Are there predictors that can determine neoadjuvant treatment responses in rectal cancer?

Sema Yılmaz Rakıcı[1], Recep Bedir[2], Celile Hatipoğlu[3]

[1]Department of Radiation Oncology, Recep Tayyip Erdoğan University School of Medicine, Rize, Turkey
[2]Department of Medical Pathology, Recep Tayyip Erdoğan University School of Medicine, Rize, Turkey
[3]Department of Public Health, Denizli Provincial Directorate of Health, Denizli, Turkey

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ABSTRACT

Background/Aims: *This study aimed to determine a predictive bioindicator that would detect the treatment response of patients diagnosed with rectal cancer and treated with neoadjuvant chemoradiotherapy (nCRT).*

Materials and Methods: *The data collected from 37 patients receiving nCRT were retrospectively evaluated. The p53 score and gene instability in MLH1 and MSH2, which are among the DNA mismatch repair (MMR) genes, were evaluated using immunohistochemical methods. The neutrophils-leukocytes ratio (NLR), carcinoembryonic antigen (CEA), and carbohydrate antigen (CA) 19-9 values were obtained as hematological parameters from computer records. The pathologic analysis of the therapy response after nCRT was classified according to the modified grading system by Ryan et al.*

Results: *The changes in the NLR, CEA, and CA19-9 values before and after treatment were statistically significant ($p \leq 0.001$ and $p=0.005$). A near significant effect of the decrease of the CEA value in the 5th week after treatment was detected on the pathological response score ($p=0.075$). The p53 mutation score in those patients with any residue was higher than the total response. Overall, 89.2% of the patients exhibited MMR positivity (stability), and 10.8% of the cases with MRM negativity (instability) had a macroscopic residue. Cases with pathological total response were MRM positive.*

Conclusion: *Consequently, in most of the patients treated with nCRT, the treatment caused tumor and nodal remission. In the prediction of this therapy response, hematological and genetic parameters, such as NLR, P53, MLH1, and MSH2, play a predictive role.*

Keywords: *Rectal neoplasms, neoadjuvant treatment, DNA mismatch repair, microsatellite instability, P53 genes, neutrophil-lymphocyte ratio*

INTRODUCTION

Currently, neoadjuvant chemoradiotherapy (nCRT) has become a standard therapy combination in local advanced-stage rectal cancer. The important benefits of nCRT are pathological downstaging or total response, cured survival, decreased local recurrence, and the possibility of conducting more sphincter protective surgeries (1). In 40%-60% of patients with advanced-stage rectal cancer who are treated with nCRT, a pathological response may be obtained to a certain degree. However, it is known that there is no effective way of predicting patients who will respond to nCRT (2). Many molecular markers have been studied, and their effects on treatment have been put forward with the purpose of determining an indicator that would have an important effect on determining patients who could benefit from the treatment (3,4). Finding predictors that would forecast the possibility of a response to nCRT before treatment could greatly contribute to the prevention of non-beneficial treatment and enhancement of survival and local control.

MATERIALS AND METHODS

Patient characteristics

Thirty-seven patients with rectal cancer who had received nCRT or radiotherapy (RT) between 2013 and 2016 at the Radiation Oncology clinic were included in the study. Ethical consent was obtained from the establishment as specified in the title page. Patients with a determined clinical stage after examination by a multidisciplinary tumor committee (Radiation Oncology, Medical Oncology, Surgery, Pathology and Radiology) and with local advanced-stage (T3/T4), lymph node uptake, or single organ metastasis were included in the study. The rectum was defined as the bowel segment between 0 and 15 cm starting from the anal verge; the section between 0 and 5 cm starting from the anal entry was classified as the distal rectum, the section between >5 and 10 cm was defined as the middle rectum, and the section between >10 and 15 cm was defined as the proximal rectum.

Corresponding Author: ***Sema Yılmaz Rakıcı; sema.rakici@erdogan.edu.tr***
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nCRT

RT included patients who were treated at the pelvis with a 45-50.4 Gy/25-28 fraction for 5 days a week in the long term. Conformal, IMRT, or VMAT was used such that the appropriate dosage distribution for target and risky organs was achieved. The concurrent chemotherapy schemes of the patients were in the form of constant intravenous infusions in 5-FU 225 mg/m^2/RT or mostly oral capecitabine 825 mg/m^2 twice a day/five times a week throughout RT.

