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The correlation between time-intensity curve parameters of transrectal contrast-enhanced ultrasound and pathological prognostic factors in rectal adenocarcinoma

Shitao Su*, Jianyuan Huang*, Xuanzhang Huang, Xigui Li, Yaoli Liu, Jun Meng, Yu Fan, Xiaoyan He, Shangyong Zhu

** the authors share the first authorship*

Department of Medical Ultrasound, First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region, China

Abstract

Aims: To investigate the correlation between time-intensity curve (TIC) parameters obtained from transrectal contrast-enhanced ultrasound (TR-CEUS) and important pathological prognostic factors in rectal adenocarcinoma. **Material and methods:** We retrospectively included 477 patients with pathologically confirmed rectal adenocarcinoma. TIC parameters were derived from preoperative dynamic TR-CEUS images. These parameters included peak intensity (PI), time to peak (TTP), mean transit time (MTT), slope (S), and area under the curve (AREA). Pathological prognostic factors included TN stage, tumor diameter, lymphovascular invasion (LVI), perineural invasion, and tumor differentiation. Spearman's correlation analysis and binary logistic regression were used to analyze the association between TIC parameters and pathological prognostic factors. **Results:** pT1-2 stages rectal carcinomas exhibited higher PI-max, PI-min, S-max, S-min, AREA-max, and AREA-min than pT3-4 stages (all $p<0.05$). pN0 stage rectal adenocarcinomas displayed higher PI-max, S-max, AREA-max, PI-ratio, S-ratio, and AREA-ratio than pN1-2 stage (all $p<0.05$). PI-ratio and S-ratio were higher in the LVI-negative and tumor diameter ≥4cm group compared to the LVI-positive and tumor diameter <4cm group, respectively ($p<0.05$). Well-differentiated rectal adenocarcinomas displayed higher PI-max, AREA-max, PI-ratio, S-ratio, and AREA-ratio than the moderate-poor differentiated group (all $p<0.05$). PI-max, S-max, AREA-max, PI-ratio, S-ratio, and AREA-ratio were negatively correlated with pN stage (all $p<0.05$). PI-ratio and S-ratio were independent predictive factors for the pN stage (OR=0.774, OR=1.048). S-ratio and AREA-ratio were independent predictive factors for tumor differentiation (OR=1.071, OR=0.911). **Conclusions:** TIC parameters derived from TR-CEUS exhibit correlations with specific pathological prognostic factors in rectal adenocarcinomas. This non-invasive method may hold promise for preoperatively assessing the prognosis of rectal adenocarcinoma patients.

Keywords: rectal adenocarcinoma; contrast-enhanced ultrasound; time-intensity curve; transrectal ultrasound; prognostic factors

Introduction

Rectal cancer is a global health concern, with an estimated 732,210 new cases and 339,022 deaths reported worldwide in 2020, highlighting its substantial impact (GLOBOCAN 2020) [1]. Strikingly, over one-third of these cases and deaths occur in China. The incidence and mortality rates of rectal cancer in China have rapidly escalated [2]. Early diagnosis is pivotal for improving prognosis in rectal cancer: the 5-year relative survival rate for stage I stands at 90%, while the 5-year relative survival rate for stage IV colorectal cancer with distant metastases is only 12% [3]. While pathological TNM stage, lymphovascular invasion (LVI), perineural invasion (PNI), and tumor differentiation are valuable prognostic indicators [4-6], these can be confirmed only post-surgery. If such critical prognostic information could be obtained preop-

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Corresponding author: Shangyong Zhu
Department of Medical Ultrasound, First Affiliated Hospital of Guangxi Medical University, 6, Shuangyong road, Nanning, 530021, Guangxi Zhuang Autonomous Region, China
Phone: +867715356899
E-mail: zhushangyong2021@sina.com

eratively, it would revolutionize treatment decisions and enhance prognostic predictability.

