Pathol. Oncol. Res. (2015) 21:83–95
DOI 10.1007/s12253-014-9791-9

RESEARCH

Association of Serum Levels of CEA, CA199, CA125, CYFRA21-1 and CA72-4 and Disease Characteristics in Colorectal Cancer

Wa Zhong · Zhong Yu · Jun Zhan · Tao Yu · Ying Lin · Zhong-Sheng Xia · Yu-Hong Yuan · Qi-Kui Chen

Received: 16 October 2013 / Accepted: 24 April 2014 / Published online: 30 May 2014

Abstract Identifying predictive biomarkers for colorectal cancer would facilitate diagnosis and treatment of the disease. This study aimed to investigate the association of the serological biomarkers CEA, CA19–9, CA125, CYFRA21–1 and CA72–4 with patient characteristics and disease outcomes in colorectal cancer. Patients (N=373) with colorectal cancer were evaluated for the association of CEA, CA19–9, CA125, CYFRA21–1, and CA72–4 pre and post-surgery and at disease recurrence with demographics, disease characteristics including pathological types, degree of differentiation, invasion depth, abdominal lymph node metastasis, TMN stage, Dukes stage, location of cancer and metastasis, and disease outcomes. It was more common for a patient to express these markers prior to surgery and at disease recurrence than following surgery. Overall, the serum levels of CEA, CA19–9, CA125, CYFRA21–1, and CA72–4 were not associated with age, gender, pathological type and location of cancer (all P-values >0.05), but were associated with the poor tumor differentiation, higher tumor invasion, greater degree of abdominal lymph node metastasis, and higher TNM and Duke stage tumors (all P-values<0.01). CEA expression was associated with older ages (median age 65 years). Multivariate analysis indicated that CEA was correlated with overall survival and none of the markers correlated with disease recurrence. The expression of CEA, CA19–9, CA125, CYFRA21–1, and CA72–4 was associated with specific disease characteristics which tended to indicated more advanced disease and disease recurrence consistent with these biomarkers being useful for detecting colorectal cancer.

Keywords Colorectal cancer · Cancer marker · TNM stage · Duke stage · Overall survival

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the third leading cause of cancer death in both men and women world-wide [1]. It is believed that proper screening could prevent the majority of these deaths. This is particularly important since early colorectal cancer is asymptomatic [2]. In the US over the past 10 years there has been progress in reducing the incidence of colorectal cancer through prevention and early detection of the disease [2]. However, in Asia there has been an increase in the morbidity and mortality due to CRC due to changes in lifestyle and diet [1]. In addition, the age of patients in Asia with CRC is decreasing which has major implications for public health.

Currently, there are a number of methods used to screen for colorectal cancer and include stool tests that primarily detect cancer, and flexible sigmoidoscopy, colonoscopy, CT, colonography, and double-contrast barium enema which detect cancer and precancerous growths [2]. Many of these methods are invasive or have limited sensitivity. Development of simple non-invasive sensitive screens for colorectal cancer would be beneficiary both for the patient and the healthcare providers [3].

Detection of cancer markers is a non-invasive method in the diagnosis of cancers. It is acceptable in patients and simple in procedures. Thus, detection of cancer markers plays important clinical roles in the early diagnosis, treatment and prognosis of CRC [4]. In the present study, five serological biomarkers (carcinoembryonic antigen [CEA], carbohydrate antigen

Wa Zhong and Zhong Yu are contributed equally.

W. Zhong · Z. Yu · J. Zhan · T. Yu · Y. Lin · Z.-S. Xia · Y.-H. Yuan · Q.-K. Chen (✉)
Department of Gastroenterology, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, No.107 Yanjiang Xi Road, Yuexiu District, Guangzhou, Guangdong 510120, People's Republic of China
e-mail: qikuichen@yahoo.com

CA19–9, CA125, CYFRA21–1, and CA72–4) which are associated with colon-cancer were analyzed to evaluate their association with patient clinical characteristics and disease progression in CRC. CEA is a serum glycoprotein and currently is the most widely used marker for colon cancer [4]. It is commonly secreted by tumors located in hollow organs and has a specificity and sensitivity of 36 and 87 %, respectively, in screening for colon cancer [4]. CA19–9 is an antigen that elevated in many types of gastrointestinal cancer including colorectal cancer, esophageal cancer, and hepatocellular carcinoma [5]. CA19–9 has a sensitivity and specificity of 23 and 96 % for colorectal cancer [6]. CA125 is a glycoprotein antigen that was first found associated with ovarian cancer [7]. It is also associated with gastric, colon, lung, pancreatic, and liver cancers, as well as, cancers of the blood. Multivariate analysis revealed that serum CEA levels (all $p<0.001$) was an independent prognostic predictors for liver metastases [8–11]. CA72–4 has a sensitivity of approximately 40 % in colorectal and gastric cancer and 50 % in ovarian cancer, with an overall specificity of more than 95 % [12]. CYFRA21–1 is a fragment of cytokeritin-19. Cytokeritin-19 is expressed in the unstratified or pseudostratified epithelium of the bronchial tress and is over expressed in many lung cancers [13–15] and has also been implicated as a marker for colorectal cancer [16,17]. In colorectal cancer at the level of 95 % sensitivity, CYFRA21–1 has a specificity of about 35.5 % [17].

Patients and Methods

This was a retrospective study of patients admitted to Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University from January 2006 to December 2007 that investigated the association of the expression of CEA, CA19–9, CA125, CYFRA21–1and CA72–4 with colorectal cancer. Patients were followed for 5 years. The study was approved by the institutional review board of Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, Guangdong, China. Informed patients' consents had been obtained in advance.

Study Patients

Eligible patients had diagnosed colorectal cancer as determined by pathology following surgery. Patients had received no preoperative chemotherapy or radiotherapy. Patients who were lost during the 5-year follow-up or died from other causes were excluded from the analysis. Patients were staged in accordance with TMN and Dukes classification.

Analysis of CEA, CA19–2, CA125, CYFRA21–1 and CA72–4

Fasting blood (2 ml) was collected from the cubital vein in the morning and serum was collected by centrifugation. All 5 markers were assessed at patient initial screening for cancer, pre and post-surgery, routine follow-up, and at disease recurrence. CEA, CA19–2, CA125, CYFRA21–1 and CA72–4 were detected using electrochemiluminescence immunoassay system (Elecsys 2010, Roche, Basel Switzerland) according to the manufacturer's instructions. The reference range was 0 to 5 ng/ml for CEA, 0 to 34 kU/L for CA19–9, 0 to 35 kU/L for CA125, 0 to 3.3 kU/L for CYFRA21–1 and 0 to 7 kU/L for CA72–4.

