The value of serum methylated septin 9 and carcinoembryonic antigen in efficacy evaluation and follow-up monitoring of colorectal cancer

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This study explored the value of the detection of serum methylated septin 9 (mSEPT9) and carcinoembryonic antigen (CEA) in the auxiliary diagnosis, curative effect evaluation, and follow-up monitoring of colorectal cancer (CRC). The diagnosis and treatment data of 208 CRC patients in the First Affiliated Hospital of Xinjiang Medical University (China) were collected from March 2019 to December 2019, and these patients were followed up. The correlation between serum CEA, mSEPT9 levels, and tumor location and size were analyzed. Serum mSEPT9 and CEA were detected before and after surgery and during follow-up after treatment to analyze the value of mSEPT9 in efficacy evaluation and follow-up monitoring. In 87 patients with CRC patients who underwent surgery, the average size of poorly differentiated tumors was the largest (25.01±14.08 cm²), which was significantly different from that of moderately differentiated tumors (P =0.039). There was a statistically significant difference in serum CEA level among different degrees of differentiation (P=0.018). The level of CEA was relatively low when tumors occurred in the transverse and ascending colon. When the level of CEA was high, negative mSEPT9 suggested that the probability of a tumor occurring in the cecum was high; positive mSEPT9 indicated that the tumor was highly likely to occur in the descending or sigmoid colon. Detection before and after surgery revealed that the level of mSEPT9 may be related to the tumor-bearing state of patients. A Follow-up study also showed that the sensitivity and specificity of mSEPT9 for recurrence and metastasis were 83.3% and 97.7%, respectively, and the sensitivity and specificity of CEA were 61.1% and 89.5%, respectively. The combined detection of mSEPT9 and CEA can indicate the location and size of colorectal cancer, while the detection of serum mSEPT9 may have clinical significance in the efficacy evaluation and follow-up monitoring of colorectal cancer.

KEY WORDS: Colorectal Cancer, mSEPT9, Recurrence, Metastasis, CEA

Introduction

Ranking third, and mortality ranking fifth [2]. Approximately 30%-50% of CRC patients will have recurrence or metastasis after radical resection, making cancer recurrence or metastasis the primary cause of death among CRC patients. Early detection and early treatment are still the primary methods for improving the therapeutic effects of treating CRC. The issue how to enhance the monitoring of postoperative Colorectal cancer (CRC) is a malignant tumor that threatens human health. Recent epidemiological studies have shown that approximately 1.8 million new cases of CRC are detected annually. The incidence of CRC ranks third in malignant tumors in the world. Approximately 881,000 people die of CRC yearly, accounting for 9.2% of all tumor deaths [1] and making CRC a serious threat to human health [1-3].
The incidence and mortality linked to CRC in China are increasing annually. According to Chinese cancer sta-

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tistics, in 2015, there were 388,000 new cases of CRC and 187,000 deaths related to the disease with the incidence of the disease ranking recurrence and metastasis is important in the treatment of colorectal cancer. Early detection of recurrence or metastasis and early treatment may improve the survival time of patients.

An existing study [4] revealed that methylation of the septin 9 (SEPT9) gene is closely related to the pathogenesis of CRC. In several studies [5-7], SEPT9 methylation in peripheral blood was detected for CRC screening and early diagnosis. Research showed that when peripheral blood SEPT9 methylation was used for screening and the early diagnosis of CRC, the sensitivity was 30%-75%, and specificity was close to 90% [3,8,9]. The methylated SEPT9 (mSEPT9) assay became the first blood-based test approved by the United States Food and Drug Administration as a CRC screening test. Carcinoembryonic antigen (CEA) is a well-established tumor marker for CRC, but its sensitivity and specificity is low. mSEPT9 was more sensitive than CEA and combined mSEPT9 and CEA was more accurate for diagnosing CRC. However, its expression in Chinese patients with colorectal cancer and its value in tumor progression or recurrence monitoring is not clear. The present study aimed to analyze the diagnostic value of mSEPT9 for CRC by following up and analyzing the diagnosis and treatment data of CRC patients. By analyzing and comparing the expression difference of mSEPT9 in CRC patients before and after surgery, as well as the status of mSEPT9 during regular follow-up and its correlation with tumor recurrence and metastasis, the investigators explored the value of serum mSEPT9 in the diagnosis, curative effect evaluation, and follow-up monitoring of CRC.

