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RESEARCH

The value of SDC2 and Septin9 combined with serum tumor markers in early diagnosis of colorectal cancer

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Abstract

Objective The aim of this study is to evaluate the significance of combined detection of Septin9 and syndecan-2 (SDC2) methylation markers and serum tumor markers for the early diagnosis of colorectal cancer.

Methods A total of 116 patients diagnosed with colorectal cancer between December 2022 and February 2024 were designated as the colorectal cancer group. Additionally, 31 patients with colorectal adenoma were assigned to the adenoma group, while 44 individuals undergoing routine physical examinations were included in the control group. Concentrations of Septin9, SDC2, fecal occult blood (FOB), and four tumor markers—carcinoembryonic antigen (CEA), carbohydrate antigen 199 (CA199), carbohydrate antigen 125 (CA125), and carbohydrate antigen 724 (CA724)—were measured. Diagnostic performance was assessed using receiver operating characteristic (ROC) curves for Septin9, SDC2, the four tumor markers, FOB, the combination of Septin9 and SDC2, and the combined use of all seven indicators (CEA, CA19-9, CA125, CA72-4, FOB, Septin9, and SDC2).

Results The colorectal cancer group exhibited the highest positive rates for Septin9, SDC2, the four tumor markers, the combined detection of Septin9 and SDC2, and the combined detection of all seven indicators, compared to both the adenoma and control groups ($P < 0.05$). The adenoma group also showed higher positive rates than the control group ($P < 0.05$). For patients with stage I–III colorectal cancer, the positive rates for the combined detection of Septin9 and SDC2 were 81.3%, 78.9%, and 90.2%, respectively, surpassing those for the combined detection of the four tumor markers (43.8%, 55.3%, and 61.0%). Additionally, the positive rates for the two-gene combination in stage III colorectal cancer were higher than those for FOB ($P < 0.05$). The sensitivity and area under the curve (AUC) for SDC2 were 73.3% and 0.855, respectively, exceeding the sensitivity and AUC for the combined four tumor markers, which were 60.3% and 0.734 ($P < 0.05$). The combined detection of the two methylated genes demonstrated a sensitivity of 86.2% and an AUC of 0.908, outperforming both FOB and the combined detection of the four tumor markers ($P < 0.05$).

Conclusion The detection of SDC2 exhibits high sensitivity for colorectal cancer, and when combined with Septin9, it significantly enhances the diagnostic accuracy for early-stage colorectal cancer, offering substantial clinical value.

Keywords Colorectal cancer · Methylation · SDC2 · Septin9

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Introduction

Colorectal cancer continues to present a significant global health challenge as a malignant disease of the digestive tract. Its development typically follows a prolonged course, beginning with normal epithelium, progressing through stages of polyp formation, non-advanced adenomas, advanced adenomas, and eventually culminating in cancer—a process that generally spans 10 to 15 years. In developed Western countries, both incidence and mortality rates have decreased in recent years due to the implementation of effective screening programs and advancements in treatment. Conversely, in China, colorectal cancer incidence and mortality rates are on the rise, largely attributable to dietary and lifestyle changes, positioning colorectal cancer as the third most common and second deadliest cancer in the country [1]. Previous research indicates that early detection of colorectal cancer, followed by timely surgical resection and standardized treatment—including adjuvant radiotherapy, chemotherapy, targeted therapy, and immunotherapy—can result in a 5-year survival rate approaching 64% [2].

Current primary screening methods for colorectal cancer include tumor markers, fecal occult blood (FOB), and endoscopic examinations. While fecal and tumor marker tests are non-invasive, simple, and have high patient compliance, their sensitivity and specificity are limited when utilized in isolation. Although endoscopic examination remains the gold standard for diagnosing colorectal cancer, it requires specialized equipment and expertise and carries risks, such as infection, along with the need for bowel preparation, which can lower patient compliance. Consequently, there is increasing interest in molecular biomarkers that are highly sensitive, specific, non-invasive, simple, quick, and suitable for large-scale screening. Recent studies have identified colorectal cancer as a malignant disease driven by molecular genetic alterations across multiple genes and stages, with abnormal DNA methylation recognized as a key pathogenic factor [3–5].

In recent years, molecular testing technology has gained widespread clinical use, with extensive research exploring DNA methylation in blood, stool, and urine for the detection of pancreatic and colorectal cancers [6]. Several methylated markers have emerged as promising as promising non-invasive cancer markers [7,8]. Prior studies have established a connection between the methylation of Septin9 and syndecan-2 (SDC2) and the development of colorectal cancer. However, the clinical application of combining these markers with conventional tumor markers for early colorectal cancer screening remains limited.

