The Combined Evaluation of Preoperative Serum CEA and Postoperative Tissue CEA as One Factor on the Prognosis of 0~IVColorectal Cancer

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Article

The Combined Evaluation of Preoperative Serum CEA and Postoperative Tissue CEA as One Factor on the Prognosis of 0~Ⅳ Colorectal Cancer

Running Title: Combined Cea as One Factor for Crc.

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Abstract: Background: The role of preoperative serum carcinoembryonic antigen (sCEA) and postopeative tissue CEA (tCEA) were widely elaborated separately for colorectal cancer (CRC). But the role of the combined sCEA and tCEA was scarcely described by now. Methods: 1757 cases of 0~IVCRC from January 2006 to January 2016 in our institution were involved. Clinicopathological features and follow up data were collected. 0 stage was combined into 0&I stages. SCEA was classified to normal and high(>10ng/ml) and tCEA were classified into three grades (+,++,+++). So combined groups were six (2x3). ANOVA and Crosstab were used to analyze continuous and counting data, Univariate and multivariate were analyzed by Cox regression. All data were analyzed by SPSS27 and survival curves with number at risk were visualized by R 4.3.1. Results: Gender, age, tumor location, tumor size, blood loss, T stage, differentiation, harvested lymph nodes, positive lymph nodes, chemotherapy, TNM stage and complication have difference between the combined CEA test (all P<0.05). ROC (Receiver Operating Characteristic Curve) of sCEA, tCEA and combined CEA have significant difference by 5 year overall survival (OS) with death as input variate (all P<0.05) in which AUC (Area Under Curve) of combined CEA was maximum, indicating the value of this study. Cox regression showed tumor location, T stage, differentiation, chemotherapy, TNM stage , tCEA and combined CEA have significances by univariate analysis but tCEA have no significance (P=0.096) in multivariate analysis about above seven variates. Furthermore 5 year OS analysis showed sCEA, tCEA and combined CEA have no significance in 0&I~IIstages and have significance in III~IV stages except for tCEA in IVstage. Conclusions: sCEA, tCEA and combined CEA have prognostic role in III~IV stages of CRC, but only combined CEA is an independent factor in III~IV stages of CRC.

Keywords: CRC; Prognosis; sCEA; tCEA ; combined CEA; ROC; AUC; OS

1. Instruction

Colorectal cancer (CRC) is one of the most common cancers worldwide that is responsible for serious damage to human health, and a reduction in the survival of affected patients. Colorectal adenocarcinoma accounts for approximately three- quarters of colorectal cancer cases[1]. Despite decades of intense research into this disease, it still proves challenging to unravel the molecular mechanisms underlying it. However, a common consensus is that CRC is a genetic disease resulting from accumulated mutations in tumor suppressor genes and oncogenes, referred to as genomic instability [2]. Tumor markers can indicate the presence of cancer, more importantly, provide information about treatment response or progression[3]. Carcinoembryonic antigen (CEA) can be elevated in CRC patients and be associated with worse prognosis with CRC[4, 5]. Carcinoembryonic

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antigen (CEA) in serum (sCEA) is widely used as a tumor marker in colorectal cancer (CRC)[6]. SCEA level was correlated with tumor stage and metastasis[7]and our previous study showed high level sCEA was associated with poor prognosis of CRC in III stage[8]. Rulan Ma,et. al[9] showed sCEA≥5ng/ml was an factor with poor prognosis of CRC. The levels of sCEA was significantly increased in the gastrointestinal tumor group compared with the healthy group[7]. Elevated preoperative s-CEA concentration, defined as > 5 ng/ml or more than two-fold higher than the normal cut-off value, is significantly associated with poorer overall and higher cancer-specific mortality in CRC patients [10]. sCEA is a clinically-established serum biomarker for CRC diagnosis [11]. Preoperatively elevated levels of sCEA were reliable predictors of postoperative high-risk recurrence in CRC and combined with TNM stage precisely identify postoperative recurrence CRC patients in stage I-III and the benefit of adjuvant chemotherapy for patients with stage II CRC[12].The expression of tissue CEA (tCEA) can be immunohistochemically assessed in colorectal mucosa and tumor tissues. t-CEA is rarely expressed in normal colorectal mucosa but is consistently found in colorectal neoplasms, with different expression patterns and intensities[10, 13, 14]. Aldilaijan AF et.al[10] found that t-CEA expression intensity and pattern correlated significantly with preoperative s-CEA level. In their study of the 7412 patients included in the present study, only 100 (1.3%) showed inverse relationships between t-CEA expression intensities and preoperative sCEA levels. Low t-CEA expression intensity in patients with high preoperative s-CEA levels may be explained by factors unrelated to malignancy, including the wide range of normal preoperative sCEA concentrations among healthy people, the effects of age and benign conditions, the high variability of liver metabolic rates, and the long half-life of glycoproteins. Our previous literature showed higher level of tCEA was associated with worse prognosis of CRC in I~III stage[8].But the prognosis of tCEA for CRC was rarely reported and Polivka J,et al[15] suggested the best prognostic value could be reached by a combination of circulating cell-free tumor DNA (ctDNA) and tumor marker CEA. The combination of CEA, carbohydrate antigen 19-9(CA19-9) and carbohydrate antigen 24-2(CA24-2) ranked the best sensitivity and specificity for colorectal cancer diagnosis[16]. Preoperative serum CA724 might serve as a potential prognostic factor for CRC patients with normal serum CEA levels[17]. But Kemper M et.al [18]pointed out only CEA was an independent prognostic factor for survival by multivariate Cox regression analysis. One other literature showed serum carbohydrate antigen 19–9 (CA19-9) with recurrence free survival (RFS) and overall survival (OS) were evaluated in patients with or without elevated sCEA[19]. So by far the value of only sCEA and tCEA have controversial issues for the prognosis of CRC. This is the aim of this study in which we assessed the prognostic values for CRC combining sCEA and tCEA. In this study we used the the factor of combined CEA to explore the prognostic values of CRC according to the different level of sCEA [normal(<10ng/ml),high(≥10ng/ml)] and different expression level of tCEA(+,++,+++) by the classification of our previous literature[8]. Therefor combined CEA has six grades (2x3). Before the study, we assessed the value of combined CEA using ROC (Receiver Operating Characteristic Curve) which showed that sCEA, tCEA and combined CEA have significant difference by 5 year overall survival (OS) with death as input variate (all $P<0.05$) in which AUC (Area Under Curve) of combined CEA was maximum, indicating that combined CEA as a variate has value and necessity compared with only sCEA and tCEA analysis for the prognostic role of CRC .

