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Research article

The predictive value of LGR for distant metastasis-free survival in locally advanced rectal cancer patients

Minghe Lv [a,b,c,d], Songsong Wu [a,b,c,e], Zhiyuan Zhang [a,b,c], Zhen Zhang [a,b,c,1,*], Juefeng Wan [a,b,c,1,**]

[a] Department of Radiation Oncology, Fudan University Shanghai Cancer Center, Shanghai, 200032, China
[b] Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, 200032, China
[c] Shanghai Clinical Research Center for Radiation Oncology Shanghai Key Laboratory of Radiation Oncology, Shanghai, 200032, China
[d] Department of Radiotherapy, Shuguang Hospital Affiliated to Shanghai University of Chinese Traditional Medicine, Zhang Heng Road, Pudong New Area, Shanghai, 201203, China
[e] Department of Radiotherapy, The Third Affiliated Hospital of Wenzhou Medical University, Zhejiang, 325200, China

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ABSTRACT

Studies show that inflammation induced by cancer is a key factor in carcinogenesis. Here, we sought to assess the relationship between patients with locally advanced rectal cancer (LARC) and the lymphocyte to neutrophil granulocyte ratio (LGR) prior to neoadjuvant chemoradiotherapy (nCRT) and distant metastasis-free survival (DMFS). Using a receiver operating characteristic (ROC) analysis of 326 LARC patients who underwent total mesorectal excision (TME) surgery and neoadjuvant chemoradiotherapy, we were able to determine the ideal LGR cutoff value. We used the Kaplan-Meier method and univariate and multivariate Cox regression to study the clinical characteristics of LARC patients in comparison between the low LGR group and the high LGR group. DMFS analysis was one of the primary clinical variables examined. We discovered that the low LGR group of LARC patients had a longer DMFS than the high LGR group. The median duration of follow-up for LARC patients was 89.4 months, with a significantly lower DMFS observed in the high LGR group compared to the low LGR group. Multivariate Cox regression analysis revealed that LARC patients with low LGR levels, early ypTNM stages, and BRAF wild had longer DMFS. LGR prior to nCRT was a critical prognostic indicator that contributed extra predictive value beyond conventional clinicopathological characteristics to predict the outcome of LARC patients receiving neoadjuvant chemoradiotherapy followed by TME surgery.

1. Introduction

As a common malignant tumor, the incidence of rectal cancer (RC) is increasing, and there is a rising number of patients diagnosed with locally advanced disease. For locally advanced rectal cancer (LARC), neoadjuvant chemoradiotherapy (nCRT) combined with

* Corresponding author. Department of Radiation Oncology, Fudan University Shanghai Cancer Center, Fudan University, Shanghai, 200032, China.
** Corresponding author. Department of Radiation Oncology, Fudan University Shanghai Cancer Center, Fudan University, Shanghai, 200032, China.
E-mail addresses: zhen_zhang@fudan.edu.cn (Z. Zhang), wjf62313172@163.com (J. Wan).
[1] Zhen Zhang and Juefeng Wan are both corresponding authors and contributed equally to this work.

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therapeutic surgery (total mesorectal excision (TME)) has become the standard treatment for preventing local recurrence in Western countries [1–4]. nCRT combined with TME can significantly reduce the local disease recurrence rate and improve the preservation rate of the anal sphincter. In our research center, the use of irinotecan combined with capecitabine in the treatment of LARC patients during radiotherapy under the state guidance targeting UGT1A1 gene, as well as the use of TME, significantly improved the pathological complete response (pCR) rate and anal sphincter retention rate of patients [5]. Studies have shown that this standard treatment has good local control, with a local recurrence rate of only 6.0%–8.7%, but fail to improve overall survival (OS) as the distant recurrence rate range from 24.3% to 36.0% [3,6]. Therefore, we need to screen the factors affecting distant metastasis, so as to identify patients prone to distant metastasis, and further to improve the overall survival of this part of patients prone to distant recurrence via a higher intensity treatment. It is urgent to elucidate the predictors of distant recurrence in neoadjuvant chemoradiotherapy patients with rectal cancer after TME surgery, in order to provide the best individualized treatment for patients.

Pro-inflammatory cytokines and chemokines in the cancer microenvironment contribute to tumor cell survival and proliferation, metastasis, angiogenesis, and disruption of adaptive immunity, all of which impact survival and prognosis. Inflammation is thought to be a signature feature of cancer initiation and progression. On the one hand, long-term inflammation produces cytokines and inflammatory factors, causes localized monocyte, platelet, and neutrophil aggregation, and promotes tumor angiogenesis and metastasis. However, an increase in monocytes and lymphocytes strengthens the body's defense against tumor invasion. There has been increasing evidence that local and systemic inflammatory responses are associated with survival prognosis of solid tumors such as rectal cancer [7], cholangiocarcinoma [8], lung cancer [9], and esophageal cancer [10]. Recent studies showed that many inflammatory indicators can be used as prognostic markers of tumor patients, such as lymphocyte to monocyte ratio (LMR) [11,12], white blood cell to monocyte ratio (WMR) [13], white blood cell to C-reactive protein ratio (WCR) [14], platelets to lymphocyte ratio (PLR) [15], and neutrophil to lymphocyte ratio (NLR) [16]. Previous studied found that elevated NLR and PLR levels can be considered as predictors of poor pathologic response, and NLR can be considered a prognosticator in patients who underwent neoadjuvant chemoradiotherapy for locally advanced rectal cancer [17]. Elevated LMR was an independent factor for better OS (hazard ratio 0.538, 95% confidence interval 0.292–0.991, P = 0.047) of LARC patients receiving chemoradiotherapy [18]. Nonetheless, there hasn't been a detailed analysis on the prognostic significance of the baseline lymphocyte to neutrophil granulocyte ratio (LGR) for the disease-free survival (DMFS) of patients with rectal cancer having neoadjuvant chemotherapy and radiation therapy followed by TME surgery.

The purpose of this study was to evaluate the predictive significance of the lymphocyte to neutrophil granulocyte ratio (LGR) prior to CRT in patients with rectal cancer following neoadjuvant chemotherapy and radiotherapy, as well as TME surgery. Additionally, we sought to analyze the risk factors for DMFS of rectal cancer patients undergoing neoadjuvant chemoradiotherapy followed by TME surgery by employing a receiver operating characteristic (ROC) curve and building a Cox regression analysis model.