The aim of this study is to assess the significance and clinical value of early colorectal cancer diagnosis by detecting serum tumor markers—carcinoembryonic antigen (CEA), carbohydrate antigen 199 (CA199), carbohydrate antigen 125 (CA125), and carbohydrate antigen 724 (CA724)—in conjunction with FOB, and the methylation of serum Septin9 and fecal SDC2 in patients with colorectal cancer and colorectal adenomas. We evaluated the levels of serum Septin9 methylation and fecal SDC2 methylation, along with routine serum tumor markers (CEA, CA199, CA125, CA724) and FOB in these two study groups as well as a control group. Additionally, we assessed the significance and clinical utility of these early diagnostic indicators for colorectal cancer, including individual tests for FOB, Septin9, and SDC2 gene methylation, as well as combined testing for four tumor markers and the combined detection of Septin9 and SDC2 methylation with all seven indicators.

Materials and methods

Study participants

This study was conducted in accordance with the STROBE guidelines, ensuring the systematic collection of clinical data and the accurate testing and analysis of samples. The study cohort consisted of patients diagnosed with either colorectal cancer or with colorectal adenoma, all of whom were admitted to the First People's Hospital of Yulin between December 2022 and February 2024. Diagnoses were confirmed through pathology or colonoscopy. The participants were divided into two groups: the colorectal cancer group and the adenoma group, respectively. Additionally, healthy individuals undergoing routine health examinations during the same period were selected to form the control group.

The inclusion criteria for the study participants were as follows: (1) a confirmed diagnosis of colorectal cancer or adenoma through pathology or colonoscopy; (2) no prior radiotherapy or chemotherapy before surgery; (3) complete clinical data. The exclusion criteria were (1) presence of other malignant tumors; (2) metastatic colorectal cancer; (3) severe impairment of other organ functions. The study was approved by the Ethics Committee of the First People's Hospital of Yulin, and all participants provided informed consent.

Methods

Collection of clinical data

Collection of serum and plasma samples

Venous blood samples were collected from all participants after an overnight fast and before any surgical intervention.

For the tumor marker assay, 2 to 3 mL of venous blood was drawn and centrifuged at 3500 rpm for 8 min to isolate the serum. For the Septin9 methylation assay, approximately 5 to 7 mL of venous blood was collected using ethylenediaminetetraacetic acid (EDTA)-K2 blood collection tubes, supplied by the reagent manufacturer (Shanghai Tellgen Life Science Co., Ltd.). This blood was then centrifuged at 2500 rpm for 10 min to obtain plasma. Both serum and plasma samples were stored at − 80 °C for subsequent analysis.

Collection of fecal specimens

For the FOB test, a fresh fecal sample weighing approximately 1 g, roughly the size of a broad bean, was collected and delivered to the laboratory within 3 h. For the fecal SDC2 test, all participants received thorough training to adhere to the manufacturer's instructions (Guangzhou Creative Biosciences Co., Ltd.) for sample collection. They were instructed to collect 4 to 5 g of well-formed, non-sloppy stool using a specialized fecal collection device and place it into a sample preservation tube containing cell preservation fluid, which protects the target nucleic acid from degradation. These samples were transported and stored at room temperature for up to 7 days. If testing was not performed immediately, the samples were thoroughly mixed and shaken before being stored at − 80 °C for future analysis.

Main reagents and instruments

Serum tumor markers including CEA, CA199, CA125, and CA724 were analyzed using the Cobas E801 fully automated chemiluminescent immunoassay system (Roche Diagnostics International Ltd., Switzerland) along with its specific reagents. Fecal SDC2 methylation was detected using a methylation detection kit for the human SDC2 gene via real-time polymerase chain reaction (PCR) (Guangzhou Creative Biosciences Co., Ltd.). Plasma Septin9 gene methylation was assessed using a DNA methylation detection kit tailored for the human Septin9 gene (real-time PCR, Shanghai Tellgen Diagnostic Technology Co. Ltd.). The amplification of fecal SDC2 and plasma Septin9 genes was performed using the Z480 real-time fluorescent quantitative PCR amplification system (Roche Diagnostics International Ltd., Switzerland) and the SLAN-96S system (Shanghai Hongshi Medical Technology Co., Ltd.), respectively.

FOB detection

The FOB test utilized a double antibody sandwich assay to qualitatively detect human hemoglobin in fecal samples. The reagent was supplied by Blue Cross Bio-Medical (Beijing) Co., Ltd. Following the manufacturer's instructions, a fecal sample approximately the size of a broad bean was diluted with an appropriate volume of normal saline. The sample end of the test strip was immersed in the diluted fecal solution, and the results were read within 5 to 10 min. A positive result was indicated by the appearance of a red band in both the test area (T line) and the control area (C line). Conversely, a negative result was indicated by the presence of a red band in the control area, with no band in the test area.

Detection of fecal SDC2 and serum Septin9 genes

The detection of SDC2 gene methylation in fecal samples and Septin9 gene methylation in plasma involved DNA extraction and conversion, followed by fluorescence PCR assays. Fecal DNA extraction was conducted as per the kit instructions, utilizing magnetic bead capture to extract SDC2 and beta-actin (ACTB) genes from human fecal samples. This process included thorough washing to remove impurities, resulting in highly purified fecal DNA. Similarly, plasma-free DNA extraction was conducted using a nucleic acid extraction kit instructions to purify free DNA in plasma. Following extraction, both fecal DNA and plasma cell-free DNA underwent sulfite conversion, during which unmethylated cytosines were converted into uracil through a deamination reaction, while methylated cytosines remained unaltered.

