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ORIGINAL ARTICLE

Clinical Value of Serum CEA, CA24-2 and CA19-9 in Patients with Colorectal Cancer

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SUMMARY

Background: **To investigate the clinical value of serum concentration of carcinoembryonic antigen (CEA), carbohydrate antigen 24-2 (CA24-2), and carbohydrate antigen 19-9 (CA19-9) in the detection of colorectal cancer (CRC).**

Methods: **The serum levels of tumor markers and *KRAS/NRAS/PIK3CA/BRAF* gene mutations were detected in patients with colorectal cancer. Clinical medical records in colorectal cancer patients were collected.**

Results: **A total of 2,281 patients were recruited in the study, included 1,578 colorectal cancer patients and 703 controls. CEA, CA24-2, and CA19-9 concentrations were significantly higher in the colorectal cancer group than in the control group. The sensitivity of these tumor markers sorted in descending order was CEA>CA19-9>CA24-2. The best specificity was CA24-2, followed by CA19-9 and CEA, with all were more than 92%. The combination of CEA, CA19-9, and CA24-2 ranked the best sensitivity and specificity for colorectal cancer diagnosis. The prediction equation excluding the risk of colorectal cancer was. Probability (normal) = Exp (-5.47 - 0.28*CEA - 0.11*CA242 + 0.001*CA199)/(1+ Exp (-5.47 - 0.28*CEA - 0.11*CA242 + 0.001*CA199)). Besides, there were no significant differences in age, gender, histology type, differentiation, depth of invasion, and TNM stage in *KRAS/NRAS*, *BRAF*, and *PIK3CA* mutations compared with wild type.**

Conclusions: **Serum CEA, CA24-2, and CA19-9 are valuable indicators for predicting the risk of colorectal cancer.**

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KEY WORDS

colorectal cancer, tumor markers, clinical value, combined detection

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LIST OF ABBREVIATIONS

CEA - carcinoembryonic antigen
CA24-2 - carbohydrate antigen 24-2
CA19-9 - carbohydrate antigen 19-9
TNM - TNM classification of malignant tumors
AUC - area under the ROC curve

INTRODUCTION

Malignant tumor diseases occurring in the mucosal epithelium and glands of the rectum and other parts of the colon, are collectively referred to as colorectal cancer [1,2]. Colorectal cancer (CRC) is one of the most common malignant tumors in clinical practice [3,4]. Because the patients' early symptoms are not obvious enough, most of the patients were already in the advanced stage when they were admitted to the hospital [5-9]. Therefore, rapid and correct diagnosis or prediction the risk of colorectal cancer is helpful for clinical treatment, so as to realize early diagnosis and treatment. In recent years, the detection of serum tumor markers has played an important role in the diagnosis of colorectal cancer and the assessment of the disease due to its high efficiency and non-invasive characteristics, and has been widely used in clinical practice [10,11]. At present, CEA and CA19-9 are tumor markers that are widely used in clinical practice. Due to the low monopositive rate in colorectal cancer, their clinical application value is limited. Besides, CA24-2 can be used for the diagnosis of colorectal cancer [12-14]. Some studies have shown that the combined detection of tumor markers can improve the detection rate of malignant tumors [15-17]. There are few studies on the combined detection of CEA, CA19-9, CA24-2 in the diagnosis of colorectal cancer, the relationship between these tumor markers, and clinicopathological characteristics of colorectal cancer patients [18].

EGFR has been reported to be overexpressed in 49% to 82% of colorectal tumors [19,20]. Cetuximab and panitumumab are monoclonal antibodies for treatment of colorectal cancer patients against EGFR, inhibiting its downstream signaling pathways. However, studies have shown that cetuximab and panitumumab are only effective in approximately 10% to 20% of colorectal cancer patients [21,22]. EGFR-mediated continuous activation of downstream signaling pathways is closely related to the formation and development of colorectal cancer. The RAS/RAF/MAPK pathway is located downstream of EGFR. At present, the research on this pathway to find predictive markers of the efficacy of these therapies is a research hotspot, such as mutations in KRAS, NRAS, BRAF, and PI3K signaling pathways [23-26]. Colorectal cancer patients with *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* mutations cannot benefit from anti-EGFR targeted therapy. Mutations of these genes are associated with tumor development, drug resistance, and prognosis.