2. Patients and methods

2.1. Patients

This work involved the retrospective analysis of a cohort of 326 patients with newly-diagnosed rectal cancer who were treated at Fudan University Shanghai Cancer Center between December 2013 and February 2022 with neoadjuvant chemoradiotherapy and TME surgery. The Medical Ethics Committee of Fudan University Shanghai Cancer Center provided ethical permission for this study. Patients who satisfied the following criteria were included in the study: (1) confirmatory pathology and immunohistochemistry of rectal cancer; (2) receipt of neoadjuvant chemotherapy and radiotherapy, as well as TME surgery; and (3) completion of clinicopathological and follow-up data. Before receiving neoadjuvant chemotherapy and radiation therapy, patients with mixed malignancies, tumors of unknown origin, or distant metastases were not eligible for this study. The ethics committee gave its approval and the study was carried out in conformity with the guidelines provided in the 2003215–1 ethical code.

2.2. Follow up and data extraction

All patients gave their informed consent, and the follow-up period ended in June 2023. The time span between the initial consultation and the date of the last follow-up or distant metastasis was used to define distant metastasis-free survival (DMFS). For every patient that was part of our investigation, the following information was gathered: Age, Sex, BMI, the preoperative levels of CA199, CA50, CA242, CA724, AFP, and CEA, cTNM stage, cMRF, cEMVI, the gene status of KRAS, BRAF, and NRAS, ypTNM stage, Tumor regression grade (TRG), Pathological complete response (pCR), Intravascular cancer thrombus (ICT), Lymphatic metastasis, Nerve invasion. Absolute lymphocyte count and absolute neutrophil granulocyte count in peripheral blood were obtained from the standard automated complete blood counts within 3 days before neoadjuvant chemoradiotherapy. We calculated the LGR using the equation LGR = absolute lymphocyte count/absolute neutrophil granulocyte count.

2.3. Statistical analysis

Fisher's exact test or the Chi-square test were used to compare categorical traits and ratios. The chance of survival was estimated using the Kaplan-Meier method, and the log-rank test was employed to evaluate group differences. Additionally, univariate and multivariate analyses were carried out using the Cox proportional-hazards regression model. P-values less than 0.05 were deemed statistically significant. All statistical analyses were conducted using Statistical Package for the Social Sciences program (SPSS Inc., Chicago, IL, version 25.0 for Windows). Furthermore, we utilized the survival, rms, and ggplot 2 packages in R (version 4.2.2) to

construct nomograms of multivariable analysis results.

3. Results

3.1. Kaplan-Meier analysis curves for distant metastasis-free survival and ROC curve analysis of the LGR in rectal patients

In this study, we conducted an analysis of distant metastasis-free survival (DMFS) and receiver operating characteristic (ROC) curve for 326 newly-diagnosed rectal cancer patients who underwent neoadjuvant chemoradiotherapy followed by TME surgery at Fudan University Shanghai Cancer Center between December 2013 and February 2022. First, as shown in Fig. 1A, we analyzed the DMFS of these patients using the Kaplan-Meier technique. In addition, we calculated the ideal cut-off value for the lymphocyte to neutrophil granulocyte ratio (LGR) before to CRT using a ROC curve to evaluate the diagnostic value. The optimum LGR cut-off value was found to be 0.325, with an area under the curve (AUC) of 0.584 (95% CI = 0.514–0.654, P = 0.026), as shown in Fig. 1B. We then separated the patients into two groups: the low LGR group (LGR≤0.325) and the high LGR group (LGR>0.325) based on the ideal cut-off value of LGR. The low LGR group showed a substantially longer DMFS than the high LGR group (log-rank chi-square = 9.277, P = 0.002), according to the Kaplan-Meier analysis of DMFS in these two groups (Fig. 1C). Additionally, the risk analysis function showed that the low LGR group had a considerably lower risk (P value was statistically significant) of distant metastasis, while the high LGR group had a higher risk (Fig. 1D).