2. Materials and Methods

2.1. Patients

A total of 2,540 CRC patients were collected in the Colorectal Surgery Department of Huzhou Central Hospital, China from January 2006 to January 2016. 783 cases were deleted for kinds of reasons such as no surgery, clinicopathological data missing, follow up data missing and dying from no primary tumors. At last, 1757 cases were involved in this study. The Routine of collecting cases as our previous literature as using the same data[20]. The inclusion criteria were as follows: patients diagnosed with CRC through colonoscopy, computed tomography (CT), and pathological tests inside or outside our hospital; no preoperative adjuvant treatment; surgery in our department; normal

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lymph node dissection indicating that ≥12 lymph nodes were detected, although a small number of samples were included in this article, and only 10–11 lymph nodes were detected; CRC-related death as a termination event; postoperative routine immunohistochemical (IHC) analysis and pathological examination for tCEA and postoperative chemotherapy determined by the National Comprehensive Cancer Network (NCCN, version 2006) guidelines. Edition of the American Joint Committee on Cancer (AJCC-8) guidelines was used to determine the TNM stage after surgery. The exclusion criteria were as follows: CRC patients with serious heart, brain, liver, and lung diseases that did not tolerate surgery; non-CRC factors leading to patient death; and follow-up data missing and/or clinicopathological data missing. Patients undergoing preoperative neoadjuvant chemotherapy and radiotherapy were also excluded. According to previous literature,0 stage was combined in to I stage using 0&Istage[21] in this study.

2.2. Follow Up

Patients were followed up once every 3 months in the first year after primary CRC surgery then every 6 months at second year, and 12 months at the remaining three years, till total 5 years. All follow up data came from our document acquired by phone and inpatient electronic medical record system (Haitai Software Version 3.0, Nanjing). Survival time was the month from primary surgery date to death date or the end of follow up time which was equal or more than 5 years. If survival time was more than 60 months it was defined as 60 months. Death from primary tumor or tumor-related disease was defined as positive event, others as censoring. So in this study only overall survival (OS) were analyzed.

2.3. Detection of Preoperative Serum CEA

For each of the involved patients, venous blood was drawn before surgery which was utilized by Shanghai Yu-ping biotechnology company kit (Shanghai, China), using double antibody one-step enzymelinked immunosorbent assay (ELISA). Experimenters added the sample, standard, and horseradish peroxidase (HRP)-labeled detection antibody, in that order, to microwells precoated with the CEA capture antibody. After an incubation period, the wells were washed. The absorbance (OD value) was measured using a microplate reader at a wavelength of 450 nm to calculate the sample concentration (the normal reference value is 0-10 ng/mL). An s-CEA level of >10 ng/mL is considered high, and ≤10 ng/mL is considered normal.

