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Superiority of fecal carcinoembryonic antigen as diagnosis marker for adenomatous polyposis coli and asymptomatic colorectal cancer

Linfang Li*, Wenshen Gu*, Xingping Wu, Yufeng Ao, Yiling Song, Xiaohui Li# and Qiuyao Zeng#

Abstract

Background: Non-invasive diagnostic tools of adenomatous polyposis coli (APC) and asymptomatic colorectal cancer (CRC) are urgently needed. Although fecal carcinoembryonic antigen (FCEA) has been documented in some studies, the diagnostic potential for the detection of APC and asymptomatic CRC has not been described yet.

Methods: This is a retrospective study. The pre-diagnostic serum carcinoembryonic antigen (SCEA) and fecal occult blood test (FOBT) levels were retrospectively analyzed in 212 patients with intestinal diseases group (IDG) and 224 controls. The levels of FCEA across all the studied groups were measured using electronic chemiluminescence immunoassay (ECLIA), and their sensitivity and specificity were used to evaluate their diagnostic potential. The individual diagnostic accuracy of the three indices, as well as their combined diagnostic potential, was compared using the receiver operating characteristic (ROC) curve and chi-square test.

Results: The FCEA had low sensitivity (50%) and high specificity (93.91%) for the diagnosis of IDG, with the area under the curve (AUC) value of 0.781. The AUC of FCEA was higher than that of SCEA for the diagnosis of APC and CRC in the APC, asymptomatic CRC, and APC + CRC-stage I patients. The AUCs of FCEA were 0.708 and 0.691 for the 'double-negative patients' and 'triple-negative patients', respectively. In addition, FCEA could diagnose 45.5% of the 'double-negative' patients, 43.3% of the asymptomatic patients, and 42.9% of the 'triple-negative' patients. The combination of FCEA and FOBT improved the diagnostic value (AUC = 0.916).

Conclusion: FCEA has been demonstrated to be a favorable diagnostic marker in intestinal diseases, especially in the APC, asymptomatic CRC, and 'double-negative' or 'triple-negative' CRC patients.

Keywords: adenomatous polyposis coli, asymptomatic colorectal cancer, FCEA, FOBT, SCEA

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Correspondence to:
Qiuyao Zeng
State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, 651 Dongfeng Road East, Guangzhou 510060, China

Department of Clinical Laboratory Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China
zengqiuy@sysucc.org.cn

Linfang Li
Xingping Wu
Yufeng Ao
Yiling Song
Xiaohui Li
Department of Clinical Laboratory Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China

State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China

Wenshen Gu
Department of Laboratory Medicine, Sun Yat-sen Memorial Hospital, Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetics and Gene Regulation, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China

*Linfang Li and Wenshen Gu contributed equally to this work.

#Xiaohui Li and Qiuyao Zeng contributed equally to this work.

Introduction

There are several screening modalities for the diagnosis of colorectal cancer (CRC), including fecal occult blood test (FOBT), serum carcinoembryonic antigen (SCEA), and flexible sigmoidoscopy and colonoscopy, all of which have their own merits and demerits.[1,2] Randomized controlled trials have shown that the FOBT reduces CRC mortality by 15–33%.[3–5] However, it has limitations, including low sensitivity for polyps, low specificity, and false positives.[6] Moreover, the detection rate for asymptomatic CRC patients is only about 13–50%.[7,8] It is known that SCEA is a significant marker for diagnosing the recurrence of cancer after surgery or drug treatment.[9–12] However, due to its low sensitivity and specificity,

it is not suitable for an early diagnosis of CRC.[13] The flexible sigmoidoscopy method reduces CRC mortality by 30%.[6] Colonoscopy is a gold-standard screening test for the diagnosis and removal of lesions. However, the use of both flexible sigmoidoscopy and colonoscopy is limited due to their invasive nature, high cost,[14,15] and poor compliance. Therefore, they are not routinely recommended for all patients.

CRC is known as 'silent disease', as many people do not develop symptoms until the disease is difficult to cure. The survival of patients is significantly affected by the stage of disease at the time of diagnosis. Therefore, diagnosis at an early stage of precancerous colorectal lesions can play a pivotal role for the improvement of treatment outcomes in patients. Most of the colorectal neoplasia are adenocarcinomas, originating from the epithelial cells of colorectal mucosa. In most cases, CRC usually develops from focal changes in benign precancerous polyps, and the full progression from polyps to cancer usually takes years, sometimes up to 10 years.[16,17] The latest guidelines demonstrate that the detection and removal of adenomatous polyposis coli (APC) can reduce mortality of CRC.[18] APC is the most common and important clinical polyp, and accounts for about half to two-thirds of all the colorectal polyps, with a high risk of developing into CRC.[19,20] However, only a few studies have reported the diagnosis of APC apart from colonoscopy and serum marker tests, which do not meet the clinical requirements.

To date, the main problem with the use of potential biomarkers for routine diagnosis of polyps and asymptomatic CRC is that they are not sufficiently sensitive or specific. Therefore, there is an urgent need to develop simple and less invasive diagnostic methods with a high sensitivity and specificity for the diagnosis of CRC patients. Fecal carcinoembryonic antigen (FCEA) has been documented in some studies, but its diagnostic value in asymptomatic CRC and APC has not been described yet.

This study aimed to systematically study the common biomarkers (FOBT and SCEA) and FCEA among patients with asymptomatic CRC and APC, as well as to compare their diagnostic efficacy in order to provide new insights into the diagnosis of these two diseases.

Materials and methods

Study subjects

All fecal samples were collected from the clinical laboratory of Sun Yat-sen University Cancer Center. From April 2019 to April 2020, we collected fecal samples from CRC and APC patients and healthy people in a consistent manner. However, fecal samples from non-gastrointestinal cancer (NGC) patients were randomly collected.