Statistical Analysis

Patients' demographics and clinical characteristics were summarized descriptively as were serological markers, including CEA, CA125, CA19–9, CA72–4, and CYFRA21–1for pre-operation, post-operation, and recurrence period. Changes in expression of serological makers during different treatment periods were determined using the McNemar test. The association of expression of serological makers with patient demographics and clinical characteristics were performed using the Mann–Whitney *U* test and Pearson Chi-square or Fisher's exact test with Yate's correction for samples in which <5 cell expressed a given marker. A step-wise Cox-regression analysis was applied to identify the associations among time related data, overall survival time, and recurrence time in comparison with patient demographics, clinical characteristics, and pre-operative marker's expression, respectively. Variables with significant associations in univariate Cox-regression analysis ($P<0.05$) were selected and analyzed using multivariate Cox-regression analysis. Moreover, Bayesian information criterion (BIC) index to evaluate which of the 5 markers may be best at predicting overall survival (smaller value is better). Hazard ratios (HR) with corresponding 95 % confidence intervals (95 %CI.) as well as *P*-values were determined by Cox-regression analysis. All statistical assessments were two-tailed and considered significantly at $P<0.05$. Kaplan-Meier analysis was used to evaluate the cumulative overall survival and disease recurrence rates. All the statistical analysis was performed using SPSS 18.0 statistics software (SPSS Inc, Chicago, IL, USA).

Results

There are 373 patients enrolled in the study and all completed the trial. The median age of was 62 years, the most common form of cancer was adenocarcinoma (85.5 %) and most tumors were well differentiated (54.2 %) (Table 1). The most common depth of tumor infiltration and abdominal lymph node metastasis was serosal layer and pareneoplasctic lymph node metastasis (40 %) followed by serosal layer alone (29.2 %). Most patients had either stage III (44.0 %) or stage II (29.0 %) cancer and had cancer in the right colon (50.9 %).

Table 1 Patient demographics and clinical characteristics

Variables	(*N*=373)
Age, median (Range) years	62 (15 to 100)
Males	214 (57.4 %)
Pathological type	
Adenocarcinoma	319 (85.5 %)
Mucinous Adenocarcinoma	51 (13.7 %)
Signet ring cell carcinoma	3 (0.8 %)
Degree of differentiation	
Poorly differentiated	46 (12.3 %)
Moderately differentiated	125 (33.5 %)
Well differentiated	202 (54.2 %)
Depth of infiltration and Abdominal lymph node metastasis[a]	
Within the intestinal wall	48 (13.0 %)
Serosal layer	108 (29.2 %)
Serosal layer + paraneoplastic lymph node metastasis	148 (40.0 %)
Serosal layer + paraneoplastic lymph node metastasis + mesenteric lymph node metastasis	66 (17.8 %)
Clinical stage	
I	50 (13.4 %)
II	108 (29.0 %)
III	164 (44.0 %)
IV	51 (13.7 %)
Lesion site	
Transverse colon cancer	39 (10.5 %)
Left colon cancer	124 (33.2 %)
Right colon cancer	190 (50.9 %)
Rectal cancer	20 (5.4 %)
Organ metastasis	
Yes	50 (13.4 %)
No	323 (86.6 %)
Recurrence	
Yes	144 (38.6 %)
No	229 (61.4 %)
Survival status	
Alive	207 (55.5 %)
Dead	166 (44.5 %)

Age were summarized as median (Range: minimum to maximum); other categorical data were as n (%)

[a] three patients were unavailable

The majority of patients had no metastasis (86.6 %) or disease recurrence (61.4 %). About half the patients (55.5 %) were still alive at the end of the 5-year follow-up period.

All the five markers, CEA, CA19–9, CA125, CA72–4, and CYFRA21–1 were present in the patient population prior to and after surgery as well as when the disease recurred (Table 2). Prior to surgery less than 50 % of the patient were positive for any of the 5 markers with the most common being CEA (48 %), CA19–9 (33.2 %) and CYFRA21–1 (29.8 %). Following surgery, the proportion of patients having these makers significantly decreased (all *P*-values<0.001) (Table 2). Disease recurrence was associated with a significant increase from post-surgery in the proportion of patients positive for the 5 markers (Table 2). CEA was the most common (87.5 %) followed by CYFRA21–1 (73.6 %) and CA19–9 (70.1 %).

Evaluation of the association of being positive for a given marker and demographics and disease characteristics found patients with positive CEA might be older than patients with negative CEA (median age: 65 vs. 59 years, $P=0.002$). Furthermore, the positive expressions of a given markers was associated with degree of tumor differentiation, depth of infiltration, clinical stage, organ metastasis, recurrence, and survival status. CA72–4 was significantly associated with lesion site (all *P*-values<0.05) (Tables 3 and 4).

Figure 1 represented the Kaplan-Meier curve of overall survival (OS) and recurrence for the 373 patients. The estimated mean of time to OS was derived as 47.1 months with a 95 %CI.=45.5 to 48.8 months. The estimated mean of time to recurrence was derived as 42.4 months with a 95 %CI.=40.0 to 44.7 months. (Fig. 1).

Univariate analysis found that overall survival was associated with patients' clinical characteristics as well as pre-operative and post-operative expression of CEA, CA125, CA19–9, CA72–4, CYFRA21–1 expressions (Table 5). Multivariate analysis showed that overall survival was associated with signet ring carcinoma, good differentiation, depth of infiltration (serosal layer plus paraneoplastic lymph node metastasis, serosal layer plus paraneoplastic lymph node and mesenteric lymph node metastasis), stage II cancer, and the presence of CEA before surgery (all *P*-value<0.05) (Table 5). Multivariate analysis did not find an association of post-operative levels of any of the 5 markers with overall survival (Table 5).

Univariate Cox-regression analysis found disease recurrence was associated with patients' clinical characteristics, and pre- and post-operative expression CEA, CA125, CA19–9, CA72–4, CYFRA21–1 expressions. Multivariate Cox-regression analysis revealed disease recurrence was associated with degree of differentiation (well differentiated), and depth of infiltration (serosal layer plus paraneoplastic lymph node metastasis, serosal layer plus paraneoplastic lymph node and mesenteric lymph node metastasis) (all *P*-value<0.05). There was no significant association for disease recurrence and pre-operative and post-operative CEA, CA125, CA19–9, CA72–4, or CYFRA21–1 expressions. (Table 6).

To further examine the role of pre- and post-operative levels of the 5 markers in predicting overall survival, we used the BIC index to evaluate which of the 5 markers may be best at predicting overall survival (smaller value is better) (see Table 7). The model was based on the multivariate model in this manuscript. Each marker was individually included in the

Table 2 Distribution of serological markers at pre- and post-surgery and at disease recurrence periods

Markers				*P*-values[a] for comparison	
	Pre-operative (n=373)	Post-operative (n=373)	Re-current (n=144)	Pre- vs. post- operative	Post- operative vs. Re-current
CEA				<0.001*	<0.001*
Positive	179 (48 %)	65 (17.4 %)	126 (87.5 %)		
Negative	194 (52 %)	308 (82.6 %)	18 (12.5 %)		
CA19–9				<0.001*	<0.001*
Positive	124 (33.2 %)	54 (14.5 %)	101 (70.1 %)		
Negative	249 (66.8 %)	319 (85.5 %)	43 (29.9 %)		
CA7–24				<0.001*	<0.001*
Positive	86 (23.1 %)	25 (6.7 %)	88 (61.1 %)		
Negative	287 (76.9 %)	348 (93.3 %)	56 (38.9 %)		
CA125				<0.001*	<0.001*
Positive	91 (24.4 %)	41 (11 %)	79 (54.9 %)		
Negative	282 (75.6 %)	332 (89 %)	65 (45.1 %)		
CYFRA21–1				<0.001*	<0.001*
Positive	111 (29.8 %)	64 (17.2 %)	206 (73.6 %)		
Negative	262 (70.2 %)	309 (82.8 %)	38 (26.4 %)		