2.4. T-CEA Immunohistochemistry

Immunohistochemical t-CEA detection is used as a method to pathologically examine CRC in our hospital. Formalin fixed and paraffin-embedded tumor specimens were cut into 5-mm-thick slices, that were then subjected to methyl dewaxing and hydration. The two-step EnVision immunohistochemistry system was used: the original anti-CEA antibody (clone No. COL-1, zm-0061; Golden Bridge Company, Beijing, China) was used in a 1:50 dilution, incubated at 4°C; two anti pv8000; finally, an examination under a microscope was performed to determine the percentage of cells positively stained for CEA. All slides were independently analyzed by two regularly trained pathologists; the third pathologist was asked to confirm the assessment in case of disagreement. All slides were observed under 200× magnifications to determine the cell density (+,++, and +++) and the corresponding proportion (≤25%,> 25&≤50% and >50%) of stained cells in different regions. From the t-CEA images shown in Figure 1 (A,B,C), the 200× magnification image was used for good clarity(Figure 1 ,Left), 800× magnification image for better clarity by Photoshop (Version 2020, Figure 1, Right).

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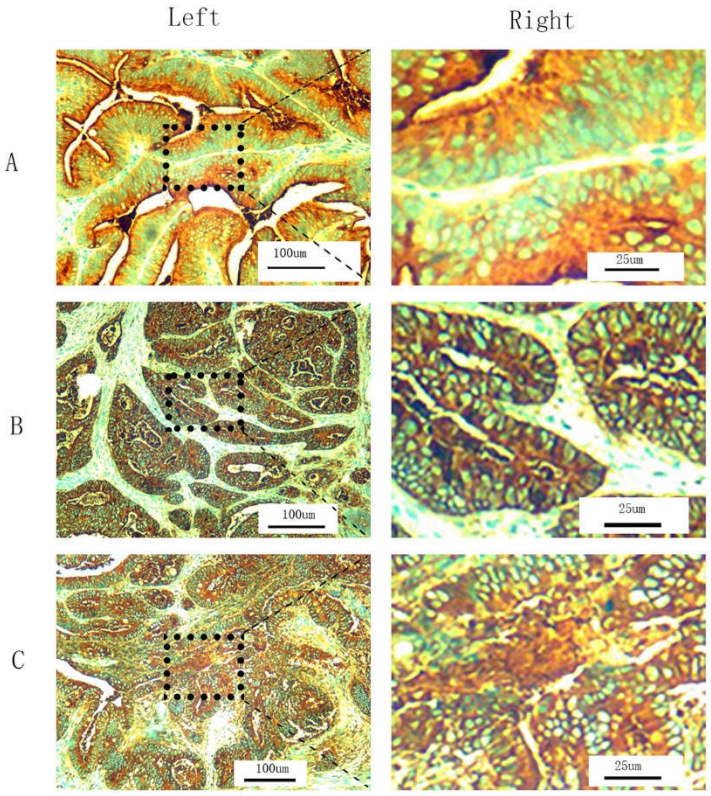

Figure 1. T-CEA immunohistochemistry. **(A)** Staining level is +; **(B)** Staining level is ++;**(C)** Staining level is +++. Left: x200 magnification under microscope(original images); Right: x800 magnification by Photoshop using the especial regions of original images.

2.5. Combined CEA Classification

According to previous sCEA and tCEA classification which have 2 grades and 3 grades especially , combined CEA was classified into 6 grades which were sCEA normal& tCEA+, sCEA normal& tCEA++, sCEA normal& tCEA+++, sCEA high& tCEA+, sCEA high& tCEA++, sCEA high& tCEA+++. So all data were divided into six groups for analysis by combined CEA classification.

2.6. Receiver Operating Characteristic Curve (ROC) Analysis

Receiver Operating Characteristic Curve (ROC) analysis was used for sCEA and tCEA and Combined CEA using death event of 5 year OS as input parameter to determine whether Combined CEA classification has necessity and priority to perform this study. AUC (Area Under Curve) analysis confirmed the value of the study.

2.7. Statistical Analysis

All clinicopathological features were analyzed by SPSS 27. ANOVA and Crosstab methods were used to analyze continuous variates and counting variates especially. Comparisons between Combined CEA groups were performed by F and X^2 test. Kaplan-Meier and Log rank test were used to perform survival analysis between sCEA and tCEA and combined CEA groups. Then Cox regression analysis were used for univariate and multivariate. 5 year OS survival curves with numbers at risk were drawn by R software (version 4.3.1) using “ggplot2”, “survival”,”survminer” packages.

3. Results

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3.1. Clinicopathological Features by Combined CEA

The percentages of combined CEA are 21.2(372/1757) for sCEA normal&tCEA+, 19.5%(343/1757) for sCEA normal&tCEA++,7.7%(136/1757) for sCEA normal&tCEA+++,13.1%(230/1757) for sCEA high&tCEA+, 24.9%(438/1757) for sCEA high&tCEA++, and 13.5%(238/1757) for sCEA high&tCEA+++. Gender has difference between combined CEA, F=12.22, P=0.032; There is significant difference about age between the groups, x2=5.37, P<0.001; significant differences between combined CEA groups, F=202.11, P<0.001, F=452.82, P<0.001, F=22.25, P<0.001, F-22.25, P<0.001, F= 160.92, P<0.001, F=58.60, P<0.001. While continuous parameters shown as median and interquartile range (IQR) have significant differences between the groups, such as age (x^2=5.37,P<0.001); tumor size (cm)(x^2=18.60,P<0.001); blood loss (ml)(x^2=3.51,P=0.004); harvested lymph nodes (no.) (x^2=6.83,P<0.001);metastatic positive lymph nodes (no.) (x^2=22.17,P<0.001). Counting data were shown as numbers and total percentage and continuous data were shown as median and IQR, details are shown in Table 1.