Hematological Analysis: By examining the computer records of the hematological parameters of patients taken before treatment, during treatment, and in the 5th week after treatment, the neutrophils-leukocytes ratio (NLR), carcinoembryonic antigen (CEA), and carbohydrate antigen (CA) 19-9 values were recorded. It was observed whether the changes happening throughout the therapy had a statistically significant effect on the therapy response.

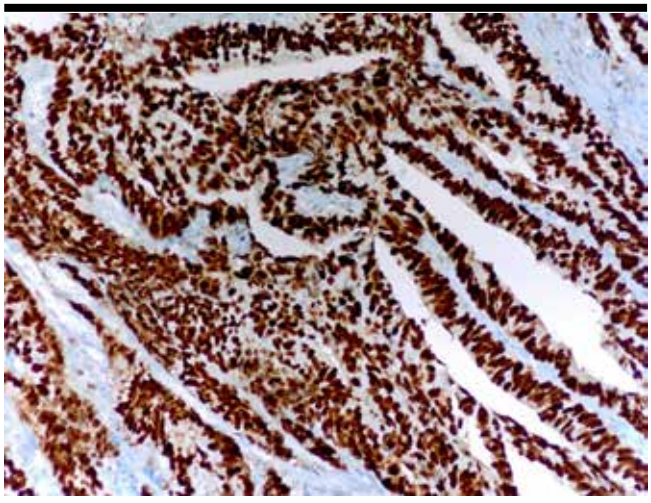

Figure 1. p53 diffuse positive adenocarcinoma (200x)

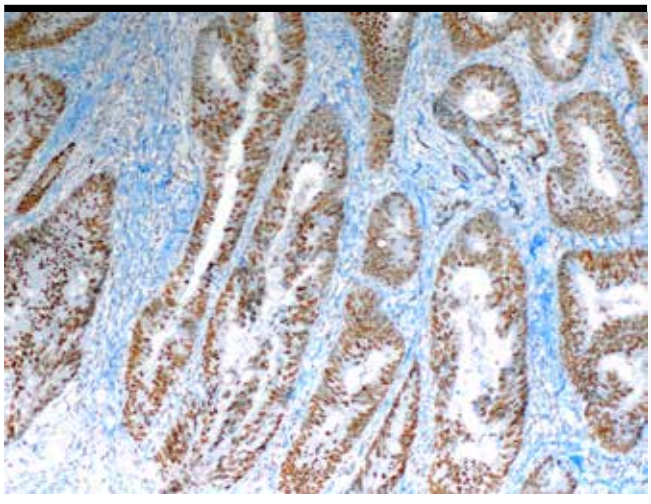

Figure 2. MLH1 positive adenocarcinoma (200x)

Genetic analysis

Paraffin blocks of biopsies appropriate for the study of MLH1 and MSH2, which had p53 mutations and mismatch repair (MMR) genes were detected from pathological blocks of patients who received neoadjuvant therapy sent for diagnosis. Sections of 4 µm taken from paraffin blocks belonging to the selected tumor were transferred to PolyLlysine-coated laminas. Staining was carried out on these tissues using an automated Immunohistochemistry device (Roche, Ventana, Benchmark, XT, USA) at our laboratory with MLH1 (Mouse monoclonal, clone G168-15, isotope IgG1/kappa, ready-to-use, 30 min. incubation, Biocare Medical, USA), MSH2 (Mouse monoclonal, clone FE11, isotope IgG1/kappa, ready-to-use, 10-15 min incubation, Biocare Medical, USA), and p53 (Mouse monoclonal, clone BP53-12, isotope IgG2a/kappa, ready-to-use, 30 min incubation, ScyTek Laboratories, Logan, USA) primary antibodies. MLH1 and MSH2 tonsil tissues were used as the tissue positive control, whereas colon adenocarcinoma was used for p53. The results were evaluated using an Olympus BX51 light microscope. For the p53 scoring, 0-10% staining was accepted as negative, 11%-50% was accepted as staining+ (weak staining), 51%-75% was accepted as staining++ (moderate staining), and over 75% was accepted as staining+++ (strong staining; Figure 1). The existence of staining in MLH1 and MSH2 was accepted as positive (stability; Figures 2 and 4), non-existence of staining was accepted as negative (instability; Figures 3 and 5). The effects of p53, MLH1, and MSH2 genetic conditions on the treatment response were examined.