Materials and Methods

General Information

A total of 208 CRC patients who met the research requirements in the First Affiliated Hospital of Xinjiang Medical University (China) from March 2019 to December 2019 were enrolled in this study. Among them, 93 patients underwent surgical treatment and 115 patients underwent other treatment.

Inclusion Criteria

The perioperativeSubjects met the following criteria: (1) patients who were initially treated in our hospital and who did not receive radiotherapy or chemotherapy; (2) patients with a pathological diagnosis of CRC; (3) patients who voluntarily participated in the study.

Inclusion criteria for postoperative follow-up patients were as follows: (1) patients who had completed surgery or adjuvant radiotherapy and chemotherapy; (2) patients with a pathological diagnosis of CRC; (3) patients who voluntarily participated in the study.

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of The First Affiliated Hospital of Xinjiang Medical University. All patients provided signed informed consent for inclusion in the research.

Exclusion Criteria

The exclusion criteria for patients in the perioperative period and during postoperative follow-up were as follows: (1) patients with malignant tumors related to other biological systems; (2) patients with immune system diseases, such as rheumatic fever, nephrotic syndrome, and rheumatoid arthritis; (3) patients undergoing other scientific experiments.

Plasma Sample Acquisition And Processing Method

We examined and collected the relevant clinical and laboratory test data one day before operation and three days after operation for the patients who underwent surgical treatment during the study period. A 10 ml peripheral venous blood sample was taken from each CRC patient before and after surgery.

According to the current CEA follow-up standard, for patients who were followed up, peripheral venous blood was withdrawn once at three months after surgery [10]. A 10 ml sample of peripheral venous whole blood was withdrawn from each patient and treated with ethylenediaminetetraacetic acid anticoagulation and could be used immediately for mSEPT9 detection. The detection process of mSEPT9 was carried out according to the instructions accompanying the EPI proColon 2.0 CE Kit (Beijing BioChain Technology Co, Ltd, China). Carcinoembryonic antigen was detected by radioimmunoassay as per the product instructions.

The technicians involved in the experiment were blinded to the clinical information of the subjects.

Statistical Methods

Experimental data were statistically analyzed using the SPSS Statistics 23.0 and Prism 5 software programs. Measurement data were expressed as mean ± standard deviation (x ± SD), and compared using a one-group t-test. Count data were expressed as a percentage (%) and compared using a chi-square (χ^2) test for data in a four-fold table. The data in min/max normalization were expressed as [0,1]. All comparison tests were two-sided; $P < 0.05$ was considered statistically significant.

Results

Tumor size was highly correlated with carcinoembryonic antigen level and the degree of tumor differentiation in colorectal cancer patients

Among the crc patients who were included in this study and received surgical treatment, the degree of tumor differentiation was determined in 87 patients. According to statistics, tumor size and preoperative CEA expression level were significantly associated with the degree of tumor differentiation (Table I, Fig. 1).

In 87 patients with CRC, the average size of poorly differentiated tumors was the largest (25.01±14.08 cm^2), which was significantly different from that of moderately differentiated tumors (P = 0.039). The serum CEA levels of patients with different pathological grades did not conform to the normal distribution. The rank sum test was used to analyze the serum CEA level and the degree of tumor differentiation. There was a statistically significant difference in serum CEA level among different degrees of differentiation (P = 0.018). The LSD method was used to compare the tumor sizes of patients with different pathological grades. The results showed that the tumor sizes of patients with low differentiation, moderate differentiation (P = 0.028) and high differentiation (P=0.017) were statistically different, and the tumor sizes of patients with moderate differentiation and high differentiation were statistically different. (Table I, Fig. 1).

The indication effect of methylated septin9 and carcinoembryonic antigen on tumors at different locations

In this study, the results of mSEPT9, CEA, and tumor size and location in patients with CRC undergoing surgical treatment were analyzed. The results revealed that mSEPT9 and CEA may be able to indicate the location of a tumor. The deviation normalization of tumor size, CEA level, and other patient data included in the study was analyzed, and the results showed that when the tumor occurred in the transverse and ascending colon, the CEA level was relatively low, and the corresponding tumor size was relatively large. When the tumor occurred in the sigmoid and descending colon, the CEA level was relatively high, and the corresponding tumor size was relatively small (Fig. 2A). When mSEPT9 was positive, the location and size of the tumor were consistent with the above situation (Fig. 2B); however, when the mSEPT9 test result was negative, the tumor volume

