically modulating Foxp3 expression through enhanced histone acetylation at its gene locus.[38] Thus, gut microbiota dysbiosis may drive systemic immunosuppression via intertwined "metabolite – epigenetic" and "S100A12-inflammatory signaling" pathways. Given the cross-sectional design, causal directions among intestinal dysbiosis, elevated S100A12/DAO, and immune alterations cannot be established; these factors likely participate in a mutually reinforcing pathogenic loop.

Taken together, our findings support a vicious cycle model that promotes colon cancer development mediated by the S100A12-DAO-microbiota-immunity axis (Figure 3). In this model, colon cancer lesions lead to increased circulating levels of S100A12 and DAO by inducing chronic inflammation and local barrier damage. The synergistic effect of the two factors disrupts intestinal microecological homeostasis, which is manifested as the expansion of opportunistic pathogenic bacteria (such as *E. coli* and *E. faecalis*) and the depletion of beneficial bacteria (such as *Bifidobacterium* and *Lactobacillus*). This dysbiosis, along with depletion of beneficial bacterial metabolites such as the short-chain fatty acid butyrate, concurs with S100A12-driven persistent inflammatory signaling to create a systemic immunosuppressive environment characterized by expansion of Treg cells, depletion of $CD4^+$ T cells, and a decreased $CD4^+/CD8^+$ ratio. Ultimately, this immunosuppressive state in turn facilitates tumor immune escape and progression, thus forming a self-reinforcing vicious cycle. This model

provides a unifying pathophysiological explanation for the strong associations between serum biomarkers, gut microbiota, and systemic immune parameters observed in this study.

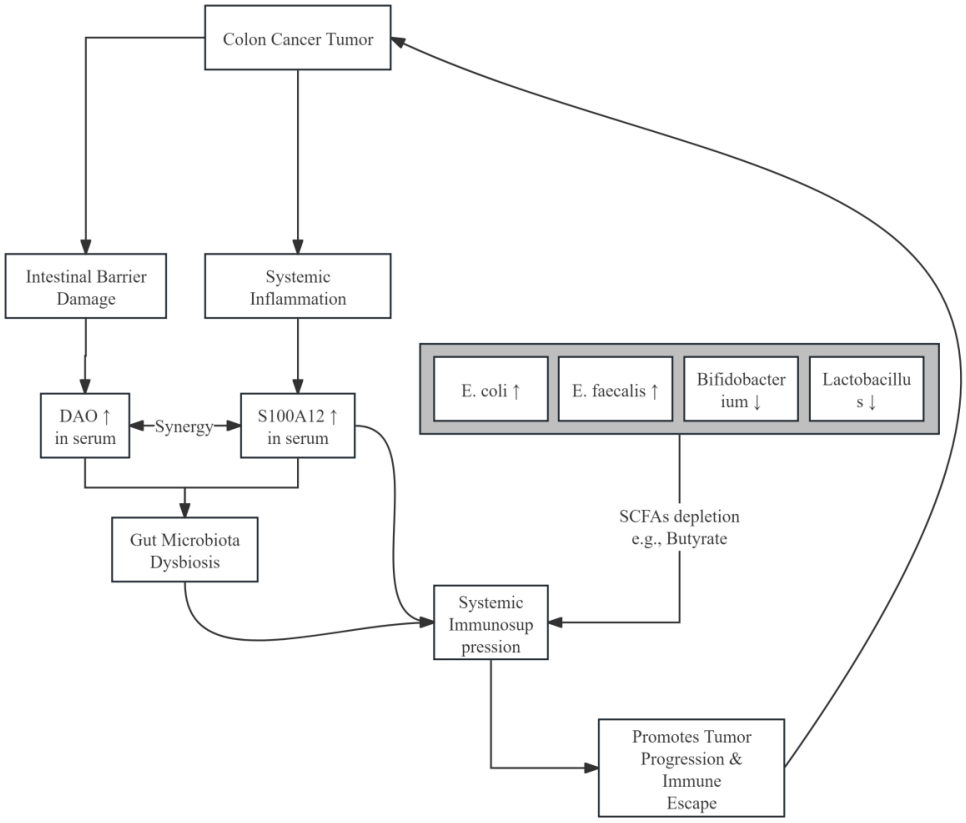

Figure 3. Schematic Representation of the S100A12-DAO-Microbiota-Immunity axis in the Pathogenesis of Colon Cancer.

