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Relationship between quantitative parameters of contrast-enhanced ultrasound and EMT-related markers and prognosis in patients with liver metastasis of colorectal cancer

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Abstract: Objective To investigate the relationship between quantitative analysis of contrast-enhanced ultrasound (CEUS) and epithelial mesenchymal transition(EMT) related markers in patients with colorectal cancer liver metastasis (CRLM), and the predictive value of CEUS for the prognosis of patients. **Methods** A total of 102 patients with CRLM admitted to the First Affiliated Hospital of Hebei North University from June 2018 to July 2021 were selected as the study objects. Logistic regression was used to analyze the correlation between CEUS quantitative parameters and prognosis, and the correlation between the expression level of EMT related markers and CEUS quantitative parameters. **Results** The 3-year overall survival rate of 102 patients with CRLM was 42.16%. The E-cadherin immunohistochemical staining scores of survival group were significantly higher , while the Vimentin, β-catenin and N-cadherin immunohistochemical staining scores were significantly lower than those of death group(P<0.05). The time to peak (TTP)and area under the curve (AUC) in survival group were significantly higher, while peak intensity (PI) was significantly lower than those in death group (P<0.05). After adjusting for confounding factors, TTP [OR=0.40, 95%CI:(0.31, 0.51), P<0.001], PI [OR=3.43, 95%CI: (2.16,5.43), P<0.001], AUC [OR=0.48, 95%CI: (0.40, 0.58), P<0.001] were significantly correlated with prognosis, and the correlation intensity of TTP, PI and AUC with prognosis showed a nonlinear dose-response relationship (P<0.001). TTP, PI and AUC of CRLM patients with different E-cadherin scores were significantly different(P<0.05). E-cadherin scores were significantly different from TTP [β=1.08, 95%CI: (0.42, 1.75), P=0.001], PI [β=-0.60, 95%CI: (-1.05,-0.22), P=0.002] and AUC [β=0.99, 95%CI: (0.19, 1.77), P=0.015] were significantly correlated. TTP and AUC increased with the increase of E-cadherin score, while PI decreased. **Conclusion** CEUS quantitative parameters TTP, PI and AUC are closely related to the prognosis of patients with CRLM, and TTP, PI and AUC may be prognostic factors of CRLM.

Keywords: Contrast-enhanced ultrasound; Colorectal cancer; Liver metastasis; Epithelial mesenchymal transformation; Prognosis

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Colorectal cancer, a general term for colon cancer and rectal cancer, is a common malignant tumor. It ranks third in incidence among all malignancies and second in cancer-related mortality rates [1]. Notably, approximately half of colorectal cancer patients experience liver metastasis during the course of the disease, which poses a significant threat to the patient's quality of life and disease prognosis [2]. Recent studies have shown that epithelial-mesenchymal transformation (EMT) plays a crucial role in the initiation, progression, and metastasis of colon cancer [3]. The occurrence of EMT is a complex process that is co-regulated by multiple signaling pathways and cytokines, and is closely associated with the expression of a series of proteins related to invasion and metastasis. This sequence of events ultimately promotes the migration and spread of cancer cells [4]. Currently, there are various methods for the early diagnosis and efficacy assessment of colorectal cancer liver metastases (CRLM), with magnetic resonance imaging and computed tomography having relatively poor real-time dynamic capability and radiation exposure [5-7]. In contrast, contrast-enhanced ultrasound (CEUS) quantitative analysis has technical advantages in dynamic observation of tumors, providing clinicians with more accurate information [8-9]. However, there are no reports on the correlation between CEUS quantitative analysis, EMT, and prognosis in CRLM patients. Therefore, this study examines the preoperative CEUS quantitative parameters in CRLM patients and analyzes their relationship with EMT-related markers and prognosis.

1 Materials and Methods

1.1 Selection of Study Subjects

A retrospective analysis was conducted on 102 patients with CRLM who were admitted to the First Affiliated Hospital of Hebei North University from June 2018 to July 2021, with ages of 38 to 70 (51.26±5.35) years.

Inclusion criteria:

(1) Pathologically confirmed diagnosis of colorectal cancer;

(2) Liver metastasis confirmed by imaging or pathological diagnosis [10];

(3) All patients underwent tumor resection surgery to obtain colorectal cancer tissue;

(4) No preoperative radiotherapy or chemotherapy;

(5) All patients provided informed consent.

Exclusion criteria:

(1) Primary liver cancer;

(2) Metastasis to the liver from other organs besides the colon and rectum;

(3) Perioperative death;

(4) Coexisting autoimmune diseases;

(5) Severe liver, kidney, or heart dysfunction;

(6) Cardiovascular and cerebrovascular diseases;

(7) Missing clinical data.

This study was approved by the Ethics Committee of the First Affiliated Hospital of Hebei North University (Ethical Approval Number: K20230168).

1.2 Research Methods

1.2.1 Ultrasound Examination

Ultrasonic examination was performed using DD70 Doppler ultrasound diagnostic equipment from DDIT Co., Ltd., equipped with a C5-1 ultrasound probe, with the frequency set at 3.5 MHz. Patients were placed in a supine position. First, a dual examination of two-dimensional ultrasound and color Doppler ultrasound was conducted to preliminarily assess the tumor's location, quantity, size, morphology, echogenicity, and blood supply. For patients with multiple lesions in the body, lesions exhibiting a necrosis rate less than 50% were prioritized as targets for CEUS examination. During CEUS examination, 2.4 mL was drawn from the contrast agent (SonoVue) suspension and rapidly injected into the patient's antecubital vein via bolus injection. Immediately afterward, 5.0 mL of saline was injected to flush the catheter. Dynamic observation of both normal liver tissue areas and metastatic lesion areas was performed for at least 3 minutes, with key focus on recording and analyzing the ultrasound manifestations during the arterial phase, portal phase, and delayed phase.

The Qlab 10.0 contrast-enhanced ultrasound quantitative analysis software was used to acquire multi-frame ultrasound imaging data reflecting the internal blood perfusion of the lesions. Regions of interest (ROI) were defined to obtain time-intensity curves (TIC). CEUS quantitative parameters were calculated, including time to peak (TTP), peak intensity (PI), ascending slope (AS), and area under the curve (AUC).

1.2.2 Immunohistochemical Analysis of Tissue Samples and Scoring

Colorectal cancer tissue was fixed in 10% formalin and processed into paraffin-embedded sections. After dewaxing and rehydration, the sections were rinsed with phosphate-buffered saline (PBS) three times for 3 minutes each. To block endogenous peroxidase activity, 3% H_2O_2 was applied for 10 minutes at room temperature, followed by rinsing with PBS three times for 3 minutes each. One drop of the corresponding primary antibodies E-cadherin (1:10,000), β-catenin (1:5,000), Vimentin (1:1,000), and N-cadherin (1:5,000) was added to each section, and incubation was performed at room temperature for 2 hours. The specific primary antibody was then diluted, and incubation was continued at 37°C for 20 minutes, followed by rinsing with PBS three times for 3 minutes each. The sections were stained with 3,3'-diaminobenzidine (DAB) and mounted with resin. Each section was observed under 400× magnification, and five random fields were selected to count 100 tumor cells. The percentage of positive tumor cells (brown-yellow) was scored as the relative intensity of protein expression. The scoring criteria for the percentage of positive tumor cells were: 0 point for <5%, 1 point for 5% to 25%, 2 points for 25% to 50%, 3 points for 50% to 75%, and 4 points for >75%.

