



# Evaluation of serum midkine and carcinoembryonic antigen as diagnostic biomarkers for rectal cancer and synchronous metastasis

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**Background:** Carcinoembryonic antigen (CEA) is a conventional biomarker in detecting rectal cancer, but the serum midkine (S-MK), identified to be a promising cancer biomarker due to its multifunctionality, in rectal cancer detection remains unknown. This study aimed to investigate the diagnostic values of S-MK and CEA in specific rectal cancer and synchronous metastasis.

**Methods:** S-MK and CEA, related to cancer status, were measured in 106 patients and 30 controls by ELISAs. Diagnostic and Prognostic values of S-MK and CEA for rectal cancer were conducted by receiver operating characteristic (ROC) curves with the analysis of sensitivity, specificity, diagnostic accuracy, positive predictive value (PPV) and negative predictive value (NPV) for differential diagnosis.

**Results:** Pre-surgical S-MK was elevated in cancer patients than the normal and benign subjects. In term of phases, S-MK and CEA of T3/T4 were both significantly higher than those in T1/T2, with further up-regulation of S-MK. The diagnostic capability of S-MK was higher than CEA and increased in integrating S-MK with CEA when differentiated rectal cancer, benign rectal polyps and normal subjects. Moreover, when evaluating metastasis predictive efficacies, the combination of S-MK and CEA showed higher accuracy than any individual parameter.

**Conclusions:** Pre-surgical S-MK is a rectal cancer indicator which is superior to CEA. It can potentially be used to monitor the prognosis of rectal cancer, and predict synchronous metastases, providing assistance to clinicians.

**Keywords:** Serum midkine (S-MK); carcinoembryonic antigen (CEA); rectal cancer; synchronous metastases

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## Introduction

Rectal cancer concerned about cancer of the large intestine and rectum is one of the most common malignancies that affect the digestive system. As with most other neoplasms, the incidence rate for rectal cancer rises dramatically with age. In the United States, an estimated 135,430 cases of rectal cancer are diagnosed in 2017, resulting in approximately 50,260 deaths (1). The key to decreasing

rectal cancer mortality is early detection, diagnosis and treatment. However, most rectal cancer patients miss the optimal treatment strategy and have poor prognosis owing to no early warning signs and symptoms. At present, the early diagnosis and prognosis of rectal cancer mainly depend on colonoscopy, imaging and other examinations with relatively high expenses. Patients exhibit very low levels of adherence on subsequent return to the hospital.

In the diagnosis and therapy of rectal cancer, molecular tumor markers have been playing an increasingly vital role. However, conventional biomarkers like carcinoembryonic antigen (CEA) are unable to satisfy the demand of patients in clinical practice. For over 40 years, American Society of Clinical Oncologists has recommended performing routine measurement of CEA to monitor local recurrence or metastatic relapse in rectal cancer patients, however, CEA increase only occurs for 38.7% of rectal cancer (2). In addition to rising in most rectal cancer, pancreatic cancer, stomach cancer, lung cancer, and breast cancer, the CEA is also seemed to be slightly elevated in some benign diseases. Therefore, it is of great significance to identify effective serum biomarkers with both satisfactory sensitivity and specificity to detect rectal cancer before surgery and to predict the prognosis.

Serum midkine (S-MK), firstly reported as the product of a retinoic acid-responsive gene, is a heparin-binding growth factor (3). It specifically advances in neuronal survival and differentiation and also has a critical role in cell growth, survival, migration, angiogenesis, and carcinogenesis (4). The expression of S-MK is up-regulated in neuroblastoma, gastrointestinal cancers, bladder cancer (5-10). However, little is known about S-MK levels in the multistep process of oncogenesis and development of rectal cancer. In 2013, Krzystek-Korpaczka *et al.* (11) published a paper describing that S-MK was higher in rectal cancer than in inflammatory bowel disease (IBD), adenoma or controls. Nonetheless, the diagnostic and prognostic values of serum S-MK have not yet been comprehensively elucidated in patients with rectal cancer.