The inclusion criteria were as follows. First, the clinicopathological data of patients, including gender, age, pathological type, tumor stage, and metastatic status, were completely recorded. Second, the tumor stage was determined according to the TNM (tumor–node–metastasis) staging system of the American Joint Committee on Cancer (AJC), *Cancer Staging Manual* (7th Edition). Third, the healthy participants were determined by the clinician to be free of tumor or obvious diseases. Fourth, all participants had FCEA and SCEA test results conducted at the hospital and undergo radiotherapy, chemotherapy, or surgical treatment. A total of 166 CRC patients, 46 APC patients, 60 NGC patients, and 164 healthy participants met the inclusion criteria.

Samples preparation and measurements

This research approach was approved by the Ethics Committee of Sun Yat-sen University Cancer Center (Approval No. GZR2020-118, Approval Date: 8 March 2020). All the participants signed a written informed consent form, and their identifications were removed from the data. This study conformed to STROBE Statement.[21]

Although this study was retrospective, the fecal samples were collected quantitatively before performing the FOBT. The fecal collection tubes (Guangzhou Forreal Biotechnology Co., Ltd, China) were used to collect 0.1 mg of fresh feces from three different locations and then added to 4 ml of buffer solution. The collected fecal samples were homogenized in a homogenizer for 2 min and then centrifuged for 10 min at 10,000 r/min. The supernatant was removed, and the sample was stored at −80°C. The samples were filtered, if necessary. Electronic chemiluminescence immunoassay (ECLIA) Kit (Roche Diagnostics GmbH, China) was utilized for quantitative

detection of CEA, following the manufacturer's instruction. The serum levels of tumor markers were determined through the use of a Cobas 6000 analyzer, as well as their supporting reagents (Roche, Germany). The normal reference value of CEA was set to <5.0 ng/ml. The FOBT was performed using fecal occult blood detection kit (colloid gold method; Jiangxi Jinhuan Medical Equipment Co., Ltd, China). The positive judgment value was set to 0.2 μg/ml.

Statistical analysis

The chi-square test and *t*-test were utilized to identify differences in gender and age between the case and control groups. The receiver operating characteristic (ROC) curve evaluated diagnostic accuracy. However, the sensitivity and specificity of 95% confidence interval were calculated to evaluate the diagnostic efficacy of FCEA. At the same time, Kruskal–Wallis *H* test and Mann–Whitney *U* rank sum test were utilized to assess differences among the different groups. The SPSS 23.0 (SPSS, UK) and GraphPad Prism 7.0 (San Diego, CA, USA) statistical software were used for data analysis. The *p* value of less than 0.05 (two-tailed) was considered statistically significant.

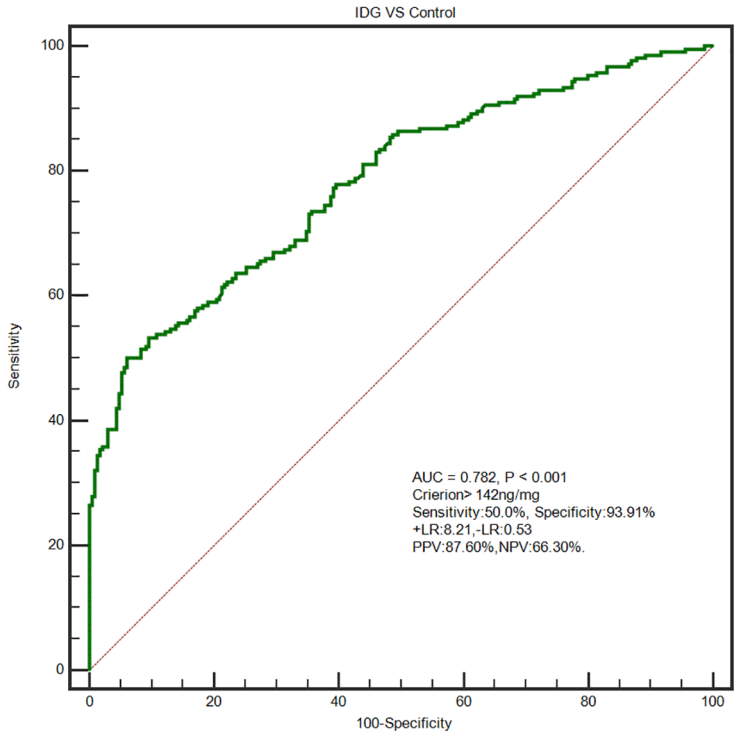

Figure 1. The ROC curve analysis of the FCEA in diagnosis of IDG. The appropriate cutoff value for the FCEA to be 142.0 ng/mg. AUC = 0.781 (95% CI: 0.534–0.640, $p < 0.001$). Intestinal diseases group (IDG): CRC + APC; Control group: healthy controls and NGC.

Results

Demographic and clinical features of the subjects

There were 212 cases in intestinal diseases group (IDG), which included 166 pathologically confirmed patients with CRC (all the CRCs were adenocarcinomas) and 46 pathologically confirmed patients with APC (there are 28 males and 18 females, with an average age of 53.13 years). The IDG comprised of 124 males and 88 females, with an average age of 57.86 ± 11.53 years. In addition, there were 224 cases in the control group, including 109 males and 115 females with an average age of 43.3 ± 12.02 years. The control group included 164 healthy participants and 60 NGC patients confirmed by pathology. The NGC group included 11 cases of head and neck tumors, 11 cases of hepatobiliary and pancreatic tumors, 9 cases of breast cancer, 9 cases of lung cancer, 9 cases of reproductive system tumors, 6 cases of esophageal cancer, 3 cases of kidney cancer, and 2 other cases. The detailed information of participants is provided in Table S1.