Tumor prognosis is intricately tied to angiogenesis, making it a crucial factor. Contrast-enhanced ultrasound (CEUS) can provide information about tumor angiogenesis quantitatively through the analysis of time intensity curve (TIC) [7]. Several studies have demonstrated that CEUS TIC parameters correlate with prognostic factors in various malignancies, such as hepatocellular carcinoma and breast cancer [8-10]. However, investigations into the relationship between CEUS TIC parameters and prognostic factors in rectal carcinoma have yielded inconclusive results. For instance, a study involving 42 colorectal cancer patients, including 33 cases of rectal cancer, found no correlation between CEUS parameters such as peak intensity (PI), mean transit time (MMT), and time to peak (TTP) with T stage. Still, a positive correlation was identified between PI, wash-in and wash-out AUC with N stage [11]. Similarly, Wang et al [12] observed significant differences in the TIC parameter "enhanced intensity" (EI) among histological grading in rectal carcinoma. Still, no statistical correlations were found between TIC parameters and TNM stage. Notably, Wang et al study included only 66 patients with rectal carcinoma. Consequently, further exploration is warranted to unravel the association between CEUS quantitative parameters and pathological prognostic factors.

Hence, the aim of our study is to investigate the correlation between absolute and relative TIC parameters in transrectal contrast-enhanced ultrasound (TR-CEUS) and pathological prognostic factors in patients with rectal adenocarcinoma. We aim to explore the potential value of CEUS quantitative parameters in preoperatively predicting pathological prognostic factors in rectal adenocarcinoma.

Material and methods

Patients

We retrospectively analyzed a cohort of 477 patients with confirmed rectal adenocarcinoma based on postoperative pathology reports. All patients underwent tumor excision at the First Affiliated Hospital of Guangxi Medical between January 2019 and April 2023. Inclusion criteria: (1) pathologically confirmed primary rectal adenocarcinoma; (2) underwent TR-CEUS examination within two weeks before surgery; (3) no neoadjuvant chemoradiation therapy before surgery; (4) age over 18 years old; (5) complete clinical, laboratory, and histopathologic data. Exclusion criteria: (1) poor ultrasonography imaging or unavailable TR-CEUS data; (2) mucinous adenocarcinoma, signet-ring cell carcinoma et al; (3) incomplete patient data; (4) patients allergic to ultrasound contrast agents. Our study received ethical approval from the Institutional Research Ethics Committee and the need for informed consent was waived.

Transrectal ultrasound (TRUS) and CEUS examination

All TRUS and CEUS examinations were conducted using the Aplio 500 scanner (Canon Medical Systems, Tokyo, Japan), equipped with a transrectal head-scanning probe operating at 5-10MHz. A single sonographer with nine years of experience in TRUS and five years of experience in CEUS for rectal tumors performed the ultrasound assessments. Patients assumed a left lateral decubitus position during the procedure, and rectal cleansing was achieved with enemas. To create an ultrasound-translucent window, 100–150 mL of sterile coupling gel was gently introduced through the anus into the rectal cavity. The probe was then maneuvered through the rectum to assess parameters such as the distance from the anal verge, largest tumor diameter, infiltration depth, and the tumor's internal blood flow.

Following the routine TRUS examination, a section containing the largest tumor size was selected for the CEUS examination. The contrast agent utilized was SonoVue™ (Bracco SpA, Milan, Italy), a lipid-coated microbubble contrast agent. It was prepared by mixing 5 ml of 0.9% normal saline and 2.4 ml of Sonovue™ before administration. The contrast agent was injected into the forearm vein as a bolus within 2 seconds, followed by a 5 ml flush of 0.9% normal saline. Dynamic CEUS images were recorded for 60 seconds and stored on the internal hard drive of the Aplio 500 scanner.

TIC analysis

CEUS TIC analysis was conducted using the built-in quantitative analysis software on the Aplio 500 scanner. To ensure objectivity, dynamic CEUS images were independently reviewed by an experienced sonographer who was unaware of clinical and pathological information. For TIC analysis, a circular region of interest (ROI) measuring 5 mm in diameter was manually placed within the maximum and minimum enhancement area at the peak phase of the CEUS images [13], denoted as ROI-max and ROI-min, respectively (fig 1). After setting the ROIs, time-intensity curves (TICs) were generated, and absolute quantitative parameters, including peak intensity (PI), time to peak (TTP), mean transit time (MTT), slope (S), and area under the curve (AREA), were derived. Each ROI-max and ROI-min was measured three times, and the average values of the quantitative parameters were calculated. Additionally, relative parameters were computed by taking the ratio of PI, TTP, MTT, S, and AREA, where the ratio was determined as relative