In terms of clinical translation, the ROC analysis of this study showed that the efficacy (AUC=0.894) of the combined diagnosis of S100A12 and DAO for CRC was significantly better than that of any single indicator. This discovery is highly consistent with the trend of similar studies, highlighting the significant advantages of multi-dimensional biomarker combinations in enhancing diagnostic accuracy. Li et al[31] reported that the AUC of S100A12 combined with sRAGE for predicting the ineffectiveness of chemotherapy in CRC was as high as 0.91. Although the clinical endpoints (chemotherapy efficacy) predicted by these studies differ from those of this study (diagnosis), they collectively confirm a core strategy: For the complex disease of CRC, combining serum markers that reflect different pathophysiological dimensions (such as inflammation, barrier function, and immune recognition) can more comprehensively capture disease information, thereby achieving better predictive or diagnostic performance. S100A12 focuses more on reflecting the systemic inflammatory state, while DAO more specifically indicates intestinal barrier damage. The two provide information from different but complementary pathophysiological dimensions, thereby achieving a more comprehensive and accurate identification of diseases. This result provides new ideas and strong experimental evidence for the development of noninvasive and convenient auxiliary diagnostic tools for CRC or screening programs for high-risk populations.

In conclusion, this study elaborated the clinical value of serum S100A12 and DAO as complementary diagnostic tools for CRC, and elaborated in depth on their close association with intestinal flora imbalance and systemic immunosuppression. These findings not only provide mechanistic insights into the "inflammation-barrier-microbiota-immune" network in CRC, but also provide new and transformative ideas for the clinical management of CRC.

Although this study achieved positive results, there are still some limitations: First, the retrospective design may have been subject to selection bias, although it was controlled for by strict criteria. All analyses were based on complete data sets, and cases with missing key data were excluded. Second, the sample size was relatively limited, and subgroup analyses based on disease stage were not performed because of limitations in sample size and stage information. Third, with respect to diagnostic validation, all analyses were based on a single cohort without internal cross-validation, which makes the reported measures of diagnostic efficacy potentially subject to a degree of optimism bias. Future studies need to be validated in multicenter prospective cohorts, and net reclassification improvement or decision curve analysis can be used to further quantify clinical utility. Fourth, long-term follow-up was not performed in this study, and the prognostic value of markers cannot be assessed. Fifth, in terms of microbiological methods, only four representative bacterial genera were included in the conventional culture method, which can effectively indicate the core characteristics of dysbiosis, but cannot fully reveal the overall diversity and complex composition of the gut microbiome. Sixth, dietary information was not collected, which may have compromised insight into the causes of dysbiosis. Finally, the immunoassays in this study were based solely on peripheral blood samples, and the results may not fully reflect the local immune microenvironment of the tumor. Future studies should employ more comprehensive omics techniques, combine multi-site samples, and incorporate environmental factors to more precisely elucidate the mechanism and clinical value of this axis.

STATEMENT OF ETHICS

The study was approved by the Institutional Review Board of the Second Affiliated Hospital of Qiqihar Medical University. The principles of the Declaration of Helsinki were strictly followed and all research procedures were in accordance with international ethical standards. (Approval number: (2025) 02-011-01).

FUNDING

This work was supported by Qiqihar City Joint Guidance Science and Technology Program Project. (Grant No. LSFGG-2025071).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

The data supporting the findings of this study can be obtained from the corresponding author, upon request.

AI ASSISTANCE DISCLOSURE

No artificial intelligence (AI) tools were used in the preparation of this manuscript.