The relationship between serum tumor markers, *KRAS/NRAS/BRAF/PIK3CA* mutation status, and clinical characteristics in Hakka patients with colorectal cancer has not been studied. The aim of this study was to explore and analyze the clinical value of serum CEA, CA24-2, and CA19-9 in patients with colorectal cancer, the relationship between CEA, CA24-2, CA19-9, *KRAS/NRAS/BRAF/PIK3CA* gene mutations, and clinical characteristics in colorectal cancer patients.

MATERIALS AND METHODS

Participants

This retrospective study included 1,578 colorectal cancer patients and 703 controls who visited Meizhou People's Hospital *(*Meizhou City, Guangdong Province, China), between January 2016 and May 2019. Inclusion criteria: (1) Both imaging and pathological examination met the diagnostic criteria for colorectal cancer; (2) No mental diseases, can cooperate with treatment independently. Exclusion criteria: (1) Not meeting the diagnostic criteria for colorectal cancer; (2) Patients with heart, lung, and other significant organ dysfunction; (3) Patients with unconsciousness and unable to communicate normally. Controls were non-colorectal cancer patients, most of whom were healthy, underwent a physical examination. This study was supported by the Ethics Committee of the Meizhou People's Hospital and was conducted based on the Declaration of Helsinki. Serum tumor markers and *KRAS/NRAS/PIK3CA/BRAF* gene mutations were detected in Meizhou People's Hospital.

Sample preparation and detection of tumor markers

Three milliliters blood samples were obtained from patients. Serum CEA, CA19-9, and CA24-2 concentrations were detected routinely by flow fluorescence method with Quantitative Detection Kit for Tumor Markers (Tellgen Life Science, Shanghai, China). According to the manufacturer's instructions, critical value of CEA, CA19-9, and CA24-2 was 5.00n g/mL, 37.00 U/mL, and 20.00 U/mL, respectively.
(http://en.tellgen.com/product/tumor_marker.htm).
The results were considered positive when any of the markers were positive, and negative when all were negative.

DNA extraction

Ten formalin-fixed and paraffin-embedded (FFPE) slices (5 μm thick per slice) were dewaxed in xylene and rehydrated in descending grades of ethanol. DNA was extracted by AmoyDx® Tissue DNA Kit (Spin Column) (Amoy Diagnostics, Xiamen, China), following the manufacturers' instructions. The concentration and purity of DNA were evaluated by NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Amplification-refractory mutation system-polymerase chain reaction (ARMS-PCR) for *KRAS/NRAS, PIK3CA*, and *BRAF* gene mutations

KRAS (exons 2, 3, and 4), *NRAS* (exons 2, 3, and 4), *PIK3CA* (exon 9 and 20), and *BRAF* (exon 15) gene status were tested using Human *KRAS/NRAS* Mutations Detection Kit, Human *PIK3CA* Mutations Detection Kit, and Human *BRAF* Mutations Detection Kit (Amoy Diagnostics Co. Ltd, Xiamen, China), respectively. PCR Amplifications were performed with an initial denaturation at 95°C for 5 minutes, followed by 15 cycles of first amplification (at 95°C for 25 seconds, 64°C for 20 seconds, and 72°C for 20 seconds) and 31 cycles of second amplification (at 95°C for 25 seconds, 60°C for 35 seconds, and 72°C for 20 seconds) in the LightCycler 480 real-time PCR system (Roche Diagnostics, Germany).