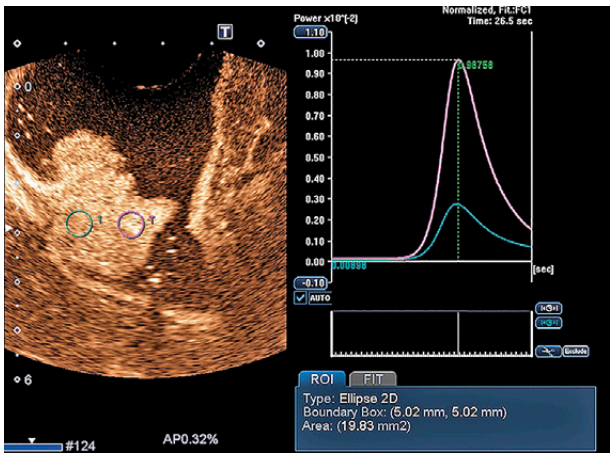

Fig 1. Methods of placing the region of interest (ROI) in the rectal lesion and the time-intensity curve (TIC) of the contrast-enhanced ultrasound (CEUS) image. Left: CEUS imaging at peak phase. Two ROIs were placed in the rectal lesion: the maximum enhancement ROI (purple) and minimum enhancement ROI (green); Right: TICs were generated from two ROIs.

parameters of ROI-max divided by relative parameters of ROI-min, for example, PI-ratio=PI-max/PI-min. This approach aimed to mitigate potential confounding factors such as rectal lesion heterogeneity and variations in contrast injection rates.

Histopathological analysis

Postoperative pathology results, including tumor diameter, depth of tumor infiltration, number of metastatic lymph nodes, tumor differentiation grading, lymphovascular invasion (LVI), and perineural invasion (PNI), were obtained from our hospital's Picture Archiving and Communication System. These pathologic examinations were conducted by an experienced pathologist. The TN stage was determined in accordance with the Eighth Edition of the AJCC Cancer Staging Manual [14]. Tumor differentiation grading was assessed based on the 2019 WHO classification of tumors of the digestive system [15]. LVI in rectal cancer was defined as the invasion of carcinoma cells into lymphatic and/or blood vessel structures [16]. PNI in rectal cancer was characterized by the invasion of carcinoma cells into any layer of the nerve sheath or perineural space [17]. PNI and LVI were detected using hematoxylin and eosin (HE) staining.

Intra-operator reliability

To assess intra-operator reliability, 200 dynamic CEUS images were randomly selected for TIC analysis two weeks apart by the same sonographer who initially analyzed the TIC parameters. The TIC analysis was conducted as previously described.

Statistical analysis

All statistical analyses were performed using SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics included mean±standard deviation (X±SD) for normally distributed variables and median (interquartile range) for non-normally distributed variables. The Mann–Whitney U-test compared TIC parameters across different groups of pathological prognostic factors. Spearman's correlation test and binary logistic regression were employed to analyze the correlation between TIC parameters and pathological prognostic factors. Predictive performance was assessed using the area under the receiver operating characteristic (ROC) curve. Intra-operator reliability was determined using the Intraclass correlation coefficient (ICC), with ICC values above 0.75 indicating good reliability. A two-tailed p value less than 0.05 was considered statistically significant.

Results

Patients

A total of 477 patients with mean age of 60.0±11.5 year with rectal adenocarcinoma were included. Among them, 282 were male. The mean tumor diameter was 3.8(1.7) cm, and the mean tumor thickness was 1.3(0.5) cm. 164 (34.4%) lesions were located in the upper rectum, 232 (48.6%) lesions were located in the mid rectum, and 81 (17%) lesions were located in the lower rectum. There were 21 (4.4%), 396 (83%) and 60 (12.6%) lesions poorly, moderately, and well differentiated, respectively. Pathological T stages were 42 (8.8%) in T1,110 (23.1%) in T2, 242 (50.7%) in T3, and 83 (17.4%) in T4. Pathologic N stages were 262 (54.9%) cases in N0, 153 (32.1%) cases in N1, and 62 (13.0%) cases in N2. LVI was positive in 28.9% of cases, and PNI was positive in 28.3% of cases.

