Clinical Diagnostic Value for Colorectal Cancer Based on Serum CEA, CA24-2 and CA19-9

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Abstract

Background To explore the clinical value of a combined detection of serum concentration of carcinoembryonic antigen (CEA), carbohydrate antigen 24-2 (CA24-2), and carbohydrate antigen 19-9 (CA19-9) for colorectal cancer (CRC). Methods The levels of serum tumor markers (CEA, CA24-2 and CA19-9) and clinical characteristics in patients with colorectal cancer were evaluated. In addition, KRAS/NRAS/PIK3CA/BRAF mutations were detected in some patients with colorectal cancer. Results A total of 2,281 patients were recruited in the study, included 1,578 colorectal cancer patients and 703 controls. The levels of CEA, CA24-2 and CA19-9 in colorectal cancer group was significantly higher than control group. The sensitivities of three individual markers were lower than 30%, which individual sensitivity of the tumor markers sorted in descending order was CEA>CA19-9>CA24-2. The specificities of three individual markers were more than 92%, and the specificity sorted in descending order was CA24-2>CA19-9>CEA. The combination of CEA+CA19-9+CA24-2 ranked the highest in sensitivity index and specificity index for colorectal cancer diagnosis. The prediction equation excluding the risk of colorectal cancer was. Probability (normal) = Exp (-5.47 - 0.28CEA - 0.11 CA242 + 0.001 CA199)/ (1+ Exp (-5.47 - 0.28CEA - 0.11 CA242 + 0.001 CA199)). There were no significant differences in age, gender, histology type, differentiation, depth of invasion and TNM stage between mutations in KRAS/NRAS, BRAF and PIK3CA genes or not, respectively. Conclusions Serum CEA, CA24-2, and CA19-9 are valuable noninvasive indicators for prediction the risk of colorectal cancer. We need to look for other, more sensitive tumor markers.

Introduction

Malignant tumor diseases occurring in the mucosal epithelium and glands of the rectum and other parts of the colon, collectively referred to as colorectal cancer, colorectal cancer by sigmoid colon and rectal junction is more common[1, 2]. Colorectal cancer (CRC) is one of the most common malignant tumors in clinical practice[3, 4]. Because the early symptoms of the patients are not obvious enough, most of the patients were already in the advanced stage when they were admitted to the hospital, which seriously threatened the life of the patients[5-9]. Therefore, the rapid and correct diagnosis or prediction the risk of colorectal cancer are helpful for clinical treatment, so as to improve the efficacy of patients.

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 mono-positive rate in colorectal cancer, their clinical application value is limited. In addition, 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], but there are few studies on the combined detection of CEA, CA19-9, CA24-2 in the diagnosis of colorectal cancer and the relationship between these tumor markers and clinicopathological characteristics of patients with colorectal cancer.

EGFR has been reported to be overexpressed in 49% to 82% of colorectal tumors[18, 19]. Cetuximab and Panitumumab are monoclonal antibodies directed against EGFR that inhibit its downstream signaling pathways for treatment of patients with colorectal cancer. Cetuximab and Panitumumab are only effective in approximately 10% to 20% of colorectal cancer patients[20, 21]. The RAS/RAF/MAPK pathway is downstream of EGFR, mutations in components of this pathway are being studied in search of predictive markers for efficacy of these therapies. Such as mutations in KRAS, NRAS, BRAF, and components of the PI3K signaling pathway, potentially exert negative effects on the response to anti-EGFR antibodies[22-25].

There has been no systematic study on the association between serum tumor markers, *KRAS/NRAS/BRAF/PIK3CA* genes status and clinical characteristics in colorectal cancer patients in Hakka population. In this study, the data of colorectal cancer patients were analyzed retrospectively, who had detected tumor markers from January 2016 to May 2019. The relationship between CEA, CA24-2, CA19-9 and *KRAS/NRAS/BRAF/PIK3CA* gene mutations were analyzed. In order to explore the value of a combined application of serum tumor markers in prediction the risk of colorectal cancer.