Statistical analysis

SPSS statistical software version 21.0 was used for data analysis. The levels of tumor markers were expressed as the means ± SD. The rank sum test was used to compare the levels of tumor markers in each group and the chi-square (χ^2) test was used to compare the rates or constituent ratios. To compare the diagnostic accuracy of detection markers in predicting pathological type of colorectal cancer, the receiver operator characteristic (ROC) curve was generated and the area under the curve was calculated. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Population characteristics

A total of 2,281 patients (59.73 ± 12.46 years) were recruited in the study, including 1,420 males (60.54 ± 11.98 years) and 861 females (58.38 ± 13.11 years). These subjects included 1,578 colorectal cancer patients and 703 controls. There were 1,529 (96.89%) patients with adenocarcinomas, 40 (2.53%) with mucinous carcinoma, and 9 (0.57%) with signet ring cell carcinoma and other types. There were 15 (0.95%), 1,480 (93.79%), and 61 (3.87%) patients with well, moderately, and poorly differentiated tumors, respectively. There were 22 (1.39%) patients who had unknown type. There were 40 (2.53%), 141 (8.94%), 827 (52.41%), and 431 (27.31%) patients with T1, T2, T3, and T4 tumors according to depth of invasion, respectively. There were 139 (8.81%) patients with unknown type. There were 604 (38.28%) patients with stages I/II, 541 (34.28%) with stage III, and 405 (25.67%) with stage IV according to the TNM classification of malignant tumors. The clinical characteristics of the subjects are presented in Table 1.

Comparison of the CEA, CA24-2, and CA19-9 concentrations in the colorectal cancer group and control group

Comparison of the CEA, CA24-2, and CA19-9 concentrations among groups, showed that the levels of CEA ($p = 0.005$) and CA24-2 ($p < 0.001$) were higher in the colorectal cancer group than the control group. There were no significant differences in serum CEA ($p = 0.908$), CA24-2 ($p = 0.870$), and CA19-9 ($p = 0.899$) levels among the adenocarcinoma group, mucinous carcinoma group, and signet ring cell carcinoma and other types. There were no significant differences in serum CEA ($p = 0.998$) and CA19-9 ($p = 0.298$) levels among the well-differentiated group, moderately differentiated group, and poorly differentiated group. However, there was a significant difference in serum CA24-2 ($p = 0.005$) level among these groups. There were no significant differences in serum CEA ($p < 0.001$), CA19-9 ($p < 0.001$), and CA24-2 ($p < 0.001$) levels for different depths of invasion (T1, T2, T3, T4) and TNM stages (I/II, III, IV), respectively, using Mann-Whitney U test (Table 2).

Specificity and sensitivity of individual tumor markers and combination of these markers for colorectal cancer

Tumor markers were detected in colorectal cancer patients and controls. The tumor marker with the best sensitivity was CEA (28.71%), followed by CA19-9 (16.92%) and CA24-2 (12.74%). Combined detection of tumor markers can improve the sensitivity, and the combination of CEA, CA19-9, and CA24-2 had the highest sensitivity index (34.92%) in colorectal cancer. The tumor markers with the best specificity was CA24-2 (96.44%), followed by CA19-9 (92.46%) and CEA (92.18%), all more than 92%. The combination of CEA, CA19-9, and CA24-2 had the highest specificity index (98.86%) in colorectal cancer. The combination of CEA, CA19-9, and CA24-2 had the highest Youden's index (0.34) (Table 3).

Comparison on ROC curves of CEA, CA24-2, and CA19-9

In colorectal cancer, it had better diagnostic accuracy and sensitivity when the positive cutoff value of CEA was 2.495 (AUROC = 0.637, $p < 0.001$, 95% CI 0.614 - 0.660), CA24-2 was 6.140 (AUROC = 0.580, $p < 0.001$, 95% CI 0.556 - 0.604), and CA19-9 was 19.630 (AUROC = 0.565, $p < 0.001$, 95% CI 0.540 - 0.590). The AUROC of these three markers in combination was 0.641 ($p < 0.001$, 95% CI 0.618 - 0.664) (Table 4 and Figure 1). Logistic regression analysis showed that the prediction equation excluding the risk of colorectal cancer was: Probability (normal) = Exp (-5.47 - 0.28*CEA - 0.11*CA242 + 0.001*CA199)/(1+ Exp (-5.47 - 0.28*CEA - 0.11*CA242 + 0.001*CA199)). When the calculated value was close to 0, the risk of colorectal cancer was higher, while the risk of colorectal cancer was lower when the value approached 1.

