Original Article
Role of blood tumor markers in predicting metastasis and local recurrence after curative resection of colon cancer

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Abstract: Aim: To investigate the prognostic value of carcinoembryonic antigen (CEA), CA199, CA724 and CA242 in peripheral blood and local draining venous blood in colon cancer patients after curative resection. Methods: 92 colon cancer patients who received curative resection were retrospectively analyzed. The CEA, CA199, CA724 and CA242 were detected in peripheral blood and local draining venous blood. Results: Metastasis or local recurrence was found in 29 (29/92, 31.5%) patients during follow-up period. 92 patients were divided into two groups: metastasis/local recurrence group (n = 29) and non-metastasis/local recurrence group (n = 63). Peripheral venous CEA, CA199, CA724 and CA242 (p-CEA, p-CA199, p-CA724 and p-CA242) were comparable between two groups ($P > 0.05$). The median draining venous CEA (d-CEA) in metastases/local recurrence group (23.7 ± 6.9 ng/ml) was significantly higher than that in non-metastases/local recurrence group (18.1 ± 6.3 ng/ml; $P < 0.05$), but marked differences were not observed in draining venous CA199, CA724 and CA242 (d-CA199, d-CA724 and d-CA242) between two groups ($P > 0.05$). The optimal cut-off value of d-CEA was 2.76 ng/ml, with the sensitivity and specificity of 90% and 40% in the prediction of metastasis or local recurrence, respectively. d-CEA correlated with tumor differentiation, T stage, TNM stage, metastasis and local recurrence. Subgroup analysis showed that, of 41 patients with stage II colon cancer, the optimal cut-off value of d-CEA was 8.78 ng/mL, and the sensitivity and specificity were 87.5% and 69.7% in the prediction of metastasis or local recurrence, respectively. Conclusion: d-CEA may be a prognostic factor for stage II colon cancer patients.

Keywords: Colon cancer, carcinoembryonic antigen, prognosis

Introduction

In Western countries, colon cancer is the third most common cancer [1]. In China, colon cancer is one of the most common carcinomas with increasing incidence over year. Currently, radical surgery is the major strategy for the therapy of colon cancer [2, 3]. However, the distant metastasis significantly affects the prognosis of these patients after radical resection. As a consequence of advanced techniques and new chemotherapeutics, the survival rate of colon cancer patients with distant metastasis and local recurrence have been greatly improved in the past decade [4, 5]. Nevertheless, the survival rate is usually complicated by the side effects of chemotherapy. This is mainly ascribed to the lack of reliable markers for the prediction of distant metastasis or local recurrence. To identify these markers will be greatly beneficial for the individualized chemotherapy of patients with a high risk for distant metastasis or local recurrence.

Among available markers for colonic cancer, carcinoembryonic antigen (CEA) is the most commonly accepted and frequently used one. The clinical value of CEA in peripheral venous blood of colon cancer patients has been investigated in many studies [6-8]. The postoperative peripheral CEA (p-CEA) is used as not only an early marker for recurrence and prognosis after radical surgery, but an indicator for the assessment of chemotherapeutic response in metastatic colon cancer patients [9]. American Joint Committee on Cancer recommends the

Table 1. Clinical characteristics of patients in this study (n = 92)

Variable	Value
Age (yr) (mean ± SD)	60.3 ± 0.8
Male/female ratio	47/45
Tumor site	
Colon	48
Rectum	44
TNM stage	
I	10
II	41
III	41

inclusion of CEA for the TNM classification [10]. However, the prognostic value of p-CEA is somewhat limited because only 45% of colon cancer patients are positive for p-CEA preoperatively [11, 12]. Some investigators propose that CEA might be hematogenously drained by portal system from cancer cells in the affected veins, and therefore CEA in draining venous blood (d-CEA) may be more useful in the prediction of liver metastasis in colon cancer patients [13, 14]. However, d-CEA has not been fully evaluated as a sensitive marker of metachronous hepatic metastasis in colorectal cancer (CRC) patients. Although less frequently used, other markers such as CA 19-9, CA242 and CA72-4 are sometime applied together with CEA in the prediction of progression and prognosis of colorectal cancer.

This study was to prospectively analyze tumor markers (CEA, CA19-9, CA242 and CA72-4) in the peripheral blood and draining venous blood of colon cancer patients after curative resection, and investigate their roles in the prediction of prognosis of colon cancer.