Fluorescent quantitative PCR assay

Amplification parameters were configured according to the specifications of the SDC2 and Septin9 genes. Interpretation of the amplification results was as follows:

SDC2 gene detection: A sample was considered positive if the cycle threshold (CT) value for the internal control gene ACTB was ≤ 36 and the CT value for the SDC2 gene was ≤ 38, accompanied by an S-shaped amplification curve. Conversely, if the ACTB gene CT value was ≤ 36 and the SDC2 gene CT value exceeded 38, the sample was classified as negative. Samples with an ACTB gene CT value > 36 or those lacking a CT value were deemed invalid and necessitated re-examination.

Septin9 gene detection: A sample was considered positive if the internal control gene ACTB had a CT value ≤ 35, and the Septin9 gene also had a CT value ≤ 35 with an S-shaped amplification curve. Conversely, the result was negative if the ACTB gene had a CT value ≤ 35 and the Septin9 gene had a CT value > 35. Samples with an ACTB gene CT value > 35 or lacking a CT value were deemed invalid and required re-examination.

Interpretation of results

The standard reference ranges for the tumor markers were as follows: CEA (0–5 ng/mL), CA19-9 (0–37 U/mL), CA125 (0–35 U/mL), and CA72-4 (0–8.2 U/mL). Any individual marker exceeding its respective threshold was considered positive. For the combined detection of methylation in fecal SDC2 and serum Septin9 genes, along with FOB and tumor markers, a parallel interpretation method was employed. A positive result in any of these tests was interpreted as a positive outcome for the combined detection.

Statistical analysis

Statistical analysis was performed using SPSS 22.0 software. Descriptive statistics, including the mean ± standard deviation ($\bar{x} \pm s$), were utilized for normally distributed continuous data. Comparisons between two groups were conducted using the *t*-test, while one-way ANOVA was employed for comparisons among multiple groups with normally distributed data. For non-normally distributed continuous data, the median and interquartile range [M (P25, P75)] were reported, and group comparisons were made using the rank sum test. Categorical data were described using frequency and constituent ratio, with group comparisons conducted using the chi-squared (χ^2) test or Fisher's exact probability method. A binary logistic regression analysis was applied to develop a combined diagnostic equation. Receiver operating characteristic (ROC) curves were plotted, and the area under the curve (AUC) was calculated to assess the diagnostic performance of the methylations of the SDC2 and Septin9 genes, FOB, four tumor markers, the combined detection of SDC2 and Septin9 methylations, and the combined detection of all seven indicators for colorectal cancer. Statistical significance was set at $P < 0.05$.

Results

Study group characteristics

The diagnostic criteria for colorectal cancer were based on the "Chinese Guidelines for the Diagnosis and Treatment of Colorectal Cancer (2020 Edition)."[9] The colorectal cancer group comprised 71 males and 45 females, aged 22 to 86 years, with a mean age of 60.4 ± 11.9 years. The adenoma group included 17 males and 14 females, aged 48 to 82 years, with a mean age of 60.5 ± 11.1 years. The control group consisted of 22 males and 22 females, aged 39 to 75 years, with a mean age of 57.7 ± 8.35 years. Colorectal cancer staging was classified according to the American Joint Committee on Cancer (AJCC) tumor node metastasis (TNM) staging system, with 16 cases in stage I, 38 in stage II, 41 in stage III, and 21 in stage IV [10]. Statistical analysis revealed no significant differences in age or gender distribution among the three groups ($P > 0.05$).

Expression levels and differences of serum tumor markers CEA, CA199, CA125, and CA724 among groups

There were no significant differences in age and gender distribution among the control, adenoma, and colorectal cancer groups ($P > 0.05$). However, statistically significant differences were observed in the expression levels of CEA and CA199 among these three groups ($P < 0.01$). Specifically, the colorectal cancer group exhibited significantly higher levels of CEA compared to both the control and adenoma groups ($P < 0.05$). Similarly, the colorectal cancer group exhibited elevated levels of CA199 compared to the adenoma group, with a statistically significant difference ($P < 0.05$). No significant differences were found between the adenoma and control groups in the levels of CEA and CA19-9 ($P > 0.05$). Furthermore, there were no significant differences in the levels of CA125 and CA724 among the three groups ($P < 0.01$), as shown in Table 1.

Positive rates of individual SDC2 or Septin9 methylation, FOB, four tumor markers, combined methylation of the two genes, and combined use of all seven indicators in colorectal cancer, adenoma, and control groups.