Table 1. Clinicopathological features by Combined CEA(n, %; median, IQR).

Variables	sCEA:normal & tCEA+	sCEA:normal & tCEA++	sCEA:normal & tCEA+++	sCEA:high & tCEA+	sCEA:high & tCEA++	sCEA:high & tCEA+++	F or x^2 test	P
Gender							12.22	0.032*
Male	186(10.6)	165(9.4)	74(4.2)	111(6.3)	209(11.9)	143(8.1)		
Femail	186(10.6)	178(10.1)	62(3.5)	119(6.8)	229(13.0)	95(5.4)		
Age(year)	67(16)	65(15)	67(21.75)	65(19.5)	67(21)	67(15)	5.37	<0.001**
Location							202.11	<0.001**
Ileocecum	41(2.3)	33(1.9)	18(1.0)	11(0.6)	7(0.4)	37(2.1)		
Right colon	30(1.7)	76(4.3)	8(0.5)	12(0.7)	38(2.2)	8(0.5)		
Transverse colon	66(3.8)	39(2.2)	21(1.2)	42(2.4)	86(4.9)	23(1.3)		
Left colon	84(4.8)	32(1.8)	36(2.0)	38(2.2)	87(5.0)	47(2.7)		
Sigmoid colon	35(2.0)	45(2.6)	12(0.7)	31(1.8)	18(1.0)	26(1.5)		
Rectum	116(6.6)	118(6.7)	41(2.3)	96(5.5)	202(11.5)	97(5.5)		
Tumor size(cm)	3.7(1.1)	4.1(1)	3.6(1)	3.5(1.2)	3.5(0.9)	4.1(1.33)	18.60	<0.001**
Blood loss(ml)	180(110)	160(150)	180(115)	180(160)	180(110)	160(52.5)	3.51	0.004**

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T stage							452.82	<0.001a***
Tis	9(0.5)	3(0.2)	3(0.2)	1(0.1)	0(0)	0(0)		
T1	23(1.3)	37(2.1)	5(0.3)	21(1.2)	20(1.1)	7(0.4)		
T2	106(6.0)	26(1.5)	30(1.7)	24(1.4)	91(5.2)	37(2.1)		
T3	53(3.0)	157(8.9)	73(4.2)	117(6.7)	177(10.1)	53(3.0)		
T4a	23(1.3)	29(1.7)	24(1.4)	66(3.8)	92(5.2)	45(2.6)		
T4b	158(9.0)	91(5.2)	1(0.1)	1(0.1)	58(3.3)	96(5.5)		
Differentiation							22.25	<0.001***
well	26(1.5)	66(3.8)	19(1.1)	31(1.8)	59(3.4)	24(1.4)		
moderate	234(13.3)	251(14.3)	75(4.3)	136(7.7)	350(19.9)	90(5.1)		
poor or undifferentiation	112(6.4)	26(1.5)	42(2.4)	63(3.6)	29(1.7)	124(7.1)		
Harvested Lymph node(no.)	14(3)	13(3)	14(3)	14(2)	14(2)	14(3)	6.83	<0.001***
Positive Lymph node(no.)	2(2)	2(6)	0(2)	2(6)	3(5)	2(5)	22.17	<0.001***
Chemotherapy							22.25	<0.001***
Yes	319(18.2)	295(16.8)	117(6.7)	200(11.4)	402(22.9)	227(12.9)		
No	53(3.0)	48(2.7)	19(1.1)	30(1.7)	36(2.0)	11(0.6)		
TNM stage							160.92	<0.001a***
0& Ⅰ	42(2.4)	30(1.7)	12(0.7)	26(1.5)	28(1.6)	9(0.5)		
Ⅱ	26(1.5)	49(2.8)	42(2.4)	28(1.6)	47(2.7)	33(1.9)		
Ⅲ	170(9.7)	133(7.6)	82(4.7)	136(7.7)	209(11.9)	145(8.3)		
Ⅳ	134(7.6)	131(7.5)	0(0)	40(2.3)	154(8.8)	51(2.9)		
Complication							58.60	<0.001***
No	357(20.3)	284(16.2)	121(6.9)	214(12.2)	421(12.2)	215(12.2)		
Yes	15(0.9)	59(3.4)	15(0.9)	16(0.9)	17(1.0)	23(1.3)		

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a means respected values <5 and using exact test; no. means numbers; IQR: interquartile range; * indicates P<0.05;**indicates P<0.01;*** indicates P<0.001.