Materials And Methods

Participants

This retrospective clinical study included 1,578 patients with colorectal cancer and 703 controls who visited Meizhou *People's Hospital (Huangtang Hospital),* Meizhou Academy of Medical Sciences, *Meizhou* Hospital Affiliated to Sun Yat-sen University between January 2016 and May 2019. This study was conducted on the basis of the Declaration of Helsinki and was supported by the Ethics Committee of the Meizhou *People's Hospital.*

Sample preparation and tumor markers detection

Three milliliters of blood samples were obtained from each subject. CEA, CA19-9 and CA24-2 were measured routinely by flow fluorescence method with Quantitative Detection Kit for Tumor Markers (Tellgen Life Science, Shanghai, China). Critical value of CEA, CA19-9 and CA24-2 was 5.00ng/mL, 37.00U/mL and 20.00U/mL. The results were considered positive when any of the markers were positive, and negative when all of the markers were negative.

DNA extraction

Ten slices of 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 and NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) was used to evaluate the quantity and quality of extracted DNA.

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

We tested mutations in the *KRAS* (exons 2, 3 and 4), *NRAS* (exons 2, 3 and 4), *PIK3CA* (exon 9 and 20) and *BRAF* (exon 15) genes using Human *KRAS/NRAS* Mutations Detection Kit, Human *PIK3CA* Mutations Detection Kit and Human *BRAF* Mutation Detection Kit (Amoy Diagnostics Co. Ltd, Xiamen, China), respectively. PCR Amplifications were performed with 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). Positive results were defined as Ct (sample)-Ct (control) < Ct (cut-off). These molecular arrays allow to identify the most important mutations of *KRAS* (codons 12, 13, 61, 117, and 146), *NRAS* (codons 12, 61, 117, and 146), *BRAF* (codons 600), and *PIK3CA* genes (codons 542, 545 and 1047).

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) curves was generated and the area under the curve was calculated. A value of *P*<0.05 was considered as statistically significant.

Results

Population characteristics

A total of 2,281 patients (1,420 males and 861 females) were recruited in the study, and 59.73±12.46 years in all subjects, which 60.54±11.98 years in males and 58.38±13.11 years in females. 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 others. There were 15(0.95%), 1,480(93.79%) and 61(3.87%) patients with well, moderately and poorly differentiated tumors, respectively. And there were 22(1.39%) patients were unknown. 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. And there were 139(8.81%) patients were unknown. 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 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*<0.001), CA24-2 (*P*<0.001) and CA19-9 (*P*<0.001) in colorectal cancer group was significantly higher than control group. There were no significant differences in serum CEA (*P*=0.741), CA24-2 (*P*=0.625) and CA19-9 (*P*=0.738) levels among adenocarcinoma group, mucinous carcinoma group and signet ring cell carcinoma and others. Detailed data are presented in Table 2.

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

In this study, tumor markers were detected in colorectal cancer patients and controls. Individual sensitivity sorted in descending order, the tumor markers were CEA (28.71%)> CA19-9 (16.92%)> CA24-2 (12.74%). Combined detection of tumor markers can improve the sensitivity, and the combination of CEA+CA19-9+CA24-2 ranked the highest in sensitivity index (34.92%) in colorectal cancer. The individual specificity of tumor markers were CA24-2 (96.44%)> CA19-9 (92.46%)>CEA (92.18%). Specificity can be improved by combining with tumor markers, and the combination of CEA+CA19-9+CA24-2 ranked the highest in specificity index (98.86%) in colorectal cancer (Table 3).

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

Comparison the accuracies when using CEA, CA24-2 and CA19-9 in diagnosis of colorectal cancer by calculating area under the ROC curve (AUROC) (Figure 1). In colorectal cancer, the AUC of CEA, CA24-2 and CA19-9 were 0.637, 0.580 and 0.565, respectively. Detailed data are presented in 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 - 0.28CEA - 0.11 CA242 + 0.001 CA199)/ (1+ Exp (-5.47 - 0.28CEA - 0.11 CA242 + 0.001 CA199)). When the calculated value was close to 0, the risk of colorectal cancer was higher. The risk of colorectal cancer was lower when the value approached 1.