Data were summarized as n (%) for a given period

[a] p-value were derived using McNemar test

* indicates significant difference between two periods. (P< 0.05×

multivariate analysis model which adjusted for patients' age, pathological type, degree of differentiation, depth of infiltration and abdominal lymph node metastasis, clinical stage, lesion site, and organ metastasis. The pre- and post—operative analyses indicated that models containing levels of CEA and CA199 or CEA and CYFRA21–1 might be better at predicting overall survival than the model which included other marker combinations including all 5 markers. (for pre-operative, BIC index: 1716.68 vs. 1730.75, respectively, and for post-operative, BIC index: 1718.56 vs. 1731.64) (Table 7). We also found that post-operative levels for each marker were positively correlated with pre-operative levels and this was independent of whether a patient was alive or dead (Table 8).

Discussion

This study evaluated the association of CEA, CA19–9, CA7–24, CA125, and CYFRA21–1 with disease characteristics, patient demographics, and disease progression in patients with CRC. We found that it was more common for a patient to express at least one of these markers prior to surgery and at disease recurrence than following surgery. In general, the presence of CEA, CA19–9, CA125, CYFRA21–1, and CA72–4 were not associated with gender, age, pathological type, and location of the cancer (all *P*-values >0.05), but were associated with poor tumor differentiation, higher tumor invasion, greater degree of abdominal lymph node metastasis, and higher TNM and Dukes stage tumors, and overall survival (all *P*-values <0.01). In contrast to the other markers, CEA was associated with older age (median 65 years of age), and multivariate analysis indicated the pre-surgery levels of only CEA correlated with overall survival. Multivariate analysis did not find an association of post-operative levels of any of the 5 markers to be significantly associated with disease recurrence or overall survival. Using the BIC Index we found that models containing pre- or post-operative levels of CEA and CA199 or CEA and CYFRA21–1 were possibly better at predicting overall survival than model which included other combination of marker or all 5 markers. These findings suggest CEA plus CA199 or CEA plus CYRFA21–1 may be useful in aiding in diagnosis, following recurrence, and monitoring patients.

Medically useful tumor markers to screen a large population at risk for a specific cancer would ideally be non-invasive, highly sensitive and specific, and inexpensive. CEA, CA199, CA125, and CA72–4 are commonly used for post-operative surveillance and monitoring treatment effect for colorectal cancer, pancreatic cancer, ovarian cancer and gastric cancer, respectively, [9,18–21]. In this study the overall positive rate of these markers prior to surgery were 48, 33.2, 23.1 %, respectively, which was similar to that reported previously [22]. In our study, the pre-operative positive rate for CEA was similar to another study which reported 43.9 % sensitivity of detecting colorectal cancer [17]. We found that CA125 was positive for 24.4 % of patient prior to surgery which was less

Table 3 The association of demographics and clinical characteristics with marker expression

Variables	All (n=373)	CEA			CA19–9			CA125		
		Negative (n=194)	Positive (n=179)	p-value	Negative (n=249)	Positive (n=124)	p-value	Negative (n=282)	Positive (n=91)	p-value
Age, median (Range) yrs	62 (15 to 100)	59 (15 to 100)	65 (18 to 89)	0.002*	61 (15 to 88)	64.5 (27 to 100)	0.305	62 (15 to 100)	64 (18 to 81)	0.283
Sex				0.187			0.121			0.768
Males	214 (57.4 %)	105 (49.1 %)	109 (50.9 %)		150 (70.1 %)	64 (29.9 %)		163 (76.2 %)	51 (23.8 %)	
Females	159 (42.6 %)	89 (56.0 %)	701 (44.0 %)		99 (62.3 %)	60 (37.7 %)		119 (74.8)	40 (25.2 %)	
Pathological type				1.000			1.000			0.145
Adenocarcinoma	319 (85.5 %)	166 (52.0 %)	153 (48.0 %)		213 (66.8 %)	106 (33.2 %)		240 (75.2 %)	79 (24.8 %)	
MucinousAdenocarcinoma	51 (13.7 %)	26 (51.0 %)	25 (49.0 %)		34 (66.7 %)	17 (33.3 %)		41 (81.4 %)	10 (19.6 %)	
Signet ring cell carcinoma	3 (0.8 %)	2 (66.7 %)	1 (33.3 %)		2 (66.7 %)	1 (33.3 %)		1 (33.3 %)	2 (66.7 %)	
Degree of differentiation				<0.001*			0.002*			<0.001*
Poorly differentiated	46 (12.3 %)	20 943.5 %)	26 (56.5 %)		22 (47.8 %)	24 (52.2 %)		21 (45.7 %)	25 (54.3 %)	
Moderately differentiated	125 (33.5 %)	45 (36.0 %)	80 (64.0 %)		78 (62.4 %)	47 (37.6 %)		91 (72.8 %)	34 (27.2 %)	
Well differentiated	202 (54.2 %)	129 (63.9 %)	73 (36.1 %)		149 (73.8 %)	53 (26.2 %)		170 (84.2 %)	32 (15.8 %)	
Depth of infiltration and Abdominal lymph node metastasis				<0.001*			<0.001*			<0.001*
Within the intestinal wall	48 (13.0 %)	39 (81.3 %)	9 (18.8 %)		42 (87.5 %)	6 (12.5 %)		47 (97.9 %)	1 (2.1 %)	
Serosal layer	108 (29.2 %)	65 (60.2 %)	43 (39.8 %)		86 (79.6 %)	22 (20.4 %)		92 (85.2 %)	16 (14.8 %)	
Serosal layer + paraneoplastic lymph node metastasis	148 (40.0 %)	68 (45.9 %)	80 (54.1 %)		92 (62.2 %)	56 (37.8 %)		109 (73.6 %)	39 (26.4 %)	
Serosal layer + paraneoplastic lymph node metastasis + mesenteric lymph node metastasis	66 (17.8 %)	20 (30.3 %)	46 (69.7 %)		28 (42.4 %)	38 (57.6 %)		31 (47.0 %)	35 (53.0 %)	
Clinical stage				<0.001*			<0.001*			<0.001*
I	50 (13.4 %)	41 (82.0 %)	9 (18.0 %)		43 (86.0 %)	7 (14.0 %)		49 (98.0 %)	1 (2.0 %)	
II	108 (29.0 %)	65 (60.2 %)	43 (39.8 %)		86 (79.6 %)	22 (20.4 %)		92 (85.2 %)	16 (14.8 %)	
III	164 (44.0 %)	76 (46.3 %)	88 (53.7 %)		102 (62.2 %)	62 (37.8 %)		119 (72.6 %)	45 (27.4 %)	
IV	51 (13.7 %)	12 (23.5 %)	39 (76.5 %)		18 (35.3 %)	33 (64.7 %)		22 (43.1 %)	29 (56.9 %)	
Lesion site				0.403			0.370			0.154
Transverse colon cancer	39 (10.5 %)	22 (56.4 %)	17 (43.6 %)		30 (76.9 %)	9 (23.1 %)		26 (66.7 %)	13 (33.3 %)	
Left colon cancer	124 (33.2 %)	70 (56.5 %)	54 (43.5 %)		83 (66.9 %)	41 (33.1 %)		95 (76.6 %)	29 (23.4 %)	
Right colon cancer	190 (50.9 %)	94 (49.5 %)	96 (50.5 %)		125 (65.8 %)	65 (34.2 %)		149 (78.4 %)	41 (21.6 %)	
Rectal cancer	20 (5.4 %)	8 (40.0 %)	12 (60.0 %)		11 (55.0 %)	9 (45.0 %)		12 (60.0 %)	8 (40.0 %)	
Organ metastasis				<0.001*			<0.001*			<0.001*
Yes	50 (13.4 %)	12 (24.0 %)	38 (76.0 %)		18 (36.0 %)	32 (64.0 %)		21 (42.0 %)	29 (58.0 %)	

