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Original Article

Prognostic Significance of Serum Biomarkers in Rectal Cancer Patients Treated with Neoadjuvant Radiotherapy Followed by Radical Surgery

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ABSTRACT

Background: The identification of predictive prognostic factors in locally-advanced rectal cancer patients is crucial before surgery. In this study, we aimed to evaluate the prognostic significance of pretreatment with various serum biomarkers in locally-advanced rectal cancer patients treated with neoadjuvant radiotherapy followed by radical surgery.

Methods: A total of 154 locally-advanced rectal cancer patients who received preoperative radiotherapy or chemoradiation followed by total mesorectal excision at the Toranomon Hospital, Tokyo from April 2010 to December 2015 were retrospectively analyzed to evaluate prognostic variables. Various blood tests, neutrophil-to-lymphocyte ratio (NLR), modified Glasgow Prognostic Score (mGPS), and serum albumin, C-reactive protein (CRP), carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), and serum p53 antibodies (s-p53-Abs) levels were evaluated before surgery for their prognostic impact. Relapse-free survival (RFS) was evaluated by the Kaplan-Meier method and differences were assessed using the log-rank test. The Cox proportional hazard model was used to assess independent predictors for RFS.

Results: While age, tumor distance from anal verge, CA19-9, and ypT stage were associated with RFS, inflammation and nutritional status such as NLR and mGPS were not. Based on multivariate analyses, patients with a shorter tumor distance from anal verge, higher CA19-9 levels and advanced ypT stage had poorer RFS. Adjuvant chemotherapy in patients with elevated CA19-9 levels demonstrated a trend toward improved RFS, although this was statistically insignificant.

Conclusions: Although neither inflammation scores nor nutritional status such as NLR and mGPS were prognostic factors, serum CA19-9 level was found to be an independent prognostic predictor of RFS.

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KEYWORDS: neutrophil-to-lymphocyte ratio, carbohydrate antigen 19-9, locally advanced rectal cancer, preoperative radiotherapy, prognostic factor

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Introduction

Locally-advanced rectal cancer is one of the greatest challenges in colorectal cancer treatment. Preoperative radiotherapy followed by total mesorectal excision is currently the standard treatment for locally-advanced rectal cancer in Western countries in order to reduce local recurrence.[1–3] However, in Japan the standard treatment, particularly for low rectal cancer, is surgery alone with lateral pelvic lymph node dissection.[4] Recently, several institutions have introduced preoperative radiotherapy to reduce local recurrence and to increase the anal sphincter preservation ratio. Although most of the patients benefited from preoperative radiotherapy, some patients remained unresponsive. Moreover, even with the use of preoperative radiotherapy, recurrence will occur in about 25% of those patients.[5] In order to predict patients' prognosis and optimize surveillance of patients, several biomarkers which can be obtained before radiotherapy should be investigated.

Carcinoembryonic antigen (CEA) is the most widely used tumor marker in colorectal cancer.[6] Although carbohydrate antigen 19-9 (CA19-9) is not specific to colorectal cancer, the simultaneous assessment of CA19-9 and CEA levels may increase the diagnostic sensitivity in colorectal cancer.[6, 7] Serum p53 antibodies (s-p53-Abs) are also one of the standard serum markers for colorectal cancer[8, 9] however their prognostic significance is controversial.[9–11] Recently, scores based on inflammation and nutritional status, such as neutrophil-to-lymphocyte ratio (NLR), modified Glasgow Prognostic Score (mGPS), C-reactive protein (CRP), and platelet count, have been reported to be predictive prognostic factors in colorectal cancer.[12–15] However, the prognostic significance of NLR in locally-advanced rectal cancer patients with preoperative treatment remains a point of debate.[16, 17]

This study aimed to investigate the prognostic significance of inflammation-based indexes, serum markers, and clinicopathological features in locally-advanced rectal cancer patients treated with preoperative radiotherapy followed by radical surgery.

Materials and Methods

Patients

In this study, 154 locally-advanced rectal cancer patients who received preoperative radiotherapy followed by total mesorectal excision at the Toranomon Hospital, from April 2010 to December 2015, were enrolled. The criteria for preoperative radiotherapy were low rectal cancer with clinical stage II or III based on the second English edition of the Japanese Society for Cancer of the Colon and Rectum.[18] Low rectal cancer is defined as a tumor located below the peritoneal reflection. Histopathological classification, staging (ypT and ypN), and tumor regression grade were determined according to the second English edition of the Japanese Society for Cancer of the Colon and Rectum. All patients were followed up by the end of March 2017.