1.2.3 Data Collection

Basic and clinical data were collected for all patients, including age, body mass index (BMI), primary tumor site, gender, liver metastasis size, primary tumor size, number of liver metastases, degree of differentiation, TNM stage [11], radical resection, and postoperative chemotherapy. Fasting venous blood samples were taken from patients the morning after admission. Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) were measured using the ELISA method. Quantitative parameters from contrast-enhanced ultrasound (CEUS), including TTP, PI, AS, and AUC, were collected. EMT-related markers in colorectal cancer tissue, such as E-cadherin, β-catenin, Vimentin, and N-cadherin, were evaluated by immunohistochemical staining and scoring. After discharge, all patients were followed up every 3 months to monitor their survival status. The follow-up endpoint was 3 years after discharge or death, and the follow-up will continue until August 2024.

1.3 Statistical Methods

Data analysis was performed using SPSS 22.0 software. Normally distributed continuous variables were expressed as $\bar{x} \pm s$, and inter-group comparisons were made using independent sample *t*-tests. Non-normally distributed continuous variables were expressed as $M(P_{25},P_{75})$, and inter-group comparisons were made using the Mann-Whitney *U* test. Categorical data were expressed as frequencies, and inter-group comparisons were made using the chi-square test. Logistic regression analysis was used to assess the relationships between CEUS quantitative parameters, EMT-related markers, and the prognosis of CRLM patients. Restricted cubic spline analysis was used to examine the strength of association between CEUS quantitative parameters and prognosis. A $P<0.05$ was considered statistically significant.

2 Results

2.1 Prognostic Factors Analysis in CRLM Patients

The 3-year overall survival rate for 102 CRLM patients was 42.16% (43/102). The study found no statistically significant differences in the prognosis of CRLM patients based on age, gender, BMI, primary tumor location, primary tumor diameter, T stage, N stage, or CA19-9 levels (P>0.05). However, patients with liver metastasis diameter ⩾ 5 cm, ⩾ 3 liver metastases, high/medium differentiation, non-curative resection, no postoperative chemotherapy, and CEA ⩾10 ng/mL had a lower 3-year survival rate (P<0.05). See **Table 1**.

2.2 Comparison of EMT-Related Marker Scores

The E-cadherin immunohistochemical staining score in the survival group was significantly higher than that in the death group. Conversely, the immunohistochemical staining scores for Vimentin, β-catenin, and N-cadherin were significantly lower in the death group (P<0.05). See **Table 2**.

Tab.1 Analysis of prognostic factors in patients with CRLM

Indicators	Cases	OS (%)	95%*CI*	χ^2 value	*P* value
Age					
<60 years	53	47.17	32.57-51.74	1.179	0.279
≥60 years	49	36.73	33.73-60.61		
Gender					
Male	55	45.45	32.29-58.61	0.532	0.466
Female	47	38.30	24.40-52.20		
BMI					
<25 kg/m²	70	44.29	32.65-55.92	0.415	0.520
≥25 kg/m²	32	37.50	20.73-54.27		
Primary tumor location					
Rectum	57	45.61	32.68-58.54	0.633	0.426
Colon	45	37.78	23.61-51.94		
Primary tumor diameter					
<5 cm	60	46.67	34.04-59.29	1.215	0.270
≥5 cm	42	35.71	21.22-50.21		
Diameter of liver metastases					
<5 cm	67	49.25	37.28-61.23	4.033	0.045
≥5 cm	35	28.57	13.60-43.54		
Number of liver metastases					
<3	50	54.00	40.19-67.81	5.641	0.018
≥3	52	30.77	18.22-43.31		
Degree of differentiation					
High/moderate	68	32.35	21.23-43.47	8.041	0.005
Poorly	34	61.76	45.43-78.10		
T stage					
T_2/T_3	71	43.66	32.13-55.20	0.217	0.641
T_4	31	38.71	21.56-55.86		
N stage					
N_0	35	40.00	23.77-56.23	0.102	0.750
N_1/N_2	67	43.28	31.42-55.15		
Radical resection					
Yes	44	59.09	44.56-73.62	9.100	0.003
No	58	29.31	17.60-41.03		
Postoperative chemotherapy					
Yes	64	56.25	44.10-68.40	13.992	<0.001
No	38	18.42	6.10-30.75		
CEA					
<10 ng/mL	50	52.00	38.15-65.85	3.897	0.048
≥10 ng/mL	52	32.69	19.94-45.44		
CA19-9					
<37 u/mL	43	48.84	33.90-63.78	1.360	0.243
≥37 u/mL	59	37.29	24.95-49.63		

2.3 Comparison of CEUS Quantitative Parameters Between Two Groups

There was no statistically significant difference in AS between the survival and death groups (P>0.05). The survival group had significantly higher TTP and AUC, and significantly lower PI compared to the death group (P<0.05). See **Table 3**.

2.4 Correlation Analysis of CEUS Quantitative Parameters and Prognosis

Statistically significant CEUS quantitative parameters between the survival and death groups were stratified (Q1-Q5), and a logistic model was established to progressively exclude confounding factors with collinearity. The model adjusted for liver metastasis diameter, number of liver metastases, differentiation, curative resection, postoperative chemotherapy, and CEA levels to eliminate the impact of confounders on prognosis. In the unadjusted model (unadjusted model), each CEUS quantitative parameter was significantly correlated with prognosis (P<0.01). After adjustment, TTP (OR=0.40, 95%CI: 0.31-0.51, P<0.01), PI (OR=3.43, 95%CI: 2.16-5.43, P<0.01), and AUC (OR=0.48, 95% CI: 0.40-0.58, P<0.01) remained significantly correlated with prognosis. As TTP, PI, and AUC increased (Q2-Q5), the trend analysis of their association showed statistically significant differences (P_{trend}<0.05). See **Table 4**.

2.5 Dose-Response Relationship Between CEUS Quantitative Parameters and Prognosis

Using TTP = 23.47 s, PI = 10.44 dB, and AUC = 732.77 as reference points, a restricted cubic spline model (4 nodes, node positions at 0.05, 0.35, 0.65, and 0.95) was established to analyze the dose-response relationship between CEUS quantitative parameters and prognosis. After adjusting for liver metastasis diameter, number of liver metastases, differentiation, curative resection, postoperative chemotherapy, and CEA levels, the relationship between TTP, PI, and AUC and prognosis showed a nonlinear dose-response relationship (nonlinear test, P<0.01). See **Figure 1**.