Surgery

Following neoadjuvant treatment, total mesorectal incision and en-bloc removal of the tumor were the preferred methods of surgical resection. It was observed that while low anterior resection (LAR) was performed in cases with sphincter protective penetration, abdominoperineal resection (APR) was performed in cases with sphincter invasion, which did not allow going down 2-4 cm into the distal of the tumor.

Pathological analysis

The analysis of pathologic treatment response following neoadjuvant treatment was carried out according to

the modified grading system by Ryan et al. (5). No observation of any cancer cells in the resection material was interpreted as total response: G0; observation of cancer cells as single occurrences or in groups was interpreted as a pathological medium response: G1; the case of fibrosis residue is more than cancer was accepted as a pathologic minimal response: G2; and the existence of the progression of widespread residue cancer was interpreted as a pathological weak response: G3. In the tumor, node, and metastasis classification, cTNM was used for clinical classification, pTNM was used for pathologic classification, and the prefix y was used as a pathological classification following nCRT. For example, ypTNM was used for staging after nCRT.

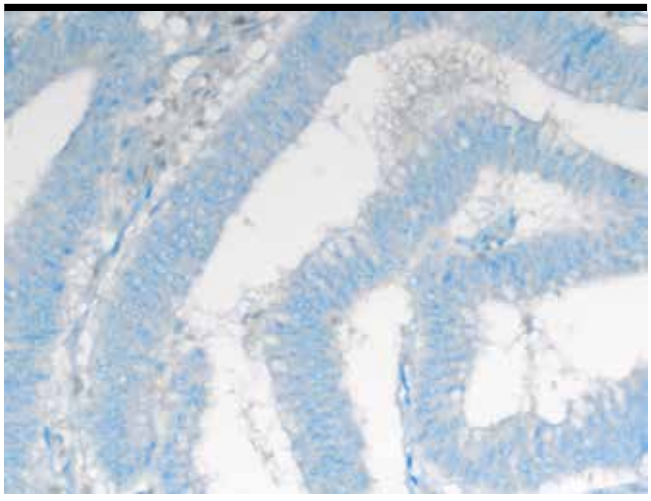

Figure 3. MLH1 negative adenocarcinoma (400x)

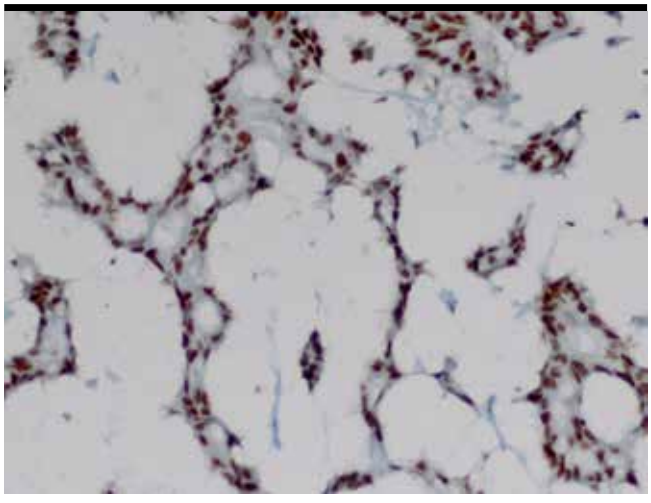

Figure 4. MSH2 positive adenocarcinoma (400x)

Statistical analysis

The statistical analyses were conducted using the Statistical Package for Social Sciences software version 17 (SPSS Inc.; Chicago, IL, USA). The variables were tested for their compliance with normal distribution. Descriptive analyses for numerical variables are given with mean, standard deviation, median, interquartile range distribution, and minimum and maximum values. Categorical variables are represented with frequency and percentage values. Group comparisons for numerical variables were carried out using the Kruskal-Wallis, Mann-Whitney U, Friedman, and Wilcoxon tests. Chi-square and Fisher tests were used for categorical variables. A p value of <0.05 was considered significant.