REFERENCES

1. El Kadmiri N. Advances in early detection of colorectal cancer: a focus on non-invasive biomarkers. Curr Drug Targets. 2021;22(9):1043-53.
2. Nambidi S, Shruthi KB, Kanimozhe SP, Banerjee A, Kondaveeti SB, Duttaroy AK, et al. A review of circular RNAs in colorectal cancer: insights into biomarker discovery and therapeutic targeting. Front Oncol. 2025;15.
3. Ionescu VA, Diaconu CC, Gheorghe G, Mihai M-M, Diaconu CC, Bostan M, et al. Gut microbiota and colorectal cancer: a balance between risk and protection. Int J Mol Sci. 2025;26(8):3733.
4. Tudorache M, Treteanu A-R, Gradisteanu Pircalabioru G, Lixandru-Petre I-O, Bolocan A, Andronic O. Gut microbiome alterations in colorectal cancer: mechanisms, therapeutic strategies, and precision oncology perspectives. Cancers. 2025;17(14):2294.
5. Kang Z, Jiang S, Fang J-Y, Chen H. Intestinal dysbiosis and colorectal cancer. Chin Med J. 2025;138(11):1266-87.
6. Palmieri O, Castellana S, Latiano A, Latiano T, Gentile A, Panza A, et al. Mucosal microbiota from colorectal cancer, adenoma and normal epithelium reveals the imprint of Fusobacterium nucleatum in cancerogenesis. Microorganisms. 2023;11(5):1147.
7. Rezaee MA, Nouri R, Hasani A, Asgharzadeh M, Sefidan FY, Hemmati F. Roles of gut microbiota in colorectal carcinogenesis providing a perspective for early diagnosis and treatment. Curr Pharm Biotechnol. 2022;23(13):1569-80.
8. Liang JQ, Zeng Y, Lau EYT, Sun Y, Huang Y, Zhou T, et al. A probiotic formula for modulation of colorectal cancer