Significant differences were observed in the positive rates of individual SDC2 or Septin9 methylation, FOB, the four tumor markers, combined methylation of the two genes, and the combined use of all seven indicators among the three groups ($P < 0.01$). The highest positive rates for individual SDC2 or Septin9 methylation, the combination of four tumor markers, the combined methylation of two genes, and the combined use of all seven indicators were observed in the colorectal cancer group compared to both the adenoma and control groups ($P < 0.05$). Moreover, these positive rates were higher in the adenoma group compared to the control group ($P < 0.05$). Positive rates of FOB were higher in the colorectal cancer group compared to both the adenoma and control groups ($P < 0.05$), with no significant difference between the adenoma and control groups ($P > 0.05$). In the colorectal cancer group, positive rates of SDC2 methylation and the combined methylation of two genes, as well as the combined use of all seven indicators, were higher than those of the combined four tumor markers ($P < 0.05$). Additionally, positive rates for the combined methylation of two genes and the use of all seven indicators were significantly higher compared to those for FOB ($P < 0.05$) (Table 2).

Table 1 Comparison of basic demographic characteristics and tumor markers among different groups

Group	Sex (case, m/f)	Age (year,$\bar{x} \pm s$)	Tumor marker [M (P_{25}, P_{75})]			
			CEA (ng/mL)	CA199 (U/mL)	CA125 (U/mL)	CA724 (U/mL)
Healthy control	22/22	57.7 ± 8.35	1.78 (1.46, 2.78)[a]	11.15 (5.89, 18.55)	11.1 (6.79, 15.78)	2.31 (1.5, 3.59)
Adenoma	17/14	61.5 ± 11.1	2.16 (1.51, 4.05)[ab]	9.21 (2.00, 19.5)[ab]	9.87 (6.51, 14.7)	1.50 (1.50, 2.93)
Colorectal Cancer	71/45	60.4 ± 11.9	5.34 (1.72, 13.35)	12.2 (6.66, 27.58)	9.99 (7.40, 16.45)	2.11 (1.5, 6.55)
statistical value	1.755	1.279	8.769	7.011	0.739	2.619
P-value	0.418	0.281	< 0.01	< 0.01	0.481	0.078

[a]$P < 0.05$ compared with the group diagnosed with colorectal cancer; [b]$P > 0.05$ compared with the healthy control group

Correlation between the pathological features of colorectal cancer and the positive rates of the individual methylation of SDC2 or Septin9, the combined methylation of the two genes, and the combined use of the seven indicators.

The positive rates of individual SDC2 or Septin9 methylation, the combined methylation of two genes, and the combined use of all seven indicators did not exhibit any significant correlation with patient age, gender, tumor location, lymph node metastasis, thrombosis in tumor vasculature, or invasion of nerves ($P > 0.05$). Similarly, there was no significant association between the positive rates of SDC2 methylation, the combined methylation of the two genes, and the combined use of all seven indicators with the maximum tumor diameter ($P > 0.05$). However, a statistically significant correlation was found between the positive rate of Septin9 methylation and the maximum tumor diameter ($P < 0.05$) (Table 3).

Comparison of positive indicators across clinical stages of colorectal *cancer*

In patients with TNM stages I–IV colorectal cancer, the positive rates for the combined detection of two gene methylations were 81.3%, 78.9%, 90.2%, and 95.2%, respectively, with no statistically significant difference among these stages ($P > 0.05$). However, when compared to the positive rates for the four tumor markers in patients with stages I–III colorectal cancer, which were 43.8%, 55.3%, and 61.0%, respectively, the positive rates for the combined gene methylation showed a statistically significant difference ($P < 0.05$). Additionally, in patients with stage III colorectal cancer, the positive rate of the combined gene methylation was higher than that of FOB ($P < 0.05$). In patients with stage IV colorectal cancer, the positive rates of Septin9 and the four tumor markers were 85.7% and 81.0%, respectively, significantly higher than those in patients with stage I colorectal cancer, where the positive rates of Septin9 and the four tumor markers were 50.0% and 43.8%, respectively ($P < 0.05$). However, across stages I–IV, there was no significant difference in the positive rate for the combined detection of two gene methylations compared to the combined use of all seven indicators ($P > 0.05$) (Table 4).

Assessment of the diagnostic efficacy of FOB, individual methylation of SDC2 or Septin9, four tumor markers, combined methylation of two genes, and combined use of seven indicators for colorectal cancer.

The diagnostic performance of the combined four tumor markers, FOB, and individual SDC2 or Septin9 methylation was assessed using the ROC curve (Table 5, Fig. 1A).