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

Among colorectal cancer patients, 458 were tested for *KRAS/NRAS* mutations, 431 for *BRAF* mutations, and 146 for *PIK3CA* mutations. Among them, 216 patients (47.2%) with *KRAS/NRAS* mutations, 11 patients (2.6%) with *BRAF* mutations and 14 patients (9.6%) with *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 *KRAS/NRAS* (+) group and *KRAS/NRAS* (-) group, *BRAF* (+) group and *BRAF* (-) group, *PIK3CA* (+) group and *PIK3CA* (-) group, respectively (Table 5).

Discussion

Colorectal cancer is the most common malignant tumor in human digestive tracts[26, 27]. With the development of researches in recent years, tumor markers have been used in the diagnosis of various tumors[28-31]. Tumor markers refer to substances synthesized and secreted by tumor cells and

released into blood, cells and body fluids, which can reflect the occurrence and development of tumor[28, 31, 32]. CEA is a proteoglycan complex produced by colorectal cancer, which is widely found in cancers of the digestive system[33, 34]. CA24-2 is a kind of salivary acidified spingolipid glycosylated chain antigen, which is expressed in human pancreatic duct cells and colonic mucosal epithelial cells. It is mainly used in clinical detection of pancreatic cancer and colorectal cancer[35]. CA19-9 is a specific tumor marker for adenocarcinoma, but it lacks organ specificity and can be expressed in various malignancies such as pancreatic cancer, gastric cancer and colorectal cancer[36, 37]. The levels of CEA, CA19-9 and CA24-2 are significant for the 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 evaluated. 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 may be of important 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 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 was not statistically significant. This is consistent with other studies[38, 39]. There are also inconsistencies with some studies[37, 40].

The sensitivity, specificity and ROC curve of these tumor markers were analyzed. The sensitivities of three individual markers were lower than 30%, which individual sensitivity of the tumor markers sorted in descending order was CEA> CA19-9> CA24-2. The specificities of three individual markers were more than 92%, and the specificity sorted in descending order was CA24-2> CA19-9> CEA. 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 *KRAS/NRAS* (+) group and *KRAS/NRAS* (-) group, *BRAF* (+) group and *BRAF* (-) group, *PIK3CA* (+) group and *PIK3CA* (-) group, respectively. It showed that mutations in *KRAS/NRAS*, *BRAF* and *PIK3CA* genes were not associated with pathological status of colorectal cancer. There are different domestic and foreign studies on the relationship between *KRAS/NRAS*, *BRAF* and *PIK3CA* gene mutation 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 have been found due to the research progress in molecular subtypes, DNA methylation, microRNA (miRNA) biogenesis and their role in colorectal cancer. 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] for diagnosis of colorectal cancer. And hsa-mir-143 and hsa-mir-145 have promising prognostic value for colorectal cancer[49]. More and more studies have confirmed that the abnormal expression of miRNA in colorectal cancer tissues and plasma is closely related to diagnosis, treatment and prognosis. However, currently, miRNA as a tumor marker is still in the research stage, and there are still many problems to be solved. For example, the detection sample sizes of some experimental researches were small, and the selected miRNA tumor markers need further experimental verification. Sensitivity and specificity also need further confirmation. 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, 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 association with advanced stage of colorectal cancer[50, 51]. But the specificity of methylation detection is low. The detection of tumor markers in peripheral blood has the advantages of less trauma, 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.

Conclusions

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. New more specific and sensitive markers for diagnosis, therapeutic effect and prognostic evaluation of colorectal cancer also needed. And we need to find it.

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Abbreviations

CEA, carcinoembryonic antigen;

CA24-2, carbohydrate antigen 24-2;

CA19-9, carbohydrate antigen 19-9;

ADK, adenocarcinomas;

TNM, TNM classification of malignant tumors;

AUC, area under the ROC curve.

Declarations

Authors' contributions

Zhixiong Zhong and Heming Wu designed the study. Hui Rao, Qingyan Huang, Zhikang Yu and Qunji Zhang 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.**Acknowledgments**The author would like to thank other colleagues whom were not listed in the authorship of Center for Precision Medicine, Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences, Meizhou Hospital Affiliated to Sun Yat-sen University for their helpful comments on the manuscript.

Tables

Table 1

Baseline characteristics of subjects.

	Colorectal cancer (n = 1578)	Controls (n = 703)	P value
Gender (male)			0.044
Male, n (%)	1004(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 (%)	1529(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 (%)	1480(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)		

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

Table 2

Different levels of tumor markers were observed among groups.