3.2. Receiver Operating Characteristic Curve (ROC) Analysis

Receiver Operating Characteristic Curve (ROC) analysis was used for sCEA and tCEA and combined CEA using death event of 5 year OS as input parameter. AUC (Area Under Curve) analysis confirmed the value of the study. AUC, 95% confidence interval (CI) and P values of sCEA and tCEA and combined CEA are as follows: ACU=0.539, CI=0.512~0.566,P=0.004; AUC=0.558,CI=0.531~0.584,P<0.001; AUC=0.566,CI=0.540~0.593,P<0.001. The outcomes show AUC of combined CEA is max in the three variates, indicating that combined CEA as an factor to study is valuable in this study (Figure 2A). In this analysis binary variate is death in 5 year OS and other variate is censoring. Mean survival time of 5 year OS in this study is 43.093 （months）, SE =0.483 and 95%CI=42.146~44.040 (Figure 2B).

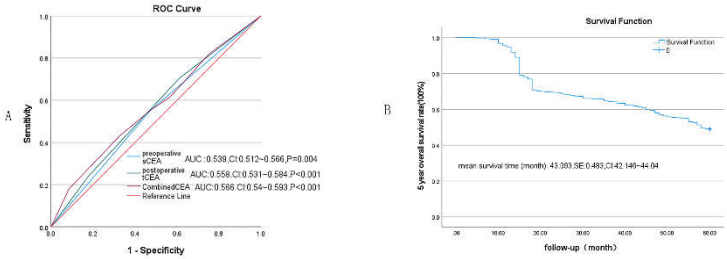

Figure 2. Receiver Operating Characteristic Curve (ROC) and 5 year overall survival curve(OS). ROC analysis: sCEA and tCEA and combined CEA are as follows: ACU=0.539, CI=0.512~0.566,P=0.004; AUC=0.558,CI=0.531~0.584,P<0.001; AUC=0.566,CI=0.540~0.593,P<0.001. **(B)** 5 year OS: Mean survival time of 5 year OS in this study is 43.093 （months）, SE =0.483 and 95%CI=42.146~44.040.

3.3. 5 Year OS Analysis by sCEA and tCEA and Combined CEA for 0&I~IV CRC of AJCC-8

As Zhang G, et. al.'s study[21], in this study, we combined 0 stage (less data and data losing in some combined CEA) to I stage and used 0&Istage.5 year OS and numbers at risk were carried out in any stage of AJCC-8. In 0&Istage , there are no significances in sCEA ,tCEA and combined CEA groups (P=0.13,0.50,0.54 respectively, Figure 3); In II stage there are no significances in sCEA ,tCEA and combined CEA groups (P=0.29,0.36,0.15 respectively, Figure 4); In III stage there are all significant difference in the three classification methods.(all P<0.001, Figure 5); In IV stage sCEA and combined CEA have significant difference (all P<0.001),but tCEA has no significant difference (P=0.24). In this stage, the group of sCEA normal&tCEA+++ is missing in Combined CEA (Figure 6).

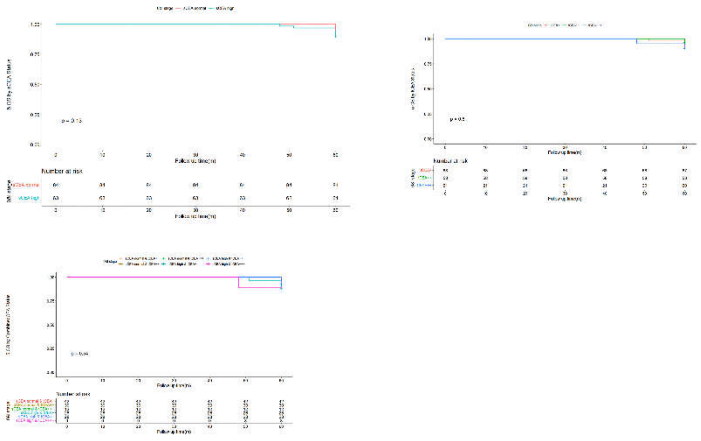

Figure 3. Comparisons for subgroups of sCEA , tCEA and combined CEA in 0&Istage.**(A)** comparison for subgroups of sCEA (P=0.13);**(B)** comparison for subgroups of tCEA (P=0.5);**(C)** comparison for subgroups of combined CEA (P=0.54).

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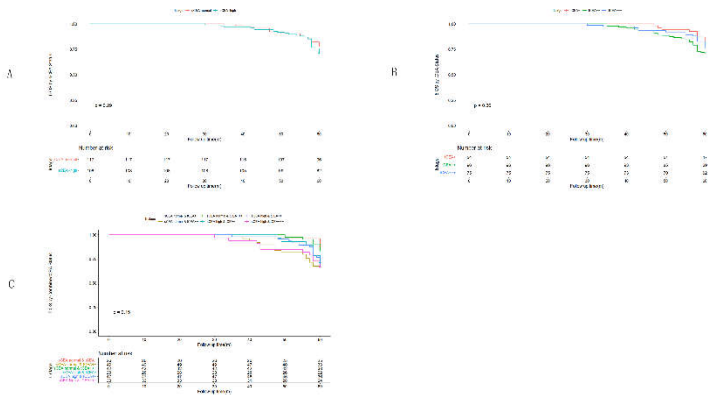

Figure 4. Comparisons for subgroups of sCEA , tCEA and combined CEA in IIstage.**(A)** comparison for subgroups of sCEA (P=0.29);**(B)** comparison for subgroups of tCEA (P=0.36);**(C)** comparison for subgroups of combined CEA (P=0.15).