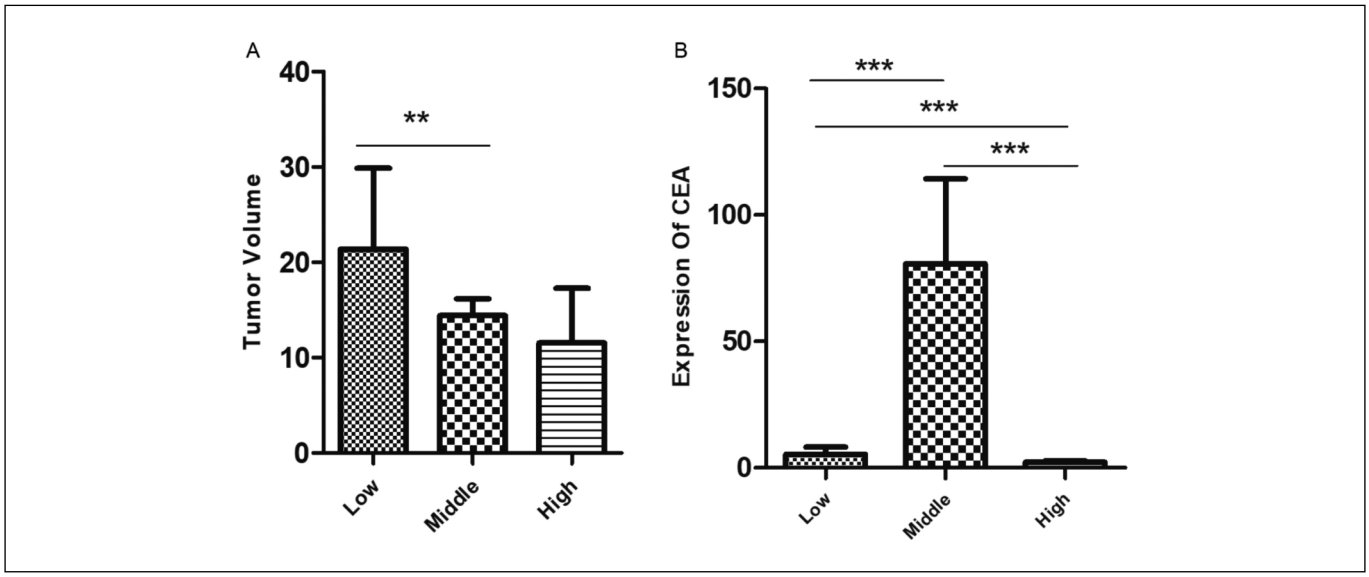

Fig. 1: Correlation between tumor volume (A), CEA expression (B) and tumor differentiation in CRC patients.

TABLE I - *Tumor size and preoperative CEA levels under different degrees of differentiation*

Degree of tumor differentiation	N	Mean tumor size (cm^2)	Mean CEA level (ng/mL)
Low differentiation	10	25.01±14.08	1.60 (1.32,2.87)
Moderate differentiation	69	13.98±15.02	3.37 (1.74,8.89)
High differentiation	8	8.15±10.88	2.44 (1.08,3.99)
F/H		3.374	8.035
P		0.039	0.018

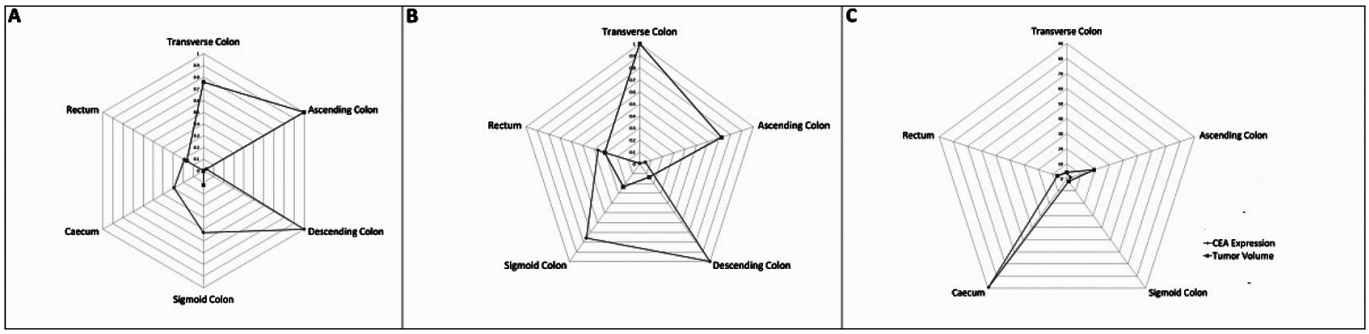

Fig. 2: Indication effects of CEA and SEPT9 on tumors at different sites; A) correlation between CEA expression and tumor size and location; B) correlation between CEA expression and tumor size and location in patients positive for SEPT9 before operation; C) correlation between CEA expression and tumor size and location in patients negative for SEPT9 before operation.