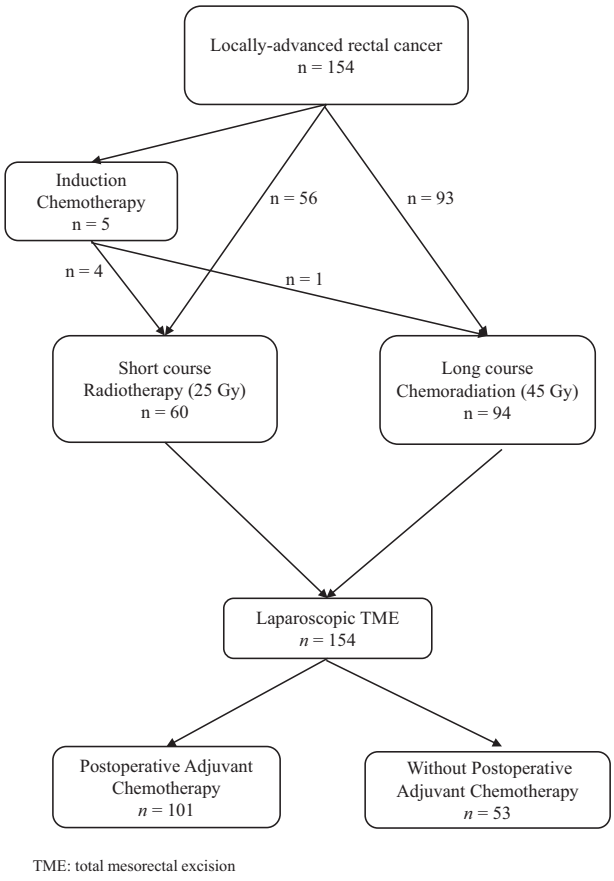

Fig. 1 Treatment regimen

Radiotherapy

Patients were treated with either short (25 Gy: 5 Gy × 5 fractions) or long (45 Gy: 1.8 Gy × 25 fractions) course irradiation. Patients treated with long course irradiation underwent concurrent chemotherapy using oral fluoropyrimidine with or without CPT-11. Five patients underwent systemic induction chemotherapy before radiotherapy. Fig. 1 shows the treatment regimen and number of patients at each treatment modality. Long course irradiation was our first choice of treatment. Short course irradia-

tion combined with induction chemotherapy was the other option for patients who potentially benefit from the short preoperative treatment period. For example, multiple nodal metastases and/or primary tumors which do not require tumor shrinkage for anal preservation were candidates for short course irradiation. Elderly patients and patients with several comorbidities were also candidates for short course irradiation.

Postoperative adjuvant therapy

Oral fluoropyrimidine was administered for six months after surgery. We recommended postoperative adjuvant therapy to all who underwent preoperative radiotherapy, followed by total mesorectal excision.

Blood analysis

Blood samples were collected from each patient before preoperative radiotherapy. The total white blood cell, neutrophil and lymphocyte counts, serum albumin concentration, and CRP, CEA, CA19-9, and s-p53-Abs levels were evaluated. NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. A cutoff value for NLR of 3.0 was used to categorize patients. In addition, 4.7 ng/mL and 36 U/mL were used as cutoff values for CEA and CA19-9, respectively, to categorize patients into those with high and low serum levels. mGPS was determined as previously described (patients with CRP $\leq$ 1 mg/dL, score 0; those with CRP $>$ 1 mg/dL and albumin $\geq$ 3.5 g/dL, score 1; and those with CRP $>$ 1.0 mg/dL and albumin $<$ 3.5 g/L, score 2).[12]

Follow-up

After surgery, patients were followed up every three months for five years or until the end of March 2017. Pelvic MRI examination was performed three months after surgery, and chest, abdominal, and pelvic CT examinations were performed six months after surgery; MRI and CT examinations were performed every six months. Total colonoscopy was performed once a year. Local and distant recurrences were defined by clinical, radiological, or histological findings. Type of recurrence was classified as distant recurrence and local recurrence. Recurrence of tumors located outside of the pelvic cavity was defined as distant recurrence. Recurrence of tumors located inside of the pelvic cavity was defined as local recurrence.