Table 3 (continued)

Variables	All (*n*=373)	CEA			CA19–9			CA125		
		Negative (*n*=194)	Positive (*n*=179)	*p*-value	Negative (*n*=249)	Positive (*n*=124)	*p*-value	Negative (*n*=282)	Positive (*n*=91)	*p*-value
No	323 (86.6 %)	182 (56.3 %)	141 (43.7 %)		231 (71.5 %)	92 (28.5 %)		261 (80.8 %)	62 (19.2 %)	
Recurrence				<0.001*			<0.001*			<0.001*
Yes	144 (38.6 %)	51 (35.4 %)	93 (64.6 %)		75 (52.1 %)	69 (47.9 %)		91 (63.2 %)	53 (36.8 %)	
No	229 (61.4 %)	143 (62.4 %)	86 (37.6 %)		174 (76.0 %)	55 (24.0 %)		191 (83.4 %)	38 (16.6 %)	
Survival status				<0.001*			<0.001*			<0.001*
Alive	207 (55.5 %)	140 (67.6 %)	67 (32.4 %)		170 (82.1 %)	37 (17.9 %)		184 (88.9 %)	23 (11.1 %)	
Dead	166 (44.5 %)	54 (32.5 %)	112 (67.5 %)		79 (47.6 %)	87 (52.4 %)		98 (59.0 %)	68 (41.0 %)	
DFS				<0.001*			<0.001*			<0.001*
Yes	166 (44.5 %)	54 (32.5 %)	112 (67.5 %)		79 (47.6 %)	87 (52.4 %)		98 (59.0 %)	68 (41.0 %)	
No	207 (55.5 %)	140 (67.6 %)	67 (32.4 %)		170 (82.1 %)	37 (17.9 %)		184 (88.9 %)	23 (11.1 %)	

Table 4 The associations of demographics and clinical characteristics with marker expression

Variables	All (*n*=373)	CA72–4			CYFRA21–1		
		Negative (*n*=287)	Positive (*n*=86)	*p*-value	Negative (*n*=262)	Positive (*n*=111)	*p*-value
Age, median (Range) years	62 (15 to 100)	62 (15 to 100)	65.5 (29 to 91)	0.177	61.0 (15 to 100)	65 (29 to 91)	0.053
Sex				0.184			0.539
Males	214 (57.4 %)	170 (79.4 %)	44 (20.6 %)		153 (71.5 %)	61 (28.5 %)	
Females	159 (42.6 %)	117 (73.6 %)	42 (26.4 %)		109 (68.6 %)	50 (31.4 %)	
Pathological type				0.348			0.830
Adenocarcinoma	319 (85.5 %)	249 (78.1 %)	70 (21.9 %)		226 (70.8 %)	93 (29.2 %)	
MucinousAdenocarcinoma	51 (13.7 %)	36 (70.6 %)	15 (29.4 %)		34 (66.7 %)	17 (33.3 %)	
Signet ring cell carcinoma	3 (0.8 %)	2 (66.7 %)	1 (33.3 %)		2 (66.7 %)	1 (33.3 %)	
Degree of differentiation				<0.001*			<0.001*
Poorly differentiated	46 (12.3 %)	24 (52.2 %)	22 (47.8 %)		22 (47.8 %)	24 (52.2 %)	
Moderately differentiated	125 (33.5 %)	89 (71.2 %)	36 (28.8 %)		80 (64.0 %)	45 (36.0 %)	
Well differentiated	202 (54.2 %)	174 (86.1 %)	28 (13.9 %)		160 (79.2 %)	42 (20.8 %)	
Depth of infiltration and Abdominal lymph node metastasis				<0.001*			<0.001*

Table 4 (continued)

Variables	All (n=373)	CA72–4			CYFRA21–1		
		Negative (n=287)	Positive (n=86)	p-value	Negative (n=262)	Positive (n=111)	p-value
Within the intestinal wall	48 (13.0 %)	47 (97.2 %)	1 (2.1 %)		46 (95.8 %)	2 (4.2 %)	
Serosal layer	108 (29.2 %)	95 (88.0 %)	13 (12.0 %)		90 (83.3 %)	18 (16.7 %)	
Serosal layer + paraneoplastic lymph node metastasis	148 (40.0 %)	109 (73.6 %)	39 (26.4 %)		99 (66.9 %)	49 (33.1 %)	
Serosal layer + paraneoplastic lymph node metastasis + mesenteric lymph node metastasis	66 (17.8 %)	34 (51.5 %)	32 (48.5 %)		25 (37.9 %)	41 (62.1 %)	
Clinical stage				<0.001*			<0.001*
I	50 (13.4 %)	49 (98.0 %)	1 (2.0 %)		48 (96.0 %)	2 (4.0 %)	
II	108 (29.0 %)	95 (88.0 %)	13 (12.0 %)		90 (83.3 %)	18 (16.7 %)	
III	164 (44.0 %)	120 (73.2 %)	44 (26.8 %)		108 (65.9 %)	56 (34.1 %)	
IV	51 (13.7 %)	23 (45.1 %)	28 (54.9 %)		16 (31.4 %)	35 (68.6 %)	
Lesion site				0.031*			0.061
Transverse colon cancer	39 (10.5 %)	30 (76.9 %)	9 (23.1 %)		29 (74.4 %)	10 (25.6 %)	
Left colon cancer	124 (33.2 %)	96 (77.4 %)	28 (22.6 %)		85 (68.5 %)	39 (31.5 %)	
Right colon cancer	190 (50.9 %)	151 (79.5 %)	39 (20.5 %)		139 (73.2 %)	51 (26.8 %)	
Rectal cancer	20 (5.4 %)	10 (50.0 %)	10 (50.0 %)		9 (45.0 %)	11 (55.0 %)	
Organ metastasis				<0.001*			<0.001*
Yes	50 (13.4 %)	23 (46.0 %)	27 (54.0 %)		16 (32.0 %)	34 (68.0 %)	
No	323 (86.6 %)	264 (81.7 %)	59 (18.3 %)		246 (76.2 %)	77 (23.8 %)	
Recurrence				<0.001*			<0.001*
Yes	144 (38.6 %)	91 (63.2 %)	53 (36.8 %)		77 (53.5 %)	67 (46.5 %)	
No	229 (61.4 %)	196 (85.6 %)	33 (14.4 %)		185 (80.8 %)	44 (19.2 %)	
Survival status				<0.001*			<0.001*
Alive	207 (55.5 %)	188 (90.8 %)	19 (9.2 %)		180 (87.0 %)	27 (13.0 %)	
Dead	166 (44.5 %)	99 (59.6 %)	67 (40.4 %)		82 (49.4 %)	84 (50.6 %)	
DFS				<0.001*			<0.001*
Yes[†]	166 (44.5 %)	99 (59.6 %)	67 (40.4 %)		180 (87.0 %)	27 (13.0 %)	
No	207 (55.5 %)	188 (90.8 %)	19 (9.2 %)		82 (49.4 %)	84 (50.6 %)	