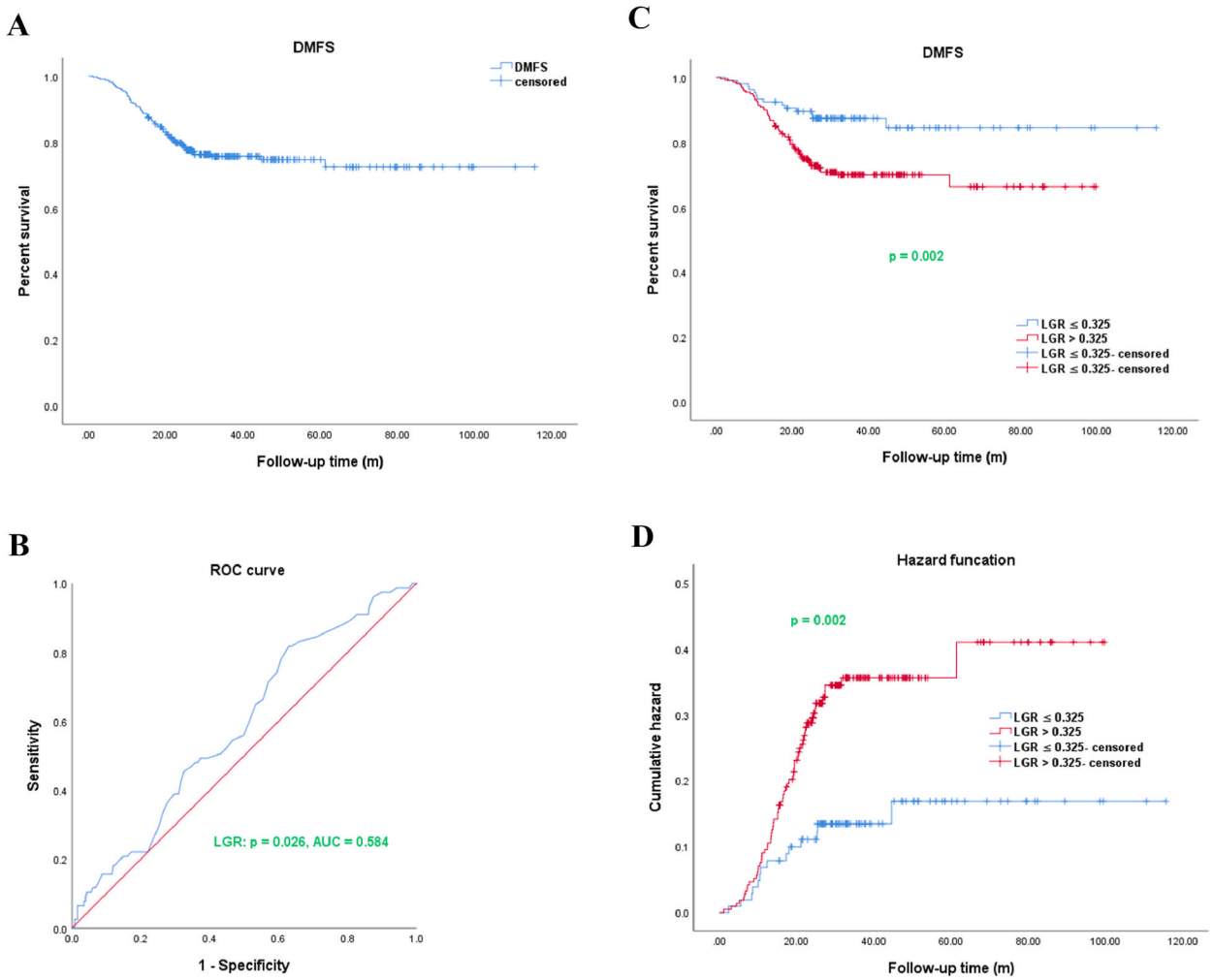

Fig. 1. Kaplan-Meier analysis curves for DMFS and ROC curve analysis of the LGR in rectal cancer patients receiving chemoradiotherapy and TME surgery. (A) The Kaplan-Meier plot of DMFS of 326 rectal cancer patients with chemoradiotherapy and TME surgery. **(B)** The LGR cut-off value for patients with rectal cancer was ascertained using the ROC. **(C)** A comparison of patients with rectal cancer withlow LGR (LGR≤0.325) and high LGR (LGR>0.325) group using DMFS analysis. (D) Comparing the survival risk analysis for DMFS between patients with rectal cancer who had low LGR (LGR≤0.325) and high LGR (LGR>0.325) group. A statistically significant value was P < 0.05. LGR, Lymphocyte to neutrophil granulocyte ratio.

Table 1
Patient characteristics (N = 326) and the association between distant metastasis and clinical characteristics.

Variable		Number	With distant metastasis n = 77	Without distant metastasis n = 249	Chi-square	P-value
Sex					0.001	0.979
	Male	224	53	171		
	Female	102	24	78		
Age (years)					2.955	0.086
	≤55	150	42	108		
	> 55	176	35	141		
BMI					2.409	0.3
	<18.5	17	2	15		
	≥18.5 and < 24	176	39	137		
	≥24	133	36	97		
cT stage					0.371	0.831
	T2	12	2	10		
	T3	253	61	192		
	T4	61	14	47		
cN stage					1.013	0.603
	N0	41	8	33		
	N1	117	31	86		
	N2	168	38	130		
cTNM stage					0.946	0.331
	II	40	7	33		
	III	286	70	216		
cMRF					2.046	0.153
	+	111	32	79		
	–	215	45	170		
cEMVI					1.255	0.263
	+	118	32	86		
	–	208	45	163		
ypT stage					7.665	**0.006**
	T1-2	160	26	134		
	T3-4	166	51	115		
ypTNM Stage					26.242	**<0.0001**
	I-II	218	33	185		
	III	108	44	64		
ypN stage					21.237	**<0.0001**
	N0	231	40	191		
	N1	66	22	44		
	N2	29	15	14		
Intravascular cancer thrombus					16.464	**<0.0001**
	Yes	53	24	29		
	No	273	53	220		
Nerve invasion		0			6.491	**0.011**
	Yes	68	24	44		
	No	258	53	205		
Tumor regression grade					8.572	**0.036**
	0	68	9	59		
	1	56	10	46		
	2	163	45	118		
	3	39	13	26		
Pathological complete response					10.749	**0.001**
	Yes	56	4	52		
	No	262	73	189		
BRAF mutation					1.467	0.226
	Yes	7	3	4		
	No	319	74	245		
NRAS mutation					2.225	0.136
	Yes	9	4	5		
	No	317	73	244		
KRAS mutation					1.285	0.257
	Yes	151	40	111		
	No	175	37	138		
AFP (ng/ml)					0.164	0.686
	<10	320	76	244		
	≥10	6	1	5		
CEA (ng/ml)					0.404	0.525
	<5.2	176	44	132		
	≥5.2	150	33	117		

(*continued on next page*)

Table 1 (*continued*)

Variable		Number	With distant metastasis n = 77	Without distant metastasis n = 249	Chi-square	P-value
CA199 (U/ml)					7.611	**0.006**
	<27	246	49	197		
	≥27	80	28	52		
CA50 (IU/ml)					8.151	**0.004**
	<25	275	57	218		
	≥25	51	20	31		
CA242(U/ml)					4.468	**0.035**
	<20	253	53	200		
	≥20	73	24	49		
CA724(U/ml)					0.124	0.725
	<6.9	271	63	208		
	≥6.9	55	14	41		
LGR					9.439	**0.002**
	≤0.325	106	14	92		
	>0325	220	63	157		

Abbreviation: LGR, Lymphocyte to neutrophil granulocyte ratio; CA199, carbohydrate antigen 199; CA50, carbohydrate antigen 50; CA724, carbohydrate antigen 724; CA242, carbohydrate antigen 242; AFP, alpha fetal protein; CEA, carcino-embryonic antigen; DMFS: distant metastasis-free survival; MRF: mesorectal fascia; EMVI: extramural vascular invasion; TRG: tumor regression grade; pCR: pathological complete response.