Diagnostic value of FCEA expression in IDG

The ROC curve analysis was performed to assess the accuracy of FCEA expression levels in IDG (Figure 1). The area under the curve (AUC) of FCEA expression was 0.781, and the cutoff value of FCEA expression was determined according to the Youden Index at >142 ng/mg to diagnose IDG. At this cutoff value, the FCEA had low sensitivity (50%) and high specificity (93.91%) for the diagnosis of IDG. The Positive Likelihood Ratio (+LR), Negative Likelihood Ratio (–LR), and Odds Ratio (OR) were 8.21, 0.53, and 15.49, respectively, while the Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were 87.6% and 66.3%, respectively.

Relationship between FCEA and clinicopathological characteristics in IDG

The relationships between FCEA and clinicopathological characteristics within the IDG are summarized in Table 1. According to the Youden Index, the IDG was divided into two sub-groups

Table 1. Relationship between the FCEA and the clinical characteristics.

Characteristic	Lower FCEA ($n = 106$) <142 ng/mg, n (%)	Higher FCEA ($n = 106$) $\geqslant 142$ ng/mg, n (%)	p value[a]
Age			
<58	49 (46.2)	57 (53.8)	0.272
$\geqslant 58$	57 (53.8)	49 (46.2)	
Gender			
Male	62 (50.0)	62 (50.0)	1.000
Female	44 (50.0)	44 (50.0)	
Location			
Rectum	44 (50.0)	44 (50.0)	0.654
Colon	55 (49.1)	57 (50.9)	
Other	7 (63.6)	4 (36.4)	
Tumor staging[b]			
I + II	33 (45.2)	40 (54.8)	0.586
III + IV	46 (49.5)	47 (50.5)	
Gross classification[b]			
Eminence type	29 (47.5)	32 (52.5)	0.868
Ulcerative type	33 (43.4)	43 (56.6)	
Infiltration type	3 (50.0)	3 (50.0)	
Histological grade[b]			
Well	2 (25.0)	6 (75.0)	0.353
Moderate	63 (47.0)	71 (53.0)	
Poor	10 (55.6)	8 (44.4)	
Tumor/polypus size			
$\leqslant 3$ cm	44 (62.0)	27 (38.0)	***0.007***
>3 cm	42 (41.2)	60 (58.8)	
Family history			
Yes	41 (54.7)	34 (45.3)	0.313
No	65 (47.4)	71 (52.6)	
Multiple polyps			
Yes	55 (53.4)	48 (46.6)	0.370
No	51 (47.2)	57 (52.8)	

(Continued)

Table 1. (Continued)

Characteristic	Lower FCEA ($n=106$) <142 ng/mg, n (%)	Higher FCEA ($n=106$) $\geqslant 142$ ng/mg, n (%)	p value[a]
Group			
APC	27 (58.7)	19 (41.3)	0.183
CRC	79 (47.6)	87 (52.4)	

Bold italics indicate significant differences ($p<0.05$). APC, adenomatous polyposis coli; CRC, colorectal cancer; FCEA, fecal carcinoembryonic antigen.
[a]The p values were calculated by using the chi-square test (χ^2 test).
[b]Only represents for the CRC group.

with a cutoff of 142 ng/mg: higher FCEA group and lower FCEA group. The clinicopathological characteristics, including age, gender, tumor location, tumor TNM stage, overall classification, histological grade, family history, and multiple polyps, were similar between the two groups. However, the IDG patients with small tumor/polypus size ($\leqslant 3$ cm) were more common in the lower FCEA group than those in the higher FCEA group ($p=0.007$; Figure S1).

Superiority of FCEA for the early diagnosis of IDG

To prove the superiority of FCEA for an early diagnosis of IDG, the IDG was divided into the following three sub-groups: APC group, CRC group, and APC + CRC-stage I group. The ROC curve analysis was performed to evaluate the accuracy of the expression levels of FCEA, FOBT, and SCEA for the diagnosis of IDG.

Among IDG compared with control subjects, the AUC of FCEA was 0.781, which was lower than that of FOBT (AUC = 0.861) and higher than that of SCEA (AUC = 0.707) (Figure 2(a)). In contrast, the different groups demonstrated different results. The AUC of FCEA was the highest (AUC = 0.704), compared with those of FOBT (AUC = 0.611) and SCEA (AUC = 0.525) in the APC group (Figure 2(b)). In the APC + CRC I group (Figure 2(d)), the AUC of FCEA (AUC = 0.729) was not significantly higher compared with that of FOBT (AUC = 0.698) ($p=0.526$), but was significantly higher compared with that of SCEA (AUC = 0.589) ($p=0.006$). However, in the CRC group, the AUC of FOBT was the highest (0.930), which was higher than those of FCEA (AUC = 0.802) and SCEA (AUC = 0.757) (Figure 2(c)). In general, the FOBT had the highest efficiency for the diagnosis of CRC, while the FCEA had higher diagnostic efficiency than that of SCEA for the early stage IDG.