Age are summarized as median (Range: minmum to maximum) andother categorical data were as n (%) for given expression markers

[a] three patients were unavailable

p-value were derived using Mann–Whitney U test in age and Pearson Chi-square or Fisher's exact test with Yate's correction if any cell number is less than 5

*indicates significant association with markers' expression. (P<0.05)

[†] Yes indicates patient died or had recurrent disease; No, indicates patients were alive and did not have recurrent disease

DFS disease free survival

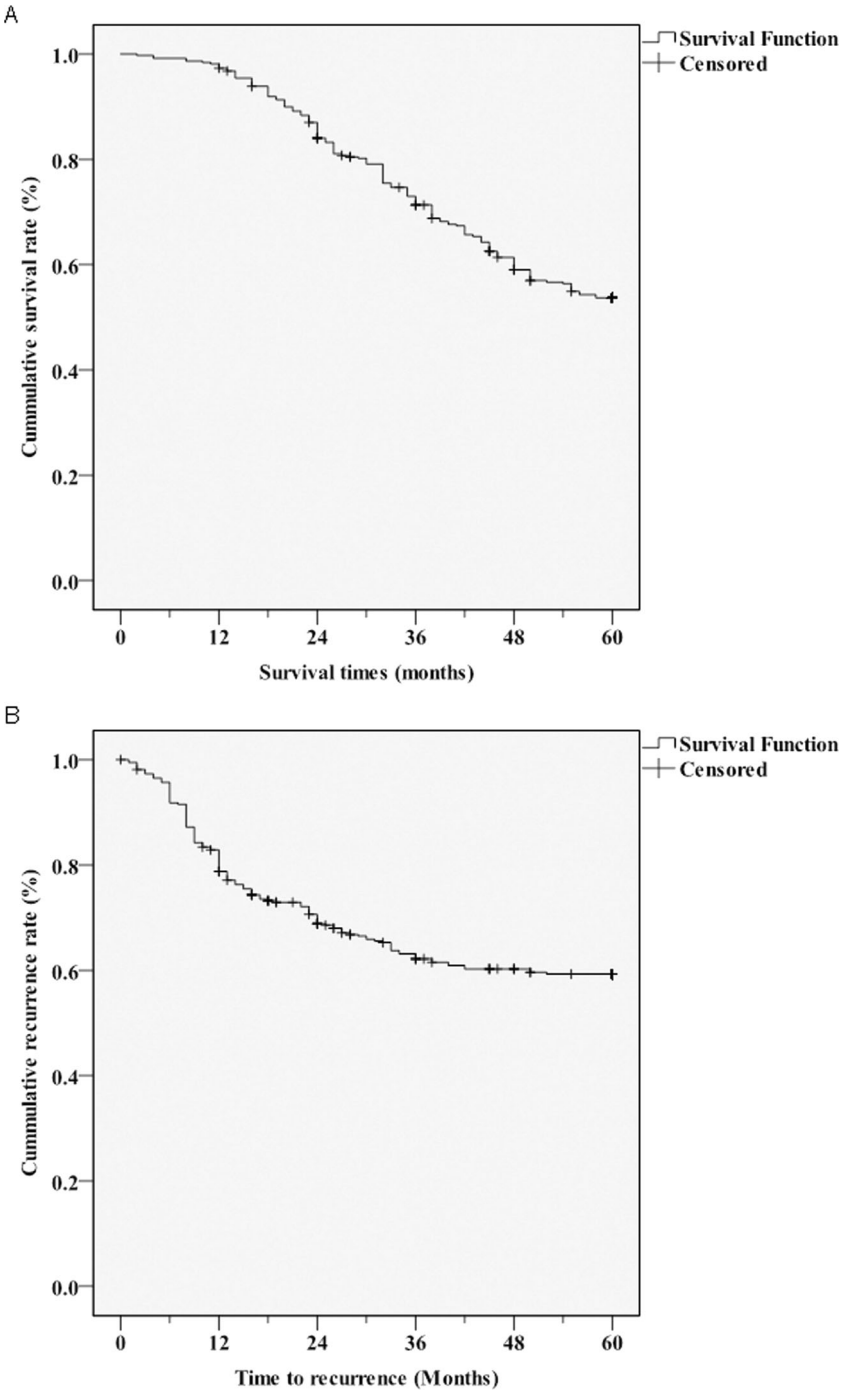

Fig. 1 Kaplan-Meier curves of (**a**) overall survival (OS) and (**b**) recurrence. The estimated mean of time to OS was derived as 47.1 months (95 %CI, 45.5 to 48.8 months). The estimated mean of time to recurrence was derived as 42.4 months (95 %CI, 40.0 to 44.7 months)

than that reported in a prior study which had a positive rate of 64.1 % [23]. The difference between the studies may reflect that the earlier study used microarrays to assay expression of the factor which is more sensitive than immunofluorescence method used in this study.

Several prior studies have also evaluated the correlation of CEA, CA125, CA19–9 with overall survival and disease recurrence [24–26]. Similar to our study, the presence of CEA has been shown previously to be predictive of increased mortality and overall survival [22,27]. In contrast to our study, multivariate analysis in one study found pre-surgery serum CA125 status and not CEA was an independent prognostic factor for overall survival (P=0.016) [26]. In addition, CA19–9 previously was shown to be an indicator of disease recurrence and overall survival, [22,27–29] and CA72–4 was associated with high recurrence rate [22]. Interestingly, the combination of being positive for CA19–9, CEA, and CA125 was a strong predictor of overall survival and patients who were positive for these 3 markers also had the highest rate of disease recurrence (100 %) [25,26]. The difference between