TABLE II - *Comparison of mSEPT9 and serum CEA in different tumor sites*

		mSEPT9			serum CEA		
		Positive %	χ^2	P	M(Q1, Q2)	H	P
Rectum	52	19(36.5)	3.391	0.495	3.43(1.68,3.38)	9.938	0.041
Descending colon	6	4(66.7)			7.05(3.47,737.14)		
Ascending colon	10	5(50.0)			1.83(1.08,2.76)		
Transverse colon	5	1(20.0)			1.54(0.94,3.59		
Sigmoid colon	14	5(35.7)			1.85(0.77,2.21)		

TABLE III - *Correlation analysis of tumor size with serum CEA level and mSEPT9*

		Mean tumor size (cm^2)	t	P
mSEPT9	Positive	19.90±14.28	2.672	0.009
	Negative	11.38±14.60		

occurring in the ascending colon was relatively large, and the CEA level was relatively high when the tumor occurred in the cecum (Fig. 2C).
These results indicate that when mSEPT9 is positive and the CEA level is relatively low, tumors are relatively large and typically occur in the transverse or ascending colon and are easily detectable. When mSEPT9 is positive and the CEA level is relatively high, the tumors primarily occurred in the sigmoid and descending colon and were relatively small. Attention should be paid to the possibility of missed detection during microscopy; when mSEPT9 is negative and the CEA level is high, the possibility of a tumor occurring in the cecum is high but these tumors are typically relatively small.

Relationship between tumor location and serum CEA, mSEPT9

There was no significant difference in mSEPT9 positive rate in patients with different tumor locations (χ^2=3.391 P=0.495), but there was a statistically significant difference in serum CEA levels in patients with different tumor locations (H=9.938 P=0.041). The tumor location was in the descending colon (Serological CEA levels were higher in patients with M=3.43) and rectum (M=7.05), (Table II).

Relationship between tumor size and serum CEA, mSEPT9

There was a weak negative correlation between tumor size and serum CEA level, and there was no statistical difference (r= –0.031, P=0.779); the tumor area of patients with positive mSEPT9 level was larger than that of negative patients, and the difference was statistically significant (t=2.672, P=0.009), (Table III).

The evaluation value of methylated septin9 expression on the tumor-bearing state of colorectal cancer patients
Postoperative blood mSEPT9 detection was carried out in CRC patients undergoing resection. Subsequent analysis revealed that 77 patients were negative and 16 patients were positive for mSEPT9 after surgery. In patients negative for mSEPT9 after surgery, cancer metastasis was found in 4 patients, and 73 patients showed no metastasis during follow-up; in patients positive for mSEPT9 after surgery, cancer metastasis was found in 13 patients, and 3 patients showed no metastasis during follow-up (Table IV).
The proportion of tumor metastasis in patients with postoperative positive mSEPT9 was 81.25%, and the pro-

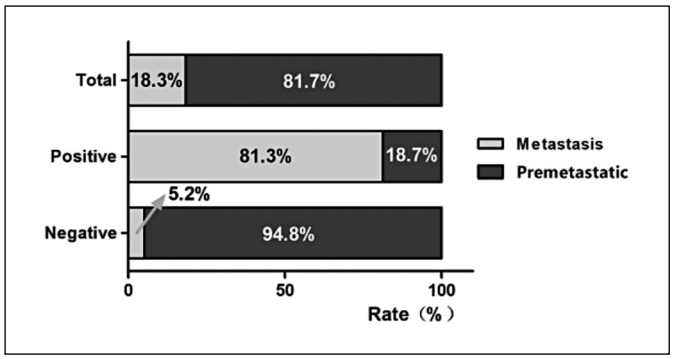

Fig. 3: Postoperative mSEPT9 results and percentage of case with tumor metastasis

portion of tumor metastasis in patients with post-operative negative mSEPT9 was 5.19%; the proportion of tumor metastasis in patients with postoperative positive mSEPT9 was much higher compared with patients with postoperative negative mSEPT9 (Fig. 3).