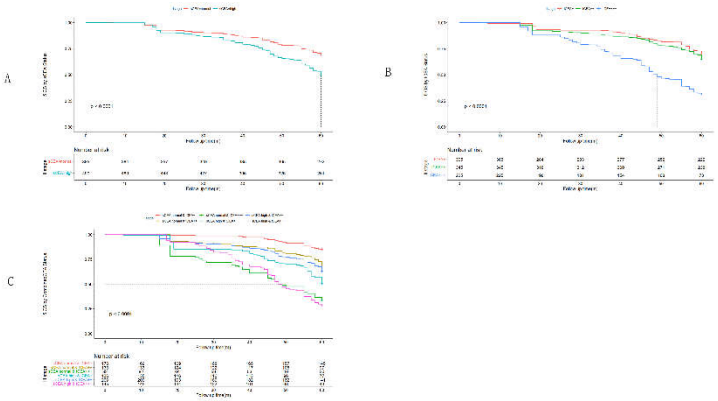

Figure 5. Comparisons for subgroups of sCEA , tCEA and combined CEA in III stage.**(A)** comparison for subgroups of sCEA (P<0.001);**(B)** comparison for subgroups of tCEA (P<0.001);**(C)** comparison for subgroups of combined CEA (P<0.001).

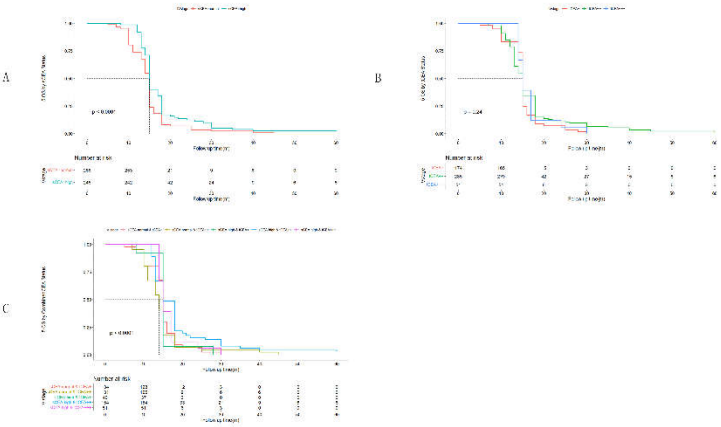

Figure 6. Comparisons for subgroups of sCEA , tCEA and combined CEA in IV stage.**(A)** comparison for subgroups of sCEA (P<0.001);**(B)** comparison for subgroups of tCEA (P=0.24);**(C)** comparison for subgroups of combined CEA (P<0.001),in subgroup of sCEA normal& tCEA+++ has no survival data.

3.4. Univariate Analysis by Cox Regression for Clinicopathological Features

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In univariate analysis, there is no significant difference about age (P=0.926) and sCEA and complication have no significant difference (P=0.55, 0.85), indicating that age ,sCEA and complication are not prognostic factors for CRC in this study. While there are significant differences about tumor location, T stage ,differentiation, chemotherapy, TNM stages, tCEA and combined CEA (tCEA a, P=0.002, all others P<0.001). Numbers, hazard ratio (HR), mean survival time and 95%CI, 5 year OS (%) and P value are all shown in Table 2.

Table 2. Univariate analysis of prognosis for colorectal cancer.

Factor	N	Hazard Ratio(HR)	Mean and 95%CI for survival time(60months)	5-year OS(%)	P value
Gender					0.296
M	888	Ref.	42.78(41.45~44.09)	47.5	
F	869	1.072	43.42(42.06~44.78)	50.5	
Location					<0.001***
Ileocecum	147	Ref.	36.93(33.42~40.49)	38.1	
Right colon	172	1.488	35.63(32.42~38.85)	37.8	
Transverse colon	277	1.521	47.40(45.24~49.56)	57.0	
Left colon	324	0.777	43.28(41.13~45.44)	48.5	
Sigmoid colon	167	1.019	45.99(42.95~49.03)	57.5	
Rectum	670	0.815	43.77(42.27~45.27)	49.1	
T stage					<0.001***
Tis	16	Ref.	60(60~60)	93.8	
T1	113	0.064	57.97(56.93~59.02)	81.4	
T2	314	0.197	42.29(40.04~44.53)	46.8	
T3	630	0.759	40.37(38.75~41.99)	39.8	
T4a	279	0.911	52.96(51.37~54.54)	69.9	
T4b	405	0.345	36.34(34.26~38.42)	39.8	
Differentiation					<0.001***
well	225	Ref.	52.88(50.77~64.98)	76.9	
moderate	1136	0.159	45.27(44.11~46.43)	56.2	
poor or undifferentiation	396	0.355	31.30(29.53~33.08)	12.6	
Chemotherapy					<0.001***
Yes	1560	Ref.	41.09(40.07~42.11)	44.0	
No	197	6.78	58.98(58.46~59.50)	88.3	
TNM stage					<0.001***
0& Ⅰ	147	Ref.	59.86(59.61~60.00)		
Ⅱ	225	0.003	58.08(57.40~58.76)		
Ⅲ	875	0.027	51.98(51.08~52.88)		
Ⅳ	510	0.060	16.40(15.76~17.04)		
Complication					0.85
No	1612	Ref.	42.95(41.96~43.94)	49	
Yes	145	1.023	44.70(41.60~47.79)	49	