Comparison of TIC parameters according to pathological prognostic factors in rectal adenocarcinomas

Table I and Table II present the absolute and relative TIC parameters, respectively, according to different pathological prognostic factors in rectal adenocarcinomas. In terms of pathological (p) T stage, significant differences were observed in absolute TIC parameters, including PI-max, PI-min, S-max, S-min, AREA-max, and AREA-min between pT1-2 and pT3-4 stages (all $p<0.05$). pT1-2 stage carcinomas exhibited higher PI, S, and AREA values compared to pT3-4 stage carcinomas. Regarding pathological N stage, several absolute and relative TIC parameters showed statistically significant differences between pN0 and pN1-2 stages (all $p<0.05$). Rectal adenocarcinomas without lymph node metastasis displayed higher absolute TIC parameters (PI-max, S-max, AREA-max, and AREA-min) and relative parameters (PI-ratio, S-ratio, AREA-ratio) than those with lymph

Table I. Comparison of absolute parameters of the TIC in different group of pathological prognostic factors

Prognostic factor	PI-max, 10E-5 AU	TTP-max, s	MTT-max, s	S-max, 10E-5 AU/s	AREA-max, 10E-5 AU.s	PI-mi, 10E-5 AU	TTP-min, s	MTT-min, s	S-min, 10E-5 AU/s	AREA-min, 10E-5 AU.s
pT stage										
T1-2 (n=152)	240.0 (537.4)	6.0 (2.6)	10.2 (5.4)	44.0 (121.3)	11202.6 (18631.4)	52.0 (68.2)	6.4 (3.1)	14.8 (18.6)	9.2 (12.2)	2941.1 (5374.2)
T3-4 (n=325)	181.4 (243.0)	6.3 (2.6)	11.0 (8.0)	33.2 (48.5)	7367.9 (9958.1)	43.4 (48.7)	6.9 (3.1)	16.4 (27.7)	7.9 (9.9)	2296.1 (3034.8)
p value	0.001*	0.519	0.221	0.001*	0.001*	0.031*	0.292	0.344	0.015*	0.006*
pN stage										
N0 (n=262)	280.2 (468.4)	6.2 (2.8)	10.3 (6.2)	52.7 (111.0)	11039.9 (18652.2)	48.3 (57.1)	6.7 (3.0)	14.9 (24.3)	8.8 (11.7)	2662.7 (3494.0)
N1-2 (n=215)	130.0 (172.8)	6.2 (2.4)	11.2 (8.8)	26.4 (34.4)	5939.3 (8961.7)	43.2 (48.0)	6.9 (3.2)	16.4 (25.0)	7.7 (9.9)	2215.7 (3073.6)
p value	<0.001*	0.820	0.086	<0.001*	<0.001*	0.078	0.845	0.500	0.096	0.029*
LVI										
Negative (n=339)	202.3 (297.1)	6.2 (2.7)	10.4 (6.8)	37.0 (70.9)	8193.1 (11432.3)	46.6 (50.5)	6.7 (3.1)	15.3 (24.3)	8.3 (9.8)	2301.3 (3347.0)
Positive (n=138)	190.8 (256.4)	6.3 (2.6)	11.4 (7.7)	33.0 (49.9)	7389.5 (11911.0)	44.9 (58.5)	6.8 (3.1)	16.1 (24.6)	8.4 (12.3)	2666.0 (3295.1)
p value	0.132	0.581	0.171	0.124	0.307	0.890	0.983	0.447	0.995	0.517
PNI										
Negative (n=342)	201.4 (297.9)	6.2 (2.6)	10.6 (6.6)	36.1 (64.4)	7954.3 (12470.3)	46.2 (58.4)	6.8 (2.9)	15.4 (27.3)	8.3 (9.8)	2442.0 (3398.6)
Positive (n=135)	189.5 (247.7)	6.1 (2.6)	11.0 (7.4)	34.5 (48.5)	7449.4 (9090.5)	47.5 (43.4)	6.7 (3.3)	15.3 (20.5)	8.4 (12.4)	2392.8 (2995.8)
p value	0.383	0.933	0.311	0.726	0.530	0.631	0.786	0.869	0.608	0.761
Tumor diameter										
≥4cm (n=214)	218.0 (300.9)	6.1 (2.6)	10.5 (5.5)	40.5 (61.5)	8918.3 (12148.9)	43.3 (46.5)	6.6 (3.1)	15.8 (28.8)	7.9 (9.7)	2402.7 (2954.0)
<4cm (n=263)	187.2 (255.4)	6.3 (2.6)	10.8 (8.4)	34.0 (53.2)	7475.3 (10974.8)	48.4 (57.1)	6.8 (3.0)	15.3 (21.3)	8.8 (11.7)	2393.6 (3605.7)
p value	0.364	0.082	0.083	0.165	0.706	0.096	0.246	0.720	0.332	0.448
Tumor differentiation										
Well (n=60)	234.2 (438.0)	6.6 (2.3)	10.2 (5.7)	44.9 (70.9)	9707.3 (16286.7)	51.4 (63.5)	6.9 (3.1)	15.7 (27.9)	9.3 (12.9)	2385.4 (3515.4)
Moderate-Poor (n=417)	195.9 (269.5)	6.2 (2.7)	10.8 (7.1)	34.9 (57.7)	7763.0 (11170.5)	45.9 (51.6)	6.8 (3.1)	15.3 (23.6)	8.2 (10.2)	2423.7 (3322.9)
p value	0.038*	0.412	0.236	0.119	0.049*	0.508	0.670	0.333	0.647	0.977