Table 1. Baseline characteristics of subjects.

	Colorectal cancer (n = 1,578)	Controls (n = 703)	p-value
Gender			**0.044**
Male, n (%)	**1,004 (63.62)**	**416 (59.17)**	
Female, n (%)	**574 (36.38)**	**287 (40.83)**	
Age, mean ± SD	**60.80 ± 11.36 (20 - 93)**	**57.32 ± 14.34 (4 - 97)**	**< 0.001**
≤ 60, n (%)	**754 (47.78)**	**399 (56.76)**	
> 60, n (%)	**824 (52.22)**	**304 (43.24)**	
Histology type			
Adenocarcinoma, n (%)	**1,529 (96.89)**		
Mucinous carcinoma, n (%)	**40 (2.53)**		
Signet ring cell carcinoma and others, n (%)	**9 (0.57)**		
Differentiation			
Well, n (%)	**15 (0.95)**		
Moderate, n (%)	**1,480 (93.79)**		
Poor, n (%)	**61 (3.87)**		
Unknown, n (%)	**22 (1.39)**		
Depth of invasion			
T1, n (%)	**40 (2.53)**		
T2, n (%)	**141 (8.94)**		
T3, n (%)	**827 (52.41)**		
T4, n (%)	**431 (27.31)**		
Unknown, n (%)	**139 (8.81)**		
TNM stage			
I/II, n (%)	**604 (38.28)**		
III, n (%)	**541 (34.28)**		
IV, n (%)	**405 (25.67)**		
Unknown, n (%)	**28 (1.77)**		

TNM - TNM classification of malignant tumors (By meticulous description of the primary tumor (T), related lymph nodes (N) and any discernible metastases (M) it is possible to analyze groups of patients in many different ways).

The association between *KRAS/NRAS*, *PIK3CA*, and *BRAF* gene status and clinical characteristics

In this study, 458 were tested for *KRAS/NRAS* mutations, 431 for *BRAF* mutations, and 146 for *PIK3CA* mutations. Among them, 216 patients (47.2%, 216/458) had *KRAS/NRAS* mutations, 11 patients (2.6%, 11/431) had *BRAF* mutations, and 14 patients (9.6%, 14/146) had *PIK3CA* mutations. There were no significant differences in age, gender, histology type, differentiation, depth of invasion, TNM stage, and tumor markers (CEA, CA24-2, and CA19-9) between the *KRAS/NRAS* (+) and *KRAS/NRAS* (-) group, the *BRAF* (+) and *BRAF* (-) group, and the *PIK3CA* (+) and *PIK3CA* (-) group (Table 5).

DISCUSSION

Colorectal cancer is one of the common malignant tumors in the human digestive tract [27,28]. Serum tumor markers have been used in the diagnosis of various tumors in recent years [29-32]. Serum tumor markers refer to substances synthesized and secreted by tumor cells and released into the blood, cells, and body fluids, reflecting the occurrence and development of the tumor [29,32,33]. CEA is a proteoglycan complex produced by cancer cells, widely found in cancers of the digestive system [34,35]. CA24-2 is mainly used in clinical detection of pancreatic cancer and colorectal cancer, which is a kind carbohydrate antigen expressed in human pancreatic duct cells and colonic mucosal epithelial cells [18]. CA19-9 is a tumor marker for adenocarcinoma, which is expressed in various malignancies such as

Table 2. Different levels of tumor markers were observed among groups.