Table 2 Detection results of four tumor markers, FOBT, SDC2, Septin9 gene methylation, and the combined seven indicators [number of cases (%)]

Group	Number of cases	Four tumor markers	FOBT	SDC2	Septin9	SDC2 + Septin9	Combined seven indicators
Healthy control	44	6 (13.6)[a]	5 (11.4)[a]	1 (2.3)[a]	1 (2.3)[a]	2 (4.5)[a]	11 (25.0)[a]
Adenoma	31	11 (35.5)[a b]	7 (22.6)[a]	12 (38.7)[a b]	7 (22.6)[a b]	13 (41.9)[a b]	20 (64.5)[a b]
Colorectal Cancer	116	70 (60.3)	72 (62.1)	85 (73.3)[c]	64 (55.2)	100 (86.2)[cd]	111 (95.7)[c]
statistical value		29.57	40.17	66.72	41.6	93.95	85.45
P-value		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

[a]$P < 0.05$ compared with the group diagnosed with colorectal cancer; [b]$P < 0.05$ compared with the healthy control group; [c]$P < 0.05$ compared with the four tumor markers; [d]$P < 0.05$ compared with the combined seven indicators

Table 3 Relationship between colorectal cancer's pathological features and the positive rates of individual methylation of SDC2 or Septin9, combined methylation of SDC2 and Septin9, and the combined seven indicators [number of cases (%)]

Clinical feature	Number of cases	Septin9	*P*-value	SDC2	*P*-value	Septin9 + SDC2	*P*-value	Combined seven indicators	*P*-value
Age			0.28		0.109		0.492		0.592
≤ 60 years	56	28 (50.0)		37 (66.1)		47 (83.9)		53 (94.6)	
> 60 years	60	36 (60.0)		48 (80.0)		53 (88.3)		58 (96.7)	
Sex			0.75		0.09		0.178		0.378
Male	71	40 (56.3)		51 (71.8)		60 (88.9)	0.505	67 (97.8)	
Female	45	24 (53.3)		34 (75.6)		40 (84.5)		44 (94.4)	
Tumor site			0.26		0.968		0.679		0.303
Colon	49	30 (61.2)		36 (73.5)		43 (87.8)		48 (98.0)	
Rectum	67	34 (50.7)		49 (73.1)		57 (85.1)		63 (94.0)	
Lymph node metastasis			0.15		0.79		0.27		0.855
No	65	32 (49.2)		47 (72.3)		54 (83.1)		62 (95.4)	
Yes	51	32 (62.7)		38 (74.5)		46 (90.2)		49 (96.1)	
Cancer-associated thrombosis			0.32		0.455		0.863		0.867
No	96	55 (57.3)		69 (71.9)		83 (86.5)		92 (95.8)	
Yes	20	9 (45.0)		16 (80.0)		17 (85.0)		19 (95.0)	
Invasion of nerves			0.55		0.979		0.361		0.335
No	90	51 (56.7)		66 (73.3)		79 (87.8)		87 (96.7)	
Yes	26	13 (50.0)		19 (73.1)		21 (80.8)		24 (92.3)	
Maximum diameter of tumor			0.01		0.199		0.081		0.059
< 5 cm	55	21 (38.2)		38 (69.1)		43 (78.2)		50 (90.9)	
≥ 5 cm	37	24 (64.9)		30 (81.1)		34 (91.9)		37 (100.0)	

Table 4 Comparison of TNM stages of colorectal cancer [number of cases (%)]

Group	Number of cases	Four tumor markers	FOBT	Septin9	SDC2	Septin9 + SDC2	Combined seven indicators
Stage I	16	7 (43.8)	10 (62.5)	8 (50.0)	10 (62.5)	13 (81.3)[a d]	14 (87.5)
Stage II	38	21 (55.3)	22 (57.9)	17 (44.7)	27 (71.1)	30 (78.9)[a d]	36 (94.7)
Stage III	41	25 (61.0)	24 (58.5)	21 (51.2)	33 (80.5)	37 (90.2)[abd]	39 (95.1)
Stage IV	21	17 (81.0)[c]	16 (76.2)	18 (85.7)[c]	15 (71.4)	20 (95.2)[d]	21 (100.0)

[a] $P < 0.05$ compared with four tumor markers; [b] $P < 0.05$ compared with FOBT; [c] $P < 0.05$, comparison between four tumor markers and Septin9 at stage IV versus stage I; [d] $P > 0.05$ compared with the combined seven indicators

Table 5 Evaluation of the detection performance of FOBT, individual methylation of SDC2 and Septin9, four tumor markers, the combination of the methylation of two genes, and the combined seven indicators in diagnosing colorectal cancer

Testing item	Sensitivity (%)	Specificity (%)	Predictive positivity (%)	Predictive negativity (%)		95% confidence interval	
Four tumor markers	60.3	86.4	92.1	45.2	0.734	0.651–0.816	$P < 0.01$
FOBT	62.1	88.6	93.5	47.0	0.754	0.674–0.833	$P < 0.01$
Septin9	55.2	97.7	98.5	58.1	0.764	0.692–0.837	$P < 0.01$
SDC2	73.3	97.7	98.8	45.3	0.855	0.796–0.914	$P < 0.01$
Septin9 + SDC2	86.2	95.5	98.0	72.4	0.908	0.857–0.960	$P < 0.01$
Combined seven indicators	95.7	75.0	91	86.8	0.853	0.774–0.933	$P < 0.01$