	CEA	CA24-2	CA19-9
Colorectal cancer (n = 1578)	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	< 0.001	< 0.001	< 0.001
Histology type			
Adenocarcinoma (n = 1529)	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 = 1480)	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

Table 3

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

Tumor markers	Sensitivity (%)	Specificity (%)	Youden's index
CEA	28.71(453/1578)	92.18(648/703)	0.21
CA24-2	12.74(201/1578)	96.44(678/703)	0.09
CA19-9	16.92(267/1578)	92.46(650/703)	0.09
CEA + CA24-2	32.19(508/1578)	98.15(690/703)	0.30
CEA + CA19-9	34.16(539/1578)	98.15(690/703)	0.32
CA24-2 + CA19-9	19.84(313/1578)	97.87(688/703)	0.18
CEA + CA24-2 + CA19-9	34.92(551/1578)	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
95% CI	0.614−0.650	0.556−0.604	0.540−0.590
P value	< 0.001	< 0.001	< 0.001
AUC	0.637	0.580	0.565
Cut-off point	2.495	6.140	19.63
Specificity (%)	50.0	39.3	30.0
Sensitivity (%)	71.7	74.1	81.2
95% CI, 95% confidence interval; AUC, area under the ROC curve.			

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

acteristic	KRAS/NRAS mutation			PIK3CA mutation			BRAF mutation		
	+ (n=216)	- (n=242)	P value	+ (n=14)	- (n=132)	P value	+ (n=11)	- (n=420)	P value
0			$0.454(c^2=0.576)$			$0.173(c^2=2.017)$			$0.762(c^2=0.251)$
	95(44.0)	115(47.5)		4(28.6)	64(48.5)		6(54.5)	197(46.9)	
	121(56.0)	127(52.5)		10(71.4)	68(51.5)		5(45.5)	223(53.1)	
...der			$0.277(c^2=1.362)$			$0.381(c^2=1.257)$			$0.537(c^2=0.037)$
...le	137(63.4)	166(68.6)		7(50.0)	86(65.2)		7(63.6)	279(66.4)	
...nale	79(36.6)	76(31.4)		7(50.0)	46(34.8)		4(36.4)	141(33.6)	
...logy type			$0.255(c^2=2.729)$			$0.760(c^2=0.549)$			$0.250(c^2=2.776)$
...enocarcinoma	212(98.1)	234(96.7)		14(100.0)	127(96.2)		10(90.9)	409(97.4)	
...cinous ...noma	4(0.9)	5(2.1)		0(9.5)	4(3.0)		1(9.1)	8(1.9)	
...net ring cell ...noma and ...rs	0(0)	3(1.2)		0(0)	1(0.8)		0(0)	3(0.7)	
...rentiation			$0.122(c^2=5.803)$			$0.202(c^2=4.616)$			$0.873(c^2=0.699)$
...ll	5(2.3)	0(0)		1(7.1)	1(0.8)		0(0)	5(1.2)	
...derate	200(92.6)	228(94.2)		13(92.9)	123(93.2)		10(90.9)	393(93.6)	
...r	9(4.2)	12(5.0)		0(0)	7(5.3)		1(9.1)	19(4.5)	
...h of invasion			$0.398(c^2=5.152)$			$0.492(c^2=2.408)$			$0.693(c^2=3.047)$
	2(0.9)	7(2.9)		0(0)	0(0)		0(0)	7(1.7)	
	17(7.9)	17(7.0)		1(7.1)	5(3.8)		2(18.2)	30(7.1)	
	127(58.8)	128(52.9)		9(64.3)	71(53.8)		5(45.5)	231(55.0)	
	51(23.6)	65(26.9)		4(28.6)	39(29.5)		2(18.2)	107(25.5)	
...stage			$0.176(c^2=4.949)$			$0.150(c^2=5.321)$			$0.382(c^2=3.064)$
I	72(33.3)	78(32.2)		4(28.6)	22(16.7)		6(54.5)	129(30.7)	
	70(32.4)	86(35.5)		8(57.1)	49(37.1)		2(18.2)	142(33.8)	
	73(33.8)	71(29.3)		2(14.3)	59(44.7)		3(27.3)	141(33.6)	
...or markers									
...EA	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
...A24-2	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
...A19-9	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