Therefore, the footing of this study was to determine the diagnostic significance and the prediction ability for synchronous metastasis of serum S-MK for rectal cancer before the radical resection. To achieve this, the study among S-MK, CEA and combination of S-MK and CEA were conducted respectively. Sensitivity, specificity, and diagnostic accuracy, positive predictive value (PPV) and negative predictive value (NPV) were evaluated by statistically analyzing and compiling the measured parameters for differential diagnosis.

## Methods

### Patients

A group of 106 subjects were investigated in this study. There were 76 individuals with rectal cancer, 30 subjects

with benign rectal polyps. Enrolled patients were treated between October 2015 and October 2017 at the surgical department of Tianjin Union Medical Center (China). Blood samples were collected at the admission or a close time-point preceding treatments.

Sera from blood donors were considered healthy according to routine blood tests. The control group enrolled 30 individuals from the cohorts in the health management department of Tianjin Union Medical Center.

Patient recruitment and sample collection were performed within the guidelines of protocols approved by the institutional review boards informed consent was obtained from all subjects. The ID/number of ethical approval is 2019C03 approved by Tianjin Union Medical Center.

### Serum parameter evaluation

CEA (reference 0–5.00 ng/mL), carbohydrate antigen 19-9 (CA19-9) (reference 0–37.00 U/mL) and carbohydrate antigen 72-4 (CA72-4) (reference 0–69.00 U/mL) assays were performed on a fully automated ADVIA Centaur analyzer (Siemens Healthcare Diagnostics, New York, USA). All these assays were conducted with respect to the chemiluminescent reaction principle.

### Serum S-MK measurement

Blood samples (5 mL) were obtained by venipuncture and immediately centrifuged at 3,000 ×g for 10 min. The serum was frozen at –80 °C until the assay was performed. We avoided repeating thawing and freezing of samples. Enzyme-linked immunosorbent assay (ELISA) (DuoSet ELISA, R&D Systems Inc., USA) was performed to measure sample S-MK level. The assay was conducted according to the manufacturer's instructions and values were reported as pg/mL. Briefly, the microplate was pre-coated with goat anti-human S-MK antibody (R&D Systems UK) and then blocked by 1% bovine serum albumin (BSA, St. Louis, USA). One hundred µL of each serum sample (1:10 dilution) or standard was incubated in a 96-well microplate (Corning UK) for 2 hours at room temperature. Firstly, biotinylated goat anti-human S-MK antibody was added and incubated with captured S-MK for 2 hours at room temperature, after washing three times. Conducting another three washes, we added 100 µL aliquots of streptavidin-conjugated horseradish-peroxidase (San Francisco, USA) which allowing reacting for 30 minutes

**Table 1** Data comparison in the malignant, benign and the control cohorts

Group* [case number]	Age (years old)	S-MK (pg/mL)	CEA (ng/mL)	CA19-9 (U/mL)	CA72-4 (U/mL)
1 [30]	64.63±11.14	219.3 (165.01–275.78)	2.43 (1.43–4.43)	6.1 (4.83–13.35)	2.49 (1.78–4.32)
2 [30]	66.50±11.43	261.85 (213.41–353.98)	3.5 (2.17–8.13)	3.25 (1.88–4.7)	2.34 (1.54–3.93)
3 [76]	62.88±9.95	380.95 (309.55–463.52)	13.64 (3.15–22.31)	6.25 (3.2–17.75)	2.03 (1.27–4.92)
T value <sub>(1),(2)</sub> <sup>#</sup>	t=-0.641	z=-2.087	z=-1.528	z=0.978	z=0.449
P value <sub>(1),(2)</sub> <sup>#</sup>	0.524	0.041	0.032	0.549	0.801
T value <sub>(1),(3)</sub> <sup>#</sup>	t=0.789	z=-7.33	z=-5.514	z=-2.391	z=-0.166
P value <sub>(1),(3)</sub> <sup>#</sup>	0.432	<0.001	<0.001	<0.001	0.868
T value <sub>(2),(3)</sub> <sup>#</sup>	t=1.616	z=-3.734	z=-4.586	z=-2.555	z=-0.258
P value <sub>(2),(3)</sub> <sup>#</sup>	0.109	<0.001	<0.001	0.013	0.797