Tab.2 Comparison of the expression levels of EMT related markers between the two groups

Group	Cases	E-cadherin	Vimentin	β-catenin	N-cadherin
Survival group	43	3.48±0.89	2.28±0.79	2.21±0.47	1.12±0.45
Death group	59	2.70±0.71	3.82±0.85	3.78±0.74	2.13±0.65
***t* value**		4.920	9.306	12.223	8.767
***P* value**		<0.001	<0.001	<0.001	<0.001

Tab.3 Comparison of CEUS quantitative parameters between the two groups

Group	Cases	TTP	PI(dB)	AS(dB/s)	AUC
Survival group	43	27.87±6.38	9.70±3.07	0.56±0.30	768.75±80.90
Death group	59	22.15±5.94	12.60±4.39	0.48±0.24	717.20±70.60
***t* value**		4.655	3.717	1.495	3.423
***P* value**		<0.001	<0.001	0.138	0.001

2.6 Comparison of CEUS Quantitative Parameters in CRLM Patients with Different EMT-Related Marker Scores

CEUS quantitative parameters (TTP, PI, AS, and AUC) were compared between CRLM patients with different EMT-related marker scores. The results are shown in **Table 5.** There was no statistically significant difference in CEUS quantitative parameters among CRLM patients with different Vimentin, β-catenin, and N-cadherin immunohistochemical staining scores (P<0.05). However, there were statistically significant differences in TTP, PI, and AUC among CRLM patients with different E-cadherin scores (P<0.05).

Tab.4 Correlation analysis of quantitative parameters of CEUS and prognosis

Variable	Uncorrected Model	Adjusted Model [a]
TTP		
Q_1(<19.15)	Reference	
Q_2(19.15-<22.23)	0.73(0.61-0.87)	0.69(0.58-0.83)
Q_3(22.23-<24.51)	0.58(0.42-0.82)	0.59(0.45-0.77)
Q_4(24.51-<27.25)	0.49(0.40-0.60)	0.50(0.43-0.58)
Q_5(≥27.25)	0.38(0.27-0.54)	0.40(0.31-0.51)
P_{trend} value	<0.001	<0.001
PI(dB)		
Q_1(<8.35)	Reference	
Q_2(8.35-<10.12)	1.31(0.89-1.92)	1.30(0.95-1.90)
Q_3(10.12-<11.26)	1.78(1.19-2.68)	1.86(1.18-2.51)
Q_4(11.26-<12.55)	2.19(1.58-3.03)	2.13(1.38-3.29)
Q_5(≥12.55)	3.49(2.35-5.18)	3.43(2.16-5.43)
P_{trend} value	<0.001	<0.001
AUC		
Q_1(<663.28)	Reference	
Q_2(663.28-<704.65)	0.80(0.62-1.02)	0.80(0.65-0.98)
Q_3(704.65-<746.80)	0.72(0.56-0.92)	0.68(0.53-0.87)
Q_4(746.80-<794.32)	0.60(0.43-0.84)	0.64(0.55-0.74)
Q_5(≥794.32)	0.49(0.38-0.64)	0.48(0.40-0.58)
P_{trend} value	0.001	<0.001

Note: a, the adjusted indicators included the diameter of liver metastases., number of liver metastases., differentiation degree, curative resection, postoperative chemotherapy, and CEA level.

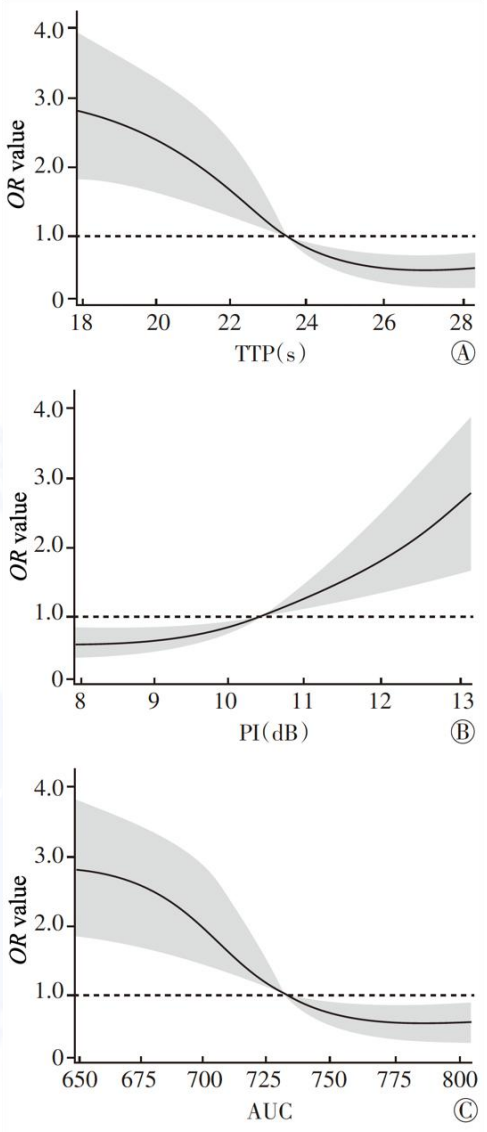

Fig.1 Association strength between CEUS quantitative parameters and prognosis in CRLM patients by restricted cubic spline model

Tab.5 Comparison of CEUS quantitative parameters in CRLM patients with different EMT related marker scores

Item	Score	TTP	PI(dB)	AS(dB/s)	AUC	Item	Score	TTP	PI(dB)	AS(dB/s)	AUC
E-cadherin	0(n=9)	19.86±5.29	13.66±3.45	0.46±0.27	688.64±70.67	**β-catenin**	0(n=8)	23.43±6.01	12.70±3.65	0.51±0.30	802.53±64.99
	1(n=7)	21.73±5.56	13.14±3.60	0.54±0.26	713.52±77.44		1(n=8)	25.25±5.26	9.83±3.43	0.60±0.23	743.25±75.57
	2(n=14)	22.45±6.27	12.52±3.31	0.52±0.21	737.60±75.54		2(n=15)	22.30±5.70	11.72±3.55	0.52±0.28	757.28±68.37
	3(n=21)	23.27±5.77	11.37±3.48	0.55±0.23	745.86±69.56		3(n=18)	24.26±5.41	10.45±3.42	0.53±0.24	753.65±66.22
	4(n=51)	25.69±5.77	10.11±3.23	0.47±0.23	776.58±75.79		4(n=53)	24.22±5.48	11.33±3.51	0.46±0.22	745.22±76.83
	F value	2.839	3.738	0.593	3.675		F value	0.509	0.978	0.807	1.121
	P value	0.028	0.007	0.668	0.008		P value	0.729	0.423	0.523	0.351
Vimentin	0(n=5)	25.47±5.93	11.27±3.38	0.52±0.22	763.28±69.43	**N-cadherin**	0(n=32)	24.54±6.26	11.53±3.27	0.49±0.27	758.63±72.83
	1(n=7)	26.86±5.62	12.53±3.44	0.48±0.20	774.36±78.98		1(n=14)	23.82±5.68	12.92±3.48	0.62±0.29	779.25±68.16
	2(n=13)	24.23±5.67	11.86±3.28	0.47±0.20	742.85±77.22		2(n=21)	24.63±5.45	10.68±3.65	0.51±0.22	743.80±64.14
	3(n=15)	23.67±5.73	10.75±3.27	0.56±0.24	757.21±68.86		3(n=19)	23.42±5.61	9.74±3.37	0.43±0.24	725.69±69.01
	4(n=62)	23.35±6.07	11.05±3.51	0.49±0.23	750.57±64.88		4(n=16)	22.70±5.93	11.58±3.75	0.47±0.21	762.13±64.10
	F value	0.669	0.475	0.375	0.306		F value	0.375	1.934	1.264	1.491
	P value	0.615	0.754	0.826	0.874		P value	0.826	0.111	0.289	0.211