Comparison between the major symptoms and three indices

IDG patients were divided into the following four groups according to their major symptoms: hematochezia (Group 1), changes in bowel habits (Group 2), abdominal pain and diarrhea (Group 3), and asymptomatic (usually detected by physical examination; Group 4). The ROC curve analysis was performed to evaluate the accuracy of expression levels of FCEA, FOBT, and SCEA in these groups (Figure 3). In the symptomatic groups (Groups 1, 2, and 3), the AUC of FCEA was lower compared with that of FOBT, but higher than that of SCEA (Figure 3(a)–(c)). However, in the asymptomatic group (Group 4), the AUC of FCEA (AUC = 0.711) was higher than that of FOBT (AUC = 0.683) and SCEA (AUC = 0.597) (Figure 3(d)). Although, the AUC of FCEA was the highest in the asymptomatic group, there was no statistically significant difference between the AUC of FCEA and that of FOBT ($p=0.593$). However, compared with that of SCEA, the difference was significant ($p=0.049$). In the symptomatic groups, FOBT exhibited the highest diagnostic efficiency (Figure 3(a)–(c)), while in the asymptomatic group (Figure 3(d)), the diagnostic efficiency of FCEA was not lower than FOBT and was higher than SCEA.

The relationship between these four groups and three indices was also analyzed. There were no significant differences in the distribution of FCEA among the four groups ($p=0.586$). However, the FOBT-positive patients, who complained of hematochezia, were very common (99.2%). Overall, 57.7% of the asymptomatic patients had

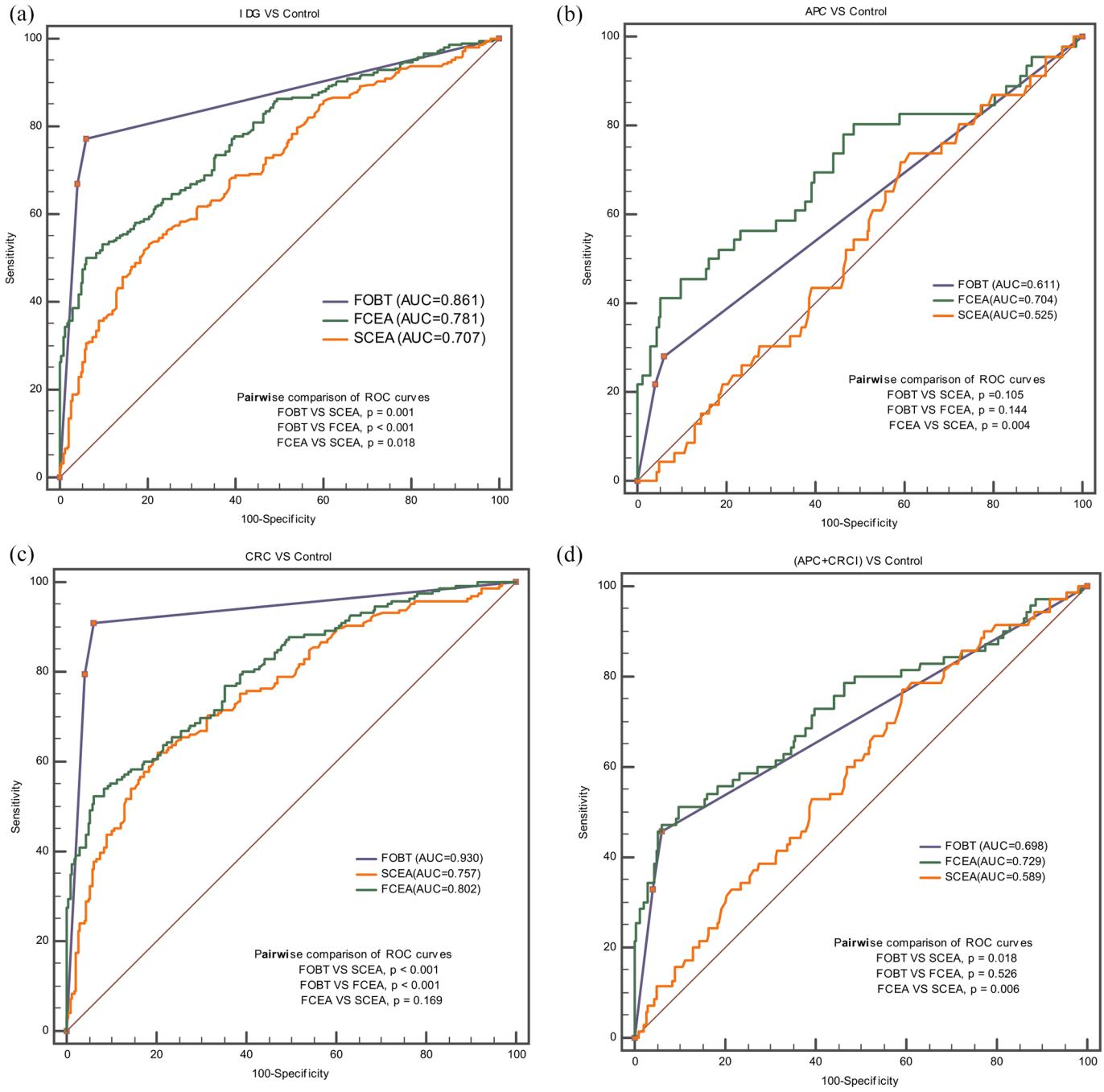

Figure 2. The ROC in different groups. All the control groups contain healthy controls and NGC.
(a) Show the AUC values of FCEA, FOBT and SCEA in IDG group. (b) Show the AUC values of FCEA, FOBT and SCEA in APC group. (c) Display THE AUC values of FCEA, FOBT and SCEA in CRC group. (d) Display THE AUC values of FCEA, FOBT and SCEA in APC+CRC I group.

false-negative results for FOBT. The FOBT showed significant biasness in differentiating among the four groups (p=0.001). In addition, the false-negative rate of SCEA in patients with hematochezia was 75.3%, while that in asymptomatic patients was 80.8%. The detailed information is listed in Table 2.