RESULTS

The clinicopathological characteristics of cases in our study are summarized in Table 1. Thirtyseven patients diagnosed with rectal cancer who had received nCRT were included in the study; 56.8% of patients were males, while 43.2% were females. The mean age of the patients was 61.7±13.3 (median, 60) years, and the age range was 27-81 years. There were 2 patients below the age of 40 years and 15 patients over the age of 65 years. The most commonly observed histopathologic type was adenocarcinoma with a rate of 89.2%. The settlement location of the tumor was 46.0% distal, 43.2% medial, and 10.8% proximal in terms of frequency in this order.

When we considered the stage before treatment, 89.2% of the cases were cT3 and 8.1% were cT4. Conversely, while the nodal stage was cN1 with a rate of 59.5%, it was cN2 with a rate of 37.8%. Before treatment, 2 patients

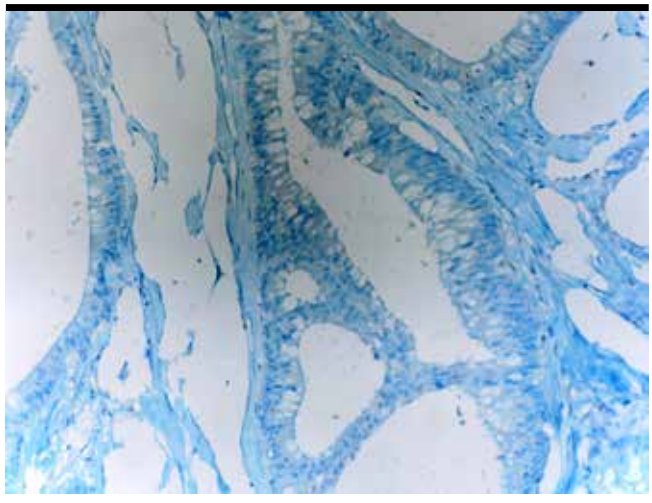

Figure 5. MSH2 negative adenocarcinoma (400x)

Table 1. Clinicopathological characteristics of patients

	n	%
Age (years)		
Mean±sd	61.7±13.3	
Median	60	
Min-max	27-81	
Sex		
Female	16	56.8
Male	21	43.2
Tumor type		
Adenocarcinoma	33	89.2
Mucinous type	1	2.7
Signet-ring cell type	1	2.7
Intramucosal carcinoma	1	2.7
Mucinous, signet-ring cell	1	2.7
Tumor localization		
Distal	17	46.0
Middle	16	43.2
Proximal	4	10.8
Pre-treatment T stage (cT)		
1	-	-
2	1	2.7
3	33	89.2
4	3	8.1
Pre-treatment nodal stage (cT)		
0	1	2.7
1A	1	2.7
1B	2	5.4
1C	19	51.4
2A	11	29.7
2B	3	8.1
2C	-	-
Metastasis existence before treatment		
Lungs	2	100.0
Radio therapy termination period (days)		
Mean±sd	50.3±61.0	
Median	41	
Surgery type		
LAR	20	55.6
APR	13	36.1
Not operated	3	8.3
Time between end of radiotherapy to surgery (weeks)		
Mean±sd	12.6±22.4	
Median	8.8	
Time between end of radiotherapy to surgery (weeks)		
<8 weeks	15	40.5
≥8 weeks	22	59.5
Pathological response grade (G)		
according to modified grading system by Ryan et al.		
Total response: G0	7	18.9
Microscopic residue: G1	11	29.7
Macroscopic residue: G2	18	48.6
Progression: G3	1	2.7
T stage after surgery (pyT)		
0	7	18.9
1	6	16.2
2	8	21.6
3	15	40.5
4	1	2.7
Number of nodes after surgery (pyN)		
0	28	75.7
1A	2	5.4
1B	1	2.7
2A	4	10.8
2B	2	5.4
Time between diagnosis-last follow-up (weeks)		
Mean±sd	96.3±58.1	
Median	93.7 (95.5)	
Time between diagnosis-surgery (weeks)		
Mean±sd	19.7±10.0	
Median	18.4 (3.8)	
Last condition after treatment		
Healthy	26	70.3
Metastasis	8	21.6
Recurrence	2	5.4
Exitus	1	2.7