	CEA	CA24-2	CA19-9
Colorectal cancer (n = 1,578)	47.89 ± 415.02	31.01 ± 166.83	69.18 ± 385.15
Controls (n = 703)	3.63 ± 14.05	6.53 ± 14.22	34.42 ± 437.64
p-value	0.005	< 0.001	0.057
Histology type			
Adenocarcinoma (n = 1,529)	48.27 ± 421.38	30.86 ± 167.41	69.84 ± 390.45
Mucinous carcinoma (n = 40)	26.91 ± 84.59	41.17 ± 164.64	55.51 ± 156.47
Signet ring cell carcinoma and others (n = 9)	6.03 ± 13.08	11.00 ± 18.70	17.95 ± 20.10
p-value	0.908	0.870	0.899
Differentiation			
Well (n = 15)	54.92 ± 134.56	8.77 ± 13.35	54.81 ± 100.31
Moderate (n = 1,480)	47.88 ± 427.68	27.79 ± 144.43	65.05 ± 376.43
Poor (n = 61)	39.64 ± 106.33	102.98 ± 441.46	157.79 ± 612.57
p-value	0.998	0.005	0.298
Depth of invasion			
T1 (n = 40)	2.32 ± 1.72	5.61 ± 4.49	13.71 ± 13.48
T2 (n = 141)	12.58 ± 61.26	6.26 ± 11.68	18.75 ± 42.25
T3 (n = 827)	17.48 ± 82.80	12.99 ± 69.22	32.37 ± 189.71
T4 (n = 431)	46.64 ± 161.87	46.45 ± 181.76	86.75 ± 247.96
p-value	< 0.001	< 0.001	< 0.001
TNM stage			
I/II (n = 604)	5.57 ± 20.39	5.74 ± 13.04	15.26 ± 39.66
III (n = 541)	12.03 ± 62.02	10.28 ± 37.73	22.52 ± 65.27
IV (n = 405)	159.33 ± 805.78	98.09 ± 316.85	214.97 ± 736.23
p-value	< 0.001	< 0.001	< 0.001

TNM - TNM classification of malignant tumors (By meticulous description of the primary tumor (T), related lymph nodes (N) and any discernible metastases (M) it is possible to analyze groups of patients in many different ways).

Table 3. Sensitivity and specificity (%) of tumor markers for colorectal cancer.

Tumor markers	Sensitivity (%)	Specificity (%)	Youden's index
CEA	28.71 (453/1,578)	92.18 (648/703)	0.21
CA24-2	12.74 (201/1,578)	96.44 (678/703)	0.09
CA19-9	16.92 (267/1,578)	92.46 (650/703)	0.09
CEA + CA24-2	32.19 (508/1,578)	98.15 (690/703)	0.30
CEA + CA19-9	34.16 (539/1,578)	98.15 (690/703)	0.32
CA24-2 + CA19-9	19.84 (313/1,578)	97.87 (688/703)	0.18
CEA + CA24-2 + CA19-9	34.92 (551/1,578)	98.86 (695/703)	0.34

Table 4. Areas under the ROC curve and predictive value of four tumor markers for colorectal cancer.

	CEA	CA24-2	CA19-9	CEA + CA24-2 + CA19-9
95% CI	0.614 - 0.660	0.556 - 0.604	0.540 - 0.590	0.618 - 0.664
p-value	< 0.001	< 0.001	< 0.001	< 0.001
AUC	0.637	0.580	0.565	0.641
Cutoff point	2.495	6.140	19.630	-
Specificity (%)	50.0	39.3	30.0	35.6
Sensitivity (%)	71.7	74.1	81.2	88.3

95% CI - 95% confidence interval, AUC - area under the ROC curve.

Table 5. Analysis of the relationship between *KRAS/NRAS, PIK3CA,* and *BRAF* gene status and clinical characteristics.