Fig. 1 A shows the ROC curves for diagnosing colorectal cancer using FOBT, the methylation of Septin9, SDC2, and four tumor markers. Figure 1 B displays the ROC curves for diagnosing colorectal cancer using FOBT, the four tumor markers, the methylation of Septin9 and SDC2, and the combined seven indicators

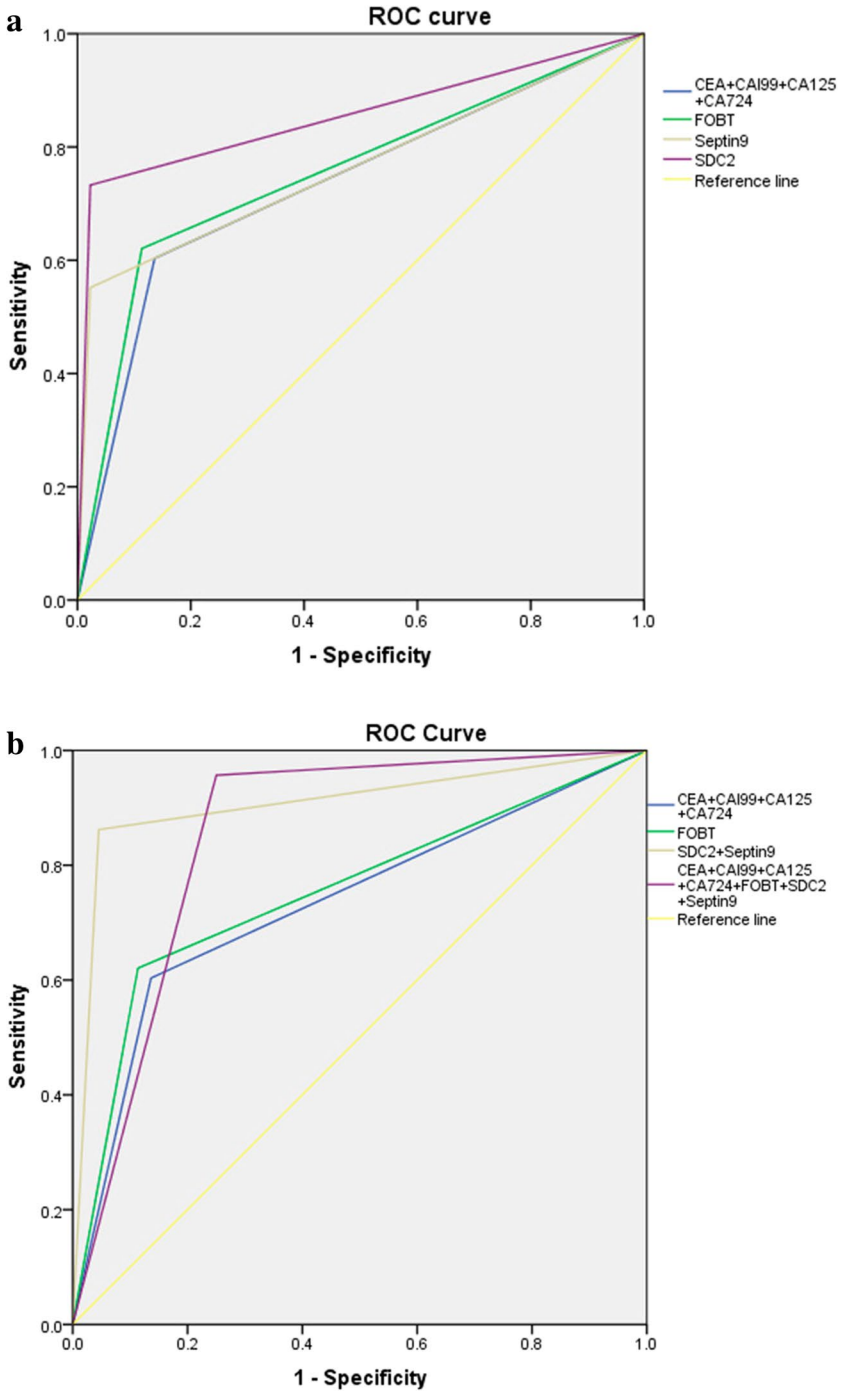

Fig. 1 **A** ROC curves for diagnosing colorectal cancer utilizing FOB, Septin9 methylation, SDC2 methylation, and four tumor markers. **B** ROC curves for colorectal cancer using FOB, four tumor markers, Septin9 and SDC2 methylation, and the combined seven indicators

The sensitivity and AUC of SDC2 methylation were 73.3% and 0.855, respectively, which were significantly higher than those for the combined four tumor markers (60.3%, 0.734, $P < 0.05$). However, this difference was not statistically significant compared to FOB (62.1%, 0.754, $P > 0.05$). Septin9 methylation demonstrated a sensitivity of 55.2% and an AUC of 0.764, which did not significantly differ from the values of FOB or the combined four tumor markers ($P > 0.05$).