pT stage – pathological T stage; *pN stage* – pathological N stage; *LVI* – lymphovascular invasion; *PNI* – perineural invasion; * p<0.05

node metastasis. No significant differences were observed in either absolute or relative TIC parameters between PNI-positive and PNI-negative groups (all p<0.05). Absolute TIC parameters did not differ significantly with LVI and tumor diameter (all p<0.05). However, relative TIC parameters PI-ratio and S-ratio showed statistical differences between LVI-positive and LVI-negative groups and between tumor diameters ≥4 cm and <4 cm (p<0.05). Well-differentiated rectal adenocarcinomas exhibited significantly higher absolute TIC parameters (PI-max and AREA-max) as well as relative parameters (PI-ratio, S-ratio, AREA-ratio) compared to the moderate-poor differentiated group (all p<0.05).

Spearman's correlation of TIC parameters with pathological prognostic factors in rectal adenocarcinomas

PI-max, S-max, AREA-max, PI-ratio, S-ratio and AREA-ratio exhibited negative correlations with pathological N stage (r=-0.345, r=-0.301, r=-0.273, r=-0.380, r=-0.331, r=-0.250). Extremely weak negative correlations were found between pathological T stage and PI-max, PI-min, S-max, S-min, AREA-max, and AREA-min (absolute value of r<0.200). PI-ratio and S-ratio showed extremely weak positive correlations with tumor diameter (r=0.164, r=0.132) and extremely weak negative correlations with LVI (r=-0.105, r=-0.104). PI-max, AREA-max, PI-ratio, S-ratio, and AREA-ratio demonstrated extremely weak negative correlations with tumor differentiation (absolute value of r<0.200) (Table III).

Binary logistic regression analysis and ROC curve of TIC parameters and pathological prognostic factors

Binary logistic regression analysis was performed to identify independent risk factors for pathological prognostic factors among the TIC parameters of CEUS. The results indicated: None of the TIC parameters were independent risk factors for pathological T stage, LVI, and tumor diameter. However, PI-ratio and S-ratio emerged as independent predictive factors for pN stage (OR=0.774 and 1.048, respectively). Additionally, S-ratio and AR-

Table II. Comparison of relative parameters of the TIC in different group of pathological prognostic factors

Prognostic factor	PI-ratio	TTP-ratio	MTT-ratio	S-ratio	AREA-ratio
pT stage					
T1-2 (n=152)	4.5 (5.2)	1.0 (0.3)	0.8 (0.6)	4.7 (7.0)	3.2 (3.7)
T3-4 (n=325)	4.1 (3.8)	0.9 (0.4)	0.7 (0.6)	4.4 (5.0)	3.0 (3.2)
p value	0.176	0.513	0.893	0.176	0.463
pN stage					
N0 (n=262)	5.3 (6.4)	1.0 (0.4)	0.7 (0.6)	5.9 (6.7)	3.8 (4.5)
N1-2 (n=215)	3.4 (2.4)	1.0 (0.4)	0.8 (0.6)	3.2 (3.6)	2.6 (2.2)
p value	<0.001*	0.834	0.240	<0.001*	<0.001*
LVI					
Negative (n=339)	4.4 (5.0)	1.0 (0.4)	0.7 (0.6)	4.6 (5.9)	3.1 (3.8)
Positive (n=138)	3.8 (3.2)	1.1 (0.5)	0.8 (0.6)	4.0 (4.1)	2.9 (2.8)
p value	0.022*	0.383	0.542	0.023*	0.061
PNI					
Negative (n=342)	4.2 (4.9)	1.0 (0.4)	0.7 (0.6)	4.7 (5.9)	3.0 (3.4)
Positive (n=135)	4.2 (3.8)	1.0 (0.4)	0.8 (0.6)	4.0 (5.0)	3.2 (3.1)
p value	0.112	0.475	0.370	0.115	0.600
Tumor diameter					
≥4cm (n=214)	4.6 (4.8)	1.0 (0.4)	0.7 (0.6)	5.0 (6.1)	3.3 (3.3)
<4cm (n=263)	3.8 (3.8)	1.0 (0.4)	0.8 (0.8)	3.9 (4.6)	2.8 (3.2)
p value	<0.001*	0.684	0. 149	0.004*	0.073
Tumor differentiation					
Well (n=60)	5.2 (5.4)	1.0 (0.3)	0.7 (0.6)	5.9 (5.4)	4.0 (4.8)
Moderate-Poor (n=417)	4.1 (4.1)	1.0 (0.4)	0.8 (0.6)	4.2 (5.1)	3.0 (3.1)
p value	0.006*	0.746	0.071	0.018*	0.005*