Figures

ROC Curve

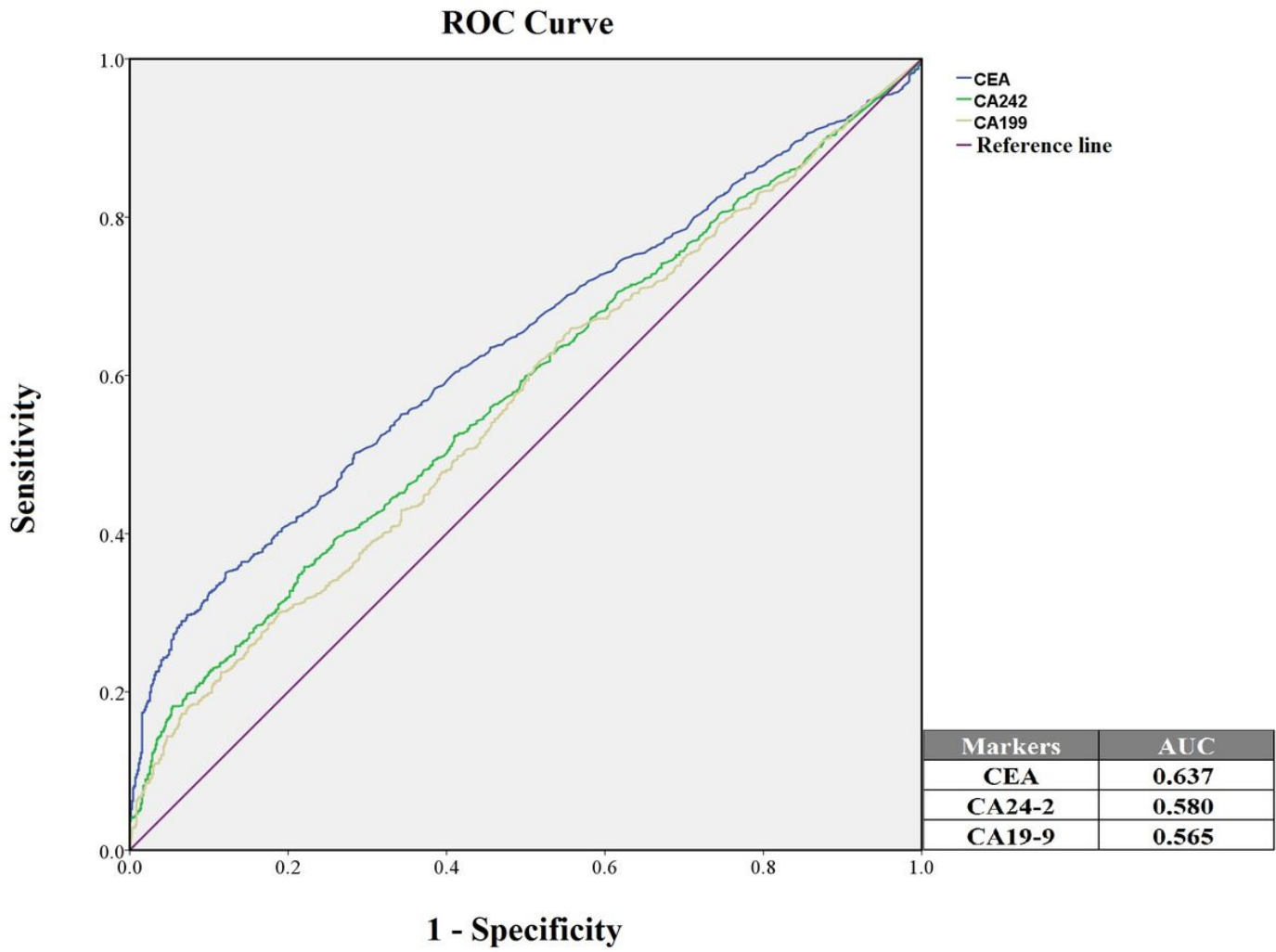| Markers	AUC
CEA	0.637
CA24-2	0.580
CA19-9	0.565

Figure 1

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