The sensitivity, specificity, and AUC of the combined methylation of the two genes and the combination of all seven indicators are presented in Table 5 and Fig. 1B, with values of 86.2%, 0.908, and 95.7% for the former and 0.853 for the latter. These values were significantly higher than those for FOB and the combined four tumor markers ($P < 0.05$). Furthermore, there was no significant difference in sensitivity, specificity, and AUC between the combination

of all seven indicators and the combined methylation of two genes for diagnosing colorectal cancer ($P > 0.05$).

Discussion

Colorectal cancer is a prevalent malignancy of the digestive tract, with global projections indicating a significant rise in incidence. By 2030, it is estimated that there will be approximately 2.2 million new cases of colorectal cancer, leading to around 1.1 million deaths worldwide [11,12]. Early detection and timely intervention in patients with early-stage colorectal cancer can result in a favorable 5-year survival rate exceeding 90%. In contrast, individuals with advanced-stage colorectal cancer, often associated with lymph node metastasis, experience a significantly reduced 5-year survival rate, approximately 10% [13].

Serum tumor markers, similar to FOB tests, have limited specificity in the diagnosis of tumors. In this study, the expression levels of four tumor markers—CEA, CA199, CA125, and CA724. Notably, CEA and CA199 expression levels demonstrated statistically significant differences across the colorectal cancer, adenoma, and control groups. Specifically, CEA levels were elevated in patients with colorectal cancer compared to both the adenoma and control groups, while no significant differences were observed between the adenoma and control groups for CEA and CA19-9. When evaluated individually, none of the four tumor markers effectively distinguished between early and advanced stages of colorectal cancer. However, when combined, the tumor markers demonstrated statistically significant differences among the three groups, with a sensitivity and specificity of 60.3% and 86.4%, respectively, and an AUC of 0.734. While combining multiple markers may enhance detection capabilities, the indiscriminate addition of markers could increase the risk of false-positive results.

DNA methylation is an early and common epigenetic modification in the initiation and progression of colorectal cancer. Recent studies have shown that DNA methylation in colorectal cancer cells can promote the proliferation, invasion, and metastasis of malignant cells [14,15]. The methylation of specific genes such as SDC2, Septin9, and BCAT1 has emerged as a novel molecular marker for colorectal cancer diagnosis [6]. The Septin9 gene, located on chromosome 17q25.3, spans approximately 2.40×10^5 bp and consists of 17 exons. This gene is widely expressed in various eukaryotic cells and plays pivotal roles in numerous physiological processes, including human cell division, proliferation, apoptosis, intracellular and extracellular substance transport, and cytoskeletal regulation [16]. Earlier studies have suggested that Septin9 may suppress normal gene expression by disrupting cytokinesis and methylating cytosine-phosphate-guanine (CpG) islands, leading to genomic instability and contributing to tumorigenesis. In recent years, Septin9 methylation testing has become increasingly utilized in screening for breast cancer, ovarian cancer, and colorectal tumors [17–20].

SDC2, a vital protein, plays an integral role in normal physiological functions, such as regulating the extracellular matrix and cellular signal transduction. However, during the onset and progression of colorectal cancer, the expression of SDC2 methylation undergoes significant alterations. Recent studies indicate that the positive rate of SDC2 methylation in colorectal cancer tissues is higher than in adjacent non-cancerous tissues throughout the development and progression of the disease [21].

In our study, the positive detection rates for colorectal cancer using four tumor markers, FOB, individual SDC2 or Septin9 methylation, combined methylation of both genes, and the combination of all seven indicators were 60.3%, 62.1%, 73.3%, 55.2%, 86.2%, and 95.7%, respectively. For the adenoma group, the corresponding positive rates were 35.5%, 22.6%, 38.7%, 22.6%, 41.9%, and 64.5%, respectively. The sensitivity of Septin9 methylation alone for diagnosing colorectal adenomas was lower than that of the four tumor markers and FOB. This could be due to the variability in gene mutation sites among individuals, which are influenced by different molecular pathways during tumorigenesis. As supported by existing literature, single-gene tests are relatively limited in diagnosing early precancerous lesions [22,23]. Furthermore, our findings indicate that the sensitivity of Septin9 methylation for detecting adenomas and colorectal cancer was not as high as previously reported [24]. The relatively modest sample size in our study may have contributed to this lower sensitivity.

This research aimed to assess the diagnostic efficacy of four tumor markers, FOB, individual SDC2 or Septin9 methylation, combined methylation of both genes, and the combination of all seven indicators for colorectal cancer. The results showed that the sensitivity, specificity, and AUC for Septin9 methylation were 55.2%, 97.7%, and 0.764, respectively, which did not differ significantly from the values for the four tumor markers (60.3%, 86.4%, and 0.734) or FOB (62.1%, 88.6%, and 0.754; $P > 0.05$). Conversely, SDC2 methylation exhibited superior detection capabilities with a sensitivity, specificity, and AUC of 73.3%, 97.7%, and 0.855, respectively, outperforming Septin9 methylation ($P < 0.05$). The enhanced performance of SDC2 may be due to the early excretion of methylated cancer cells into the feces during colorectal cancer development, which is facilitated by intestinal peristalsis and may occur before their appearance in blood or urine. The use of a specialized cell preservation solution may further optimize the capture efficiency of target genes. Conversely, the weaker detection performance of Septin9 methylation in feces could be attributed to differences in the timing and quantity of gene excretion.