pT stage – pathological T stage; *pN stage* – pathological N stage; *LVI* – lymphovascular invasion; *PNI* – perineural invasion; *p<0.05

Table III. Spearman correlations between TIC parameters and pathological prognostic factors

Prognostic factors	TIC parameters	r	p value
pT stage	PI-max	-0.151	0.001
	PI-min	-0.099	0.031
	S-max	-0.150	0.001
	S-min	-0.112	0.015
	AREA-max	-0.151	0.001
	AREA-min	-0.127	0.006
pN stage	PI-max	-0.345	<0.001
	S-max	-0.301	<0.001
	AREA-max	-0.273	<0.001
	AREA-min	-0.100	0.029
	PI-ratio	-0.380	<0.001
	S-ratio	-0.331	<0.001
	AREA-ratio	-0.250	<0.001
LVI	PI-ratio	-0.105	0.022
	S-ratio	-0.104	0.023
Tumor diameter	PI-ratio	0.164	<0.001
	S-ratio	0.132	0.004
Tumor differentiation	PI-max	-0.095	0.038
	AREA-max	-0.090	0.049
	PI-ratio	-0.127	0.005
	S-ratio	-0.109	0.017
	AREA-ratio	-0.129	0.005

pT stage – pathological T stage; *pN stage* – pathological N stage; *LVI* – lymphovascular invasion

EA-ratio were identified as independent predictive factors for tumor differentiation (OR = 1.071 and 0.911, respectively) (Table IV). The predictive performance of TIC parameters in pN stage and tumor differentiation were further evaluated using ROC curve analysis (Table V). The ROC analysis revealed that, for the differentiation pN stage, the cut-off point for PI-ratio was 5.1 with an AUC of 0.720 (95%CI: 0.675–0.765), while the cut-off point for S-ratio was 3.9 with an AUC of 0.692 (95%CI: 0.645–0.739). Concerning tumor differentiation, the cut-off point for S-ratio was 4.1 with an AUC of 0.595 (95%CI: 0.526–0.664) and the cut-off point for AREA-ratio was 3.7 with an AUC of 0.612 (95%CI: 0.536–0.688).

Intra-operator reliability

The ICC of PI-max, TTP-max, MTT-max, S-max, AREA-max, PI-min, TTP-min, MTT-min, S-min, AREA-min, PI-ratio, TTP-ratio, MTT-ratio, S-ratio and AREA-ratio were 0.975, 0.938, 0.975, 0.967, 0.990, 0.991, 0.962, 0.881, 0.883, 0.884, 0.857, 0.804, 0.841, 0.934 and 0.924, respectively.

Discussion

As the incidence and mortality of rectal carcinoma in China have been on the rise, prognostic indicators for rectal carcinoma deserve further exploration. Predicting and identifying relevant pathological prognostic factors preoperatively will facilitate individualized and precise treatment of patients with rectal carcinoma. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is commonly used for preoperative evaluation of rectal cancer, offering quantitative information about tumor angiogenesis through perfusion parameters [18]. However, studies on the correlation between DCE-MRI perfusion parameters and pathological prognostic factors in rectal cancer have yielded inconclusive results. Kim et

Table IV. Binary logistic regression analysis of TIC parameters and prognostic factors