3 Discussion

The formation and migration of tumors begin with the disruption of their connections with neighboring cells, followed by the breach of the basal membrane barrier, infiltration into surrounding stromal tissues, and ultimately integration into the blood circulation system, leading to the spread of cancer cells [12]. Some scholars believe that the metastatic mechanism of tumors is closely related to EMT. EMT, as the biological process where epithelial cells transform into mesenchymal cells, is widely involved in various life stages of organisms, including embryonic development, tissue repair, and the occurrence and progression of malignant tumors. Especially in the occurrence and progression of malignant tumors, EMT promotes the loss of epithelial characteristics and the acquisition of mesenchymal cell traits by affecting the morphology and intercellular adhesion properties of tumor cells. This process constitutes a key pathological mechanism through which EMT facilitates tumor invasion and metastasis. Ultimately, the migratory and invasive abilities of cells are significantly enhanced, allowing them to penetrate surrounding tissues, achieve local infiltration, and disseminate to distant sites via the blood or lymphatic system [13]. In this study, we compared the expression of various EMT-related markers in patients with different prognoses of CRLM. We found that the immunohisto-chemical staining score for E-cadherin in the survival group was significantly higher than that in the death group, while the immunohistochemical staining scores for Vimentin, β-catenin, and N-cadherin were significantly lower in the death group. Vimentin is mainly localized in the cytoplasm of mesenchymal cells, where its primary role is to support the cell structure and maintain the overall stability of tissues. As a key marker of malignant tumors and EMT, Vimentin is positively expressed in mesenchymal-origin cells. Therefore, if Vimentin is detected as positively expressed in epithelial cells, it suggests that EMT has occurred in the tumor [14]. E-cadherin is a crucial cell membrane adhesion protein that plays a vital role in maintaining the polarity and integrity of epithelial cells. Under normal physiological conditions, E-cadherin regulates cell-to-cell adhesion and interactions between cells and the extracellular matrix, ensuring the integrity and stability of tissue structures. However, once the expression level of E-cadherin decreases, it leads to weakened or even lost adhesion between like cells, which not only interferes with the development and morphological formation of normal tissues but also causes the loss of cell polarity in malignant tumor cells, enabling the tumor cells to acquire invasive growth ability [15]. The adhesive activity of E-cadherin on the cell surface is regulated through a mechanism that transmits signals from inside the cell to the outside. This process may include conformational changes similar to integrin regulation of homophilic adhesion bonds. When cadherin types change from E-type to N-type, i.e., when E-cadherin is transformed into N-cadherin, it marks the process of tumor cells undergoing EMT. This transformation directly leads to a significant reduction in intercellular adhesion, followed by an increase in cell motility and a marked enhancement in the cell's ability to invade and metastasize. β-catenin binds with E-cadherin to form the E-cadherin/β-catenin complex, which plays a crucial role in establishing cell polarity, stabilizing intercellular adhesion, and maintaining tissue structural morphology. However, when the quantity of this complex decreases, it directly weakens the adhesion strength between cells, making tumor cells more prone to detachment from the primary site, thereby increasing their risk of diffusion and metastasis [16-17].

For patients with colorectal cancer liver metastasis, surgical resection is a treatment that can significantly improve their survival rate. Currently, the evaluation of treatment plans generally refers to the solid tumor efficacy evaluation standards, which rely on imaging techniques such as MRI and CT to track changes in the tumor's anatomical structure. However, it is worth noting that, in the short term after surgery, the tumor's size often does not show significant changes, which somewhat limits the accuracy of evaluating tumor efficacy based solely on anatomical structural changes [18]. Recent studies have found that before obvious changes in the tumor's anatomical structure, CEUS can capture dynamic changes in blood flow perfusion within the tumor, suggesting that CEUS may be an effective monitoring method. This study found that TTP and AUC were significantly higher in the survival group, while the PI was significantly lower in the survival group compared to the death group. CEUS quantitative parameters TTP, PI, and AUC were significantly correlated with prognosis. The relationship between TTP, PI, and AUC and prognosis followed a nonlinear dose-response relationship. CEUS can extract both enhancement intensity (such as PI and AUC) and time-related information (such as TTP) from the TIC. These parameters are based on a series of key assumptions, one of which is that signal intensity is proportional to microbubble concentration. Since TTP is related to blood flow velocity, and PI and AUC are related to blood volume, it can be inferred that in the survival group, the lesion's blood volume is reduced, and the number of microbubbles entering the lesion decreases [19]. Zhang *et al.* [20] found in their study on the expression differences of EMT markers in various ultrasound manifestations of breast cancer that ultrasound features can serve as a non-invasive way to predict the expression levels of EMT markers in breast cancer patients. Our study found that different E-cadherin staining scores in CRLM patients showed statistically significant differences in CEUS quantitative parameters (TTP, PI, and AUC). As the E-cadherin staining score increased, TTP and AUC increased, while PI decreased. These CEUS quantitative parameters were correlated with the expression levels of EMT-related markers in CRLM patients.

However, there are certain limitations in this study.

All data were derived from a single medical center, which may introduce some bias. The study population was limited to Chinese Han people, without including other ethnic groups or populations from other countries, so the generalizability of the results needs further validation. The study lacks prognostic data beyond three years, and the depth and breadth of the analysis could be further enhanced. These aspects can be improved in future research.

In summary, CEUS quantitative parameters are related to the expression levels of EMT markers in CRLM patients. CEUS quantitative parameters can to some extent reflect the expression of EMT markers. TTP, PI, and AUC are associated with prognosis in CRLM patients, and these parameters may serve as prognostic predictors for CRLM.