Superiority of FCEA for diagnosing the 'double-negative patients' and 'triple-negative patients'

Clinically, the negative FOBT and SCEA <5ng/ml can still be seen in the APC and even CRC patients. In this study, the FOBT-negative and SCEA-negative patients were defined as 'double-negative patients'.

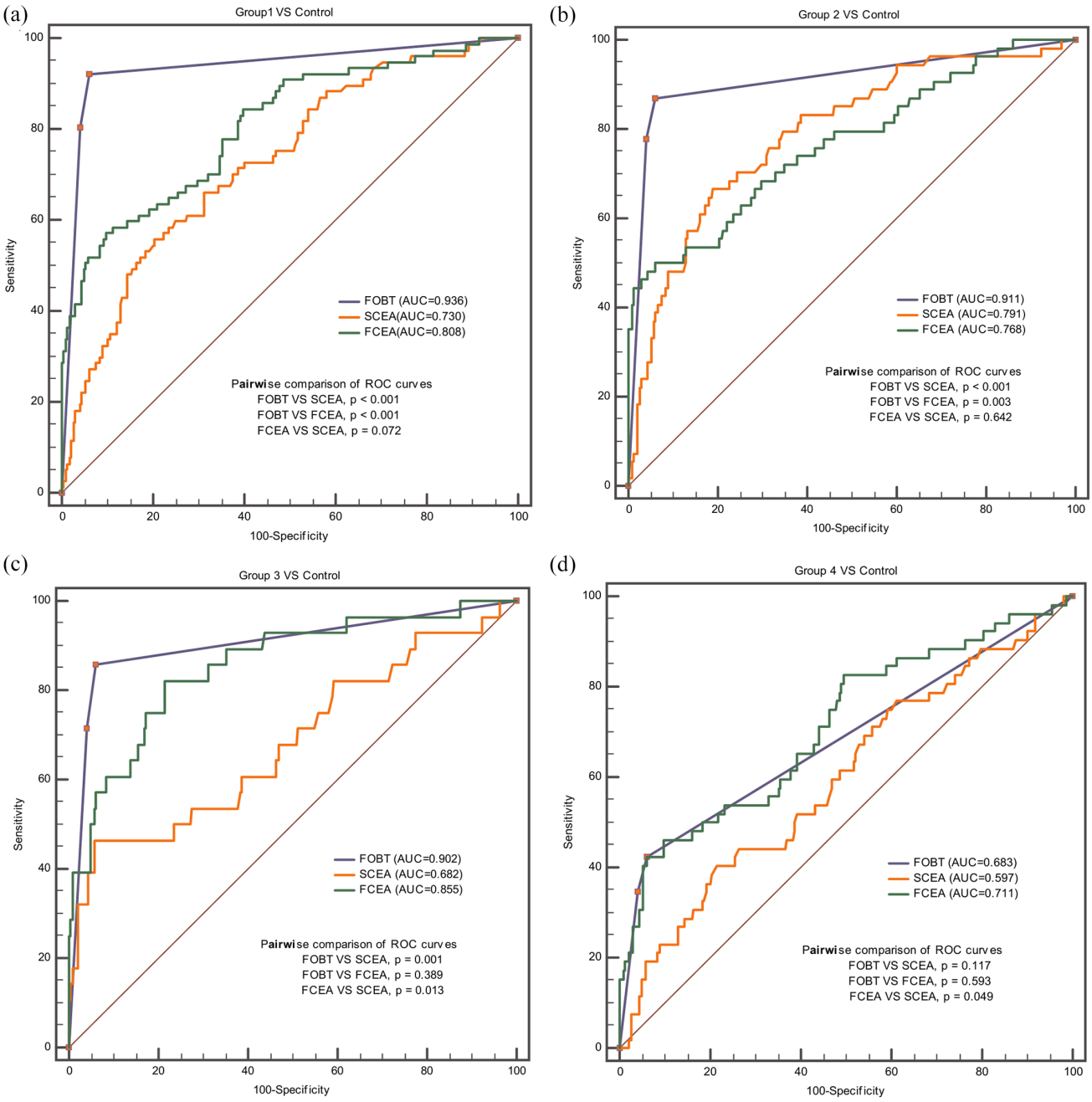

Figure 3. The ROC in different groups. All the control groups contain healthy controls and NGC. (a) Group 1 stands for patients with hematochezia. (b) Group 2 stands for patients with the change of character. (c) Group 3 stands for patients with abdominal pain. (d) Group 4 stands for asymptomatic patients.

This study explored the relationship between FCEA and two traditional markers, including FOBT and SCEA, for the diagnosis of IDG. The study group was divided into the following four sub-groups, including the CRC group ($n=166$), APC group ($n=46$), asymptomatic CRC group ($n=52$), and control group ($n=224$). Among the 166 CRC patients, 15 patients had a negative FOBT test, which accounted for 9.0% of the total. On the contrary, 106 patients had SCEA <5 ng/ml, which accounts for 63.9% of the total. Moreover, 7.2% of the CRC patients were 'double-negative patients'. FCEA could diagnose 46.7% of the FOBT-negative patients, 49.1% of

Table 2. Relationship between the complaints and three indices.