risk via reducing CRC-associated bacteria. Cells. 2023;12(9):1244.
9. Nigam M, Mishra AP, Deb VK, Dimri DB, Tiwari V, Bungau SG, et al. Evaluation of the association of chronic inflammation and cancer: insights and implications. Biomed Pharmacother. 2023;164:115015.
10. Jalalvand M, Enayati S, Akhtari M, Madreseh E, Jamshidi A, Farhadi E, et al. Blood regulatory T cells in inflammatory bowel disease, a systematic review, and meta-analysis. Int Immunopharmacol. 2023;117:109824.
11. Hanahan D, Michielin O, Pittet MJ. Convergent inducers and effectors of T cell paralysis in the tumour microenvironment. Nat Rev Cancer. 2024;25(1):41-58.
12. Chen X, Ma Z, Yi Z, Wu E, Shang Z, Tuo B, et al. The effects of metabolism on the immune microenvironment in colorectal cancer. Cell Death Discov. 2024;10(1).
13. Xie L, Fang J, Yu J, Zhang W, He Z, Ye L, et al. The role of $CD4^+$ T cells in tumor and chronic viral immune responses. MedComm. 2023;4(5).
14. Guo X, Xu L, Nie L, Zhang C, Liu Y, Zhao R, et al. B cells in head and neck squamous cell carcinoma: current opinion and novel therapy. Cancer Cell Int. 2024;24(1).
15. Wang X, Luo Y, Zhou Q, Ma J. The roles of S100A8/A9 and S100A12 in autoimmune diseases: mechanisms, biomarkers, and therapeutic potential. Autoimmun Rev. 2025;24(12):103920.
16. Chen J, Huang Y, Wang B, Lu Y, Jian J, Tang J, et al. Characterization of S100A12 from nile tilapia (Oreochromis niloticus) and its roles on inflammatory responses. Fish Shellfish Immunol. 2022;131:401-7.
17. Hou FEI, Wang L, Wang H, Gu J, Li M, Zhang J, et al. Elevated gene expression of S100A12 is correlated with the predominant clinical inflammatory factors in patients with bacterial pneumonia. Mol Med Rep. 2015;11(6):4345-52.
18. Yang X, Zhang W, Wang L, Zhao Y, Wei W. Metabolite-sensing GPCRs in rheumatoid arthritis. Trends Pharmacol Sci. 2024;45(2):118-133.
19. Chien Y-C, Chen J-N, Chen Y-H, Chou R-H, Lee H-C, Yu Y-L. Epigenetic silencing of miR-9 promotes migration and invasion by EZH2 in glioblastoma cells. Cancers. 2020;12(7):1781.
20. Wang J, He M, Yang M, Ai X. Gut microbiota as a key regulator of intestinal mucosal immunity. Life Sci. 2024;345:122612.
21. Shi L, Li Y, Liu Y, Jia H. Alterations of gut microbiota and cytokines in elevated serum diamine oxidase disorder. Medicine. 2022;101(50).
22. Nong K, Qin X, Liu Z, Wang Z, Wu Y, Zhang B, et al. Potential effects and mechanism of flavonoids extract of Callicarpa nudiflora Hook on DSS-induced colitis in mice. Phytomedicine. 2024;128:155523.
23. Efremova I, Maslennikov R, Medvedev O, Kudryavtseva A, Avdeeva A, Krasnov G, et al. Gut microbiota and biomarkers of intestinal barrier damage in cirrhosis. Microorganisms. 2024;12(3):463.
24. Yu Y, Zhao W, Yang M, Wu B, Yuan X. Tumor-promoting gut microbes in colorectal cancer: mechanisms and translational perspectives. Int J Med Sci. 2026;23(1):63-75.
25. Chaturvedi P, Pathak R, Dayal R, Parihar H, Kathireshan AK, Tirumalai PS. Colorectal cancer mitigation through probiotics: current evidence and future directions. Curr Microbiol. 2025;82(8):339.
26. Hospital Authority of National Health Commission of the People's Republic of China; Chinese Society of Oncology CMA. Chinese protocol of diagnosis and treatment of colorectal cancer of the National Health Commission (2023 edition). Chin J Pract Surg. 2023;43(6):1005-2208.
27. Sobhani I, Tap J, Roudot-Thoraval F, Roperch JP, Letulle S, Langella P, et al. Microbial dysbiosis in colorectal cancer (CRC) patients. PLoS One. 2011;6(1):e16393.
28. Gianotti L, Braga M, Fortis C, Soldini L, Vignali A, Colombo S, et al. A prospective, randomized clinical trial on perioperative feeding with an arginine-, omega-3 fatty acid-, and RNA-enriched enteral diet: effect on host response and nutritional status. JPEN J Parenter Enteral Nutr. 1999;23(6):314-20.
29. Liu Y, Lau HC, Cheng WY, Yu J. Gut microbiome in colorectal cancer: clinical diagnosis and treatment. Genomics Proteomics Bioinformatics. 2023;21(1):84-96.
30. Maecker HT, Rinfret A, D'Souza P, Darden J, Roig E, Landry C, et al. Standardization of cytokine flow cytometry assays. BMC Immunol. 2005;6:13.
31. Li Y, Yang J, Wu Q. Correlation between serum S100A12 and sRAGE levels with intestinal flora imbalance and chemotherapy effect in patients with colon cancer. Anhui Med Pharm J. 2024;28(6):1170-3.
32. Qiao SB, Niu ML, Liang WT, Zhang LJ, Chen X, Zhu YK. Analysis of serum S100A12, soluble advanced glycation end products receptor, and gut microbiome in elderly patients with colorectal cancer. World J Gastrointest Oncol. 2025;17(6):106393.
33. Shu P, Zhao L, Wagn J, Shen X, Zhang X, Shen S, et al. [Association between serum levels of S100A8/S100A9 and clinical features of colorectal cancer patients]. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2016.

34. Luo CT, Li MO. Transcriptional control of regulatory T cell development and function. Trends Immunol. 2013;34(11):531-9.
35. Zhong T, Sun S, Zhao M, Zhang B, Xiong H. The mechanisms and clinical significance of CD8(+) T cell exhaustion in anti-tumor immunity. Cancer Biol Med. 2025;22(5):460-80.
36. Testa U, Pelosi E, Castelli G. Colorectal cancer: genetic abnormalities, tumor progression, tumor heterogeneity, clonal evolution and tumor-initiating cells. Med Sci (Basel). 2018;6(2).
37. Snezhkina AV, Kudryavtseva AV, Kardymon OL, Savvateeva MV, Melnikova NV, Krasnov GS, et al. ROS generation and antioxidant defense systems in normal and malignant cells. Oxid Med Cell Longev. 2019;2019:6175804.
38. Parada Venegas D, De la Fuente MK, Landskron G, González MJ, Quera R, Dijkstra G, et al. Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. Front Immunol. 2019;10:277.