These results suggest that the detection results of msept9 after surgery may be related to the tumor-bearing state of patients. The msept9 may be associated with CRC incidence, postoperative recurrence and tumor metastasis.

The monitoring value of methylated septin9 and carcinoembryonic antigen in the follow-up of colorectal cancer after treatment

Among 208 CRC patients who underwent routine follow-up after surgery and/or other treatments, 34 were positive for mSEPT9. Imaging examination revealed that 30 of these patients had recurrence and metastasis, and 4 had no recurrence or metastasis; 174 patients were negative for mSEPT9, 6 of whom were confirmed to have recurrence and metastasis by other examinations, and 168 negative mSEPT9 patients had no recurrence or metastasis, based on imaging and endoscopy. The sensitivity and specificity of mSEPT9 for recurrence and metastasis were 83.3% and 97.7%, respectively. Among the 40 patients positive for CEA, 22 were confirmed to have recurrence and metastasis while 18 showed no signs of recurrence or metastasis. Among the 168 patients negative for CEA, 14 were diagnosed with recurrence and metastasis after examination; 148 were found to not have recurrence or metastasis. The sensitivity and specificity of CEA were 61.1% and 89.5%, respectively.

These results suggest that, in the monitoring of recurrence and metastasis among CRC patients, the sensitivity and specificity of mSEPT9 are higher compared with CEA (Table V).

The expressions of mSEPT9 and CEA were further compared in 36 patients with recurrence and metastasis. The results revealed no significant difference in the diagnosis value of recurrence and metastasis between CEA and mSEPT9 ($P > 0.05$), (Table VI).

The above results indicate that mSEPT9 shows higher sensitivity and specificity compared with CEA for predicting postoperative recurrence and metastasis. However, there was no significant difference in the diagnostic value between the two after recurrence and metastasis.

Discussion

Colorectal cancer is a malignant tumor type that poses serious risks to human health. With the continuous development of medical technology, traditional Chinese medicine is also widely used in the treatment of tumors [11-13]. At present, the management of CRC primarily takes the form of comprehensive treatment that is based on a surgical approach, where the strategy is to formulate a

TABLE IV - *Postoperative mSEPT9 test results and incidence of tumor metastasis*

Postoperative mSEPT9	Number of cases with tumor metastases	Number of cases without tumor metastases	Incidence of metastasis (%)
Positive	13	3	81.25
Negative	4	73	5.19
Total	17	76	18.28

TABLE V - *Expression of mSEPT9 and CEA in postoperative monitoring of recurrence and metastasis of colorectal cancer*

Index	Expression status	Detection object: Patients with recurrence and metastasis	Detection object: Patients without recurrence and metastasis	Sensitivity (%)	Specificity (%)
mSEPT9	Positive	30	4	83.3	97.7
	Negative	6	168		
CEA	Positive	22	18	61.1	89.5
	Negative	14	154		

TABLE VI - *Expression of mSEPT9 and CEA in CRC patients with recurrence and metastasis*

CEA	mSEPT9 +	–	Total	χ^2	P
+	20	4	24	2.57	0.180
–	10	2	12		

systematic treatment plan according to the patient's pathological grade, relevant risk factor evaluation, and gene test results. However, the five-year survival rate of CRC patients receiving standard treatment is only 45%-50%. This reflects that the current treatment effect is not ideal. Exploring and integrating more comprehensive and accurate monitoring indicators, establishing a prognosis monitoring system, and improving the effect of the precision treatment of CRC is an important concern at present.

An existing study revealed mSEPT9 to be closely related to the pathogenesis of CRC and a novel marker in the diagnosis and treatment evaluation of CRC. The septin 9 gene is located on chromosome 17 and is responsible for encoding the SEPTIN9 protein, which can inhibit cancer by regulating cell growth and preventing cell division from happening too fast or curb division and proliferation in an uncontrolled manner [14]. Methylation of the CpG sequence domain of the septin9 gene can eliminate the normal polarity of cells, leading to disordered cell division regulation and rendering cells cancerous. The results of a related study revealed that septin9 promoter had a high methylation level in CRC patients, while the methylation level of SEPT9 was fundamentally not high among the normal population and other patient groups [15]. The septin9 methylation level in peripheral blood can be used for CRC screening and early diagnosis.