The survival study results and clinical features of patients with rectal cancer in the low and high LGR groups 326 individuals in all, with a median age of 57.5 years (range: 33–76 years), were newly diagnosed with rectal cancer. 31.3% of the population was female, and 68.7% was male. Out of 326 patients, 133 (40.8%) were overweight and 17 (5.2%) were thin. There were 111 (34%) patients with mesorectal fascia (MRF) invasion and 118 (36.2%) patients with extramural vascular invasion (EMVI). In 326 rectal cancer patients, there were 151 (46.3%) patients with KRAS mutation, 9 (2.8%) patients with NRAS mutation, and 7 (2.1%) patients with BRAF mutation. The levels of CEA of 150 (46%) patients were normal, while 176 (54%) patients were abnormal. Of the patients, 246 (75.5%) had normal levels of CA199, while 80 (24.5%) had abnormal ones. Six patients (1.8%) and fifty-five patients (16.9%) had increased CA724 levels and AFP levels, respectively. 51 patients (15.6%) had elevated CA50 levels while 73 patients (22.4%) had elevated CA242 values. Using the eighth edition TNM stage, 286 patients (87.7%) were classified as III stage, while 40 patients (12.3%) were classified as II stage. Based on the ideal cut-off value of the preoperative LGR, 326 patients with rectal cancer were divided into low LGR and high LGR groups. Furthermore, based on follow-up data, these patients were categorized into two groups: one for distant metastasis and the other for non-distant metastasis. The results used the Chi-sequare test indicated that the baseline level of LGR, the baseline level of CA199, CA50, and CA242, ypTNM stage, intravascular cancer thrombus (ICT), nerve invasion, tumor regression grade (TRG), and pathological complete response (pCR) were statistically significant factors related to the condition of distant metastasis in rectal cancer patients (Table 1). Additionally, we discovered that the median disease-free survival (DMFS) for patients with rectal cancer were 89.4 months (95% CI (84.0–94.9)). More significantly, the DMFS of low LGR group (100.7 months, 95% CI (93.2–108.3)) was markedly higher ($p = 0.002$) than the high LGR group (73.3 months, 95% CI (67.4–79.1)). Table 2 displayed the 1-year, 3-year, and 5-year total disease-free survival (DMFS) rates of 326 patients with rectal cancer, which were, respectively, 91.7%, 77.0%, and 76.4%. In the low LGR group, the 1-year, 3-year, and 5-year DMFS were, in turn, 93.4%, 87.7%, and 86.8%. The DMFS of patients with rectal cancer in the high LGR group was considerably greater than that of patients in the low LGR group, with the 1-year, 3-year, and 5-year DMFS being, respectively, 90.9%, 71.8%, and 71.4%. Consequently, we deduced that LGR might function as a unique and predictive marker for the DMFS of patients with rectal cancer who underwent neoadjuvant chemoradiotherapy and TME surgery.

3.2. Univariate regression analysis for DMFS in rectal cancer patients

To investigate the potential risk factors for distant metastasis of in patients with rectal cancer receiving neoadjuvant chemoradiotherapy and radical operation, we employed the Cox univariate regression model to examin the impact of clinical characteristics on DMFS. The findings indicated that DMFS in patients with rectal cancer receiving neoadjuvant chemoradiotherapy and TME surgery

Table 2
Basic clinical characteristics of survival analysis of rectal cancer patients in low and high LGR group.

Group	Median survival				P value	DMFS (%)		
	Estimated value	Standard error	95% Confidence interval			1-year	3-year	5-year
			Inferior limit	Upper limit				
LGR≤0.325	100.7	3.84	93.2	108.3	**0.002**	93.4	87.7	86.8
LGR >0325	73.3	2.97	67.4	79.1		90.9	71.8	71.4
Total	89.4	2.78	84.0	94.9		91.7	77.0	76.4

Abbreviation: LGR, Lymphocyte to neutrophil granulocyte ratio; DMFS, Distant metastasis-free survival.

Table 3
Univariate analysis for DMFS in rectal cancer patients with neoadjuvant chemoradiotherapy and TME surgery.

Variable		Number	Relative risk	95.0% Confidence interval		P value
				Inferior limit	Upper limit	
LGR						
	≤0.325	106	1			
	>0.325	220	2.393	1.341	4.273	**0.003**
Tumor regression grade						
	0–1	124	1			
	2–3	202	1.873	1.115	3.149	**0.018**
pCR						
	No	262	1			
	Yes	56	0.245	0.089	0.670	**0.006**
cT						
	2	12	1			
	3	253	1.048	0.328	3.341	0.937
	4	61	0.995	0.286	3.465	0.994
cN						
	0	41	1			
	1	117	1.411	0.648	3.070	0.386
	2	168	1.163	0.542	2.495	0.698
cTNM stage						
	II	40	1			
	III	286	1.435	0.659	3.123	0.363
cMRF						
	No	215	1			
	Yes	111	1.425	0.905	2.242	0.126
cEVMI						
	No	208	1			
	Yes	118	1.343	0.853	2.113	0.203
Intravascular cancer thrombus						
	No	273	1			
	Yes	53	2.799	1.727	4.537	**<0.0001**
Nerve invasion						
	No	258	1			
	Yes	68	1.82	1.124	2.948	**0.015**
Sex						
	male	224	1			
	female	102	0.969	0.598	1.570	0.898
Age (years)						
	≤55	150	1			
	> 55	176	1.525	0.973	2.389	0.065
BMI						
	<18.5	17	1			
	≥18.5 and < 24	176	2.051	0.495	8.498	0.322
	≥24	133	2.601	0.626	10.811	0.189
ypT						
	T1-2	160	1			
	T3-4	166	1.939	1.208	3.111	**0.006**
ypN						
	N0	231	1			
	N1	66	2.017	1.198	3.397	**0.008**
	N2	29	3.489	1.925	6.323	**<0.0001**
ypTNM stage						
	I-II	218	1			
	III	108	3.047	1.939	4.789	**<0.0001**
KRAS						
	Wild	175	1			
	Mutation	151	1.303	0.833	2.038	0.246
NRAS						
	Wild	317	1			
	Mutation	9	0.498	0.182	1.363	0.175
BRAF						
	Wild	319	1			
	Mutation	7	3.248	1.185	8.904	**0.022**
AFP (ng/ml)						
	<10	320	1			
	≥10	6	0.645	0.090	4.643	0.663
CEA (ng/ml)						
	<5.2	176	1			
	≥5.2	150	0.840	0.535	1.320	0.451

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Table 3 (*continued*)

Variable	Number	Relative risk	95.0% Confidence interval		P value
			Inferior limit	Upper limit	
CA199 (U/ml)					
<27	246	1			
≥27	80	1.947	1.223	3.098	**0.005**
CA242(U/ml)					
<20	253	1			
≥20	73	1.697	1.048	2.749	**0.032**
CA50 (IU/ml)					
<25	275	1			
≥25	51	2.159	1.297	3.595	**0.003**
CA724(U/ml)					
<6.9	271	1			
≥6.9	55	1.193	0.679	2.097	0.539

Abbreviation: LGR, Lymphocyte to neutrophil granulocyte ratio; CA199, carbohydrate antigen 199; CA50, carbohydrate antigen 50; CA724, carbohydrate antigen 724; CA242, carbohydrate antigen 242; AFP, alpha fetal protein; CEA, carcino-embryonic antigen; DMFS: distant metastasis-free survival; MRF: mesorectal fascia; EMVI: extramural vascular invasion; TRG: tumor regression grade; pCR: pathological complete response.

was significantly impacted by baseline levels of LGR, CA199, CA242, and CA50, TRG, pCR, ICT, nerve invasion, ypTNM stage, and BRAF mutation (Table 3). Specially, we found that the high levels of LGR, CA199, CA242, and CA50, high TRG, ICT (+), nerve invasion (+), advanced ypTNM stage, and BRAF mutation were risk factors associated with distant metastasis, while pCR was protective factor. The DMFS of patients with normal levels of CA199, CA242, and CA50 was found to be longer than that of patients with higher levels, as illustrated in Fig. 2A–L. On the other hand, the DMFS of the LGR high group was shorter than that of the LGR low group. Additionally, we discovered that although pCR was linked to the longer DMFS, high TRG, ICT (+), nerve invasion (+), advanced ypTNM stage, and BRAF mutation were clearly connected with the shorter DMFS.