\*: group 1 = normal control group, group 2 = rectal polyps group, group 3 = rectal cancer group; <sup>#</sup>, analyzed by independent samples t-test. S-MK, serum midkine; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; CA72-4, carbohydrate antigen 72-4.

in a dark place. After plate washing, substrate solutions (1:1 mixture of H<sub>2</sub>O<sub>2</sub> and tetramethylbenzidine) were added to the wells (100 µL per well) for a 20-minute reaction. Finally, 1 mol/L H<sub>2</sub>SO<sub>4</sub> (stop solution) was added (50 µL per well), and the optical densities of the wells were measured at 450 nm with a Multiskan MS plate Reader (Labsystems, Helsinki, Finland). After creating a standard curve by a four-parameter logistic curve-fit method, concentrations of the samples were determined. All specimens were tested blinded and in duplicate.

### Statistical analysis

Quantitative data with normal distribution were presented as mean ± standard deviation, and other data was characterized by median (min–max). Comparisons between paired groups were made using the paired *t*-test. Either Pearson's (normally distributed data) or Spearman's rank (skewed or categorical data) correlation test was used for correlation analysis. The influence of age was determined with the Chi-square test. All calculated P values were two-sided and P<0.05 was generally considered significant.

Receiver operating characteristic (ROC) curves were drawn to evaluate the discriminative power of a given parameter. Once areas under ROC curves (AUC) were determined, optimal cut-off values were selected, and sensitivity, specificity, diagnostic accuracy, PPV and NPV for differential diagnosis were assessed, respectively.

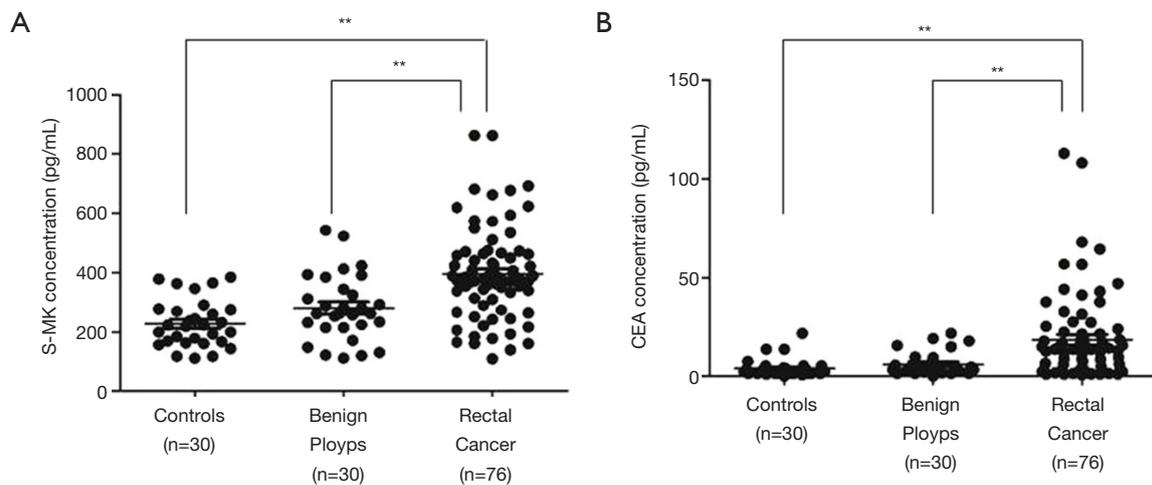
All statistical analyses were carried out using SPSS 23.0 software (SPSS Incorporated, Chicago, Illinois, USA).

### Results

#### *Both S-MK and CEA had good prognostic values to differentiate rectal cancer, benign rectal polyps and normal controls*

The pre-surgical S-MK levels and pre-surgical CEA in patients with malignant rectal cancer (380.95 pg/mL, 309.55–463.52 pg/mL) were remarkably higher than that with benign rectal polyps (261.85 pg/mL, 213.41–353.98 pg/mL) or healthy controls (219.3 pg/mL, 165.01–275.78 pg/mL) (*Table 1*, P<0.001). Similarly, the pre-surgical CEA level in the malignant rectal cancer group (13.64 ng/mL, 3.15–22.31 ng/mL) was significantly greater than that in the benign rectal polyps group (3.5 ng/mL, 2.17–8.13 ng/mL) and the control group (2.43 ng/mL, 1.43–4.43 ng/mL) (*Table 1*, P<0.001). We also measured CA19-9, showing a similar significant difference between the malignant and benign groups, malignant and normal groups but no obvious difference between benign groups and control groups. Other parameters like CA7-24 and age showed no significant differences among the groups. Scatter graphs of S-MK and CEA were plotted to demonstrate the intergroup differences as well (*Figure 1*).