At present, there are few reports on the value of mSEPT9 in perioperative efficacy evaluation and the follow-up monitoring of CRC. This study revealed that in CRC patients, tumor size was decreased with an increase in tumor differentiation, and the level of CEA was also highly correlated with the degree of tumor differentiation. There was a significant difference in CEA level between moderately, highly, and poorly differentiated tumors.

This study also revealed that the detection results of CEA and mSEPT9 may indicate the location and size of a tumor. When mSEPT9 was positive and the CEA level was relatively low, the tumors were relatively large and generally occurred in the transverse or ascending colon; when mSEPT9 was positive and the CEA level was relatively high, the tumors typically occurred in the sigmoid and descending colon but were relatively small. When mSEPT9 was negative and the CEA level was high, the possibility of a tumor occurring in the cecum was high but the tumors were relatively small.

After comprehensively analyzing these findings, the investigators infer that the combined detection of plasma mSEPT9 and CEA can assist in the diagnosis of CRC and help establish the location and size of CRC tumors. Furthermore, the combined analysis of mSEPT9 and CEA levels could also help to locate tumors and reduce the missed detection rate of local tumors in cases of CRC.

Further study revealed that among the 93 colorectal cancer patients without distant metastasis before surgery, the proportion of tumor metastasis was 81.25% in patients with postoperative mSEPT9 positive. The proportion of tumor metastasis in patients with post-operative negative mSEPT9 was 5.19%. These results indicate that mSEPT9 is associated with tumor load in patients. A positive mSEPT9 result may indicate the patient as being more likely to have a tumor.

The level of mSEPT9 in postoperative patients can be used as an evaluation standard for evaluating the effect of surgical treatment. In 208 patients who underwent surgery or follow-up, the sensitivity and specificity of plasma mSEPT9 for recurrence and metastasis were 83.3% and 97.7%, respectively. The sensitivity and specificity of CEA for recurrence and metastasis were 61.1% and 89.5%, respectively. These results indicated that in the monitoring of recurrence and metastasis among CRC patients, the sensitivity and specificity of mSEPT9 were higher compared with CEA.

In recent years, mSEPT9 has been widely studied. Several theories have been developed related to the way of blood entry, among which "active release and the necrosis of tumor cells" is broadly accepted, i.e., mSEPT9 is actively released from tumor cells into the blood circulation and can promote the transformation of susceptible cells into cancer cells through a mechanism similar to transfection. This phenomenon is currently known as the "gene transfer" theory [16].

In terms of the diagnostic value of mSEPT9 for CRC, the positive expression rate of mSEPT9 in the plasma of CRC patients was high, and the positive rate of mSEPT9 in healthy controls was very low [5]. Sun et al [17] included 29 studies involving 10,486 subjects (3,202 CRC patients and 7,284 controls) in a meta-analysis of mSEPT9 related to the diagnosis and evaluation of CRC. The results revealed that the total sensitivity and specificity of mSEPT9 were 0.74 (95% CI 0.61-0.84) and 0.96 (95% CI 0.95-0.97), respectively. Grützmann et al [18] investigated the level of mSEPT9 in 252 CRC patients and 102 healthy controls and showed that the sensitivity and specificity of mSEPT9 in detecting CRC were 72% and 90%-93%, respectively.

For the evaluation of the therapeutic value of mSEPT9, researchers found similar results for the diagnostic value in a related study on the evaluation of the curative effect of surgery on CRC [19-21].

These studies revealed that the level of plasma mSEPT9 was significantly decreased after surgical treatment in

CRC patients. Accordingly, the investigators speculate that mSEPT9 can be used as a valuable index for judging the curative effect of surgery on CRC.
Methylated SEPT9 is also of great significance regarding the value of follow-up detection. Tham conducted a five-year prospective cohort study involving 150 CRC patients [22] and revealed that the high methylation level of mSEPT9 in serum was an independent predictor of tumor recurrence and tumor-specific poor survival. In addition, a study reported that [20] patients with continuous post-operative positive mSEPT9 were highly correlated with impending recurrence or metastasis (within one year), which may be an indicator of poor prognosis. Song et al [19] showed that when 82 CRC patients were followed up for 21 months, in the univariate Cox proportional hazards analysis of patients, those with positive mSEPT9 had a higher risk of death than those with negative mSEPT9. These results suggest that mSEPT9 has prognostic value for CRC patients. Methylated SEPT9 can be used as a monitoring index for prognosis, recurrence, and metastasis following surgical therapy for CRC.
In summary, the detection of serum mSEPT9 and CEA may have clinical significance in the diagnosis, tumor location, efficacy evaluation, and post-operative follow-up of CRC. However, the current study had a small sample size. To further explore the clinical application value of plasma mSEPT9 detection, multi-center and large-sample clinical research is needed. As such, the value of mSEPT9 detection in the diagnosis and treatment of CRC still requires additional investigation.