Characteristic	*KRAS/NRAS* mutation		p-value	*PIK3CA* mutation		p-value	*BRAF* mutation		p-value
	+ (n = 216)	- (n = 242)		+ (n = 14)	- (n = 132)		+ (n = 11)	- (n = 420)	
Age			0.454 (χ^2 = 0.576)			0.173 (χ^2 = 2.017)			0.762 (χ^2 = 0.251)
≤ 60	95 (44.0)	115 (47.5)		4 (28.6)	64 (48.5)		6 (54.5)	197 (46.9)	
> 60	121 (56.0)	127 (52.5)		10 (71.4)	68 (51.5)		5 (45.5)	223 (53.1)	
Gender			0.277 (χ^2 = 1.362)			0.381 (χ^2 = 1.257)			0.537 (χ^2 = 0.037)
Male	137 (63.4)	166 (68.6)		7 (50.0)	86 (65.2)		7 (63.6)	279 (66.4)	
Female	79 (36.6)	76 (31.4)		7 (50.0)	46 (34.8)		4 (36.4)	141 (33.6)	
Histology type			0.255 (χ^2 = 2.729)			0.760 (χ^2 = 0.549)			0.250 (χ^2 = 2.776)
Adenocarcinoma	212 (98.1)	234 (96.7)		14 (100.0)	127 (96.2)		10 (90.9)	409 (97.4)	
Mucinous carcinoma	4 (0.9)	5 (2.1)		0 (0)	4 (3.0)		1 (9.1)	8 (1.9)	
Signet ring cell carcinoma and others	0 (0)	3 (1.2)		0 (0)	1 (0.8)		0 (0)	3 (0.7)	
Differentiation			0.122 (χ^2 = 5.803)			0.202 (χ^2 = 4.616)			0.873 (χ^2 = 0.699)
Well	5 (2.3)	0 (0)		1 (7.1)	1 (0.8)		0 (0)	5 (1.2)	
Moderate	200 (92.6)	228 (94.2)		13 (92.9)	123 (93.2)		10 (90.9)	393 (93.6)	
Poor	9 (4.2)	12 (5.0)		0 (0)	7 (5.3)		1 (9.1)	19 (4.5)	

Table 5. Analysis of the relationship between *KRAS/NRAS, PIK3CA,* and *BRAF* gene status and clinical characteristics (continued).

Characteristic		*KRAS/NRAS* mutation		p-value	*PIK3CA* mutation		p-value	*BRAF* mutation		p-value
		+ (n = 216)	- (n = 242)		+ (n = 14)	- (n = 132)		+ (n = 11)	- (n = 420)	
	Depth of invasion			0.398 (χ^2 = 5.152)			0.492 (χ^2 = 2.408)			0.693 (χ^2 = 3.047)
	T1	2 (0.9)	7 (2.9)		0 (0)	0 (0)		0 (0)	7 (1.7)	
	T2	17 (7.9)	17 (7.0)		1 (7.1)	5 (3.8)		2 (18.2)	30 (7.1)	
	T3	127 (58.8)	128 (52.9)		9 (64.3)	71 (53.8)		5 (45.5)	231 (55.0)	
	T4	51 (23.6)	65 (26.9)		4 (28.6)	39 (29.5)		2 (18.2)	107 (25.5)	
	TNM stage			0.176 (χ^2 = 4.949)			0.150 (χ^2 = 5.321)			0.382 (χ^2 = 3.064)
	I/II	72 (33.3)	78 (32.2)		4 (28.6)	22 (16.7)		6 (54.5)	129 (30.7)	
	III	70 (32.4)	86 (35.5)		8 (57.1)	49 (37.1)		2 (18.2)	142 (33.8)	
	IV	73 (33.8)	71 (29.3)		2 (14.3)	59 (44.7)		3 (27.3)	141 (33.6)	
	Tumor markers									
	CEA	71.56 ± 521.72	84.16 ± 878.08	0.854	76.88 ± 179.59	56.78 ± 169.74	0.676	6.43 ± 8.48	92.79 ± 765.09	0.709
	CA24-2	41.43 ± 199.58	30.58 ± 169.00	0.529	11.91 ± 13.12	58.27 ± 252.05	0.494	36.58 ± 63.54	37.82 ± 191.65	0.983
	CA19-9	106.45 ± 763.37	57.00 ± 342.04	0.363	43.46 ± 93.97	66.23 ± 233.29	0.719	28.12 ± 31.50	85.90 ± 605.48	0.752

TNM - TNM classification of malignant tumors (By meticulous description of the primary tumor (T), related lymph nodes (N) and any discernible metastases (M) it is possible to analyze groups of patients in many different ways).