3.3. Multiple regression analysis for DMFS in rectal cancer patients

In order to further identify risk variables and further show the influencing factors that were statistically significant in the univariate regression model analysis for distant metastases of rectal cancer patients, the Cox multiple regression model analysis was employed in this section. Table 4 presented the results of our analysis indicating that patients with rectal cancer who had high LGR levels ($p = 0.008$, HR = 2.224, 95%CI (1.233–4.013)), ypTNM stage (III) ($p = 0.011$, HR = 2.649, 95%CI (1.255–5.591)), and BRAF mutation ($p = 0.006$, HR = 4.456, 95%CI (1.548–12.828)) were at risk factors for distant metastasis. Multiple regression analysis results, as illustrated in Fig. 3A–D, also showed that patients in the high LGR group had a shorter disease-free survival (DMFS) than patients in the low LGR group. On the other hand, patients in the early patients (ypTNM stage (I-II)) had a longer DMFS than patients in the advanced rectal cancer patients (ypTNM stage (III)).Additionally, we discovered that the longer DMFS was linked to ICT (−), but the BRAF mutation was clearly associated with the shorter DMFS.

3.4. Creation and use of the nomogram

The construction of a Nomogram diagram to assess the significant factors influencing the distant metastasis-free survival (DMFS) of rectal cancer patients in a multivariate regression model represented an important step in understanding the risk factors associated with this condition. The Nomogram diagram incorporated risk coefficients for various indexes which were screened from a multifactor Cox regression model, including ypTNM stage, ICT, pCR, TRG, BRAF, the baseline level of CA199, CA50, CA724, and the level of LGR. This comprehensive tool enabled the estimation of risk factors affecting DMFS and survival chance for each individual rectal cancer patient. Fig. 4 showed the nomogram diagram. The following were the steps to using the Nomogram: finding the patient's value for each predictor, drawing a line up to the vertex reference line from each predictor, adding the points from each predictor, finding the sum at the total point reference line, and drawing a line down from the total point line are the first five steps in the process. The 1-, 3-, and 5-year survival rates as well as the median survival probability might be acquired by means of this procedure. According to the above instructions for the use of the Nomogram, we found that rectal cancer patients with an early ypTNM stage, negative ICT, absence of nerve invasion, pCR, wild-type BRAF, normal levels of CA199, CA50, and CA724, and a low level of LGR were associated with lower risk and greater prognosis.

4. Discussion

The second most frequent cause of cancer-related deaths in the US is colorectal cancer (CRC). According to estimates, colorectal cancer (CRC) ranks fifth in cancer-related fatalities and is the third most frequent malignancy in China [19]. 60–75% of colorectal cancer patients have rectal cancer, which put the publi's health and quality of life in grave jeopardy, particularly in cases of locally advanced rectal cancer (LARC). The standard treatment for patients with LARC is neoadjuvant chemoradiotherapy (nCRT) and TME surgery. However, postoperative distant metastasis obviously affects the overall survival prognosis of LARC patients. In addition, there