Riassunto

Questo studio ha esplorato il valore del livello della settina sierica metilata 9 (mSEPT9) e dell'antigene carcinoembrionico (CEA) nell'ausilio diagnostico, nella valutazione dell'effetto curativo e nel monitoraggio di follow-up del cancro del colon-retto (CRC).
Sono stati raccolti da marzo 2019 a dicembre 2019 i dati diagnostici e dell'esito del trattamento di 208 pazienti affetti da CRC nel primo ospedale affiliato della Xinjiang Medical University (Cina) seguiti nel followup. È stata analizzata la correlazione tra CEA sierico, livelli di mSEPT9 e la sede e dimensioni del tumore. L'mSEPT9 e il CEA sierici sono stati rilevati prima e dopo l'intervento chirurgico e durante il follow-up dopo trattamento per analizzare il valore dell'mSEPT9 nella valutazione dell'efficacia e nel monitoraggio del follow-up.
In 87 pazienti con CRC sottoposti a intervento chirurgico, la dimensione media dei tumori, scarsamente differenziati, era maggiore (25.01±14.08 cm^2), significativamente diversa da quella dei tumori moderatamente differenziati ($P^{™}$=0,039). È stata osservata una differenza statisticamente significativa nel livello sierico di CEA tra i diversi gradi di differenziazione (P=0,018).
Il livello di CEA era relativamente basso quando si trattava di tumori nel colon trasverso e ascendente. Quando il livello di CEA era alto, il mSEPT9 negativo suggeriva che la probabilità che si verificasse un tumore nel cieco fosse alta; mSEPT9 positivo indicava l'elevata probabilità che la sede era nel colon discendente o nel sigma. Il dosaggio prima e dopo l'intervento chirurgico ha rivelato che il livello di mSEPT9 può essere correlato allo stato di tumore dei pazienti.
Lo studio di follow-up ha anche mostrato che la sensibilità e la specificità di mSEPT9 per recidiva e metastasi erano rispettivamente dell'83,3% e del 97,7% e che la sensibilità e la specificità del CEA erano rispettivamente del 61,1% e dell'89,5%.
Il rilevamento combinato di mSEPT9 e CEA può indicare la posizione e le dimensioni del cancro del colon-retto, mentre il rilevamento di mSEPT9 sierico può avere un significato clinico nella valutazione dell'efficacia e nel monitoraggio di follow-up del cancro del colon-retto.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: *Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries*. CA Cancer J Clin, 2018; 68:394-424.

2. Zheng RS, Sun KX, Zhang SW, Zeng HM, Zou XN, Chen R, Gu XY, Wei WQ, He J: *Report of cancer epidemiology in China, 2015*. Chin J Oncol, 2019; 41:19-28*, in chinese.*

3. Tóth K, Galamb O, Spisák S, Wichmann B, Sipos F, Valcz G, Leiszter K, Molnár B, Tulassay *Z*: *The influence of methylated septin 9 gene on RNA and protein level in colorectal cancer.* Pathol Oncol Res, 2011; 17:503-9.

4. Jones PA, Baylin SB: *The epigenomics of cancer*. Cell, 2007; 128:683-92.

5. Li Y, Hui LY, Wang YW: *Diagnostic evaluation of Septin9 methylated DNA detection in the screening of colorectal cancer*. Clin Res Pract, 2018; 3:1-3, in chinese.

6. Xie L, Jiang X, Li Q, Sun Z, Quan W, Duan Y, Li D, Chen T: *Diagnostic value of methylated Septin9 for colorectal cancer detection.* Front Oncol, 2018; 8:247.

7. Zhang M, He Y, Zhang X, Zhang M, Kong L: *A pooled analysis of the diagnostic efficacy of plasmic methylated septin-9 as a novel biomarker for colorectal cancer*. Biomed Rep, 2017; 7:353-60.