Table 2. Multivariate analysis of risk factors of mortality in cirrhotic patients with UGIB

	G0		G1, G2, and G3		
	n	%	n	%	p
MMR positivity (stability)	7	21.2	26	78.8	0.570
MRM negativity (instability)	0	0	4	100.0	

had single solid metastasis. After treatment, pyT0 was at 18.9%, pyT1-2 disease was at 37.8%, pyT3 was at 40.5%, and pyT4 was present in 1 patient. After treatment, while there was pyN0 disease in 75.7%, pyN1 disease was present in 8.1% and pyN2 disease was present in 16.2% pa-

Table 3. Hematological parameters

	Before RT		After RT		5th week after RT		
	Mean±sd	Median	Mean±sd	Median	Mean±sd	Median	p
NLR	2.5±1.0	2.2(1.5)	7.9±4.4	7.4(5.6)	5.5±4.1	4.4	<0.001
CEA	8.2±10.7	4.4(6.1)	-	-	6.9±24.6	2.2	<0.001
CA19-9	53.2±115.4	16.1(36.7)	-	-	17.5±32.1	7.0	0.005

tients. At the T stage after treatment, a great remission occurred, and this situation was more drastically experienced at the nodal staging. No significant relationship was detected between tumor regression and tumor placement at the distal, proximal, or medial (p=0.984) locations. While no patients below the age of 40 years had total, all 18 patients with G0 and G1 were over the age of 40 years.

While the RT dose was 50.4 Gy for 91.9%, it was 45 Gy for 3 patients. During RT, 28 patients orally took capecitabine, whereas 7 took 5-FU, and 2 did not take any concurrent CT. The mean time for RT completion was 50.3±61.0 days, and while 40.5% completed this time under 8 weeks, 59.5% completed it in 8 weeks or longer. The mean time passing between diagnosis and surgery was 19.7±10.0 (median, 18.4) weeks; 55.6% of the patients were given LAR, 36.1% were given APR, whereas the rate of patients who did not undergo surgery was 8.3%. While the patients with a pathologic response score of G0 was 18.9%, the G1, G2, and G3 rates were 48.6%, 29.7%, and 2.7%, respectively.

Although the mean p53 score was 85.8±26.4 for those who were classified as G0 and G1, it was found to be 79.4±30.0 for those who were classified as G2 and G3. The p53 mutation rate was higher for G0 and G1 patients; however, this difference was not statistically significant (p=0.380).

While MLH1 and MSH2 were positive for 89.2% of the cases (33 patients), 10.8% (4 patients) showed negative staining (MMR instability). No significant effect of the MMR gene condition was detected on the total response or residue presence (p=0.570). However, in 4 patients with MRM instability, macroscopic residue was detected (Table 2). Out of these patients, 3 had taken capecitabine and 1 had taken 5-fluorouracil. Moreover, 7 cases with G0 were MMR positive.

The NLR, CEA, and CA 19-9 values of our cases are shown in Table 3. The mean NLR values were found to be 2.5±1.0 (median, 2.2) prior to RT, 7.9±4.4 (median, 7.4) after RT, and 5.5±4.1 (median, 4.4) in the 5th week after RT. The differences among these were statistically significant (p<0.001). When the NLR values in the 5th week after RT were compared with those between 'G0 and G1' and 'G2 and G3;' these were 5.6±5.4 and 5.4±2.6, respectively. Although NLR was higher for G0 and G1, no statistically significant effect on the pathological response score was found.