Table 5 Univariate and multivariate Cox-regression analysis of overall survival

Variables	Univariate			Multivariate I[a]			Multivariate II[b]		
	HR	95 % CI.	*P*-value	HR	95 % CI.	*P*-value	HR	95 % CI.	*P*-value
Age, years	0.996	(0.986, 1.006)	0.384	0.997	(0.986, 1.007)	0.526	1.003	(0.992, 1.014)	0.565
Sex									
Males vs. females	0.881	(0.649, 1.195)	0.415	NA					
Pathological type									
MucinousAdenocarcinoma vs. Adenocarcinoma	1.159	(0.751, 1.787)	0.505	1.116	(0.693, 1.797)	0.653	1.175	(0.725, 1.906)	0.512
Signet ring cell carcinoma vs. Adenocarcinoma	6.366	(2.015, 20.108)	0.002*	4.296	(1.210, 15.253)	0.024*	4.317	(1.218, 15.309)	0.024*
Degree of differentiation									
Moderately vs. poorly differentiated	0.538	(0.366, 0.792)	0.002*	0.706	(0.461, 1.081)	0.109	0.880	(0.569, 1.361)	0.566
Well vs. poorlydifferentiated	0.170	(0.11, 0.259)	<0.001*	0.348	(0.217, 0.558)	<0.001*	0.387	(0.242, 0.617)	<0.001*
Depth of infiltration and Abdominal lymph node metastasis									
Serosal layer vs. Within the intestinal wall	2.537	(0.739, 8.706)	0.139	1.650	(0.474, 5.737)	0.431	1.851	(0.534, 6.422)	0.332
Serosal layer + paraneoplastic lymph node metastasis vs. Within the intestinal wall	15.169	(4.794, 47.997)	<0.001*	20.453	(5.526, 75.704)	<0.001*	21.009	(5.696, 77.495)	<0.001*
Serosal layer + paraneoplastic lymph node metastasis + mesenteric lymph node metastasis vs. Within the intestinal wall	37.511	(11.714, 120.12)	<0.001*	17.505	(5.108, 59.989)	<0.001*	25.000	(7.444, 83.964)	<0.001*
Clinical stage									
II vs. I	2.651	(0.772, 9.098)	0.121	0.437	(0.234, 0.788)	0.006*	0.497	(0.277, 0.892)	0.019*
III vs. I	16.256	(5.148, 51.328)	<0.001*	ND			ND		
IV vs. I	56.304	(17.412, 182.072)	<0.001*	ND			ND		
Lesion site									
Transverse colon cancer vs. rectal cancer	0.027	(0.008, 0.085)	<0.001*	1.313	(0.604, 2.857)	0.492	1.506	(0.686, 3.305)	0.308
Left colon cancer vs. rectal cancer	0.068	(0.039, 0.118)	<0.001*	1.277	(0.682, 2.391)	0.444	1.524	(0.796, 2.917)	0.204
Right colon cancer vs. rectal cancer	0.404	(0.290, 00,565)	<0.001*	1.347	(0.733, 2.475)	0.338	1.671	(0.887, 3.148)	0.112
Organ metastasis									
Yes vs. No metastasis	6.686	(4.699, 9.512)	<0.001*	ND			ND		
Pre-operative									
CEA, positive vs. negative	3.401	(2.449, 4.723)	<0.001*	1.698	(1.129, 2.552)	0.011*	NA		
CA19–9, positive vs. negative	3.453	(2.539, 4.698)	<0.001*	1.333	(0.828, 2.145)	0.236	NA		
CA125, positive vs. negative	3.475	(2.544, 4.748)	<0.001*	1.183	(0.797, 1.756)	0.405	NA		
CA72–4, positive vs. negative	3.960	(2.886, 5.434)	<0.001*	0.679	(0.386, 1.193)	0.178	NA		
CYFRA21–1, positive vs. negative	4.271	(3.133, 5.821)	<0.001*	1.824	(0.951, 3.500)	0.071	NA		
Post-operative									
CEA, positive vs. negative	5.337	(3.830, 7.437)	<0.001*	NA			1.696	(0.921, 3.124)	0.090

Table 5 (continued)

Variables	Univariate			Multivariate I[a]			Multivariate II[b]		
	HR	95 % CI.	*P*-value	HR	95 % CI.	*P*-value	HR	95 % CI.	*P*-value
CA19–9, positive vs. negative	3.862	(2.708, 5.508)	<0.001*	NA			1.280	(0.718, 2.280)	0.403
CA125, positive vs. negative	3.428	(2.335, 5.033)	<0.001*	NA			0.767	(0.423, 1.391)	0.383
CA72–4, positive vs. negative	2.359	(1.426, 3.900)	0.001*	NA			0.698	(0.394, 1.237)	0.218
CYFRA21–1, positive vs. negative	5.464	(3.929, 7.599)	<0.001*	NA			1.762	(0.945, 3.287)	0.075

Results were represented as hazard ratio (HR) with corresponding 95 % confidence interval (95 %CI.) and *p*-values. Variables with significant association in univariate analysis model ($P<0.05$) were selected and put into multivariate analysis model

There were co-linearity among clinical stage, Depth of infiltration and Abdominal lymph node metastasis, and Organ metastasis in the multivariate analysis model

[a, b] The multivariate [a] I and [b] II cox-regression models were considering with [a] pre-operative markers and [b] post-operative markers, separately

*indicates significant association with OS. ($P<0.05$)

ND not derived

NA not assessed

Table 6 Univariate and multivariate Cox-regression analysis of disease recurrence

Variables	Univaraite			Multivaraite I[a]			Multivaraite II[b]		
	HR	95 % CI.	*P*-value	HR	95 % CI.	*P*-value	HR	95 % CI.	*P*-value
Age, years	0.996	(0.985, 1.006)	0.430	0.995	(00,984, 1.005)	0.317	0.999	(0.988, 1.010)	0.840
Sex									
Males vs. females	0.873	(0.629, 1.212)	0.418	NA			NA		
Pathological type									
MucinousAdenocarcinoma vs. Adenocarcinoma	0.932	(0.575, 1.512)	0.776	NA			NA		
Signet ring cell carcinoma vs. Adenocarcinoma	3.380	(0.828, 13.804)	0.090	NA			NA		
Degree of differentiation									
Moderately vs. poorly differentiated	0.647	(0.421, 0.995)	0.048*	0.907	(0.576, 1.428)	0.673	1.072	(0.676, 1.701)	0.767
Well vs. poorlydifferentiated	0.206	(0.129, 0.329)	<0.001*	0.364	(0.218, 0.608)	<0.001*	0.404	(0.242, 0.672)	<0.001*
Depth of infiltration and Abdominal lymph node metastasis									
Serosal layer vs. Within the intestinal wall	0.428	(0.214, 1.479)	0.180	1.569	(0.450, 5.463)	0.479	1.759	(0.506, 6.107)	0.374
Serosal layer + paraneoplastic lymph node metastasis vs. Within the intestinal wall	6.120	(3.529, 10.612)	<0.001*	6.998	(1.870, 26.195)	0.004*	7.149	(1.899, 26.919)	0.004*

Table 6 (continued)