Both levels of S-MK and CEA in T3/T4 phases were higher than those in T1/T2 phases, more significant in S-MK (P=0.007) than CEA (P=0.019). No marked correlation was observed between S-MK, CEA and nodal involvement, or distant metastasis (*Table 2*). Both levels of S-MK and CEA gradually increased along with the stage. S-MK was higher in stage III/IV comparing to stage I/II



**Figure 1** Scatter graphs were plotted to demonstrate the inter-group differences of pre-surgical serum midkine (A) and carcinoembryonic antigen (B) in patients as well as in control subjects. \*\*, P<0.001.

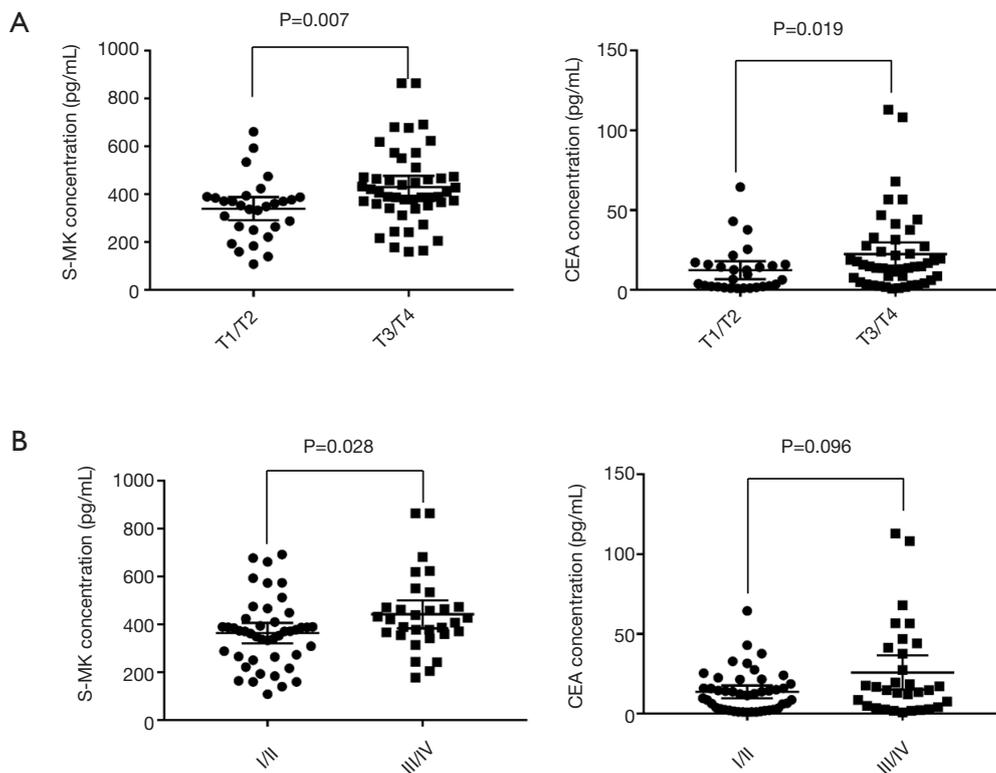
**Table 2** Relationship between serum midkine, carcinoembryonic antigen and clinicopathological variables in patients with rectal cancer