While the mean CEA value prior to RT was 8.2±10.7 (median, 4.4), it was 6.9±24.6 (median, 2.2) in the 5th week after RT. The difference was found to be statistically significant (p<0.001). While the mean CEA decrease in the 5th week after RT was 2.1±1.2 (median, 1.9) for those with a pathological response score of G0 or G1, it was found to be 11.8±35.0 (median, 2.5) for those who had scores of G2 or G3. A near significant effect of the decrease in CEA values in the 5th week after RT was found (p=0.075).

While the mean CA19-9 value before RT was 53.2±115.4 (median, 16.1), it was 17.5±32.1 (median, 7.0) in the 5th week after RT. This difference was statistically significant (p=0.005). However, no significant effect of CA19-9 decrease on the pathologic response was detected.

The mean time from diagnosis until surgery was 17.8±4.5 (median, 17.5) weeks for G0 and G1, whereas it was 21.5±13.2 (median, 19.2) weeks for G2 and G3. Although the prolongation of the time before surgery was observed more for G2 and G3 patients, there was no statistically significant difference (p=0.176). The mean time of completion of RT was 38.7±8.8 (median, 38.5) days for G0 and G1, whereas it was 61.3±84.3 (median, 42.0) days for G2 and G3. Although the RT period was longer for G2 and G3, there was no significant difference (p=0.211). While the mean time between completion of RT and surgery was 8.5±3.9 (median, 7.1) weeks for G0 and G1, it was 16.4±30.9 (median, 9.5) weeks for G2 and G3. Although this period was shorter in G0 and G1, the difference was not statistically significant (p=0.158). While the response rate was higher for G0 and G1 (68.8%) when this period was shorter than 8 weeks, it was higher for G2 and G3 (66.7%) when this period was shorter than 8 weeks. The difference was found to be near statistical significance (p=0.071).

The mean follow-up rate was 96.3±58.1 (median, 93.7) weeks. While 70.3% of the patients were observed to be healthy without local or remote metastasis, 21.6% had remote metastasis and 5.4% had local recurrence. Conversely, 1 patient was lost because of non-disease causes.

DISCUSSION

Rectal cancer is one of the leading causes of deaths associated with cancer in the world (6). In recent years, despite rapid developments in surgery, RT, CT, and molecular therapy, treatment results are still not promising due to local recurrence or distal metastasis (7). Knowing high-risk patients with planned neoadjuvant therapy in advance is important in terms of determining tumor recurrences and inclinations toward poor prognosis. nCRT probably increases the survival rate and local disease control in patients with rectal cancer. However, these benefits significantly vary among individuals. After the neoadjuvant treatment of locally advanced rectal cancer, tumor reduction may be provided in up to 60% of cases, and a pathological complete response can be additionally provided in 8%-20% of the cases (8,9). There is a wide range of responses ranging from pathological complete response to tumor progression in patients receiving the same type of treatment. Finding a predictive marker is important in terms of calculating a probability for preventing the application of an unnecessary and toxic treatment. Moreover, a successful biological token should be able to predict patients who are likely to respond to treatment or even patients who can have a pathological complete response. However, at present, a useful predictive token is not available in clinical practice. To date, p53 has been the most frequently studied response indicator in rectal cancer (10). The p53 analysis results in studies have shown that although p53 is an important regulator, it is not the main indicator of tumor radiosensitivity (11). Generally, the presence of wild-type p53 indicates radiation or chemotherapy sensitivity and that of mutated p53 indicates possible radio- and chemoresistance (12). However, there are conflicting results on this topic. Although the absence of a p53 mutation in a tumor biopsy before treatment indicates that there is a predictive factor for a pathologic complete response (13), Esposito et al. suggested that there is a positive correlation between the expression of p53 and creation of better pathologic response to nCRT (14). Another study has proposed that in predicting the pathological response to nCRT, p21 expression instead of p53 expression can be used as a more effective method (15). In our study, the p53 mutation rate was found to be higher in the G0 and G1 cases than the G2 and G3 cases; however, this difference was not statistically significant.