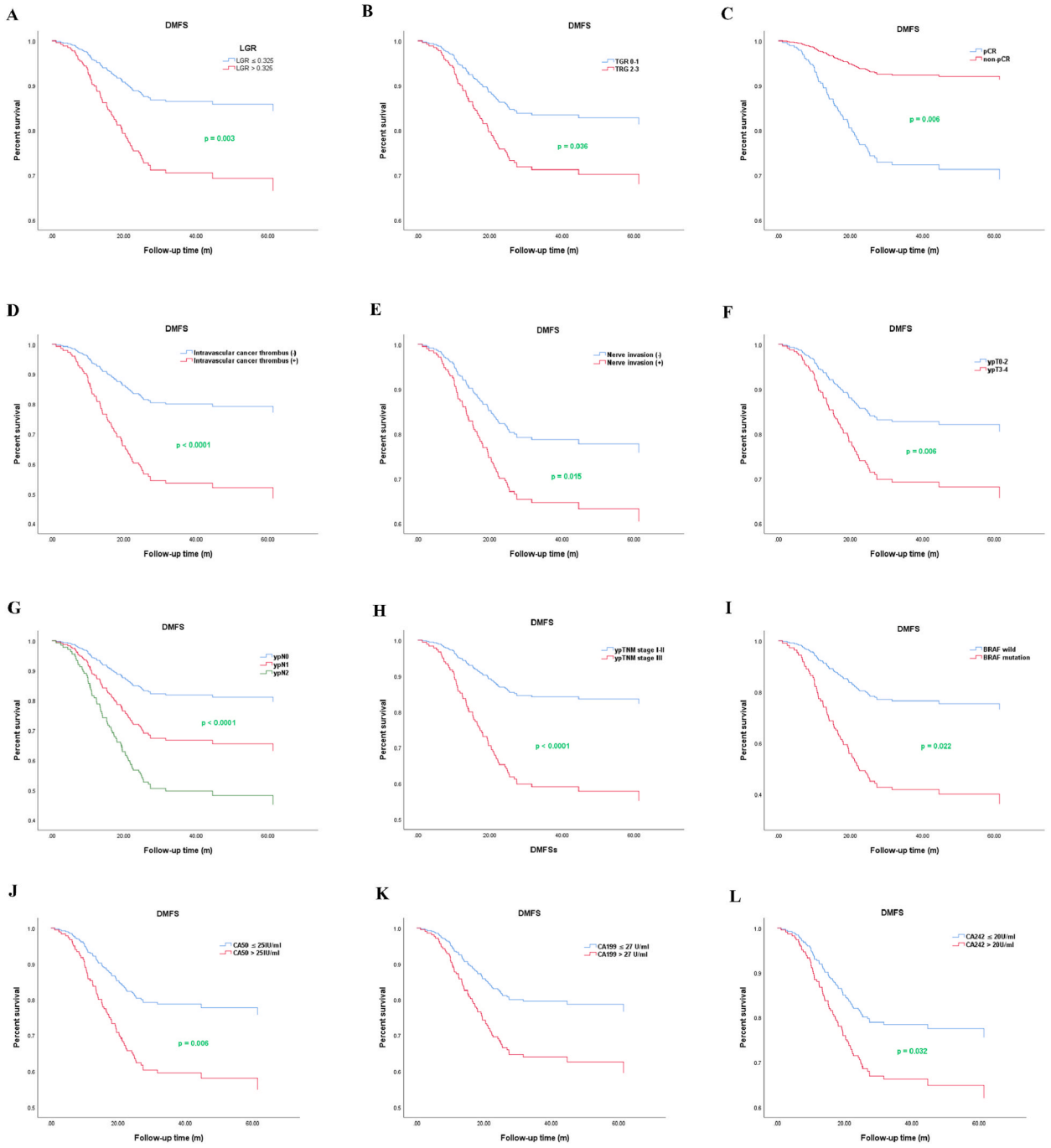

Fig. 2. Patients with rectal cancer receiving neoadjuvant chemoradiotherapy and TME had Kaplan-Meier survival curves for DMFS in univariate regression analysis. The significant indexes, including LGR **(A)**, TRG **(B)**, pCR **(C)**, intravascular cancer thrombus **(D)**, nerve invasion **(E)**, ypT **(F)**, ypN **(G)**, ypTNM stage **(H)**, BRAF **(I)**, CA50 **(J)**, CA199 **(K)**, and CA242 **(L)**, of univariate regression analysis for DMFS were showed by Kaplan-Meier survival curves. $P < 0.05$ was statistically significant. LGR, Lymphocyte to neutrophil granulocyte ratio; TRG, Tumor regression grade; pCR, Pathological complete response; CA50, carbohydrate antigen 50, CA199, carbohydrate antigen 199, CA242, carbohydrate antigen 242; CEA, carcino-embryonic antigen.

is still a lack of effective markers to predict the distant metastasis of LARC patients undergoing nCRT and TEM surgery. Therefore, in this study, we explored the predictive value of a novel marker (LGR: lymphocytes to neutrophils granulocyte radio) for distant relapse-free survival (DMFS) in rectal cancer patients undergoing neoadjuvant chemoradiotherapy and TME surgery, in order to screen high-risk patients for higher intensity therapy, thereby reducing the risk of distant metastasis and improving overall survival.

Table 4
Multivariate analysis for DMFS in rectal cancer patients with neoadjuvant chemoradiotherapy and TME surgery.

Variable		Number	Relative risk	95.0% Confidence interval		P value
				Inferior limit	Upper limit	
LGR						
	≤0.325	106	1			
	>0.325	220	2.224	1.233	4.013	**0.008**
Tumor regression grade						
	0–1	124	1			
	2–3	202	1.164	0.631	2.146	0.627
pCR						
	No	262	1			
	Yes	56	0.399	0.128	1.243	0.113
Intravascular cancer thrombus						
	No	273	1			
	Yes	53	1.707	0.964	3.025	0.067
Nerve invasion						
	No	258	1			
	Yes	68	1.217	0.706	2.096	0.480
ypT						
	T1-2	160	1			
	3–4	166	0.874	0.497	1.536	0.639
ypN						
	N0	231	1			
	N1	66	0.672	0.311	1.455	0.313
	N2	29	1.018	0.451	2.298	0.965
ypTNM stage						
	I-II	218	1			
	III	108	2.649	1.255	5.591	**0.011**
BRAF						
	Wild	319	1			
	Mutation	7	4.456	1.548	12.828	**0.006**
CA199 (U/ml)						
	<27	246	1			
	≥27	80	1.317	0.562	3.091	0.526
CA242(U/ml)						
	<20	253	1			
	≥20	73	0.886	0.425	1.844	0.746
CA50 (IU/ml)						
	<25	275	1			
	≥25	51	1.707	0.760	3.837	0.196

Abbreviation: LGR, Lymphocyte to neutrophil granulocyte ratio; CA199, carbohydrate antigen 199; CA50, carbohydrate antigen 50; CA724, carbohydrate antigen 724; CA242, carbohydrate antigen 242; AFP, alpha fetal protein; CEA, carcino-embryonic antigen; DMFS: distant metastasis-free survival; TRG: tumor regression grade; pCR: pathological complete response.

Inflammation plays a significant role in cancer development and progression. Systemic inflammatory responses can promote processes such as angiogenesis, inhibition of apoptosis, and DNA damage, which are associated with cancer growth [20]. Several studies have shown that pro-inflammatory factors and chemokines in the immunological microenvironment, as well as inflammatory markers in the peripheral blood, significantly affect patients' prognoses with solid tumors [21,22]. By assessing various circulating blood cell counts, including neutrophil, lymphocyte, platelet, monocyte, and C-reactive protein (CRP), we can calculate several inflammatory-based scores such as neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), and lymphocyte-to-C-reactive protein ratio (LCR). It has been reported in the literature that neutrophils play a two-sided role in the occurrence and development of tumors [23]. On the one hand it promotes tumor progression by promoting angiogenesis, tumor cell proliferation, metastasis, and evasion of immune responses, on the other hand, it inhibits tumors by removing pathogens and repairing tissues [24]. In addition, under the action of chemokines and cytokines secreted by tumor cells, tumor stromal cells and immune cells, monocytes in blood are recruited around tumor cells and become tumor-related macrophages (TAM). TAM, a macrophage infiltrating into the tumor, plays a "double-edged sword" role in the occurrence and development of the tumor [25]. Due to the reversible and adjustable polarization of macrophages to M1 and M2 types, M1-dominated macrophages play an anti-tumor role in the early stage, while M2-dominated macrophages play a role in promoting tumor development, metastasis and invasion in the middle and late stage [26–28]. Through their ability to nourish tumor stem cells, induce angiogenesis, facilitate cell proliferation, and elude immune surveillance, platelets have been linked to the growth of tumors [29]. Additionally, a relative decrease in lymphocyte count may indicate a poor cell-mediated cancer immune response. Serum CRP levels are a good predictive biomarker for different forms of cancer when paired with other indicators, as they indicate systemic inflammatory response [30]. Previous studies have suggested that NLR, as an inflammatory index, can serve as a prognostic factor in hepatocellular carcinoma [31], breast cancer [32], and colorectal cancer [33]. In our study, we observed that lymphocyte-to-neutrophil granulocyte ratio (LGR) was associated with distant metastasis in rectal cancer patients who received neoadjuvant chemoradiotherapy