Variable	S-MK (pg/mL)	P value	CEA	P value
Tumor depth		0.007		0.019
T1/T2	298.33 (184.62–340.93)		6.71 (1.77–13.01)	
T3/T4	398.84 (305.68–453.9)		12.65 (5.39–21.83)	
N factor				
N0	298.33 (184.62–340.93)	0.323 (N0 vs. N1)	11.25 (5.29–18.79)	0.156 (N0 vs. N1)
N1	405.37 (254.63–447.21)	1.000 (N1 vs. N2)	10.16 (2.18–24.28)	0.428 (N1 vs. N2)
N2	398.03 (350.91–512.99)	0.146 (N0 vs. N2)	11.65 (2.85–21.54)	0.838 (N0 vs. N2)
M factor		0.221		0.111
M0	298.33 (184.62–340.93)		14.47 (6.27–23.24)	
M1	405.37 (236.42–727.15)		18.63 (2.85–59.39)	
Stage		0.028		0.096
I/II	298.33 (184.62–340.93)		10.32 (1.03–25.59)	
III/IV	426.55 (360.72–471.12)		17.03 (3.15–30.84)	

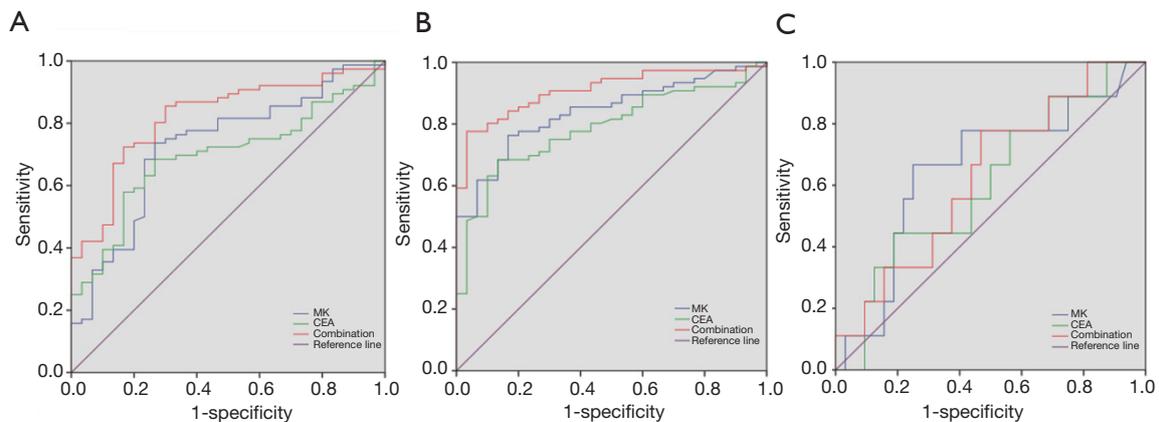
[426.55 (360.72–471.12) vs. 298.33 (184.62–340.93) pg/mL, P=0.028]. CEA was also higher in stage III/IV than in stage I/II but with no significantly difference [17.03 (3.15–30.48) vs. 10.32 (1.03–25.59) ng/mL, P>0.05 (Table 2)]. In order to investigate the relationship between S-MK, CEA and clinicopathological variables, the scatter graphs were plotted (Figure 2).

We compared the diagnostic efficacy of pre-surgical

S-MK and CEA, individually and in combination, analyzing in two separate assays. In the first analysis, differentiation between rectal cancer and rectal polyps was carried out and the ROC curve was drawn in Figure 3A. The area under the curve of S-MK was higher than that of CEA but smaller than integrating the two (Table 3). Furthermore, when the optimal cut-off values were determined (328.32 pg/mL for S-MK, 5.91 ng/mL for CEA and 449.23 for S-MK + CEA),



**Figure 2** The value serum midkine and carcinoembryonic antigen in different phases (A) and in different stages (B).



**Figure 3** Diagnostic and prognostic values of serum midkine in rectal cancer, Receiver operating characteristic curves were drawn to assess diagnostic capabilities of pre-surgical serum midkine, pre-surgical carcinoembryonic antigen and combination detection of serum midkine and carcinoembryonic antigen. (A) Rectal cancer and normal control participants; (B) Rectal cancer and benign rectal polyps; (C) A receiver operating characteristic curve was drawn to determine diagnostic capabilities of pre-surgical S-MK, pre-surgical carcinoembryonic antigen and combination detection of serum midkine and carcinoembryonic antigen to discern whether or not metastases existed in patients with rectal cancer.