Patients and methods

Patients

This study was approved by the Ethics Committee of Cancer Institution of Peking University. From 2005 to 2007, 92 patients meeting the inclusion criteria were recruited into present study: (1) Patients were diagnosed with colon adenocarcinoma by pathological examination; (2) cancers located up to 12 cm from the anal verge; (3) distant metastasis was excluded by imaging examinations; (4) patients underwent radical surgery. All the patients

underwent curative surgery in the Department of Colorectal Surgery of Cancer Hospital of Peking University. Operations were performed by one or two experienced colorectal surgeons using the 'non-touch' technique. Written informed consent was obtained from each patient before study.

Patients meeting following criteria were excluded: 1) patient received prior chemotherapy or radiotherapy; 2) patient had a history (within 5 years) of malignant tumor; 3) patient had hepatic, biliary, or inflammatory bowel disease which affected CEA test; 4) patient had unresectable colon cancer.

Measurement of tumor markers

Peripheral blood samples were collected by peripheral venipuncture intraoperatively. The draining venous blood was collected via the tributary vein catheter immediately after laparotomy, but before cancer manipulation. Blood was collected from following draining veins: inferior mesenteric vein for cancers in the descending colon and sigmoid colon, middle colic vein for cancers in the transverse colon, and superior mesenteric vein for cancers in the ascending colon.

The tumor markers were measured with electrochemiluminescent assay using Roche Diagnostic reagent kits and an Elecsys 2010 analyzer (Roche Diagnostics, Basel, Switzerland). The reference value was 5 ng/ml (normal: ≤ 5 ng/ml; abnormal: > 5 ng/ml) for CEA, 37 U/ml (normal: ≤ 37 ng/ml; abnormal: > 37 ng/ml) for CA19-9, 6.7 U/ml (normal: ≤ 6.7 ng/ml; abnormal: > 6.7 ng/ml) for CA72-4 and 20 U/ml (normal: ≤ 20 ng/ml; abnormal: > 20 ng/ml) for CA242.

Follow-up

Patients were followed up at an interval of three months within first two years and thereafter at an interval of six months in the following three years. At each follow up, physical examination, detection of serum CEA, CA19-9, CA242 and CA72-4, routine blood test, and serum chemistry profiling were done. Proctoscopy, abdominal ultrasonography, abdomen and pelvis CT, and chest radiography were performed once every 6-12 months. The primary endpoints were metastasis and local recurrence.

Table 2. Four tumor markers in the peripheral venous blood

Tumor marker	Metastasis/local recurrence (n = 29)	Non-metastasis/local recurrence (n = 63)	P value
CEA	18.1 ± 6.3	9.2 ± 2.0	0.08
CA199	37.8 ± 11.3	37.2 ± 7.7	0.83
CA724	10.0 ± 4.6	24.7 ± 18.8	0.97
CA242	29.0 ± 8.5	23.4 ± 5.2	0.58

Table 3. Four tumor markers in the draining venous blood

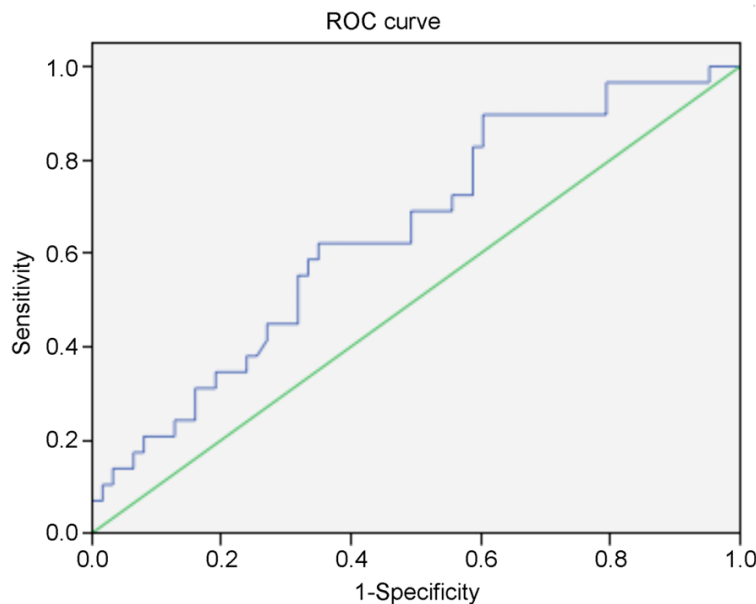| Tumor marker	Metastasis/local recurrence (n = 29)	Non-metastasis/local recurrence (n = 63)	P value
CEA	23.7 ± 6.9	11.1 ± 2.1	0.02
CA199	41.2 ± 14.6	32.5 ± 7.2	0.61
CA724	8.7 ± 4.5	20.6 ± 17.1	0.98
CA242	30.1 ± 10.1	23.0 ± 4.8	0.52