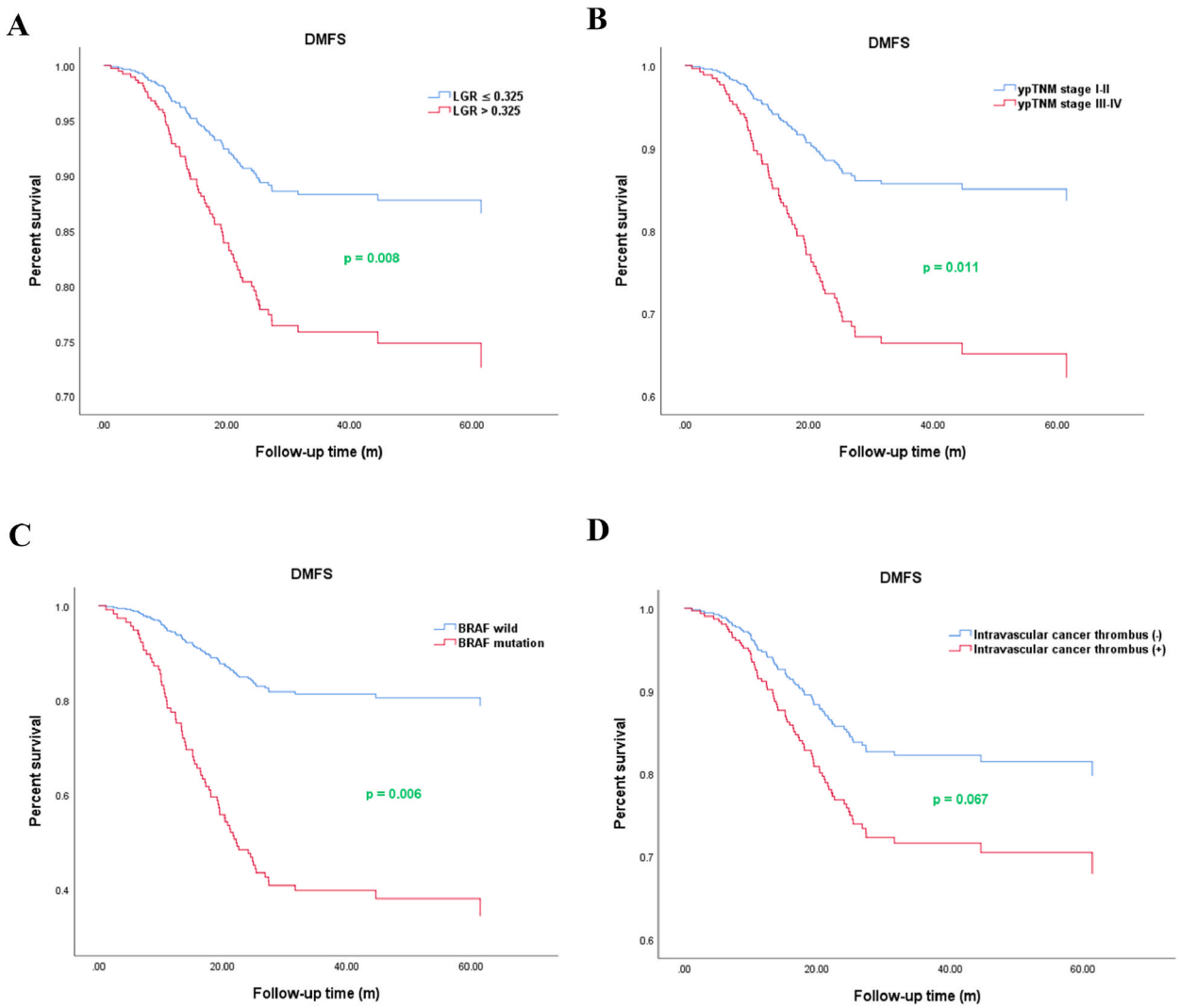

Fig. 3. Patients with rectal cancer receiving neoadjuvant chemoradiotherapy and TME had Kaplan-Meier survival curves for DMFS in multiple regression analysis. The significant indexes, including LGR **(A)**, ypTNM stage **(B)**, BRAF **(C),** and intravascular cancer thrombus **(D)**, of univariate regression analysis for DMFS were showed by Kaplan-Meier survival curves. $P < 0.05$ was statistically significant. LGR, Lymphocyte to neutrophil granulocyte ratio.

and TME surgery. The results indicated that rectal cancer patients in the low LGR group had a longer DMFS compared to those in the high LGR group.

Our results analyzed by using the multifactor Cox regression model revealed that in addition to the level of LGR significantly affecting the DMFS of rectal cancer patients undergoing neoadjuvant chemoradiotherapy and TME surgery, BRAF mutation status and postoperative pathological TNM stage were also close to the development of distant metastases in rectal cancer patients. The results of Cox univariate regression and Chi-square test also showed that LGR level, BRAF mutation and pathological stage had significant influence on DMFS. This indicated that the use of three different statistical methods can achieve cross-validation of the impact of our key variables on the outcome. According to a study from China, BRAF mutation was significantly associated with advanced TNM ($P < 0.001$), more distant metastases ($P = 0.025$), and worse overall survival (OS, $P < 0.001$; multivariate HR = 4.2, $P = 0.004$) in colorectal cancer patients [34]. In addition, another study showed that one common mutation BRAF gene (most commonly V600E substitution) occurs in ~10% of patients with metastatic CRC (mCRC) and is a marker of poor prognosis [35]. Therefore, the prognosis of locally advanced rectal cancer patients with BRAF mutation screened by genetic testing may be improved by timely further therapy after receiving concurrent chemoradiotherapy and TME surgery. Of course, the later the surgical pathological stage of rectal cancer patients after neoadjuvant chemoradiotherapy were, the greater the likelihood of distant metastasis was, and naturally they also need more active therapy to maximize the overall survival. The results of our study also gave corresponding tips for rectal patients undergoing neoadjuvant chemoradiotherapy and TME surgery. For example, the results of univariate Cox regression analysis found that high TRG, ICT (+), nerve invasion (+), advanced ypTNM stage, and BRAF mutation were all obviously associated with the shorten DMFS, while