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sCEA					0.55
normal	851	Ref.	42.91(41.49~44.33)	53.0	
high	906	0.879	43.27(42.00~44.53)	45.3	
tCEA					0.002**
+	601	Ref.	44.30(42.65~45.94)		
++	784	0.762	41.27(39.8~42.75)		
+++	372	0.985	44.99（43.22~46.76）		
CombinedCEA					<0.001***
sCEA:normal & tCEA+	372	Ref.	42.92(40.73~45.10)	57.0	
sCEA:normal & tCEA++	343	0.413	40.06(37.74~42.37)	46.6	
sCEA:normal & tCEA+++	136	0.833	50.07（47.43~52.72）	58.1	
sCEA:high & tCEA+	230	0.509	46.57（44.14~48.99）	53.9	
sCEA: high & tCEA++	438	0.599	42.30（40.40~44.21）	48.2	
sCEA:high & tCEA+++	238	0.742	41.85（39.60~44.11）	31.5	

Classified clinicopathological data were analyzed for univariate regression. Using Kaplan-Meier to analyze Number, mean ,95%CI survival time, and Cox regression（input） to analyze Harzard Ratio (HR), P value. *P<0.05,**P<0.01,***P<0.001.

3.5. Multivariate Analysis by Cox Regression for Clinicopathological Features

Seven parameters which have significant difference in univariate analysis were analyzed by multivariate analysis further. The outcomes showed that chemotherapy and tCEA have no significant difference (P=0.433,0.096),while the parameters of tumor location, T stage, differentiation, TNM stage, combined CEA have significant differences (all P<0.001). So this study documents that only combined CEA is independent prognostic factor for CRC while sCEA and tCEA are not. The details of analysis are shown in Table 3 which include comparison, ward and P value.

Table 3. Multivariate analysis of prognosis for colorectal cancer.

Factor	HR	95%CI for HR	Ward	P
Location			29.05	<0.001***
Ileocecum	Ref.			
Right colon	0.850	0.623~1.159		
Transverse colon	0.577	0.427~0.779		
Left colon	0.722	0.547~0.954		
Sigmoid colon	0.480	0.341~0.675		
Rectum	0.821	0.636~1.061		
T stage			95.93	<0.001***
Tis	Ref.			
T1	0.174	0.015~1.986		

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T2	0.280	0.025~3.118		
T3	0.565	0.050~6.399		
T4a	0.306	0.027~4.472		
T4b	0.220	0.020~2.470		
Differentiation			190.18	<0.001***
well	Ref.			
moderate	2.142	1.572~2.917		
poor or undifferentiation	6.794	4.806~9.605		
Chemotherapy			0.61	0.433
Yes	Ref.			
No	0.779	0.417~1.454		
TNM stage			954.18	<0.001***
0& Ⅰ	Ref.			
Ⅱ	4.789	1.062~21.604		
Ⅲ	9.632	2.194~42.292		
Ⅳ	267.44	60.944~1173.58		
tCEA			4.68	0.096
+	Ref.			
++	4.831	0.664~35.174		
+++	1.635	0.142~18.789		
CombinedCEA			32.67	<0.001***
sCEA:normal & tCEA+	Ref.			
sCEA:normal & tCEA++	0.475	0.065~3.488		
sCEA:normal & tCEA+++	1.176	0.100~13.824		
sCEA:high & tCEA+	1.242	0.921~1.674		
sCEA: high & tCEA++	0.266	0.036~1.958		
sCEA:high & tCEA+++	1.275	0.111~14.606		

Factors which have significance in univariate analysis were analyzed by multivariate regression analysis. Cox regression was used for multivariate analysis. *P<0.05,**P<0.01,***P<0.001.