Conflict of Interest None

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·论 著·

超声造影定量参数与结直肠癌肝转移患者上皮间质转化相关标志物及预后的关系

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摘要：目的 探讨超声造影（CEUS）定量分析与结直肠癌肝转移（CRLM）患者上皮间质转化（EMT）相关标志物的关系，及对患者预后的预测价值。**方法** 选择2018年6月至2021年7月河北北方学院附属第一医院收治的102例CRLM患者作为研究对象，采用logistic回归分析CEUS定量参数与预后的相关性，及EMT相关标志物表达水平与CEUS定量参数的相关性。**结果** 102例CRLM患者3年总生存率为42.16%，按随访结束时的生存状态，将患者分为生存组（n=43）和死亡组（n=59）。生存组患者E-cadherin免疫组化染色评分显著高于死亡组，Vimentin、β-catenin及N-cadherin免疫组化染色评分显著低于死亡组（P<0.05）；生存组患者达峰时间（TTP）、曲线下面积（AUC）显著高于死亡组，峰值强度（PI）显著低于死亡组（P<0.05）。调整混杂因素后，TTP（OR=0.40，95%CI：0.31~0.51，P<0.01）、PI（OR=3.43，95%CI：2.16~5.43，P<0.01）、AUC（OR=0.48，95%CI：0.40~0.58，P<0.01）与预后显著相关，TTP、PI及AUC与预后的关联强度均呈非线性剂量-反应关系（非线性检测，P<0.01）。不同E-cadherin评分CRLM患者TTP、PI及AUC差异有统计学意义（P<0.05），E-cadherin评分与TTP（β=1.08，95%CI：0.42~1.75，P=0.001）、PI（β=−0.60，95%CI：−1.05~−0.22，P=0.002）及AUC（β=0.99，95%CI：0.19~1.77，P=0.015）显著相关，随着E-cadherin评分增加，TTP、AUC升高，PI则降低。**结论** CEUS定量参数TTP、PI及AUC与CRLM患者预后密切相关，TTP、PI及AUC可能成为CRLM预后预测因子。

关键词： 超声造影；结直肠癌肝转移；上皮间质转化；预后

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Relationship between quantitative parameters of contrast-enhanced ultrasound and EMT-related markers and prognosis in patients with liver metastasis of colorectal cancer

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Abstract: Objective To investigate the relationship between quantitative analysis of contrast-enhanced ultrasound (CEUS) and epithelial mesenchymal transition (EMT) related markers in patients with colorectal cancer liver metastasis (CRLM), and the predictive value of CEUS for the prognosis of patients. **Methods** A total of 102 patients with CRLM admitted to The First Affiliated Hospital of Hebei North University from June 2018 to July 2021 were selected as the study objects. Logistic regression was used to analyze the correlation between CEUS quantitative parameters and prognosis, and the correlation between the expression level of EMT related markers and CEUS quantitative parameters. **Results** The 3-year overall survival rate of 102 patients with CRLM was 42.16%. According to the survival status at the end of the follow-up, the patients were divided into the survival group (n=43) and the death group (n=59). The E-cadherin immunohistochemical staining scores of survival group were

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significantly higher, while the Vimentin, β-catenin and N-cadherin immunohistochemical staining scores were significantly lower than those of death group (P<0.05). The time to peak (TTP) and area under the curve (AUC) in survival group were significantly higher, while peak intensity (PI) was significantly lower than those in death group (P<0.05). After adjusting for confounding factors, TTP [OR=0.40, 95%CI(0.31, 0.51), P<0.01], PI[OR=3.43, 95%CI(2.16, 5.43), P<0.01], AUC[OR=0.48, 95%CI(0.40, 0.58), P<0.01] were significantly correlated with prognosis, and the correlation intensity of TTP, PI and AUC with prognosis showed a nonlinear dose-response relationship (P<0.01). TTP, PI and AUC of CRLM patients with different E-cadherin scores were significantly different (P<0.05). E-cadherin scores were significantly different from TTP [β=1.08, 95%CI(0.42, 1.75), P=0.001], PI [β=−0.60, 95%CI(−1.05, −0.22), P=0.002] and AUC [β=0.99, 95%CI(0.19, 1.77), P=0.015] were significantly correlated. TTP and AUC increased, while PI decreased, with the increase of E-cadherin score. **Conclusion** CEUS quantitative parameters TTP, PI and AUC are closely related to the prognosis of patients with CRLM, and TTP, PI and AUC may be prognostic factors of CRLM.

Keywords: Contrast-enhanced ultrasound; Colorectal cancer liver metastases; Epithelial mesenchymal transformation; Prognosis

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结直肠癌是一种较为常见的恶性肿瘤，其发病率在所有恶性肿瘤中居第三位，而在癌症相关死亡率方面则位列第二[1]。值得注意的是，约有半数结直肠癌患者在病程中会发生肝转移，对患者的日常生活质量和疾病预后构成了重大威胁[2]。研究显示上皮间质转化（epithelial mesenchymal transformation，EMT）在结肠癌的发生、进展及转移的各个关键阶段均扮演了重要的角色[3]。EMT的发生是一个复杂过程，受到多种信号通路及细胞因子的协同调控，且与一系列与侵袭和转移相关的蛋白质表达密切相关。这一系列事件最终促进了癌细胞的迁移与扩散[4]。目前临床上对结直肠癌肝转移（colorectal cancer liver metastases，CRLM）的早期诊断及疗效评估方法较多，其中磁共振成像和计算机断层摄片术因其实时动态性较差，且具有放射性[5-7]。而超声造影（contrast-enhanced ultrasound，CEUS）定量分析在肿瘤的动态观察方面具有技术优势，能够为临床医师提供较为准确的信息[8-9]。但目前针对CRLM患者CEUS定量分析与EMT及预后相关性研究未见报道，因此本研究对CRLM患者术前CEUS定量参数进行检测，分析其与EMT相关标志物及预后的关系，现报道如下。

1 资料与方法

1.1 *研究对象* 选择河北北方学院附属第一医院在2018年6月至2021年7月收治的102例CRLM患者作为研究对象进行回顾性分析，年龄38~70（51.26±5.35）岁。纳入标准：（1）经病理学证实为结直肠癌；（2）经影像学或病理学诊断证实有肝转移[10]；（3）患者均接受肿瘤切除术治疗，获取结直肠癌组织；（4）术前未经放疗、化疗；（5）患者均知情同意本研究。排除标准：（1）原发性肝癌；（2）除结直肠外其他脏器肿瘤转移至肝脏；（3）围手术期死亡；（4）合并自身免疫系统疾病；（5）合并严重的肝、肾、心功能异常；（6）心脑血管疾病；（7）临床资料缺失。本研究经医院伦理委员会批准（伦理批号：K20230168）。

1.2 *研究方法*

1.2.1 超声检查 采用德润特DD70彩色多普勒超声诊断设备，配备C5-1型超声探头，频率为3.5 MHz。患者取仰卧体位，首先进行二维超声与彩色多普勒超声的双重检查，以初步评估肿瘤的位置、数量、大小、形态、回声特性及血供应情况等。对于体内存在多个病灶的患者，优先选择坏死率低于50%的病灶作为CEUS检测的目标。进行CEUS检查时，从造影剂（SonoVue）混悬液中抽取2.4 mL，通过团注方式快速注入患者的肘前静脉内，紧接着推注5.0 mL生理盐水以冲洗导管，对肝脏的正常组织区域及转移病灶区域进行至少3 min的动态观察，重点记录并分析动脉期、门脉期及延迟期的超声表现。采用Qlab 10.0超声造影定量分析软件获取病灶内部血流灌注情况的多帧超声影像资料。设定感兴趣区，获取时间-强度曲线（time-intensity curve，TIC），计算CEUS定量参数，包括达峰时间（time to peak，TTP）、峰值强度（peak intensity，PI）、上升斜率（ascending slope，AS）和曲线下面积（area under the curve，AUC）。