**Table 3** Differentiation between rectal cancer and rectal polyps according to the receiver operating characteristic curve-related data

Variable	S-MK (pg/mL)	CEA (ng/mL)	S-MK + CEA
Area under the curve	0.727	0.694	0.821
Optimal cut-off value	328.32	5.91	449.23
Sensitivity (%)	73.68	68.42	71.05
Specificity (%)	73.33	80	83.33
Accuracy (%)	73.58	71.7	74.53
PPV (%)	87.5	89.66	91.53
NPV (%)	52.38	50	53.19

S-MK, serum midkine; CEA, carcinoembryonic antigen; PPV, positive predictive value; NPV, negative predictive value.

**Table 4** Differentiation between rectal cancer and normal control according to the receiver operating characteristic curve-related data

Variable	S-MK (pg/mL)	CEA (ng/mL)	S-MK + CEA
Area under the curve	0.839	0.787	0.91
Optimal cut-off value	299.14	5.69	420.591
Sensitivity (%)	76.32	68.42	77.63
Specificity (%)	83.33	86.67	96.67
Accuracy (%)	78.3	73.58	83.02
PPV (%)	92.06	92.86	98.33
NPV (%)	58.14	52	63.04

S-MK, serum midkine; CEA, carcinoembryonic antigen; PPV, positive predictive value; NPV, negative predictive value.

the sensitivity, specificity, diagnostic accuracy, PPV, and NPV of S-MK + CEA were all higher than corresponding values of isolated S-MK or CEA. Subsequently, a differentiation analysis between rectal cancer and normal control participants was conducted. *Table 4* demonstrated a larger area under the ROC curve of S-MK compared to the CEA under-curve area, but lower than the combination of S-MK and CEA (*Figure 3B*). Quantitative values of optimal cut-off values, the sensitivity, specificity, diagnostic accuracy, PPV, and NPV of S-MK, CEA and S-MK + CEA were also shown in *Table 4*.

#### ***Both S-MK and CEA had good predictive values for metastatic existence***

Totally 41 of the 75 rectal cancer patients with TxN0M0 were continuously followed up for 12 months to appraise the completeness of metastasis existed, once every month. Based on the 12-month follow-up information, the 41 patients were further allocated into two groups according to

whether with metastasis (lymph node, lung, liver or skeletal metastasis). As shown in *Table 5*, 9 patients had metastasis with 7 lymph node metastasis, 1 liver metastasis and 1 skeletal metastasis. Comparisons of these two groups were performed. S-MK, CEA, and CA19-9 in metastasis group were higher than those in no metastasis group but with no statistical significance ( $P>0.05$ ).

The predictive efficacies of those parameters for the synchronous metastasis were tested by the ROC curve (*Figure 3C*) and it demonstrated good predictive capabilities of S-MK + CEA, with diagnostic accuracy around 80%, higher than that of S-MK and CEA individually (*Table 6*).

#### **Discussion**

S-MK, also known as neurite growth-promoting factor 2 (NEGF2), is a heparin-binding growth factor that was initially discovered during the retinoic acid-induced differentiation of embryonic tumor cells. Since the first report in 1988 (3), several pieces of literature relating to

**Table 5** Data comparison in no metastases and metastases group

Group* [case number]	Age (years old)	S-MK (pg/mL)	CEA (ng/mL)	CA19-9 (U/mL)	CA72-4 (U/mL)
1 [32]	64±9.98	365.61 (269.91–392.09)	10.99 (2.21–15.93)	5.4 (3.12–8.5)	1.9 (1.22–3.75)
2 [9]	66 (63.5–79)	409.98 (322.84–489.11)	13.78 (5.81–25.8)	6.3 (2.9–18.1)	1.4 (1.21–3.34)
T value <sub>(1);(2)</sub> <sup>#</sup>	t=-1.411	z=-0.934	z=-0.86	z=-0.62	z=0.26
P value <sub>(1);(2)</sub> <sup>#</sup>	0.230	0.164	0.359	0.676	0.793

\*: group 1 = no metastases group, group 2 = metastases group; #, analyzed by independent samples t-test. S-MK, serum midkine; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; CA72-4, carbohydrate antigen 72-4.