Figure 1. ROC curve for the prediction of recurrence and metastasis on the basis of d-CEA.

Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences Version 15.0 software (SPSS Inc., Chicago, Ill, USA). Continuous variables are expressed as mean ± standard deviation (SD). Student's t test, chi-square test and Wilcoxon test were used for comparisons of clinicopathological parameters and serum CEA. The optimal cut-off values of tumor markers as prognostic variables were determined according to the receiver-operator characteristic (ROC) analysis. A value of $P < 0.05$ was considered statistically significant.

Results

Demographics and clinico-pathological features

A total of 92 patients were reviewed (**Table 1**). The median age was 60.3 years (range: 29-87 years) and 51.1% (47/92) of patients were male. Colon cancer was found in the ascending colon in 44% (34/92) of patients, transverse colon in 8% (8/92), and descending and sigmoid colon in 22% (22/92). The median operation time was 130 min (range: 60-330 min). According to the TNM staging system, colon cancer was classified as stage I in 10.8% (10/92) of patients, stage II in 44.6% (41/92) and stage III in 44.6.% (41/92).

Follow-up

All the patients were received scheduled post-operative follow-up as mentioned previously. The follow-up period ranged from 4 months to 71 months. Metastasis or local recurrence was noted in 29 patients during follow-up period, including 3 patients with local recurrence, 8 with liver metastasis, 4 with lung metastasis, 1 with both liver and lung metastases, 3 with bone metastasis, 2 with distant lymph node metastasis, and 8 with multi-organs metastases. All the patients were divided into two groups: metastases/local recurrence group (n = 29) and non-metastases/local recurrence group (n = 63).

Tumor markers in peripheral blood

In the peripheral venous blood, CEA, CA199, CA724 and CA242 (p-CEA, p-CA199, p-CA724 and p-CA242) were detectable in 38%, 25%, 22.8% and 29.3% of patients, respectively. The

Table 4. Clinicopathological features between high d-CEA group (d-CEA ≥ 2.76 ng/m, n = 64) and low d-CEA group (d-CEA < 2.76 ng/m, n = 28)

Clinicopathological features	d-CEA < 2.76 (n = 28)	d-CEA ≥ 2.76 (n = 64)	P value
Sex			
Male	10	37	0.051
Female	18	27	
Age	60.1 ± 2.2	60.5 ± 1.5	0.442
Tumor morphology			
Ulcerative type	21	50	0.617
Fungated type	7	12	
Constrictive type	0	2	
Tumor diameter			
≤ 4.5 cm	21	31	0.018
> 4.5 cm	7	33	
Tumor differentiation			
Well to moderately	23	53	0.577
Poorly	5	11	
T stage			
T1 + T2	10	5	0.002
T3 + T4	18	59	
Regional lymph node metastasis			
Yes	8	33	0.041
No	20	31	
Vascular embolus			
Yes	3	14	0.204
No	25	50	
Metastases or local recurrence			
Yes	3	26	0.001
No	25	38	

median p-CEA, p-CA199, p-CA724 and p-CA242 were 18.1 ± 6.3 ng/ml, 37.8 ± 11.3 U/ml, 10.0 ± 4.6 U/ml and 29.0 ± 8.5 U/ml in metastasis/local recurrence group, respectively and 9.2 ± 2.0 ng/ml, 37.2 ± 7.7 U/ml, 24.7 ± 18.8 U/ml and 23.4 ± 5.2 U/ml in non-metastasis/local recurrence group, respectively. These tumor markers were comparable between two groups in peripheral venous blood ($P > 0.05$) (**Table 2**).