1.2.2 组织样本免疫组化分析及判定 10%的甲醛固定结直肠癌组织，经常规石蜡切片经烤片后，脱蜡至水，用磷酸缓冲盐溶液（phosphate buffered saline，PBS）冲洗3 min×3次。3%的H_2O_2阻断内源性过氧化

物酶，室温下孵育约10 min，再用PBS冲洗3 min×3次。每张切片分别加入1滴相应的第一抗体E-钙黏蛋白(E-cadherin)(1∶10 000)、β-catenin(1∶5 000)、Vimentin(1∶1 000)、N-cadherin(1∶5 000)，室温孵育2 h。稀释特异性一抗，37 ℃孵育20 min，PBS冲洗3 min×3次。3，3-二氨基联苯胺显色，常规树胶封片。每张切片在400倍下随机观察5个视野，并计数100个肿瘤细胞。采用阳性肿瘤细胞(棕黄色)百分比评分作为蛋白表达的相对强度。阳性肿瘤细胞比例：0分<5%，1分为5%~25%，2分为>25%~50%，3分为>50%~75%，4分>75%。

1.2.3 资料收集 收集患者的基本资料和临床资料，记录患者年龄、身体质量指数(body mass index，BMI)、原发肿瘤部位、性别、肝转移灶直径、原发肿瘤直径、肝转移灶数、分化程度、TNM分期[11]、根治性切除及术后化疗；患者入院次日早晨空腹采静脉血，采取ELISA法测定癌胚抗原(carcino-embryonic antigen，CEA)、糖蛋白抗原19-9(carbohydrate antigen 19-9，CA19-9)；收集CEUS定量参数TTP、PI、AS及AUC；检测结直肠癌组织EMT相关标志物包括E-cadherin、β-catenin、Vimentin及N-cadherin免疫组化染色评分；出院后对所有患者进行随访，每3个月1次，了解患者生存状态，患者出院3年或死亡作为随访终点，随访截至2024年8月。

1.3 统计学方法 采用SPSS 22.0软件分析数据。正态分布的计量资料以$\bar{x}\pm s$表示，组间比较采用独立样本t检验；非正态分布的计量资料以$M(P_{25},P_{75})$表示，组间比较采用Mann-Whitney U检验。计数资料以例表示，组间比较采用χ^2检验。Logistic回归分析CEUS定量参数与EMT相关标志物关系及其与CRLM患者预后的相关性。采用限制性立方样条分析CEUS定量参数与预后关联强度。$P<0.05$为差异有统计学意义。

2 结 果

2.1 CRLM患者预后影响因素分析 102例CRLM患者3年总生存率为42.16%(43/102)，研究发现年龄、性别、BMI、原发肿瘤部位、原发肿瘤直径、T分期、N分期及CA19-9水平对CRLM患者预后影响差异无统计学意义($P>0.05$)；肝转移灶直径≥5 cm、肝转移灶数≥3个、高/中分化、未根治性切除、术后未化疗及CEA≥10 ng/mL的患者预后3年生存率更低($P<0.05$)。见表1。

2.2 两组EMT相关标志物评分比较 按随访结束时的生存状态，将患者分为生存组(n=43)和死亡组(n=59)。生存组患者E-cadherin免疫组化染色评分显著高于死亡组，Vimentin、β-catenin及N-cadherin免疫组化染色评分则显著低于死亡组($P<0.05$)。见表2。

2.3 两组CEUS定量参数对比 生存组和死亡组患者AS差异无统计学意义($P>0.05$)，生存组患者TTP、AUC显著高于死亡组，PI则显著低于死亡组($P<0.05$)。见表3。

2.4 CEUS定量参数与预后的相关性分析 将生存组和死亡组差异有统计学意义的CEUS定量参数逐层划分(Q1~Q5)，建立logistic模型逐步排除存在共线性的混杂因素，最终校正肝转移灶直径、肝转移灶数、分化程度、根治性切除、术后化疗、CEA水平，以消除混杂因素对预后的影响。在未经调整的模型(未校正模型)中，各CEUS定量参数与预后显著

表1 CRLM患者预后影响因素分析

Tab.1 Analysis of prognostic factors in patients with CRLM

项目	例数	生存率(%)	95%*CI*	χ^2值	*P*值
年龄					
<60岁	53	47.17	32.57~51.74	1.179	0.279
≥60岁	49	36.73	33.73~60.61		
性别					
男	55	45.45	32.29~58.61	0.532	0.466
女	47	38.30	24.40~52.20		
BMI					
<25 kg/m^2	70	44.29	32.65~55.92	0.415	0.520
≥25 kg/m^2	32	37.50	20.73~54.27		
原发肿瘤部位					
直肠	57	45.61	32.68~58.54	0.633	0.426
结肠	45	37.78	23.61~51.94		
原发肿瘤直径					
<5 cm	60	46.67	34.04~59.29	1.215	0.270
≥5 cm	42	35.71	21.22~50.21		
肝转移灶直径					
<5 cm	67	49.25	37.28~61.23	4.033	0.045
≥5 cm	35	28.57	13.60~43.54		
肝转移灶数					
<3个	50	54.00	40.19~67.81	5.641	0.018
≥3个	52	30.77	18.22~43.31		
分化程度					
高/中分化	68	32.35	21.23~43.47	8.041	0.005
低分化	34	61.76	45.43~78.10		
T分期					
T_2/T_3期	71	43.66	32.13~55.20	0.217	0.641
T_4期	31	38.71	21.56~55.86		
N分期					
N_0期	35	40.00	23.77~56.23	0.102	0.750
N_1/N_2期	67	43.28	31.42~55.15		
根治性切除					
是	44	59.09	44.56~73.62	9.100	0.003
否	58	29.31	17.60~41.03		
术后化疗					
是	64	56.25	44.10~68.40	13.992	<0.001
否	38	18.42	6.10~30.75		
CEA					
<10 ng/mL	50	52.00	38.15~65.85	3.897	0.048
≥10 ng/mL	52	32.69	19.94~45.44		
CA19-9					
<37 u/mL	43	48.84	33.90~63.78	1.360	0.243
≥37 u/mL	59	37.29	24.95~49.63		

相关（P<0.01），调整后，TTP（OR=0.40，95%CI：0.31~0.51，P<0.01）、PI（OR=3.43，95%CI：2.16~5.43，P<0.01）、AUC（OR=0.48，95%CI：0.40~0.58，P<0.01）仍与预后显著相关，随着TTP、PI及AUC的提升（Q2~Q5），其关联效应趋势性检验差异均有统计学意义（$P_{趋势}$<0.05）。见表4。

2.5　CEUS定量参数与预后关联强度的剂量-反应关系　分别以TTP=23.47 s、PI=10.44 dB、AUC=732.77 dB·s作为参考点，建立限制性立方样条模型（节点数为4，节点位置为0.05、0.35、0.65、0.95）分析各CEUS定量参数与预后的剂量-反应关系，结果调整肝转移灶直径、肝转移灶数、分化程度、根治性切除、术后化疗、CEA水平后，TTP、PI及AUC与预后的关联强度均呈非线性剂量-反应关系（非线性检测，P<0.01）。见图1。