Statistical analysis

The patients' database was prospectively maintained using standardized fields. All statistical analyses were performed using the IBM SPSS Statistics version 24.0 Software Package (International Business Machines [IBM] Corp., Armonk, NY). Differences between groups were analyzed using Student's *t*-test for continuous variables, or Fisher's exact or χ^2 test for categorical variables. The Cox regression analysis was performed using disease recurrence as an outcome with a significance level of $P < 0.05$. The relapse-free survival (RFS) was defined as the time from the date of surgery to any recurrence. RFS was evaluated by the Kaplan-Meier method, and differences were assessed using the log-rank test. Covariates that were significant at $P < 0.05$ were included in the multivariate Cox proportional hazard model to assess independent predictors for RFS. The results of Cox model analysis were reported using the hazard ratio and 95% confidence interval.

Results

Patients' characteristics and tumor recurrence

Table 1 shows the clinicopathological characteristics of patients. Among a total of 25 patients with recurrence, 20 patients developed distant recurrence, two patients developed local recurrence and three patents developed both types of recurrence. The tumor distance from anal verge, CA19-9, ypT and ypN stages was significantly associated with tumor recurrence, whereas the tumor distance from CEA, s-p53-Abs, platelet count and mGPS was not significantly associated.

Relationship between NLR, mGPS, and RFS

RFS curves were compared by focusing on inflammation-based indexes (Fig. 2). No statistically significant differences were observed between the high and low NLR groups (Fig. 2A). The normal mGPS group demonstrated a relatively better survival than the high mGPS group; however, the difference was not statistically significant (Fig. 2B).

Comparison of RFS between patients with high- and low-serum biomarker levels

RFS curves were compared by focusing on serum biomarker levels (Fig. 3). Although the low CEA group demonstrated a relatively better survival than the high CEA group, the difference was not statistically significant ($P = 0.199$; Fig. 3A). The low CA19-9 group displayed a significantly better survival than the high CA19-9 group ($P = 0.033$; Fig. 3B). The low and high s-p53-Abs groups exhibited similar survival ($P = 0.694$; Fig. 3C).

Multivariate analysis for RFS

Among the various clinicopathological variables evaluated in the univariate analysis (Table 1), age, tumor dis-

Table 1 Patients' characteristics

	All ($n=154$)	Patients with recurrence	*P-value*
Age, years			0.025
>65	88	9	
≤65	66	16	
Gender			0.465
Male	100	18	
Female	54	7	
Tumor distance from anal verge, cm			0.032
>5	42	11	
≤5	112	14	
CEA, μg/L			0.199
≥4.7	77	10	
<4.7	72	15	
Unknown	5		
CA19-9, U/mL			0.033
>36	118	16	
≤36	33	9	
Unknown	3		
Serum p53 antibody, U/mL			0.694
>1.30	82	19	
≤1.30	38	6	
Unknown	34		
Platelet, ×1000 /μL			0.962
>370	142	23	
≤370	12	2	
NLR			0.763
>3	98	17	
≤3	45	8	
Unknown	11		
mGPS			0.444
0	132	23	
1, 2	22	2	
Histological type			0.42
tub1-2	138	22	
por sig muc	16	3	
ypT stage			0.003
ypT1-2	66	4	
ypT3-4	88	21	
ypN stage			0.001
ypN−	108	11	
ypN+	46	14	
Tumor regression grade			0.523
G1	92	17	
G2, 3	62	8	
Postoperative adjuvant chemotherapy			0.311
−	53	7	
+	101	18	
Recurrence			
Distant		20	
Local		2	
Both		3	

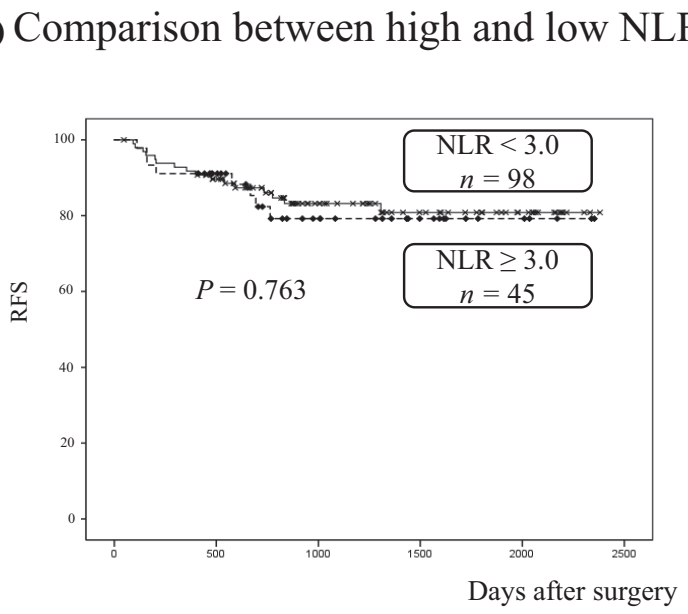

Fig. 2 Relapse-free survival curves. A; comparison between high and low neutrophil-lymphocyte ratio. B; comparison between modified Glasgow Prognostic Score 0, 1 and 2.