Our study also examined the correlation between various pathological features in patients with colorectal cancer, including gender, age, tumor location, vascular tumor thrombus, nerve invasion, lymph node metastasis, and tumor size (the tumor size was unknown in 24 cases). The results indicated that the positive rates of SDC2 or Septin9 methylation, the combination of both methylations, and the combination of all seven indicators were not significantly associated with gender, age, thrombosis in tumor vasculature, invasion of nerves, or lymph node metastasis ($P > 0.05$). These findings suggest that gene methylation may precede histopathological changes, making it a potential predictive tool for colorectal cancer through methylation detection, particularly in large-scale population screening efforts. However, a notable correlation ($P < 0.05$) was observed between tumor size and Septin9 methylation. This suggests that smaller tumors may release fewer methylated Septin9 molecules into the bloodstream, leading to delayed detection and potentially impacting the early diagnosis of colorectal cancer.

Furthermore, the positivity rates for the combined methylation of two genes in patients with colorectal cancer at TNM stages I–IV were 81.3%, 78.9%, 90.2%, and 95.2%, respectively, with no statistically significant differences ($P > 0.05$). Recent studies have demonstrated that methylated markers offer greater sensitivity and specificity in diagnosing colorectal cancer compared to traditional FOB and tumor marker tests, indicating significant potential for large-scale screening [25,26].

However, it is important to recognize that the diagnostic capacity of a single methylation site may be limited.[27] For instance, the detection rates of hyperplastic and dysplastic polyps, as well as early adenomas are relatively low when relying solely on a single methylated marker. In our study, the positivity rate for adenomas was 22.6% when assessed with Septin9 alone, but it increased significantly to 41.9% when combined with SDC2 methylation. Similarly, the positivity rates for the combined methylation of two genes in patients with stage I–III colorectal cancer were 43.8%, 55.3%, and 61.0%, respectively, significantly higher than the rates achieved with four tumor markers alone ($P < 0.05$). Colorectal cancer development likely involves multiple genes, and a single marker may not fully capture the complexity of the molecular pathways involved. Thus, enhancing detection capabilities through a combination of multiple markers is essential. Prior studies have highlighted substantial improvements in sensitivity and specificity by integrating multiple markers targeting different gene loci in stool samples [25].

However, combining multiple indicators does not automatically guarantee improved detection performance. In our study, combining four tumor markers, FOB, Septin9, and SDC2 methylation resulted in detection sensitivities, specificities, and AUCs of 95.7%, 75.0%, and 0.853, respectively. These results did not differ significantly from those obtained with the combined methylation of two genes alone. It is crucial to consider the complementary nature of the detection performance when integrating multiple indicators. Additionally, there is a risk that increasing the number of indicators could lead to more false positives, which warrants caution in combined testing.

In summary, the detection of fecal SDC2 methylation demonstrates significant diagnostic potential for the early detection of colorectal cancer, establishing itself as a novel molecular marker for early screening. When combined with plasma Septin9 methylation, this approach enhances sensitivity and specificity, making it particularly valuable for large-scale population screening and improving detection rates for early diagnosis of colorectal cancer.

However, this study has several limitations that should be acknowledged. The participants were exclusively from a single region, which may introduce sample bias, and the relatively small sample size further limits the generalizability of the findings. Future studies should consider multi-center collaborations that include diverse ethnic populations and a larger number of cases, particularly those involving precancerous lesions and colorectal cancer, to validate the diagnostic efficacy of methylation markers such as SDC2 and Septin9. Such efforts could pave the way for a novel, simple, non-invasive, and highly accurate method for the clinical diagnosis of colorectal cancer.

All authors have contributed significantly to the manuscript and declare that the work is original and has not been submitted or published elsewhere.

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Authors' contributions Conception and design of the research: Chao-Shi Zou,Yan-Ping Liu Acquisition of data: Dong-Xu Wang,Ming-Qiang Li,Yan-Ping Liu Analysis and interpretation of the data: Yu-Ling Xie,Yi Chen,Ming-Qiang Li Statistical analysis: Kang-hai Liu,Zhi-Le Su Obtaining financing: Chao-Shi Zou Writing of the manuscript: Yu-Ling Xie,Dong-Xu Wang,Zhi-Le Su Critical revision of the manuscript for intellectual content: Kang-hai Liu,Yi Chen All authors read and approved the final draft.

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Data availability The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of The First People's Hospital of Yulin (YLSY-IRB-KY-2021009). A written informed consent was obtained from all participants.

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