Index	Hematochezia ($n=77$)	Changes in bowel habits ($n=55$)	Abdominal pain and diarrhea ($n=28$)	Asymptomatic ($n=52$)	p value[a]
FCEA					
<142 ng/mg ($n=106$)	37 (48.1)	27 (49.1)	12 (42.9)	30 (56.7)	0.586
$\geqslant 142$ ng/mg ($n=106$)	40 (51.9)	28 (50.9)	16 (57.1)	22 (43.3)	
FOBT					
Positive ($n=164$)	71 (99.2)	47 (85.5)	24 (85.7)	22 (42.3)	***0.001***
Negative ($n=48$)	6 (7.8)	8 (14.5)	4 (14.3)	30 (57.7)	
SCEA					
<5 ng/ml ($n=150$)	58 (75.3)	35 (63.6)	15 (53.5)	42 (80.8)	***0.034***
$\geqslant 5$ ng/ml ($n=62$)	19 (24.6)	20 (36.4)	13 (46.5)	10 (19.2)	

Bold italics indicate significant differences ($p<0.05$). FCEA, fecal carcinoembryonic antigen; FOBT, fecal occult blood test; SCEA, serum carcinoembryonic antigen.
[a]The p values were calculated by using the chi-square test (χ^2 test).

the SCEA-negative patients, and 50.0% of the 'double-negative patients'. In contrast, the false-positive rates of both the FOBT and SCEA in the 224 patients of the control group were 6.2%, and the 'double positive patients' (both FOBT and SCEA were positive) accounted for 0.9%. However, 64.3% of the FOBT-false positive patients, 85.7% of SCEA-false positive patients, and 50.0% of 'double positive patients' had lower values compared with the critical value of FCEA.

In particular, the FOBT and FCEA had different efficiencies, independent of each other, in order to diagnose positive and negative patients in the control group ($p<0.001$). These results suggest that the combination of FOBT and FCEA to diagnose CRC was more effective. Detailed information is listed in Table 3 and presented in Figure S2.

The 'double-negative patients', who had no obvious clinical symptoms, were defined as 'triple-negative patients'. As previously mentioned, the AUC of asymptomatic patients diagnosed by FCEA was 0.711, and the AUCs of 'double-negative patients' and 'triple-negative patients' were 0.708 and 0.691, respectively (Figure S3). In the cases, where both the FOBT and SCEA were invalid, the FCEA still shows an obvious diagnostic significance. For the 'double-negative patients', FCEA was mainly found in the colon, early stage, multiple and small polyps, and asymptomatic patients (Table S2). Although these patients were more difficult to be diagnosed, the diagnosis rate of FCEA was still relatively high (45.5%, 20/44). Besides, the age, gender, tumor location, tumor TNM staging, family history, multiple polyps, and symptoms were found to be similar between the lower and higher FCEA groups. However, the 'double-negative patients' with multiple and small size polyps ($\leqslant 3$ cm) were more common in the lower FCEA group than those in the higher FCEA group ($p=0.005$). It is worth mentioning that 24 (11.3%) subjects showed negative results for all three indicators (FCEA, FOBT, and SCEA).

For asymptomatic patients, the lower FCEA levels were more common in the elderly patients with multiple and small size polyps ($\leqslant 3$ cm) in their colon ($p<0.05$) (Table S3). Both the gender and family history showed similar results between the lower and higher FCEA groups. Although tumor staging had no relationship with the levels of FCEA, most asymptomatic CRC patients were diagnosed in the early stage (13/17, 72.2%).

Table 3. Relationship between FCEA, FOBT, and SCEA.

Variables		Detectable rate (%)	FCEA <142 ng/mg	FCEA ⩾142 ng/mg	*p* value[a]
CRC group (*n* = 166)			*n* = 79	*n* = 87	
FOBT	Negative (*n* = 15)	9.0%	8 (53.3)	7 (46.7)	0.641
	Positive (*n* = 151)	91.0%	71 (47.0)	80 (53.0)	
SCEA	<5 ng/ml (*n* = 106)	63.9%	54 (50.9)	52 (49.1)	0.250
	⩾5 ng/ml (*n* = 60)	30.1%	25 (41.7)	35 (58.3)	
FOBT Negative and SCEA <5 ng/ml (*n* = 12)		7.2%	6 (50.0)	6 (50.0)	
APC group (*n* = 46)			*n* = 27	*n* = 19	
FOBT	Negative (*n* = 33)	71.7%	18 (54.5)	15 (45.5)	0.362
	Positive (*n* = 13)	28.3%	9 (69.2)	4 (30.8)	
SCEA	<5 ng/ml (*n* = 44)	95.6%	26 (59.1)	18 (40.9)	0.798
	⩾5 ng/ml (*n* = 2)	4.4%	1 (50.0)	1 (50.0)	
FOBT Negative and SCEA <5 ng/ml (*n* = 32)		69.5%	18 (56.3)	14 (43.7)	
Asymptomatic of CRC group (*n* = 52)			*n* = 30	*n* = 22	
FOBT	Negative (*n* = 30)	57.7%	17 (56.7)	13 (43.3)	0.861
	Positive (*n* = 22)	42.3%	13 (59.1)	9 (40.9)	
SCEA	<5 ng/ml (*n* = 42)	80.8%	25 (59.5)	17 (40.5)	0.584
	⩾5 ng/ml (*n* = 10)	19.2%	5 (50.0)	5 (50.0)	
FOBT Negative and SCEA <5 ng/ml (*n* = 28)		53.8%	16 (57.1)	12 (42.9)	
Control group (*n* = 224)			*n* = 209	*n* = 15	
FOBT	Negative (*n* = 210)	93.8%	200 (95.2)	10 (4.8)	***<0.001***
	Positive (*n* = 14)	6.2%	9 (64.3)	5 (35.7)	
SCEA	<5 ng/ml (*n* = 210)	93.8%	197 (93.8)	13 (6.2)	0.241
	⩾5 ng/ml (*n* = 14)	6.2%	12 (85.7)	2 (14.3)	
FOBT Positive and SCEA ⩾5 ng/ml (*n* = 2)		0.9%	1 (50.0)	1 (50.0)	

Bold italics indicate significant differences ($p < 0.05$). APC, adenomatous polyposis coli; CRC, colorectal cancer; FCEA, fecal carcinoembryonic antigen; FOBT, fecal occult blood test; SCEA, serum carcinoembryonic antigen.
[a]The *p* values were calculated by using the chi-square test (χ^2 test).