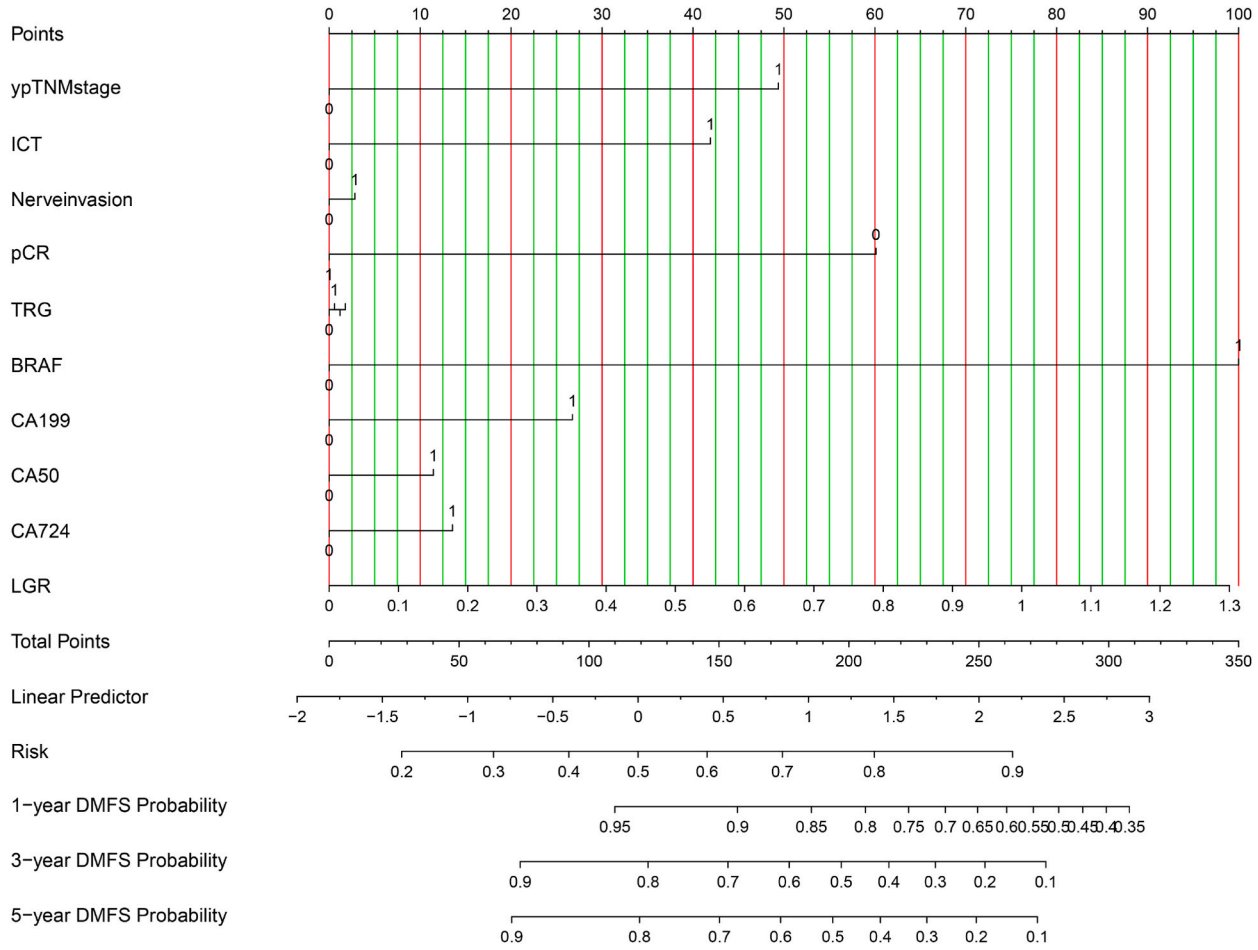

Fig. 4. A nomogram for forecasting the likelihood of survival in patients with rectal cancer after neoadjuvant chemoradiotherapy plus TME surgery. Drawing a line straight up to the point reference line yielded the value of each predictor when using the nomogram. To find the expected probability of node positivity, the points were added together and a line was drawn down from the total points line. (ypTNM stage I-II = 0, ypTNM stage III = 1; intravascular cancer thrombus (ICT (−)) = 0, ICT (+) = 1; Nerve invasion (−) = 0, Nerve invasion (+) = 1, Not-pCR = 0, pCR = 1; BRAF wild = 0, BRAF mutation = 1; CA199 ≤ 27 U/ml = 0, CA199 > 27U ng/ml = 1; CA50 ≤ 25 IU/ml = 0, CA50 > 25 IU/ml = 1; CA242 ≤ 20 U/ml = 0, CA242 ≥ 20 ng/ml = 1); LGR, Lymphocyte to neutrophil granulocyte ratio.

pCR was related to the longer DMFS, which was mutually corroborated with a large number of previous research results [36,37].

However, the results are susceptible to confounding effects because our investigation is limited to a single-center retrospective analysis. To confirm our findings, multi-center involvement is also necessary, and the sample size needs to be raised even more. Further fundamental research, such as cell culture and the creation of animal models, will be required in the future to confirm our results on several fronts.

5. Conclusion

To sum up, our study was the first to retrospectively analyze the relationship between LGR and DMFS in patients with LARC undergoing neoadjuvant chemoradiotherapy and TME surgery. We found evidence supporting a longer DMFS in LARC patients with a low LGR level, early ypTNM stage, and wild-type BRAF. Furthermore, in order to predict the DMFS rate and provide new information for LARC patients undergoing neoadjuvant chemoradiotherapy and TME surgery, we first created a nomogram plot to graphically represent the contribution of significant variables for DMFS in multivariate regression analysis.

Ethics approval and consent to participate

The study's ethical code, 2003215–1, was approved by the Fudan University Shanghai Cancer Center's ethics committee. Every patient gave their informed permission.

Consent for publication

All the authors declare their consent to publish the manuscript.

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Data availability statement

Data included in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Minghe Lv: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Songsong Wu:** Validation, Investigation, Formal analysis, Data curation. **Zhiyuan Zhang:** Validation, Formal analysis. **Zhen Zhang:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Juefeng Wan:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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List of abbreviations

LARC	Locally advanced rectal cancer
LGR	Lymphocyte to neutrophil granulocyte ratio
CA199	carbohydrate antigen 199
CA50	carbohydrate antigen 50
CA724	carbohydrate antigen 724
CA242	carbohydrate antigen 242
AFP	alpha fetal protein
CEA	carcino-embryonic antigen
DMFS	distant metastasis-free survival
MRF	mesorectal fascia
EMVI	extramural vascular invasion
TRG	tumor regression grade
pCR	pathological complete response
ROC:	Receiver operator characteristic
AUC	Area under curve

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