Prognostic factors	TIC parameters	B	p value	Exp(B)	95% CI of Exp(B)	
					Lower limit	Upper limit
pN stage	PI-ratio	-0.257	<0.001	0.774	0.681	0.879
	S-ratio	0.047	0.050	1.048	1.000	1.099
Tumor differentiation	S-ratio	0.068	0.048	1.071	1.001	1.146
	AREA-ratio	-0.094	0.006	0.911	0.852	0.973

B – regression coefficient; *Exp(B)* – odds ratio; *CI* – confidence interval; *pN stage* – pathological N stage

Table V. Diagnostic efficiency of TIC parameters for differentiating pN stage and tumor differentiation

Prognostic factors	TIC parameters	Cut-off value	Sensitivity (%)	Specificity (%)	AUC	95% CI		p value
						Lower limit	Upper limit	
pN stage	PI-ratio	5.1	82.9	52.7	0.720	0.675	0.765	<0.001
	S-ratio	3.9	60.0	69.1	0.692	0.645	0.739	<0.001
Tumor differentiation	S-ratio	4.1	45.7	71.7	0.595	0.526	0.664	0.018
	AREA-ratio	3.7	61.4	56.7	0.612	0.536	0.688	0.005

AUC – Area under the ROC; *CI* – confidence interval; *pN stage* – pathological N stage

al [19] reported that perfusion parameters K^{trans} showed a significant difference between the tumor differentiation, the TN stage showed no significant differences for all perfusion parameters ($p>0.05$). Hong et al [20] found perfusion parameters Erise was negatively correlated with N stage, DCE-MRI parameters did not show significant correlations with T stage. However, none of the DCE-MRI perfusion parameters showed significant correlation with TN stage in another study [21]. TRUS is another valuable tool for TN staging in rectal carcinoma, a meta-analysis showed that TRUS was superior to MRI in overall T staging [22]. CEUS, a technique capable of detecting low-velocity microvascular signals, provides a novel means to assess tumor angiogenesis and perfusion quantitatively based on the CEUS time-intensity curve. Previous studies have explored the relationship between CEUS quantitative parameters and differentiating between benign and malignant rectal lesions, with promising results [23,24]. However, few studies have investigated the link between CEUS quantitative parameters and pathological prognostic factors, and these studies often had limited sample sizes. In our study, we demonstrated the excellent intra-operator reliability of all TIC parameters, which indicates that the quantitative TR-CEUS TIC parameters are objective.

The post-surgical pathological TNM stage is considered the main prognostic factor and the most important determinant of treatment decisions for rectal carcinoma, such as postoperative adjuvant therapy [5]. Previous researches showed TIC parameters correlations with N stage but not with T stage or TIC parameters correlation with the histological grade but not with TNM stage [11,12]. Our results were different as we found that N0 stage rectal adenocarcinomas had higher absolute parameters and relative parameters than the N1-2 stages. Several TIC parameters were negatively correlated with pathological N stage. Relative parameters PI-ratio and S-ratio were independent predictors of lymph node metastasis in rectal adenocarcinomas. Our findings indicate that TIC parameters may have predictive value for post-surgical pathological lymph node metastasis status. This aligns with research in other malignancies. For example, Mori et al [13] found that PI-ratio of lymph-node metastasis positive group was significantly higher than the lymph-node metastasis negative group in clinically node-negative breast cancer patients. Ling et al [25] evaluated TIC parameters of nasopharyngeal carcinoma patients with enlarged cervical lymph nodes, PI and AUC in benign cervical lymph nodes were significantly higher than that in the malignant nodes. This further supports the potential utility of CEUS quantitative parameters in assessing lymph node involvement. Furthermore, T1-2 stage rectal adenocarcinomas displayed higher TIC parameters than T3-4 stages. The observed correlation between TIC parameters and pathological TN stage suggests that tumor perfusion varies with TN stage. Although some of the correlation coefficients were relatively small, this may signify that other factor besides angiogenesis influence TN stage.