tance from anal verge, and CA19-9 were evaluated by multivariate analysis for RFS (Table 2). Age (P = 0.021), short tumor distance from anal verge (P = 0.011), and high serum CA19-9 level (P = 0.010) were independent risk factors for a poor RFS.

Comparison of clinicopathological factors between high and normal CA19-9 groups

Because CA19-9 was selected as an independent prognostic factor for RFS, various clinicopathological factors were evaluated to compare high and normal CA19-9 levels (Table 3). High CEA (P = 0.002) and advanced tumor depth (P = 0.038) were significantly associated with high serum CA19-9 levels.

Impact of serum CA19-9 levels and postoperative adjuvant chemotherapy on patients' RFS

A total of 99 patients received postoperative adjuvant chemotherapy, and the high CA19-9 group demonstrated a relatively poor RFS compared with the normal CA19-9

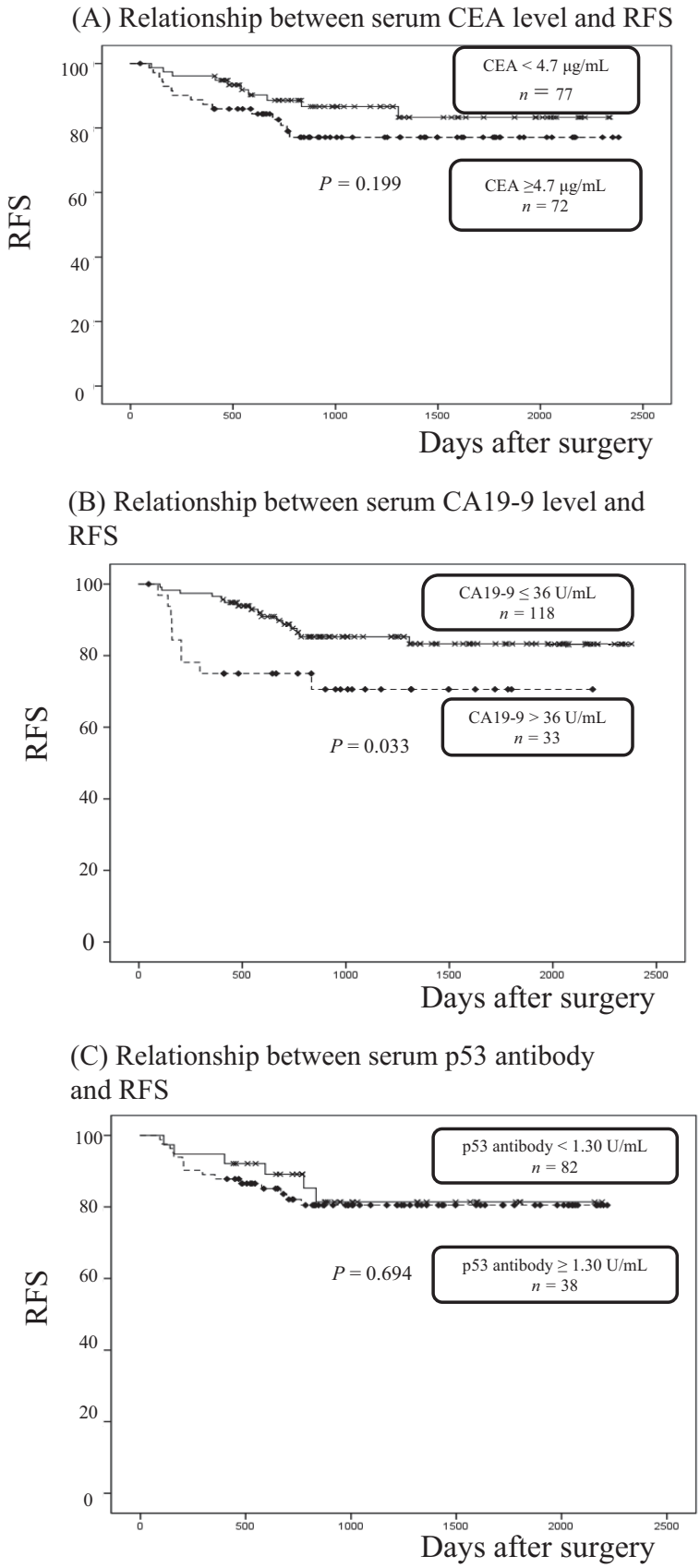

Fig. 3 Relapse-free survival curves. A; comparison between high and normal CEA groups. B; comparison between high and normal CA19-9. C; comparison between serum p53 antibodies positive and negative groups.