Tumor markers in draining venous blood

In the draining venous blood, the median d-CEA was significantly higher (23.7 ± 6.9 ng/ml) in metastasis/local recurrence group than that in non-metastasis/local recurrence group (18.1 ± 6.3 ng/ml; $P < 0.05$). The median d-CA199, d-CA724 and d-CA242 were 41.2 ± 14.6 U/ml, 8.7 ± 4.5 U/ml and 30.1 ± 10.1 U/ml, respec-

tively, in metastasis/local recurrence group and 32.5 ± 7.2 U/ml, 20.6 ± 17.1 U/ml and 23.0 ± 4.8 U/ml, respectively, in non-metastasis/local recurrence group. There were no marked differences in these tumor markers between two groups ($P > 0.05$; **Table 3**).

*Prediction of recurrence and metastasis according to d-CEA (**Figure 1**)*

The area under the ROC curve (AUC) was 0.646 for d-CEA. The Youden index was the highest when the cut-off value of d-CEA was 2.76 ng/ml. The corresponding sensitivity and specificity were 90% and 40%, respectively, in the prediction of metastasis or local recurrence. When the cut-off value of d-CEA was 5 ng/ml, the sensitivity and specificity were 62% and 56%, respectively.

Clinicopathological features between high d-CEA group (d-CEA ≥ 2.76 ng/ml, n = 64) and low d-CEA group (d-CEA < 2.76 ng/ml, n = 28)

When the cut-off value of d-CEA was 2.76 ng/ml, patients were divided into two groups: high d-CEA group (d-CEA ≥ 2.76 ng/m, n = 64) and low d-CEA group (d-CEA < 2.76 ng/m, n = 28). The clinicopathological features were compared between two groups (**Table 4**). When compared with low d-CEA group, high d-CEA significantly correlated with the cancer diameter ($P = 0.018$), invasion depth ($P = 0.002$), regional lymph node metastasis ($P = 0.041$), advanced UICC stage ($P = 0.001$), and metastasis or local recurrence ($P = 0.001$).

Subgroup analysis of stage II colon cancer patients

In addition, 41 patients with colon cancer at stage II were further investigated. There were 22 men and 19 women with a mean age of 61 years (range: 32-87 years). d-CEA (13.70 ±
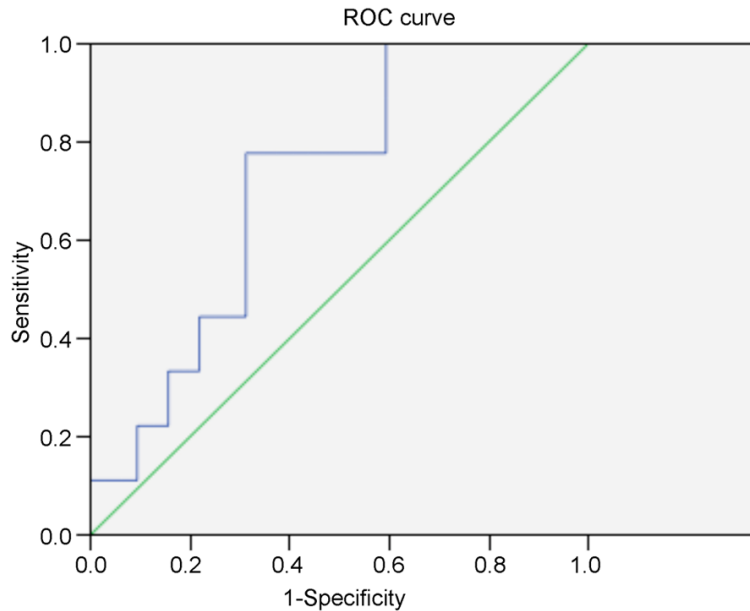
ROC curve

Figure 2. ROC curve for the prediction of recurrence and metastasis on the basis of d-CEA in Stage II colon cancer patients.

18.71 ng/ml) was significantly higher than p-CEA (9.94 ± 15.26 ng/ml) ($P < 0.05$). Metastasis and/or local recurrence were found in 8 patients. The 5-year disease-free survival (DFS) rate was 80.5%. According to the ROC curve, the optimal cut-off value of d-CEA was 8.78 ng/mL, with the sensitivity and specificity of 87.5% and 69.7% in the prediction of metastasis or local recurrence, respectively. The AUC was 0.758 ($P = 0.025$) (**Figure 2**).