Combined diagnostic efficiency of the three tumor markers in IDG

The AUC of combined three tumor markers was 0.916, which was higher than that of FOBT and SCEA (AUC = 0.886; Figure 4). Interestingly, the AUC of the other two ROC curves, including FCEA and FOBT, were the same, irrespective of the presence of SCEA (AUC = 0.916). The sensitivity of FOBT + FCEA levels and FOBT + FCEA + SCEA levels were same, reaching up to 90.63%, which was higher than that of FOBT + SCEA (76.89%). However, their specificity

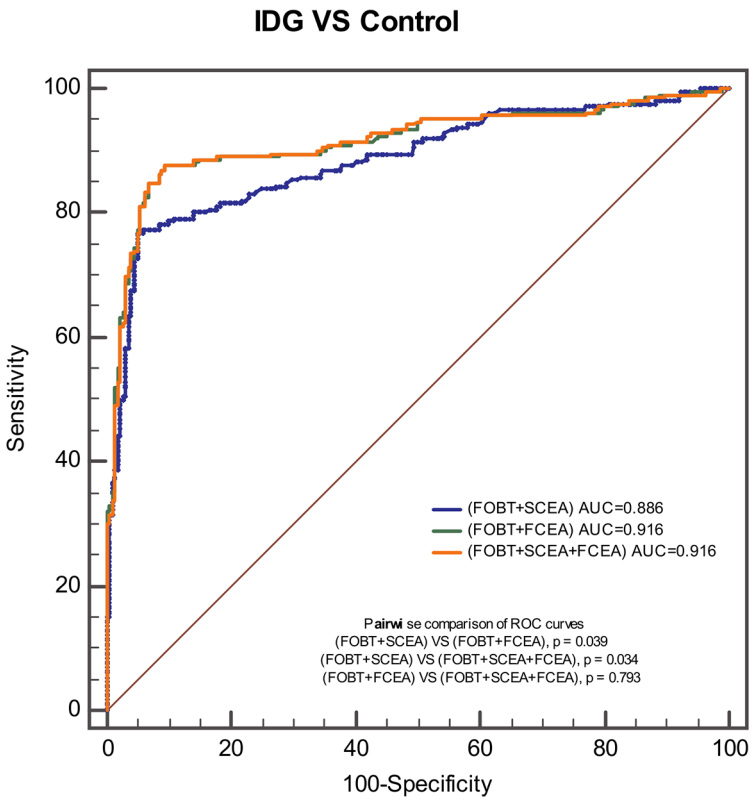

Figure 4. Combined diagnostic value of FCEA, FOBT, and SCEA in patients with intestinal diseases. The AUCs were FCEA + FOBT + SCEA (0.916) = FCEA + FOBT (0.916) > FOBT + SCEA (0.886).

(87.74%) was lower than that of FOBT + SCEA (95.09%), suggesting that in the diagnosis of IDG, the detection of SCEA could be omitted.

Discussion

The FOBT is a well-known and widely used routine clinical test, which is easy to operate, cheap, and non-invasive. However, some unavoidable factors can lead to false-negative or false-positive results in diagnosis. Another limitation of this test is that the lesions, not only in the colorectal part but also in any other part of gastrointestinal tract, might lead to the appearance of hemoglobin and result in a false-positive result. In addition, similar to a previous study, this study showed low sensitivity of FOBT for the diagnosis of adenomas (28.3%).[22] In this study, among the 46 patients diagnosed with APC, 71.7% (33/46) and 95.6% (44/46) of the patients were FOBT-negative and SCEA-negative, respectively. More importantly, 69.5% (32/46) of the patients showed negative results for both methods. Therefore, the low sensitivity of FOBT and SCEA makes them unsuitable for the diagnosis of APC. The high sensitivity for diagnosis is always recommended, but it may not always be a good thing. The false-positive FOBT and/or SCEA results might cause unnecessary panic for the patients undergoing medical examinations. In this study, 6.2% of the patients showed false-positive results for FOBT or SCEA, while 0.9% of the patients showed false-positive results for both the FOBT and SCEA. Fortunately, 40–45% and 60–80% of false-negative and false-positive patients, respectively, can be distinguished using FCEA as diagnostic marker. These results were appreciated, not due to a lack of means to distinguish between APC and CRC but rather due to a lack of appropriate non-invasive methods in order to detect precancerous lesions among patients.

In terms of clinical symptoms, the CRC is considered to be a 'silent disease' because some patients might have no obvious symptoms at the time of diagnosis. In the studied subjects, about one-third of CRC patients were presented to the clinic with stool bleeding symptoms, and less than half of them had other gastrointestinal-related symptoms. However, more than 20% of people had no symptoms and were only screened for diseases *via* routine physical examinations. Interestingly, the FOBT was significantly associated with fecal bleeding ($p < 0.001$), but more than half of the asymptomatic patients had a negative outcome. In this study, more than 80% of the asymptomatic patients had significant negative SCEA results ($p = 0.034$). Among the 52 asymptomatic patients, 57.7% (30/52) were FOBT-negative, and 80.8% (42/52) had SCEA concentrations below 5 ng/ml. Unfortunately, among APC patients, 53.8% (28/52) of patients showed negative results for the 'double-negative patients'. In other words, the FOBT and SCEA were not suitable for the diagnosis of asymptomatic patients, and new markers are urgently needed to meet clinical needs. Fortunately, this study discovered such a diagnostic method in order to screen asymptomatic patients using FCEA. The FCEA could diagnose about 50% of the FOBT- or SCEA-negative patients, as well as the APC or asymptomatic patients, who were negative for both. It should be noted that, except for APC, the diagnostic efficacy of FCEA in the CRC stage I patients was higher than those of FOBT and SCEA, suggesting that the FCEA was more suitable for the early diagnosis of CRC patients.