Variables	Univaraite			Multivaraite I[a]			Multivaraite II[b]		
	HR	95 % CI.	*P*-value	HR	95 % CI.	*P*-value	HR	95 % CI.	*P*-value
Serosal layer paraneoplastic lymph node metastasis + mesenteric lymph node metastasis vs. Within the intestinal wall	8.415	(4.617, 15.338)	<0.001*	6.526	(1.883, 22.625)	0.003*	8.975	(2.613, 30.824)	<0.001*
Clinical stage									
II vs. I	2.437	(0.706, 8.419)	0.159	1.317	(0.712, 2.436)	0.380	1.459	(0.778, 2.735)	0.239
III vs. I	15.442	(4.889, 48.771)	<0.001*	ND			ND		
IV vs. I	19.817	(5.992, 65.539)	<0.001*	ND			ND		
Lesion site									
Transverse colon cancer vs. rectal cancer	0.737	(0.322, 1.685)	0.737	NA			NA		
Left colon cancer vs. rectal cancer	0.723	(0.354, 1.475)	0.723	NA			NA		
Right colon cancer vs. rectal cancer	0.731	(0.366, 1.463)	0.731	NA			NA		
Yes vs. No metastasis	2.593	(1.703, 3.948)	<0.001*	ND			ND		
Pre-operative									
CEA, positive vs. negative	2.748	(1.947, 3.879)	<0.001*	1.477	(0.971, 2.247)	0.069	NA		
CA19–9, positive vs. negative	2.666	(1.915, 3.712)	<0.001*	1.201	(0.728, 1.981)	0.474	NA		
CA125, positive vs. negative	2.729	(1.939, 3.840)	<0.001*	1.288	(0.866, 1.916)	0.212	NA		
CA72–4, positive vs. negative	3.057	(2.164, 4.317)	<0.001*	0.729	(0.393, 1.353)	0.317	NA		
CYFRA21–1, positive vs. negative	3.252	(2.329, 4.540)	<0.001*	1.784	(0.909, 3.501)	0.902	NA		
Post-operative									
CEA, positive vs. negative	3.717	(2.559, 5.397)	<0.001*	NA			1.834	(0.993, 3.388)	0.053
CA19–9, positive vs. negative	2.275	(1.487, 3.481)	<0.001*	NA			0.89	(0.481, 1.649)	0.712
CA125, positive vs. negative	2.286	(1.448, 3.610)	<0.001*	NA			0.94	(0.509, 1.734)	0.842
CA72–4, positive vs. negative	1.823	(1.008, 3.297)	0.047*	NA			0.742	(0.372, 1.479)	0.396
CYFRA21–1, positive vs. negative	3.770	(2.593, 5.480)	<0.001*	NA			1.555	(0.819, 2.955)	0.177

Results were represented as hazard ratio (HR) with corresponding 95 % confidence interval (95 %CI.) and *p*-values. Variables with significant association in univariate analysis model ($P<0.05$) were selected and put into multivariate analysis model

There were co-linearity among clinical stage, Depth of infiltration and Abdominal lymph node metastasis, and Organ metastasis in the multivariate analysis model

[a, b] The multivariate [a] I and [b] II cox-regression models were considering with [a] pre-operative markers and [b] post-operative markers, separately

*indicates significant association with OS. ($P<0.05$)

ND not derived

NA not assessed

Table 7 Validate the pre- and post-operative markers associated with overall survival

Included markers[a]	For pre-operative markers				For post-operative markers			
	HR	95 % CI.	*P*-value	BIC	HR	95 % CI.	*P*-value	BIC
CEA, positive vs. negative	2.119	(1.479, 3.035)	<0.001*	1,718.33	2.545	(1.760, 3.680)	<0.001*	1,713.18
CA19–9, positive vs. negative	2.011	(1.436, 2.817)	<0.001*	1,719.46	1.971	(1.308, 2.970)	0.001*	1,726.25
CA125, positive vs. negative	1.583	(1.099, 2.280)	0.014*	1,730.06	1.610	(1.035, 2.502)	0.034*	1,731.74
CA72–4, positive vs. negative	1.782	(1.249, 2.541)	0.001*	1,726.06	1.026	(0.590, 1.784)	0.928	1,735.96
CYFRA21–1, positive vs. negative	2.203	(1.538, 3.155)	<0.001*	1,717.50	2.472	(1.727, 3.539)	<0.001*	1,713.43
CEA + CA19–9				1,716.68				1,718.56
CEA, positive vs. negative	1.769	(1.205, 2.597)	0.004*		2.337	(1.510, 3.617)	<0.001*	
CA199, positive vs. negative	1.652	(1.154, 2.364)	0.006*		1.202	(0.736, 1.961)	0.462	
CEA + CA125				1,722.11				1,719.05
CEA, positive vs. negative	1.995	(1.378, 2.888)	<0.001*		2.603	(1.718, 3.943)	<0.001*	
CA125, positive vs. negative	1.329	(0.911, 1.939)	0.14		0.944	(0.574, 1.551)	0.819	
CEA + CA72–4				1,722.35				1,718.31
CEA, positive vs. negative	1.885	(1.263, 2.813)	0.002*		2.642	(1.810, 3.855)	<0.001*	
CA72–4, positive vs. negative	1.319	(0.891, 1.952)	0.167		0.781	(0.446, 1.366)	0.386	
CEA + CYFRA21–1				1,716.89				1,716.38
CEA, positive vs. negative	1.684	(1.127, 2.516)	0.011*		1.702	(0.928, 3.120)	0.086	
CA211, positive vs. negative	1.724	(1.159, 2.564)	0.007*		1.652	(0.914, 2.986)	0.097	
CEA + CA19–9 + CYFRA21–1				1,721.11				1,722.25
CEA, positive vs. negative	1.652	(1.106, 2.470)	0.014*		1.682	(0.908, 3.114)	0.098	
CA19–9, positive vs. negative	1.37	(0.854, 2.196)	0.191		1.063	(0.633, 1.787)	0.817	
CYFRA21–1, positive vs. negative	1.379	(0.821, 2.315)	0.225		1.616	(0.868, 3.010)	0.130	
CEA + CA19–9 + CA125 + CA72–4 + CYFRA21–1				1730.75				1731.64
CEA, positive vs. negative	1.698	(1.129, 2.552)	0.011*		1.696	(0.921, 3.124)	0.090	
CA19–9, positive vs. negative	1.333	(0.828, 2.145)	0.236		1.280	(0.718, 2.280)	0.403	
CA125, positive vs. negative	1.183	(0.797, 1.756)	0.405		0.767	(0.423, 1.391)	0.383	
CA72–4, positive vs. negative	0.679	(0.386, 1.193)	0.178		0.698	(0.394, 1.237)	0.218	
CYFRA21–1, positive vs. negative	1.824	(0.951, 3.500)	0.071		1.762	(0.945, 3.287)	0.075	

Results were represented as hazard ratio (HR) with corresponding 95 % confidence interval (95 %CI.) and *p*-values

[a] Markers were selected and put into multivariate analysis model with adjusting patients' age, pathological type, degree of differentiation, depth of infiltration and abdominal lymph node metastasis, clinical stage, lesion site, and organ metastasis. The corresponding BIC index (smaller is better) for each model was presented accordingly

There were co-linearity among clinical stage, Depth of infiltration and Abdominal lymph node metastasis, and Organ metastasis in the multivariate analysis model

*indicates significant association with OS. ($P<0.05$)

Table 8 Correlation analysis between pre- and post-operative marker levels

Markers	All ($n=373$)		Alive ($n=207$)		Dead ($n=166$)	
	Coefficient of correlation	*p*-value	Coefficient of correlation	*p*-value	Coefficient of correlation	*p*-value
CEA	0.863	<0.001*	0.806	<0.001*	0.886	<0.001*
CA19–9	0.895	<0.001*	0.908	<0.001*	0.885	<0.001*
CA125	0.930	<0.001*	0.896	<0.001*	0.947	<0.001*
CA72–4	0.912	<0.001*	0.874	<0.001*	0.878	<0.001*
CYFRA21–1	0.901	<0.001*	0.827	<0.001*	0.937	<0.001*

Correlation analysis was analyzed using Spearman's correlation analysis and represented as coefficients of correlation and corresponding *p*-value

* indicates significant correlation between pre- and post-operative measurements for each marker ($p<0.05$)

ours and other studies may reflect methods of analysis, study design, and patient population.