**Table 6** Differentiation between no metastases and metastases group according to the receiver operating characteristic curve-related data

Variable	S-MK (pg/mL)	CEA (ng/mL)	S-MK + CEA
Area under the curve	0.655	0.604	0.628
Optimal cut-off value	389.11	17.34	516.2
Sensitivity (%)	66.67	44.44	77.78
Specificity (%)	75	81.25	77.27
Accuracy (%)	73.17	73.17	77.42
PPV (%)	42.86	40	58.33
NPV (%)	88.89	83.87	89.47

S-MK, serum midkine; CEA, carcinoembryonic antigen; PPV, positive predictive value; NPV, negative predictive value.

S-MK expression have been published. Jones *et al.* reported that the average level of S-MK was around 253 pg/mL in healthy human subjects (12). Consistently, our control healthy participants had a mean level of 255.01 pg/mL for the S-MK expression.

It is reported that S-MK has five biological functions: (I) improve angiogenesis and mitosis; (II) increase the prothrombinase activity of aortic endothelial cells; (III) promote neuronal survival and induce neurite outgrowth (13); (IV) rescues Wilms' tumor cells from apoptosis (14); and (V) exhibit chemotaxis to neutrophils (15). Specifically, the latter two may contribute to the infiltration and survival of tumor cells. In normal human tissue, the expression of S-MK is highly restricted, and low levels of S-MK can be detected in the kidneys, lungs, stomach, ovary and thyroid. However, high levels of S-MK are observed in at least 20 malignant tumors, such as breast cancer, lung cancer, esophageal cancer, and glial cell tumors. Expression conformity of S-MK in plasma is in accordance with that in tissue.

In recent years, investigators have evaluated S-MK to differentiate malignancy from benignancy in various pathological conditions. Kishida *et al.* reviewed the involvement of S-MK in neuroblastoma tumorigenesis, and

they concluded that S-MK was expressed in chemoresistant cells and was involved in anticancer drug-resistance. In contrast, neuroblastoma cells were associated with a high level of S-MK expression and that an S-MK targeted therapy could exert a vital role in these cells (16). Ren *et al.* found that S-MK was overexpressed in the cytoplasm of esophageal squamous cell carcinoma (ESCC). Noticeably, the high intensity of S-MK was found abundant in vessels and the invading border of the tumors. Moreover, it was concluded that S-MK expression was correlated with tumor cell differentiation with more intensely expressed in well-differentiated tumors (76.9%) than in moderately and poorly differentiated tumors (43.1% and 41.2%, respectively) (17). As a tumor biomarker, S-MK showed an obviously higher sensitivity compared with alpha-fetoprotein (AFP) in the investigation of hepatocellular carcinoma (HCC). In another wide-ranging study, Zhu *et al.* showed that the sensitivity of serum S-MK was much higher than that of AFP (86.9% *vs.* 51.9%) with similar specificities (83.9% *vs.* 86.3%). Even in very early stages, S-MK held a two times higher sensitivity than AFP (80% *vs.* 40%) (18). Sun *et al.* further explained that S-MK promotes HCC metastasis by elevating anoikis resistance of circulating

tumor cells. Activation of PI3K/Akt/NF- $\kappa$ B/TrkB signaling by midkine-activated anaplastic lymphoma kinase (ALK) is responsible for the anoikis resistance (19).

For rectal cancer, S-MK expression in colorectal tumors (low-grade dysplasia, adenomas with high-grade dysplasia (HGD) and invasive adenocarcinomas) was significantly higher than in the normal mucosa. Ulcerative colitis (UC)-associated lesions (regenerative mucosa of UC, UC-associated dysplasia and UC-associated adenocarcinoma) had similar variations, with higher S-MK expression than in the normal mucosa (20). Ye *et al.* had suggested that S-MK expression level was significantly elevated in carcinomas than that in normal tissues by Northern blotting. S-MK immunostaining was positive in the adenomas with moderate- and severe-grade dysplasia and in the carcinomas, but not in mild-grade dysplasia or in normal tissues (21). Krzystek-Korpacka *et al.* reported that the average multiple of S-MK expression in cancerous versus noncancerous tissue was 30.1. Higher S-MK levels express in stages III/IV *vs.* stages I/II of rectal cancer, while conversely in the colon cancer. Tissue expression of S-MK was positively correlated with its serum levels (22).