2.6　不同EMT相关标志物评分CRLM患者CEUS定量参数对比　将不同EMT相关标志物评分CRLM患者CEUS定量参数TTP、PI、AS及AUC进行对比，结果如表5所示，不同Vimentin、β-catenin及N-cadherin免疫组化染色评分CRLM患者各项CEUS定量参数差异无统计学意义（P>0.05），不同E-cadherin评分CRLM患者TTP、PI及AUC差异有统计学意义（P<0.05），各组间AS差异无统计学意义（P>0.05）。E-cadherin评分与TTP（β=1.08，95%CI：0.42~1.75，P=0.001）、PI（β=−0.60，95%CI：−1.05~−0.22，P=0.002）及AUC（β=0.99，95%CI：0.19~1.77，P=0.015）显著相关，随E-cadherin评分增加，TTP、AUC随之升高，PI随之降低。

表2　两组EMT相关标志物表达水平对比　（$\bar{x}\pm s$）

Tab.2　Comparison of the expression levels of EMT related markers between the two groups　（$\bar{x}\pm s$）

组别	例数	E-cadherin	Vimentin	β-catenin	N-cadherin
生存组	43	3.48±0.89	2.28±0.79	2.21±0.47	1.12±0.45
死亡组	59	2.70±0.71	3.82±0.85	3.78±0.74	2.13±0.65
t值		4.920	9.306	12.223	8.767
P值		<0.001	<0.001	<0.001	<0.001

表3　两组患者CEUS定量参数对比

Tab.3　Comparison of CEUS quantitative parameters between the two groups

组别	例数	TTP(s)[a]	PI(dB)[a]	AS[b]	AUC(dB·s)[a]
生存组	43	27.87±6.38	9.70±3.07	0.55(0.36,0.77)	768.75±80.90
死亡组	59	22.15±5.94	12.60±4.39	0.47(0.32,0.64)	717.20±70.60
t/Z值		4.655	3.717	0.854	3.423
P值		<0.001	<0.001	0.372	0.001

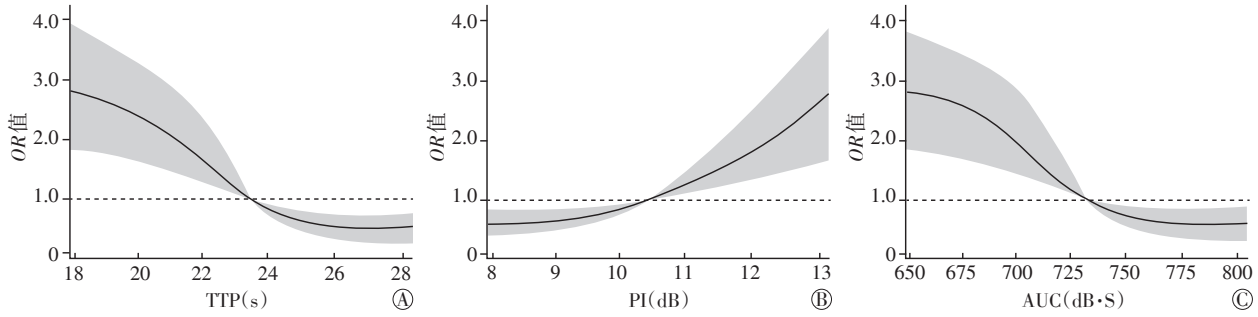
注：[a]为以$\bar{x}\pm s$表示；[b]为以$M(P_{25},P_{75})$表示。

表4　CEUS定量参数与预后的相关性分析

Tab.4　Correlation analysis of quantitative parameters of CEUS and prognosis

变量	OR(95%CI)	调整后OR(95%CI)[a]
TTP(s)		
Q1(<19.15)	参照	
Q2(19.15~<22.23)	0.73(0.61~0.87)	0.69(0.58~0.83)
Q3(22.23~<24.51)	0.58(0.42~0.82)	0.59(0.45~0.77)
Q4(24.51~<27.25)	0.49(0.40~0.60)	0.50(0.43~0.58)
Q5(≥27.25)	0.38(0.27~0.54)	0.40(0.31~0.51)
$P_{趋势}$值	<0.001	<0.001
PI(dB)		
Q1(<8.35)	参照	
Q2(8.35~<10.12)	1.31(0.89~1.92)	1.30(0.95~1.90)
Q3(10.12~<11.26)	1.78(1.19~2.68)	1.86(1.18~2.51)
Q4(11.26~<12.55)	2.19(1.58~3.03)	2.13(1.38~3.29)
Q5(≥12.55)	3.49(2.35~5.18)	3.43(2.16~5.43)
$P_{趋势}$值	<0.001	<0.001
AUC(dB·s)		
Q1(<663.28)	参照	
Q2(663.28~<704.65)	0.80(0.62~1.02)	0.80(0.65~0.98)
Q3(704.65~<746.80)	0.72(0.56~0.92)	0.68(0.53~0.87)
Q4(746.80~<794.32)	0.60(0.43~0.84)	0.64(0.55~0.74)
Q5(≥794.32)	0.49(0.38~0.64)	0.48(0.40~0.58)
$P_{趋势}$值	0.001	<0.001

注：[a]调整肝转移灶直径、肝转移灶数、分化程度、根治性切除、术后化疗、CEA水平。



注：A，TTP与预后的剂量-反应关系；B，PI与预后的剂量-反应关系；C，AUC与预后的剂量-反应关系。

图1　限制性立方样条模型分析CRLM患者CEUS定量参数与预后关联强度

Fig.1　The association strength between CEUS quantitative parameters and prognosis in CRLM patients by restricted cubic spline model

表5 不同EMT相关标志物评分CRLM患者CEUS定量参数对比

Tab.5 Comparison of CEUS quantitative parameters in CRLM patients with different EMT related marker scores