There are several limitations to our study which include the small sample size and the retrospective nature of the study. Since CYFRA21-1had not investigated as a marker for colorectal cancer before, further study had been warranted. In addition, analysis of the combination of markers and their relationship to disease characteristics, overall survival, and recurrence rate may give additional insight in the use of these markers in helping physicians following histoligical diagnosis in making clinical judgments on the patient status, and aid in monitoring treatment outcomes..

Conclusion

In conclusion, the cancer markers CEA, CA199, CA125, CYFRA21–1 and CA72–4 may potentially be useful in diagnosing and predicting treatment outcomes in patients with colorectal cancer. However, further analysis is needed to devise assays with these markers that have high sensitivity and specificity.

Acknowledgments This study was funded by the Science and technology and social development project of Guangdong Province (No.2012B031800030).

Conflict of Interest The authors declare that they have no conflicts of interest.

References

1. Jemal A, Bray F, Center MM et al (2011) Global cancer statistics. CA Cancer J Clin 61(2):69–90
2. Colorectal cancerfacts & figures: 2010–2013. http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-028323.pdf. Accessed: August 16 2013
3. Liu X, Cai H, Wang Y (2012) Prognostic significance of tumour markers in chinese patients with gastric cancer. ANZ J Surg. doi:10.1111/j.1445-2197.2012.06287.x
4. McKeown E, Nelson DW, Johnson EK et al (2014) Current approaches and challenges for monitoring treatment response in colon and rectal cancer. J Cancer 5(1):31–43
5. Perkins GL, Slater ED, Sanders GK et al (2003) Serum tumor markers. Am Fam Physician 68(6):1075–1082
6. Goldberg EM, Simunovic LM, Drake SL et al (1989) Comparison of serum ca 19–9 and cea levels in a population at high risk for colorectal cancer. Hybridoma 8(5):569–575
7. Bast RC Jr, Klug TL, St John E et al (1983) A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med 309(15):883–887
8. Zhang D, Yu M, Xu T et al (2013) Predictive value of serum cea, ca19–9 and ca125 in diagnosis of colorectal liver metastasis in chinese population. Hepatogastroenterology 60(126):1297–1301
9. Ohuchi N, Takahashi K, Matoba N et al (1989) Comparison of serum assays for tag-72, ca19–9 and cea in gastrointestinal carcinoma patients. Jpn J Clin Oncol 19(3):242–248
10. Shitara M, Yoshioka H, Sakurabayashi I et al (1988) fundamental studies and the reference values of cancer associated antigen tag-72 (ca72–4). Rinsho Byori 36(10):1177–1181
11. Kobayashi H (1989) The clinical usefulness of serum ca72–4 analysis in patients with ovarian cancer. Nihon Sanka Fujinka Gakkai Zasshi 41(5):585–589
12. Guadagni F, Roselli M, Cosimelli M et al (1995) Ca 72–4 serum marker–a new tool in the management of carcinoma patients. Cancer Investig 13(2):227–238
13. Kosacka M, Jankowska R (2009) Comparison of cytokeratin 19 expression in tumor tissue and serum cyfra 21–1 levels in non-small cell lung cancer. Pol Arch Med Wewn 119(1–2):33–37
14. Pang L, Wang J, Jiang Y et al (2013) Decreased levels of serum cytokeratin 19 fragment cyfra 21–1 predict objective response to chemotherapy in patients with non-small cell lung cancer. Exp Ther Med 6(2):355–360
15. Pujol JL, Grenier J, Daures JP et al (1993) Serum fragment of cytokeratin subunit 19 measured by cyfra 21–1 immunoradiometric assay as a marker of lung cancer. Cancer Res 53(1):61–66
16. Holdenrieder S, Stieber P, Liska V et al (2012) Cytokeratin serum biomarkers in patients with colorectal cancer. Anticancer Res 32(5):1971–1976
17. Wild N, Andres H, Rollinger W et al (2010) A combination of serum markers for the early detection of colorectal cancer. Clin Cancer Res 16(24):6111–6121
18. Kornek GV, Depisch D, Rosen HR et al (1992) Comparative analysis of ca72–4, ca195 and carcinoembryonic antigen in patients with gastrointestinal malignancies. J Cancer Res Clin Oncol 118(4):318–320
19. Posner MR, Mayer RJ (1994) The use of serologic tumor markers in gastrointestinal malignancies. Hematol Oncol Clin North Am 8(3):533–553
20. Ueda T, Shimada E, Urakawa T (1994) The clinicopathologic features of serum ca 19–9-positive colorectal cancers. Surg Today 24(6):518–525
21. Filella X, Molina R, Grau JJ et al (1992) Prognostic value of ca 19.9 levels in colorectal cancer. Ann Surg 216(1):55–59
22. Zheng CX, Zhan WH, Zhao JZ et al (2001) The prognostic value of preoperative serum levels of cea, ca19–9 and ca72–4 in patients with colorectal cancer. World J Gastroenterol 7(3):431–434
23. Streppel MM, Vincent A, Mukherjee R et al (2012) Mucin 16 (cancer antigen 125) expression in human tissues and cell lines and correlation with clinical outcome in adenocarcinomas of the pancreas, esophagus, stomach, and colon. Hum Pathol 43(10):1755–1763
24. Zhang D, Yu M, Xu T et al (2013) Predictive value of serum cea, ca19–9 and ca125 in diagnosis of colorectal liver metastasis in chinese population. Hepatogastroenterology 60(126):1301
25. Yang XQ, Chen C, Wang FB et al (2011) Preoperative serum carcinoembryonic antigen, carbohydrate antigen19–9 and carbohydrate antigen 125 as prognostic factors for recurrence-free survival in colorectal cancer. Asian Pac J Cancer Prev 12(5):1251–1256
26. Yang XQ, Li Y, Chen C et al (2011) Preoperative serum carbohydrate antigen 125 level is an independent negative prognostic marker for overall survival in colorectal cancer. Med Oncol 28(3):789–795
27. Forones NM, Tanaka M (1999) Cea and ca 19–9 as prognostic indexes in colorectal cancer. Hepatogastroenterology 46(26):905–908
28. Nakayama T, Watanabe M, Teramoto T et al (1997) Ca19–9 as a predictor of recurrence in patients with colorectal cancer. J Surg Oncol 66(4):238–243
29. Shimono R, Mori M, Akazawa K et al (1994) Immunohistochemical expression of carbohydrate antigen 19–9 in colorectal carcinoma. Am J Gastroenterol 89(1):101–105