Discussion

A large number of clinical studies have been conducted to identify biomarkers to predict the recurrence of CRC. However, several commonly used tumor markers, (such as CEA, CA199, CA724 and CA242) are not effective to predict the local recurrence and metastasis of CRC due to their low sensitivity and specificity [8, 15-20]. Some researchers attempted to increase the sensitivity by combining some tumor markers together, but the significance was limited [7, 21].

In the present study, four tumor markers were investigated. Consistent with previous findings, our results suggested that CA199 and CA724 were not associated with the recurrence and metastasis of CRC. Morita et al reported that there was no clinical evidence to support the

use of CA199 ($P = 0.23$) in the prediction of prognosis and recurrence of CRC based on a multivariate analysis. In contrast, CEA correlated well with recurrence with the OR of 32.0 ($P < 0.0001$) [17]. Therefore, they did not recommend the routine use of CA199 in staging and monitoring of CRC. In another study, Park et al found that among patients with recurrent CRC, increase in CA199 was observed in only 7.8% of patients with normal CEA during follow up period. Thus, they concluded that CA199 was an independent prognostic factor for recurrence of CRC [18]. Similarly, a postoperative follow-up study showed CA199 had poor sensitivity and specificity in the detection of recurrence, especially in patients with a normal pre-operative CA199 [8]. Taken together, these findings suggested that, even in patients with a high preoperative CA199, CEA might be able to fill the role of CA199. When compared with CEA and CA199, the sensitivity of CA724 is considerably lower in the evaluation of locoregional recurrence of CRC [15]. CA724 was more often tested together with other markers to predict the progression or recurrence of CRC.

CA242 has been reported to play a role in the diagnosis of CRC. In a follow-up study on 185 CRC patients, results showed the pre-operative serum CA242 was associated with TNM stage, lymph node metastasis and tumor invasion depth, and thus investigators concluded that CA242 could be used as an independent prognostic factor for the overall survival (OS) of CRC patients [20]. Another study also reported that CA 242 was a better maker than CEA and CA199 in the pre-operative staging [16]. In our study, the median CEA in peripheral venous blood was 29.0 ± 8.5 ng/ml in patients with recurrence/metastasis, which was slightly higher than that in non-metastasis/recurrence group (23.4 ± 4.8 ng/ml; $P = 0.58$).

In our study, the positive rates of CEA, CA199, CA724 and CA242 were 38.0%, 25.0%, 22.8% and 29.3%, respectively, in the peripheral

venous blood, and had no relationship with recurrence and metastasis of colon cancer ($P > 0.05$). Therefore, the pre-operative CEA, CA199, CA724 and CA242 in the peripheral venous blood are not suitable in the prediction of recurrence or metastasis of colon cancer.

The role of tumor markers in the evaluation of development, progression and metastasis of CRC has been studied extensively. Despite that, the clinical implication of these tumor makers in predicting the prognosis and recurrence is still limited. This is mainly ascribed to the low specificity of these tumor makers in peripheral blood. The levels of tumor markers in the peripheral venous blood are affected by a lot of factors, such as the type of tumor cells, absorption of lymphatic system, metabolism of the liver, and dilution of peripheral venous blood. When compared with markers in peripheral blood, these makers in the draining venous blood are more important since they directly reflect the biological information of the cancer. Of all the CRC makers, CEA has been clinically used in the diagnosis, monitoring of recurrence, evaluation of therapeutic efficacy and prediction of prognosis of CRC [8, 15, 17-19] despite its low specificity. Until recently, several studies suggest that d-CEA maybe a better prognostic maker than p-CEA. In patients with CRC, CEA in the portal blood was significantly higher than that in the peripheral blood. Importantly, the elevated CEA in the portal and peripheral blood was tightly associated with the vascular involvement and cancer invasion depth in the colorectal wall. Their findings suggested that CEA is hematogenously drained by the portal system from cancer cells in the invasive veins, not by the thoracic duct of lymphatic system. The d-CEA in the portal blood is more important than p-CEA in predicting the vascular involvement and liver metastasis [14]. Other studies also suggest that d-CEA, not p-CEA, greater than 5 ng/ml is closely associated with a poor prognosis and a high risk for post-operative recurrence and metastasis in CRC patients. Collectively, these studies imply that d-CEA is an independent risk factor of post-operative metastasis and recurrence [22-25]. However, in recent years, other studies reveal there is no difference between p-CEA and d-CEA in patients without vascular invasion [26]. Haraguchi et al examined CEA of peripheral blood and draining venous blood from 119

patients with CRC. Their results indicated the 5-year survival rate was 81.5% and 80.2% for patients with normal p-CEA and d-CEA (≤ 5 ng/ml), respectively, and 68.4% and 71.1% for those with abnormal p-CEA and d-CEA (> 5 ng/ml), respectively, showing no statistical significance. These findings suggest that d-CEA is not a predictor of metachronous hepatic metastasis and that measuring p-CEA is sufficient in the monitoring of CRC [27].