In this study, both S-MK and CEA were significantly higher in rectal cancer patients than other individuals (Table 1). Their blood levels increased significantly along with tumor depth and stage, but the S-MK level increased more significantly than CEA (Table 2). An optimal cut-off value of 328.32 pg/mL for S-MK was set to differentiate malignant from rectal polyps, which produced the sensitivity and diagnostic accuracy of 73.68% and 73.58%, respectively. These data were better than the results from CEA (Table 3). In the analysis to differentiate rectal cancer from normal participants without any rectal disease, a cut-off value of 299.14 pg/mL for S-MK was determined. The sensitivity and diagnostic accuracy determined by S-MK were higher than the parameters calculated from CEA (Table 4). The diagnostic sensitivity, specificity and accuracy of combined detection were higher than that of the single assay (Table 4). These results proved the efficacy of S-MK to be used as a promising early diagnostic biomarker for rectal cancer screening. Furthermore, the joint detection entails better pre-surgical diagnosis.

S-MK could serve as a practical marker for cancer screening, but also helpful in the evaluation of prognosis. The application value of S-MK as a prognostic biomarker was listed in a number of studies by the Kaplan-Meier curve research. According to Ota *et al.*, oral squamous cell carcinoma patients with high S-MK concentrations showed

a lower 5-year survival rate than patients in low S-MK groups. Even in early-stage tumor, the increased S-MK expression was strongly associated with poor survival (23). Maeda *et al.* first reported the association between S-MK and pancreatic head carcinoma. Patients with positive S-MK (53.3%) had a significantly lower 5-year survival rate than those with a negative one. The authors also revealed that S-MK expression was an independent prognostic factor (24). In the study by Rawnaq *et al.*, S-MK concentrations were significantly higher in the gastrointestinal stromal tumor with recurrence compared with those without. For 400 pg/mL as a threshold value, patients with S-MK levels higher than the threshold showed a significantly worse 5-year recurrence-free survival (25). According to Ikematsu *et al.*, there was a striking correlation between high plasma S-MK and poor prognosis for human neuroblastoma with a threshold value of 900 pg/mL (26). For rectal cancer, Krzystek-Korpacka *et al.* showed that higher S-MK levels express in stage N1 *vs.* stages N0 of rectal cancer, while conversely in the colon cancer (22).

The above studies indicate that S-MK and CEA in blood could be good biomarkers for the diagnosis of rectal cancer and prediction of synchronous metastasis. We found that significantly elevated expressions of S-MK and CEA in rectal cancer. Results showed good diagnostic accuracies of S-MK, CEA, S-MK + CEA for the purpose of differential diagnosis by ROC (Figure 3A,B, Tables 3,4). Furthermore, we divided the rectal cancer patients into two subgroups according to the status of metastases. ROC analyses also demonstrated good diagnostic abilities of the combination of S-MK and CEA to distinguish whether metastases existed (Figure 3C, Table 6).

There are two limitations in this study, which warrant further researches. Firstly, we recruited a relatively small number of cases and conducted a retrospective study. Therefore, a direct cause-effect link between activations of MK and CEA activities cannot clearly be shown through our analysis. Studies on the transcriptional regulation of these factors are needed to further investigate. Secondly, since rectal cancer has a relatively good survival rate, a longer time follow-up study is needed in the following studies.

## Conclusions

Pre-surgical S-MK level as the rectal cancer indicator was superior to CEA, it may play a significant role in rectal cancer carcinogenesis and progression. Though this study did not confirm the role of S-MK for the prognosis, it did

partially substantiate the correlation between high S-MK and tumor metastasis. The results also proved the potential usefulness of combined detection of S-MK and CEA for the diagnosis of rectal cancer, which might improve the diagnosis of rectal cancer.

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### Footnote

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2020.02.43>). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Patient recruitment and sample collection were performed within the guidelines of protocols approved by the institutional review boards informed consent was obtained from all subjects. The ID/number of ethical approval is 2019C03 approved by Tianjin Union Medical Center.

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