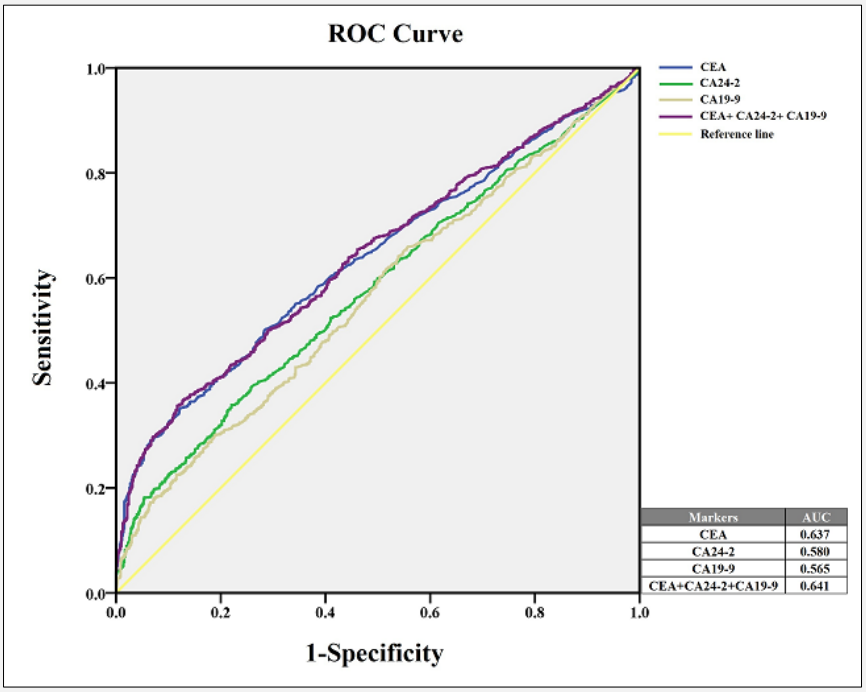

Figure 1. ROC Curves of CEA, CA24-2, and CA19-9 for colorectal cancer.

pancreatic cancer, gastric cancer, and colorectal cancer [36,37]. The levels of CEA, CA19-9, and CA24-2 are significant for diagnosis and prognosis monitoring of colorectal cancer.

In this study, three common serum markers (CEA, CA19-9, and CA24-2) of colorectal cancer were evaluated separately and jointly. The serum concentrations of CEA, CA24-2, and CA19-9 in colorectal cancer patients were significantly different from those in the control group, suggesting that CEA, CA24-2, and CA19-9 have clinical value in the diagnosis of colorectal cancer. By analyzing the relationship between serum CEA, CA19-9, and CA24-2 and clinical TNM staging, it was found that the serum concentrations of CEA, CA19-9, and CA24-2 changed with the degree of infiltration and TNM staging, showing a positive correlation. The concentration of CA24-2 was negatively correlated with the degree of tumor differentiation. The differences between CEA, CA19-9 concentration and tumor differentiation degree were not statistically significant. This is consistent with other studies [38,39]. There are also inconsistencies with some studies [37,40].

The sensitivities of these three individual markers were lower than 30%, with individual sensitivities of the tumor markers sorted in descending order as CEA>CA19-9>CA24-2. The best specificity was CA24-2, followed by CA19-9 and CEA, all were more than 92%. The sensitivities and specificities of tumor markers can be improved by combination of these markers. Although the combined detection of CEA, CA24-2, and CA19-9 can improve the ability to predict the risk of colorectal cancer, there were no significant differences compared with the single detection of CEA, CA24-2, and CA19-9.