In our study, among frequently-used tumor markers, d-CEA was significantly higher (23.7 ± 6.9 ng/ml) in patients with recurrence or metastasis than in those without relapse of colon cancer (11.1 ± 2.1 ng/ml, $P = 0.02$), suggesting that d-CEA is associated with recurrence and metastasis of colon cancer. This association was not found in d-CA199, d-CA242 and d-CA724.

Furthermore, ROC curve was employed to determine the cut-off value of d-CEA in predicting the recurrence and metastasis of colon cancer after operation. The AUC of d-CEA was 0.646 with the sensitivity of 90% and the specificity of 40%. The sensitivity and specificity of d-CEA were higher than those in the study of Haraguchi et al (73.4% and 30%, respectively, in predicting liver metastasis) [27]. However, the specificity of d-CEA was only 40% in the prediction of metastasis or local recurrence, and thus its clinical application is limited.

Moreover, we further investigate the prognostic role of d-CEA in patients with colon cancer at stage II. ROC curve showed that a d-CEA cut-off value of 8.78 ng/ml was indicative of post-operative recurrence and metastasis in colon cancer patients, and that, when the AUC was 0.758, the corresponding sensitivity and specificity were 87.5% and 69.7%, respectively, in the prediction of metastasis or local recurrence. Therefore, d-CEA may be a useful factor for the prediction of prognosis of stage II colon cancer patients.

Patients with stage II colon cancer has a relatively good prognosis after surgery alone, with the 5-year survival rate of approximately 80% [28]. The available studies have demonstrated that adjuvant chemotherapy can bring a 2% to 4% increase in absolute survival [29-31]. Adjuvant chemotherapy is only used in patients with stage II colon cancer characterized by poor

prognosis (obstruction, perforation, emergent admission, T4 stage, resection of less than 12 lymph nodes, and poor histology). However, eventually 20% to 25% of stage II colon cancer patients will die of recurrence or distant metastasis [32]. Thus, to identify more reliable prognostic factors is important to precisely stratify stage II colon cancer patients into high risk group and low risk group (risk for recurrence and distant metastasis). Serum p-CEA is the most widely accepted tumor marker for CRC. Nevertheless, there is still controversy surrounding the indications for adjuvant chemotherapy in stage II colon cancer patients when the patient has an elevated pre-operative serum p-CEA. The American Society of Clinical Oncology (ASCO) suggest the evidence is insufficient to support the use of pre-operative p-CEA in the determination of adjuvant therapy in colon cancer patients, especially in those with stage II colon cancer [29]. Investigators have found some molecular and biological factors are related to poor prognostic features. These factors include 1) tumor budding, 2) abnormal expression of vascular endothelial growth factor (VEGF) and integrin, 3) microRNA expression profile, and 4) loss of Bcl-2 expression [12, 33-36]. However, detection of these factors is usually complicated and costly, which significantly limits the wide application of these factors in clinical practice.

Our results showed that d-CEA was a simple and cost-effective factor that can be used to predict the prognosis of patients with stage II colon cancer with relatively high sensitivity and specificity. d-CEA may directly reflect secretion and proliferation of tumor cells and provide more information about the risk for recurrence and distant metastasis, which is useful for the determination of adjuvant chemotherapy and intensive surveillance in patients with stage II colon cancer.

In this study, we investigated prognostic role of CEA, CA199, CA724 and CA242 in peripheral blood and draining venous blood in colon cancer patients. Our results showed d-CEA has a better predictive value, especially in stage II colon cancer patients. d-CEA may serve as a simple and cost-effective factor to identify patients who need adjuvant chemotherapy and intensive surveillance. Future clinical trials should be prospectively conducted in multiple centers to evaluate whether high-risk patients with colon cancer can benefit from adjuvant chemotherapy.

Disclosure of conflict of interest

None.

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