Table 2 Multivariate analysis for relapse-free survival

	HR	95% CI	*P-value*
Age, years (>65/≤65)	2.636	0.969-5.363	0.021
Tumor distance from anal verge (>5 cm/≤5 cm)	2.828	1.040-6.496	0.011
Serum CA19-9 (≥36 U/ml /<36 U/ml)	2.960	1.085-6.589	0.010

Table 3 Comparison of clinicopathological factors between high and normal CA19-9 groups

	CA19-9≤36 U/mL n=118	CA19-9>36 U/mL n=33	*P-value*
Age, years			0.356
>65	64	22	
≤65	54	11	
Gender			0.551
Male	74	22	
Female	44	11	
Tumor distance from anal verge, cm			0.455
>5	65	18	
≤5	53	15	
CEA			0.002
≥4.7	60	10	
<4.7	58	23	
Serum p53 antibody, U/mL			0.694
>1.30	38	13	
≤1.30	53	16	
Unknown	27	4	
Platelet count, ×1000/μL			0.553
>370	108	30	
≤370	10	3	
NLR			0.326
>3	78	21	
≤3	34	12	
Unknown	7	0	
mGPS			0.597
0	103	29	
1, 2	15	4	
Histological type			0.47
tub1-2	104	30	
por sig muc	14	3	
ypT stage			0.038
ypT1-2	51	11	
ypT3-4	67	22	
ypN stage			0.056
ypN−	88	19	
ypN+	30	14	
Tumor regression grade			0.474
G1	74	21	
G2, 3	44	12	
Postoperative adjuvant chemotherapy			0.109
−	37	15	
+	81	18	