LVI and PNI are critical factors in assessing the prognosis of rectal carcinoma and are strongly associated with postoperative disease-free survival, recurrence, and lymph node metastasis [26-28]. Preoperative identification of LVI and PNI is of great significance for predicting outcome of patients with rectal cancer undergoing surgery. While some research has explored the potential of CEUS in predicting LVI, quantitative parameters were not included. For example, Zhou et al [29] developed a nomogram for predicting LVI in primary breast cancer patients based on 2D and qualitative CEUS characteristics but did not incorporate quantitative CEUS parameters. Similarly, a radiomics model based on gray ultrasound images showed promise in predicting LVI in rectal cancer [30]. However, our study represents a novel exploration of the relationship between TIC parameters and LVI and PNI in primary rectal adenocarcinomas. We found that the relative parameters of TIC, PI-ratio and S-ratio, were significantly higher in patients with LVI than those without LVI. Although the correlation was extremely weak, this suggests a potential association between TR-CEUS quantitative parameters and LVI in rectal cancer. In contrast, we did not observe significant differences in TIC parameters between PNI-positive and PNI-negative groups in our study. This may indicate that TR-CEUS quantitative parameters are not effective preoperative predictors of PNI in rectal carcinoma. However, it is important to note that our sample size may have limited our ability to detect subtle differences, and further investigation with larger cohorts could provide more insights into this relationship.

Tumor size is critical in rectal carcinoma management, impacting postoperative complications and the likelihood of achieving a complete pathologic response following neoadjuvant chemoradiotherapy [31]. In two trials of the correlation between quantitative parameters on CEUS and pathological prognostic factors in breast cancer, PI, MMT and TTP showed no statistical differences in different tumor diameter [32]. Our study identified a potential link between TIC parameters and tumor size in rectal carcinoma. Specifically, we found that patients with tumors >4 cm had higher relative TIC parameters, PI-ratio and S-ratio, than those with smaller tumors. Although extremely weak, the positive correlation between PI-ratio, S-ratio, and tumor diameter suggests

that TIC parameters may vary with tumor size. Notably, the manual drawing of ROIs within the tumor lesions, rather than covering the entire lesion, may have influenced these results. The ROI included the entire lesion may be more representative of the tumor vascularity [11].

Furthermore, tumor differentiation serves as a crucial prognostic factor in rectal carcinoma. Wang et al [12] observed a negative correlation between CEUS quantitative parameter enhanced intensity (EI) of rectal cancer and histological grade. They noted that EI was higher in poorly differentiated tumors compared to well-differentiated ones. Our findings, however, diverge from theirs: well-differentiated rectal adenocarcinomas exhibited higher absolute TIC parameters (PI-max and AREA-max) as well as relative parameters (PI-ratio, S-ratio, AREA-ratio) than the moderate-poor differentiated group. Binary logistic regression analysis indicated that S-ratio and AREA-ratio emerged as independent predictive factors for tumor differentiation. However, the prediction performances of S-ratio and AREA-ratio were relatively low (AUC<0.7). This might be attributed to the considerable variation in sample size across different tumor differentiation grades. Further investigations involving a larger number of patients may yield more significant results. Nonetheless, CEUS TIC parameters hold promise as noninvasive indicators for identifying tumor differentiation in rectal cancer.

Our study has illuminated the potential of absolute and relative time-intensity curve (TIC) parameters obtained through TR-CEUS in establishing correlations with specific pathological prognostic factors in rectal adenocarcinomas. While these findings represent an important step toward non-invasive and objective preoperative prognostication, there are several limitations that must be acknowledged. First, the retrospective nature of this study may introduce case selection bias, warranting further investigations with a prospective approach. Second, the small size of the region of interest (ROI) could potentially influence TIC parameter results, given the heterogeneous and chaotically allocated nature of tumor vascularization. Including the entire lesion may yield more representative results. Third, while the biplane transrectal ultrasound probe provides cross-sectional and longitudinal images for visualizing rectal lesions in two dimensions, the use of a single-plane head-scanning probe instead of a biplane probe is also a limitation of this study. Finally, for enhanced relevance, CEUS TIC parameters should be compared with microvessel density or other imaging methods for assessing tumor vascularity (e.g., CDFI, MFI, etc.). In addition, molecular biological indicators such as Ki-67, HER-2, vascular endothelial growth factor (VEGF), KRAS and BRAF gene mutation status should be included to obtain better relevance in future studies.

Conclusions

Our findings provide valuable insights into the potential of TR-CEUS quantitative parameters in assessing pathological prognostic factors such as lymph node metastasis, pathological T stage, lymphovascular invasion, and tumor differentiation. This non-invasive approach holds promise for improving preoperative prognostication and personalized treatment planning for patients with rectal adenocarcinomas. Future studies with larger sample sizes and a multicenter approach are essential to strengthen these findings and enhance their clinical utility.

Conflict of interest: none

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