There were no significant differences in age, gender, histology type, differentiation, depth of invasion and TNM staging between the *KRAS/NRAS* (+) group and *KRAS/NRAS* (-) group, the *BRAF* (+) group and *BRAF* (-) group, and the *PIK3CA* (+) group and *PIK3CA* (-) group. It showed that mutations in *KRAS/NRAS*, *BRAF* and *PIK3CA* genes were not associated with colorectal cancer's pathological status. There were different results in some studies on the relationship between *KRAS/NRAS*, *BRAF*, and *PIK3CA* gene status and pathological characteristics in colorectal cancer, which may be related to the detection method, sample size, and regional differences [41-43].

In recent years, many new biomarkers of colorectal cancer, such as DNA methylation and microRNA (miRNA), have been found in different molecular subtypes, and their role in colorectal cancer have been studied. These biomarkers can be used for diagnosis, individualized therapeutic effect, and prognostic evaluation of colorectal cancer [44]. For example, hsa-mir-183-5p and hsa-mir-21-5p [45], hsa-mir-30a [46], hsa-mir-96 [47],

hsa-mir-21 [48] are used for the diagnosis of colorectal cancer. Hsa-mir-143 and hsa-mir-145 have prognostic evaluation value for colorectal cancer patients [49]. More and more studies have confirmed that the abnormal expression of miRNAs in colorectal cancer tissues and plasma is closely related to disease development. However, there are still many problems to be solved for miRNA to be a valuable tumor marker. Sensitivity and specificity also need further confirmation. In addition, there is still no clear reference range for miRNA as a tumor marker. If peripheral blood miRNA is used as a tumor marker in clinic, the reference range should be established and a standardized system should be established to ensure the accuracy and repeatability of miRNA detection. An increasing level of methylated DNA was associated with advanced colorectal cancer [50,51]. However, the specificity of methylation detection is low.

The detection of tumor markers in peripheral blood has the advantages of repeatability and simultaneous detection of multiple indicators, which is an ideal method to determine tumor prognosis and follow-up. In gastrointestinal tumors, CEA, CA-199, and CA-242 are commonly used as tumor markers to monitor the occurrence, recurrence, and disease changes of tumors. On the whole, we need to find more specific and sensitive markers for diagnosis, therapeutic evaluation, and prognosis of colorectal cancer.

There are several strengths in this study. This is one of the largest sample size studies regarding the clinical value of serum CEA, CA24-2, and CA19-9 in patients with colorectal cancer. This is the first study about the relationship of *KRAS/NRAS/PIK3CA/BRAF* gene status and levels of serum tumor markers. There are some limitations to this study that should be noted. First, tumors are a kind of multifactorial disease caused by genetic and environmental factors. As a retrospective case control analysis, the limitations of the original data included in this study constrained assessment of potential gene-environment interactions. Second, this study's sample size is not very large, which may lead to some deviations in the results. The patient selection bias could not be completely eliminated despite our best efforts. This study should include a study that enrolled more CRC patients of different types and stages, combined with follow-up information, to better understand relevant studies in CRC patients in China.

CONCLUSION

There is no single serum marker sensitive enough for diagnosis and screening of colorectal cancer. Serum CEA, CA24-2, and CA19-9 are valuable noninvasive indicators for prediction the risk of colorectal cancer. Furthermore, we need to find new more specific and sensitive markers for diagnosis, therapeutic effect, and prognostic evaluation of colorectal cancer.

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Authors' Contributions:
Zhixiong Zhong and Heming Wu designed the study. Hui Rao, Qingyan Huang, and Zhikang Yu performed the experiments. Heming Wu and Hui Rao collected clinical data. Heming Wu and Hui Rao analyzed the data. Heming Wu prepared the manuscript. All authors were responsible for critical revisions, and all authors read and approved the final version of this work.

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The study was approved by the Ethics Committee of Medicine, Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences, Meizhou Hospital Affiliated to Sun Yat-Sen University.

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Declaration of Interest:
The authors declare that they have no competing interests.

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