It has been suggested that the FCEA, combined with FOBT, was more suitable for the diagnosis of APC and asymptomatic patients. The AUC of FCEA, combined with FOBT, is similar as that of the combined three indicators, suggesting that the SCEA can be ignored while conducting diagnostic tests. For most gastrointestinal tumors, the tumor marker CEA is a well-known and widely used diagnostic marker. However, due to a lack of sensitivity and specificity, its clinical application prospects are not optimistic.[23] CEA is formed in the cytoplasm and can be detected from a variety of body fluids, including serum, cerebrospinal fluid, urine, and feces. Unlike blood tumor markers, the stool, which consists of undigested food, endogenous secretions, microbiota, and exfoliated host cellular components, is an ideal disposition for the non-invasive evaluation of whole bowel environment, regarding CRC and its biological effects on epithelial cells.[24] In addition, the FCEA tests require only one or two stool samples, and do not require dietary or medication restrictions, thereby increasing the ease of use. The FCEA is present in higher concentrations than SCEA, especially at early stages, and a previous study suggested the use of FCEA for CRC diagnosis,[25] which is similar to our results.

The FCEA showed following characteristics in CRC diagnosis: first, low expression in NGC and high expression in APC (Figure S4); second, not affected by clinical symptoms, tumor stage, tumor location, and tumor differentiation; and third, even small tumors could produce enough FCEA. These characteristics might be due to the following reasons:[25–27] cancer tissues are prone to necrosis and shedding, and are continuously renewed and released. Therefore, it is easy for them to enter the intestinal cavity and get discharged with feces. In addition, after the production of CRC cells, the CEA is transported from the portal vein to liver and then decomposed, thereby decreasing the CEA contents in blood. Finally, through the quantitative collection of fecal samples and freeze–thaw according to the standard procedure, CEA in the exfoliated cancer cells can be completely released, thereby improving positivity rate of CEA detection, and ensuring standardization of experiment and accuracy of results.

This study had some limitations too. First, some healthy individuals were excluded from the group due to hemorrhoids, leading to the high specificity of FOBT in the diagnosis of IDG. Second, this research was conducted at a single center and was limited in number. The results of this study need to be further verified in a multicentered study with a larger sample size. Finally, this study only compared the diagnostic efficacy of FCEA and two other traditional indicators (FOBT and SCEA) in intestinal diseases, while other biochemical, immune, and blood routine indicators were not included in this study. In the future, we would combine these parameters to build a predictive model for obtaining better clinical practicability.

Nevertheless, the diagnostic sensitivity of FCEA in CRC varied among published literature. In the past, the fecal quantification was difficult,[28] and composition of the control group was relatively simple. However, in this study, the control group comprised of a large number of healthy people and NGC patients, making these results more reliable.

When summarizing the results of previous studies, attention should be paid to the following two issues, including quantification of stool samples with a simple method and the effects of NCA-2 (a C-terminal truncated form of CEA) on the diagnostic efficacy of CEA in CRC.[25–29] In this study, a quantitative fecal sampling tube was used to simplify the quantification method, and the rude advanced electrochemical luminescence instrument and supporting reagent calibration system made the results more convincing. Meanwhile, it was undeniable that antibodies used in Roche ECLIA system reacted with CEA and meconium antigen, which was a non-specific cross-reacting antigen NCA (normal feces contain both CEA and NCA antigens[30]). Most CRC cells synthesized NCA more actively than normal colonic mucosa. The results of this study confirmed that the CEA contents in fecal samples were greater than those in serum, which may be due to results of NCA-antigen reaction. The CEA is a complex family, containing 29 genes, 18 of which are expressed. Seven of these 18 genes belong to the CEA subgroup, and 11 belong to the pregnancy-specific glycoprotein sub-group. At this stage, the CEA family could not be investigated at the molecular level, but this study showed advantages of FCEA in the early diagnosis of CRC.

Summary

FCEA is not a new diagnostic marker, but it has not been investigated in asymptomatic CRC and APC patients. This study showed that the FCEA could be used as a potential diagnostic marker by comparing its diagnostic efficacy with two conventional methods (FOBT and SCEA) in CRC and APC patients, and suggested that the FCEA could be used to compensate for the detection defects of FOBT and SCEA. As a diagnostic panel, the combined FOBT and FCEA measurements improved their diagnostic ability to detect IDG. However, this study was based on a single-center data, and the sample size was not large enough. Therefore, a larger validation across multiple centers and different research groups is needed before it can be used as a routine clinical diagnostic tool.

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Author contributions

Linfang Li: Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Writing-original draft, Writing-review & editing.

Wenshen Gu: Conceptualization, Formal analysis, Investigation, Methodology, Writing-review & editing.

Xingping Wu: Investigation, Methodology, Writing-review & editing.

Yufeng Ao: Data curation, Formal analysis, Software, Writing-review & editing.

Yiling Song: Formal analysis, Writing-review & editing.

Xiaohui Li: Data curation, Methodology, Software, Writing-original draft.

Qiuyao Zeng: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing-review & editing.

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ORCID iD

Linfang Li https://orcid.org/0000-0002-2336-9885

Availability of data and materials

The authenticity of this article has been validated by uploading the key raw data onto the Research Data Deposit (RDD) public platform (www.researchdata.org.cn) with the approval RDD number as RDDB 2020000987.

Supplemental material

Supplemental material for this article is available online.

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