Mismatch repair genes help repair genetic damages, and in their absence, genetic errors that cannot be repaired accumulate and cause microsatellite instability and intestinal carcinogenesis (16,17). MMR genes also take on important roles at immune system checkpoints and code the programmed cell death protein-1. There are studies that have suggested that MMR genes are important predictive tokens for predicting survival advantages and treatment responses (18,19). It was stated that the absence of MMR is associated with a positive prognosis and poor response to fluoropyrimidine-based adjuvant therapy, and fluorouracil-based adjuvant chemotherapy is useful in patients with a microsatellite-stable stage II or III colon cancer; however, it is not useful for tumors that indicate microsatellite instability (17). In a more recent study, a relationship could not be found between the absence of MMR and prediction of a treatment response of fluoropyrimidine-based nCRT in rectal cancers (20). In our study, while in 89.2% of MLH1 and MSH2 patients, the MMR genes were positive, 10.8% had negative staining (instability). No significant effect of the MMR gene status was detected on the treatment response. However, all 4 patients with MRM instability had macroscopic residues. Three of these patients had received capecitabine and one had received fluoropyrimidine-based chemotherapy. It was stated that MMR instability is related to fluoropyrimidine resistance (17); thus, in relation to this, we believe that it is also be related to RT resistance. Moreover, all the cases with a pathological complete response were MMR positive. For statistically significant results, studies with higher number of patients are needed.

Neutrophils-leukocytes ratio, which is calculated through dividing the number of neutrophils by the number of lymphocytes, increases as a general immune response to various stress stimuli (21). In general, lymphopenia is accepted as corrupted cellmediated immunity, whereas neutrophilia is accepted as a response to systematic inflammation (22). In newly diagnosed cancer patients, high values of NLR could reflect inflammation developed against tumors and increasing silicon levels. Increased NLR is significantly related to a poor differentiation of tumor and high CEA levels (23). In a metaanalysis involving 19 studies including 10259 patients, a high NLR before treatment was found to be correlated with a poor survival and liver metastasis in localized colorectal cancer patients, and it was suggested that it is a useful biomarker in determining patients who would benefit from the adjuvant therapy (24). In fact, while

high NLR at the start of therapy is associated with a high grading of the tumor and its aggressiveness, NLR levels that increase during therapy could be associated with the treatment response of the tumor. In our study, NLR was found to be significantly higher in the 5th week of therapy compared with that before treatment.

Although high rates of NLR before treatment are related to poor prognosis, increasing NLR could be induced by the therapy process. In our study, NLR was higher in the total pathologic and microscopic response (G0 and G1) cases. These contradictory results could be explained with the survival ability of tumors against the cytotoxic effects of chemoradiation and that besides the specific phenotype of the tumor, each patient has unique genetic and immunological characteristics.

This study was retrospective and had a small number of participants that limited its impact.

In conclusion, nCRT causes tumor and nodal remission in most of the treated patients. Owing to a market that could determine in advance the patients who would benefit from this treatment, changes can be made in treatment plans. Hematological and genetic parameters, such as NLR, P53, MLH1, and MSH2, could carry a predictive role in determining the response to treatment. However, we could say that studies in the literature, including ours, have not found a predictive molecular marker whose response is sufficiently strong to provide a clinical benefit to nCRT. Eventually, with the integration of various parameters, including clinicopathological, hematological, and genetic characteristics, a biopredictor could be developed by considering the mechanical connections of tumor biology and disease heterogeneity.

Ethics Committee Approval: Ethics committee approval was received for this study from the Local Ethics Committee of Recep Tayyip Erdoğan University (Decision Date: February 23, 2018; Decision No.: 2018/39).

Informed Consent: Written informed consent was obtained from all the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - S.Y.R., R.B.; Design - S.Y.R., R.B.; Supervision - S.Y.R., R.B., C.H.; Resources - S.Y.R., R.B.; Materials - S.Y.R., R.B., C.H.; Data Collection and/or Processing - S.Y.R., R.B., C.H.; Analysis and/or Interpretation - S.Y.R., R.B., C.H.; Literature Search - S.Y.R.; Writing Manuscript - S.Y.R., R.B., C.H.; Critical Review - S.Y.R.

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