4. Discussion

sCEA is widely used before and after CRC operation[22]. CEA is a glycoprotein found by Gold and Freedman in colon cancer tissues, which was then applied as a CRC tumor marker[23]. sCEA expression was correlated with the CRC prognosis and was mainly used for disease follow-up and as a treatment response indicator[24]. Most patients (64.7%, 101/156) had increased sCEA levels in the serum[22].In the study, sCEA high level is only 51.6% (906/1757).The percentage of tCEA of

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(+,++,+++) is 34.2% (601/1757),44.6%（784/1757）and 21.2%(372/1757) respectively in this study. In CRC, CEA expressed following the disruption of normal tissue structure and the loss of polarization of neoplastic cells is secreted into the blood stream, eventually resulting in an increase in sCEA concentration[25].TCEA expression patterns have been described as apicoluminal (AL), diffuse-cytoplasmic (DC), or a combination of the two. The DC pattern and high levels of expression have been associated with tumor aggressiveness, including Lymphovascular invasion (LVI)[10, 26].Because s-CEA level is neither sufficiently sensitive nor specific as a screening tool for CRC[27],We performed this study. One recent study is similar to ours [10].We combined sCEA and tCEA to combined CEA as one new factor for further analysis.

In this paper, the percentages of sCEA normal&tCEA++, sCEA high&tCEA++ are higher than other groups in combined CEA classification as one new factor. Gender , tumor location, T stage, differentiation, chemotherapy, TNM stage, complication, age, tumor size, blood loss, harvested lymph nodes, metastatic positive lymph nodes are associated with the new factor indicating that there is value using the factor to analyze clinicopathological features. Although many previous studies have reported a lack of correlation between preoperative s-CEA levels and t-CEA expression[8, 28, 29], one literature confirmed the relationship[30].For these reasons , we used combined CEA as one new factor.

High preoperative s-CEA level is prognostic of poor survival in patients with CRC[31, 32]. High-intensity t-CEA expression was significantly associated with with higher tumor recurrence rates[33]. To analyze the 5 year OS furtherly, we performed ROC analysis for sCEA and tCEA and combined CEA. The outcome showed the AUC of combined CEA was bigger than only sCEA and tCEA , indicating that combined CEA as one new factor to analyze 5 year OS is valuable too. In this paper there are no significant differences of 5 year OS in 0&I stage and II stage for sCEA and tCEA which is not similar to our previous study[8]. The difference may be caused by recruited more patients in this study. There is also no difference of 5 year OS in 0&I stage and II stage for combined CEA. But in advanced CRC, such as III stage and IV stage there are significant difference for the three factors except for tCEA in IV stage. The reason is unclear. But this can be considered that assessing the prognosis only using tCEA might have defects. The ability of tCEA expression intensity to predict recurrence was especially noticeable among patients with low preoperative sCEA levels, with patients having high-intensity t-CEA expression showing significantly higher rates of recurrence regardless of low preoperative s-CEA level[10]. When divided into four subgroups based on both preoperative s-CEA level and t-CEA expression intensity, DFS was worse in groups with high-intensity t-CEA expression regardless of preoperative s-CEA levels. Taken together, these findings suggest that t-CEA expression intensity plays a complementary role as an adjunctive measurement of preoperative s-CEA level[10]. In this paper ,tCEA normal &CEA+ has a better 5 year OS and ,tCEA high &CEA+++ has a worse 5 year OS in III stage and IV stage compared with other groups in combined CEA.(Figure 5, Figure 6)

Our previous study showed sCEA is not independent factor while tCEA is for CRC ofI~III stage by multivariate analysis [8].Because the role of only preoperative serum for CRC has controversial issues, combined preoperative CEA and other tumor biomarkers are suggested[17, 19, 34-40]. In this study of more recruited patients in 0&I~IV, sCEA and tCEA are prognostic factors but not independent factors in CRC of III~IV stages while combined CEA is an independent factor in advanced CRC by univariate and multivariate analysis.

While our paper has some limitations such as no genetic analysis, data is old, the kits testing preoperative sCEA (ref. 0~10ng/ml) and tCEA were not the newest. And determination of t-CEA expression patterns is subjective, as these evaluations are related to the depth of CEA distribution. So now parts of pathologists in our hospital only describe whether tCEA is expressed in the process of tCEA immunohistochemistry.

5. Conclusion

SCEA, tCEA and combined CEA have prognostic role in III~IV stages of CRC, but only combined CEA is an independent factor in III~IV stages of CRC while they have no prognostic role in

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0&I~IIstages .Combined CEA can be considered as one new factor to assess the prognosis for CRC. In future we can combined postoperative sCEA ,recurrence CEA (rCEA),facal CEA , Carbohydrate antigen199(CA199), Carbohydrate antigen724 (CA724) and other tumor biomarkers with tCEA or preoperative sCEA to explore the prognostic role of CRC to avoid the defects of single testing above biomarkers.

Declarations

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Ethics statement: The current study was carried out according to the ethical guidelines of the 2013 Declaration of Helsinki and was approved by the ethics committee of Huzhou Central Hospital (No.*********). Written informed consent was obtained from every patient to use their tissue samples and medical records for research purposes.

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