项目	评分	TTP	PI(dB)	AS(dB/s)	AUC(dB·s)
E-cadherin	0(*n*=9)	19.86±5.29	13.66±3.45	0.46±0.27	688.64±70.67
	1(*n*=7)	21.73±5.56	13.14±3.60	0.54±0.26	713.52±77.44
	2(*n*=14)	22.45±6.27	12.52±3.31	0.52±0.21	737.60±75.54
	3(*n*=21)	23.27±5.77	11.37±3.48	0.55±0.23	745.86±69.56
	4(*n*=51)	25.69±5.77	10.11±3.23	0.47±0.23	776.58±75.79
	*F*值	2.839	3.738	0.593	3.675
	*P*值	0.028	0.007	0.668	0.008
Vimentin	0(*n*=5)	25.47±5.93	11.27±3.38	0.52±0.22	763.28±69.43
	1(*n*=7)	26.86±5.62	12.53±3.44	0.48±0.20	774.36±78.98
	2(*n*=13)	24.23±5.67	11.86±3.28	0.47±0.20	742.85±77.22
	3(*n*=15)	23.67±5.73	10.75±3.27	0.56±0.24	757.21±68.86
	4(*n*=62)	23.35±6.07	11.05±3.51	0.49±0.23	750.57±64.88
	*F*值	0.669	0.475	0.375	0.306
	*P*值	0.615	0.754	0.826	0.874
β-catenin	0(*n*=8)	23.43±6.01	12.70±3.65	0.51±0.30	802.53±64.99
	1(*n*=8)	25.25±5.26	9.83±3.43	0.60±0.23	743.25±75.57
	2(*n*=15)	22.30±5.70	11.72±3.55	0.52±0.28	757.28±68.37
	3(*n*=18)	24.26±5.41	10.45±3.42	0.53±0.24	753.65±66.22
	4(*n*=53)	24.22±5.48	11.33±3.51	0.46±0.22	745.22±76.83
	*F*值	0.509	0.978	0.807	1.121
	*P*值	0.729	0.423	0.523	0.351
N-cadherin	0(*n*=32)	24.54±6.26	11.53±3.27	0.49±0.27	758.63±72.83
	1(*n*=14)	23.82±5.68	12.92±3.48	0.62±0.29	779.25±68.16
	2(*n*=21)	24.63±5.45	10.68±3.65	0.51±0.22	743.80±64.14
	3(*n*=19)	23.42±5.61	9.74±3.37	0.43±0.24	725.69±69.01
	4(*n*=16)	22.70±5.93	11.58±3.75	0.47±0.21	762.13±64.10
	*F*值	0.375	1.934	1.264	1.491
	*P*值	0.826	0.111	0.289	0.211

3 讨 论

肿瘤的形成与迁移起始于破坏其与邻近细胞间连接，随后突破基底膜的屏障，渗透至周围间质组织，进而融入血液循环系统，导致癌细胞扩散[12]。有学者认为肿瘤的转移机制与EMT关系密切。EMT作为上皮细胞向间质细胞转变的生物学现象，广泛涉及生物体的多个生命活动阶段，包括胚胎发育、组织修复以及恶性肿瘤的发生与进展。特别是在恶性肿瘤的发生发展中，EMT通过影响肿瘤细胞的形态及细胞间的黏附特性，促使细胞丧失其上皮特性，转而获得间质细胞的特征。这一过程构成了EMT促进肿瘤侵袭与转移的关键病理机制。最终，细胞的迁移与侵袭能力显著提升，得以穿透周围组织，实现局部浸润，并通过血液或淋巴系统实现远处转移[13]。本研究对不同预后CRLM患者各项EMT相关标志物表达情况进行对比发现，生存组患者E-cadherin免疫组化染色评分显著高于死亡组，Vimentin、β-catenin及N-cadherin免疫组化染色评分则显著低于死亡组。Vimentin主要定位于间充质细胞的细胞质内，其核心作用是支撑细胞结构并维护组织的整体稳定性。作为恶性肿瘤及EMT的关键标识，Vimentin在间质来源细胞中呈阳性表达，因此，若在上皮细胞中检测到Vimentin的阳性表达，则提示该肿瘤已发生了EMT[14]。E-cadherin是一种至关重要的细胞膜黏附蛋白，对于维持上皮细胞的极性及其完整性至关重要。在正常生理条件下，E-cadherin负责调控细胞间的黏附以及细胞与基质间的相互作用，确保细胞组织结构的完整性和稳定性。然而，一旦E-cadherin的表达水平下调，就会导致同种细胞间的黏附力减弱甚至丧失，这不仅会干扰正常组织的发育与形态建成，而且在恶性肿瘤细胞中，这种变化还会导致细胞极性的丧失，赋予肿瘤细胞以侵袭性生长的能力[15]。E-cadherin在细胞表面的黏附活性是通过一种从细胞内部向外传递的调控机制来进行调节的，这一过程可能包含了类似于整合素调节方式的同性黏合键的构象变化调整。当钙黏蛋白的种类从E型转变为N型，即E-cadherin转化为N-cadherin时，这标志着肿瘤细胞正在经历EMT的过程。这一转变直接导致细胞间的黏附能力显著减弱，随之而来的是细胞运动性的增加，以及细胞在侵袭和转移方面的能力得到显著增强。β-catenin与E-cadherin结合成E-cadherin/β-catenin复合体，它在确立细胞极性、稳固细胞间的黏附连接以及维护组织结构的形态方面扮演着举足轻重的角色。然而，当这一复合体的数量减少时，它会直接削弱细胞间的黏附强度，使得肿瘤细胞更加容易从原发部位脱离，从而增加了其扩散转移的风险[16-17]。

针对结肠癌肝转移的患者，手术切除是一种能够显著提升其生存率的治疗手段。当前，治疗方案的评价普遍参考实体瘤疗效评价标准，该标准依赖于MRI和CT等影像学技术来追踪肿瘤解剖学结构上的变化。然而，值得注意的是，在手术后的短期内，肿瘤的大小往往尚未发生明显的改变，这在一定程度上限制了仅通过解剖学结构变化来评估肿瘤疗效的准确性[18]。近期研究发现，在肿瘤解剖学结构发生明显变化之前，CEUS技术能够捕捉到肿瘤内部血流灌注的动态变化，提示CEUS可能是一种有效的监测方法。本研究发现生存组患者TTP、AUC显著高于死亡组，PI则显著低于死亡组，CEUS定量参数TTP、PI及AUC与预后显著相关，TTP、PI及AUC与预后的关联强度均呈非线性剂量-反应关系，CEUS检测获

得TIC可提取增强强度(如PI、AUC)及时间相关(如TTP)两种信息。这些参数分析依赖于一系列关键假设,其中之一是信号强度与微泡浓度呈比例。由于TTP与血流速度相关,PI、AUC与血容量相关,信号强度与微泡浓度呈比例,可以推断存活组患者病灶血容量减小、进入病灶内的微泡数目减少[19]。张海见等[20]在EMT标志物在不同乳腺癌超声征象下表达水平的差异性研究表明,超声征象可作为无创性预测乳腺癌患者EMT标志物表达水平的方式。本研究发现不同E-cadherin染色评分CRLM患者CEUS定量参数TTP、PI及AUC差异有统计学意义,随E-cadherin染色评分增加,TTP、AUC随之升高,PI则随之降低,CEUS定量参数与CRLM患者EMT相关标志物表达水平具有一定相关性。但本研究尚存在一定的局限性,所有数据均来自同一医疗中心,可能存在一定偏倚;研究对象仅限于中国汉族人群,未涉及其他民族或其他国家的人群,因此结果的普适性有待进一步验证;研究缺少3年以后预后相关数据,分析结果的深度和广度将进一步提升,可在后续研究中加以改进。

综上所述,CEUS定量参数与CRLM患者EMT相关标志物表达水平存在一定联系,CEUS定量参数在一定程度上可体现EMT标志物的表达情况。CEUS定量参数TTP、PI及AUC与CRLM患者预后相关,TTP、PI及AUC可能成为CRLM预后预测因子。

利益冲突　无

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