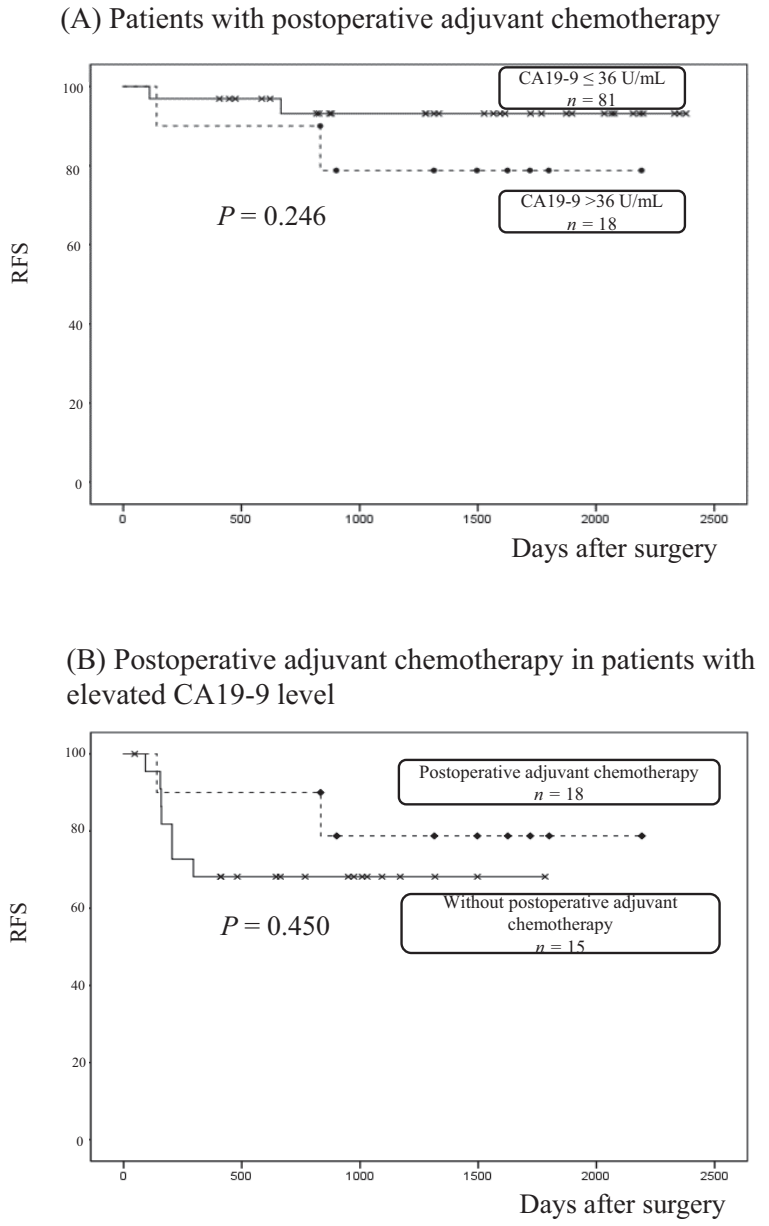

Fig. 4 The relationship between serum CA19-9 level and RFS of patients. A; comparison between high and normal CA19-9 in patients treated with postoperative adjuvant chemotherapy. B; comparison between patients with high CA19-9 levels treated with and without postoperative adjuvant chemotherapy.

group, with no statistical significance (P = 0.246; Fig. 4A). In the high CA19-9 group, although the difference was not statistically significant, the presence of postoperative adjuvant chemotherapy seemed to improve RFS (P = 0.450; Fig. 4B).

Discussion

In our study, age, tumor distance from anal verge, CA 19-9, ypT stage, and ypN stage were associated with RFS, whereas inflammation and nutritional status, such as s-p53-Abs, NLR and mGPS, were not. Certainly, ypT stage and ypN stage have a significant association with poor prognosis, but these factors are only known post-surgery. Based on multivariate analyses on factors obtained before preoperative radiotherapy, patients with an older age, shorter tumor distance from anal verge, and high CA19-9 levels have a poorer RFS than the other groups. Adjuvant chemotherapy in patients with high CA19-9 levels slightly

improved RFS, although the difference was not statistically significant.

Previous studies have not considered the prognostic impact of inflammation scores. Based on 1,788 colon cancer patients and 1,943 rectal cancer patients, Chiang et al. concluded that preoperative NLR influences disease-free survival in colorectal cancer patients.[19] In their study, although elevated NLR (> 3) was associated with worse outcomes of both colon and rectal cancers, the difference was larger in colon cancer than in rectal cancer. However, our study demonstrated that NLR was not associated with RFS of rectal cancer. This discordance is partially explained by the effects of preoperative radiation/chemoradiation, which may improve RFS, particularly of rectal cancer, with high NLR.

Regarding the prognostic significance of s-p53-Abs, previous reports have shown discordant results.[9–11] Suzuki et al. have suggested that serum titer levels potentially affect patients' oncological outcomes.[11] Unfortunately in our study there was no precise information regarding monitoring the titer to evaluate its prognostic significance. Similar to a previous study on oncological outcomes in metastatic colorectal cancer patients,[20] patients in this study did not display any unfavorable effect of s-p53-Abs on the treatment response to preoperative therapy.

Zhang et al. have reported that high CA19-9 levels are associated with poor survival in rectal cancer patients surgically-treated with neoadjuvant chemoradiation.[21] Consistent with the study by Zhang et al., we also found that patients with high CA19-9 levels demonstrated a significantly poor RFS. CA19-9 is an antigen expressed by the glycosylated MUC1 protein, which plays an essential role in cancer invasion by enhancing cell adhesion and by indirectly promoting angiogenesis.[22] Patients of such a subgroup with high CA19-9 may be good candidates for postoperative adjuvant therapy. There was a slight tendency toward improved RFS after postoperative adjuvant chemotherapy, although it had no statistically significant difference.

Our study had certain limitations. First, the number of patients was relatively small, and this was a single-institutional study. Second, this study was a retrospective study, with a relatively short follow-up period. Finally, the management of locally-advanced rectal cancer significantly varied among doctors because of evolving treatment regimens and heterogeneity of patient preferences.

In conclusion, our study suggests that serum CA19-9 levels are useful prognostic predictors of RFS in locally-advanced rectal cancer patients treated with preoperative radiotherapy, whereas s-p53-Abs, NLR, and mGPS levels are not. These results might be affected by the biological impact of preoperative radiotherapy. Further investigation is needed to clarify the impact of postoperative adjuvant therapy on patients treated with neoadjuvant radiotherapy.

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Conflicts of interest: The authors have no conflicts of interest.

Ethical statement: This study was approved by the Institutional Review Board of the Toranomon Hospital (#1507).

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