8. Jin P, Kang Q, Wang X, Yang L, Yu Y, Li N, He YQ, Han X, Hang J, Zhang J, Song L, Han Y, Sheng JQ: *Performance of a second-generation methylated SEPT9 test in detecting colorectal neoplasm.* J Gastroenterol Hepatol, 2015; 30:8303-3.

9. Church TR, Wandell M, Lofton-Day C, Mongin SJ, Burger M, Payne SR, Castaños-Vélez E, Blumenstein BA, Rösch T, Osborn N, Snover D, Day RW, Ransohoff DF: *PRESEPT Clinical Study Steering Committee, Investigators and Study Team: Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer.* Gut, 2014; 63:317-25.

10. Collins JF, Lieberman DA, Durbin TE, Weiss DG: *Veterans affairs cooperative study #380 group: Accuracy of screening for fecal occult blood on a single stool sample obtained by digital rectal examination: A comparison with recommended sampling practice*. Ann Intern Med, 2005; 142:81-5.

11. Tang M, Huang LL, Du QQ, Yan C, Gu AD, Yang JL, et al: *Ginsenoside 3 -O-Glc-DM (C3DM) enhancesthe antitumor activity of Taxol on Lewis lung cancer by targeting the interleukin-6/Jak2/STAT3 and interleukin-6/AKT signaling pathways.* World J Tradit Chin Med, 2020; 6:432-40.

12. Shen XY, Su CY, Yan YY, Zhang LL, Guo QR, Chen HB, et al: *A study on the mechanism of bruceine D in the treatment of non-small cell lung cancer H1299 cells.* World J Tradit Chin Med, 2020; 6:500-07.

13. Bailly C: *Xihuang pills, a traditional chinese preparation used as a complementary medicine to treat cancer: An updated review.* World J Tradit Chin Med, 2020; 6:152-62.

14. Burrows JF, Chanduloy S, McIlhatton MA, Nagar H, Yeates K, Donaghy P, Price J, Godwin AK, Johnston PG, Russell SE: *Altered expression of the septin gene, SEPT9, in ovarian neoplasia*. J Pathol, 2003; 201:581-8.

15. Zhang LY, Chen P, Gao P, Shen W: *Study of early diagnosis of colorectal cancer by analysis of stool SEPT9 Gene Methylation*. J Med Res, 2016; 45:142-144, 152, in chinese.

16. García-Olmo DC, Domínguez C, García-Arranz M, Anker P, Stroun M, García-Verdugo JM, García-Olmo D: *Cell-free nucleic acids circulating in the plasma of colorectal cancer patients induce the oncogenic transformation of susceptible cultured cells.* Cancer Res, 2010; 70:560-7.

17. Sun G, Meng J, Duan H, Zhang D, Tang Y: *Diagnostic assessment of septin9 DNA methylation for colorectal cancer using blood detection: A meta-analysis*. Pathol Oncol Res, 2019; 25:1525-34.

18. Grützmann R, Molnar B, Pilarsky C, Habermann JK, Schlag PM, Saeger HD, Miehlke S, Stolz T, Model F, Roblick UJ, Bruch HP, Koch R, Liebenberg V, Devos T, Song X, Day RH, Sledziewski AZ, Lofton-Day C: *Sensitive detection of colorectal cancer in peripheral blood by septin 9 DNA methylation assay*. PLoS One, 2008; 3:e3759.

19. Song L, Guo S, Wang J, Peng X, Jia J, Gong Y, Yang B, Xiao W, Dong C, Liu H, Li Y: *The blood mSEPT9 is capable of assessing the surgical therapeutic effect and the prognosis of colorectal cancer*. Biomark Med, 2018; 12:961-73.

20. Fu B, Yan P, Zhang S, Lu Y, Pan L, Tang W, Chen S, Chen S, Zhang A, Liu W: *Cell-free circulating methylated SEPT9 for non-invasive diagnosis and monitoring of colorectal cancer*. Dis Markers, 2018; 2018:6437104.

21. Xia CQ, Xu ZF: *Monitoring role of blood plasma Septin 9 methylation in the treatment of colorectal carcinoma*. Chin J Coloproctol, 2019; 39:6-8, in chinese.

22. Tham C, Chew M, Soong R, Lim J, Ang M, Tang C, Zhao Y, Ong SY, Liu Y: *Postoperative serum methylation levels of TAC1 and SEPT9 are independent predictors of recurrence and survival of patients with colorectal cancer*. Cancer, 2014; 120:3131-41.