Original Article

Blood Biomarkers in the Prediction of Response to Chemoradiation in Rectal Cancer

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Key Words
Rectal cancer;
Chemoradiation;
Blood biomarkers

Purpose. Chemoradiation has been shown to downgrade rectal cancer. Hemoglobin level (Hb), neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), albumin (Alb), and lactate dehydrogenase (LDH) were assessed to predict response prior to chemoradiation.
Methods. In total, 222 rectal cancer patients received chemoradiotherapy. We retrospectively examined the relationship between hemoglobin level, neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, albumin, LDH and response to chemoradiotherapy.
Results. Forty-eight patients were defined as poor responders according to their pathologic report. Highneutrophil/lymphocyte ratio (NLR > 3), hypoalbuminemia (Alb < 3.5 g/dL), high LDH level (LDH > 200 U/L), high platelet/lymphocyte ratio (PLR > 150), and anemia (Hb < 10.0 g/dL) were classified as predictors of poor response. The results of our study did not show any difference in these markers. Preoperative NLR showed a slight difference between patients with a good response patients (2.51) and patients with a poor response (3.01), as analyzed by tumor regression grading. However, the difference did not reach statistical significance (p = 0.057).
Conclusions. Lower preoperative NLR might lead to more favorable tumor regression grading. However, the cut-off point will require validation in a prospective, stratified study before it can be accepted into clinical practice.
[*J Soc Colon Rectal Surgeon* (*Taiwan*) *2018;29:8-14*]

Rectal adenocarcinoma accounts for about one-third of all colorectal cancers. Locally advanced rectal cancer is defined by tumor invasion into the mesorectum (T3-T4) or spreading to regional lymph nodes (N+). Currently, the standard treatment for locally advanced rectal cancer is preoperative chemoradiotherapy (CRT) followed by surgery (total mesorectal excision).[1] This multimodality treatment has led to reduced local recurrence rates.[2-4] Tumor regression grade (TRG) represents the pathological characteristics of post-CRT tumor response. Better TRG results in improved prognosis. In a previous study, the 5-year crude disease-free survival rate for pathological complete response was 83.3% compared with 65.6% for those without pathological complete response.[2] However, radiotherapy can also cause several side effects including disturbances in sexual, urinary, and anorectal function.[5] Moreover, radiotherapy may result in increased risk of anastomosis leakage, wound infection, and some cases develop distant metastases during concurrent chemoradiotherapy (CCRT). Therefore, it is important to identify surrogate markers to predict

Received: April 1, 2017. Accepted: November 16, 2017.
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radiotherapy response, and prevent rectal cancer patients receiving CRT if they are not likely to receive any benefits, only side effects.

Systemic inflammation had been found to be associated with treatment response.[6-9] Previous studies have shown that neutrophil-lymphocyte ratio (NLR) is a valuable marker to predict outcomes. This study analyzed the relationship between inflammation markers and TRG in order to find reliable markers to predict response to radiotherapy.

Materials and Methods

Clinical data

We reviewed the database of the Taipei Veterans General Hospital from 2000 to 2010. Two hundred and twenty-two patients were enrolled. All patients had histologically confirmed rectal adenocarcinoma. The clinical stage was determined using computed tomography (CT), or magnetic resonance imaging (MRI) according to the American Joint Committee on Cancer (AJCC) classification system (7th edition). The clinical T2 stage with image lymphadenopathy was considered as locally advanced tumor, and was enrolled into this study. The regimen was 45 Gy radiation in 25 fractions and oral tegafur-uracil (UFUR) three times a day. Operation with total mesorectal excision (TME) was performed 6 to 8 weeks after CCRT. Blood sample were obtained before chemoradiotherapy, and analyzed for white blood cell, neutrophil, lymphocyte, and platelet counts, as well as hemoglobin (Hb), serum lactate dehydrogenease (LDH), and albumin levels. NLR and platelet-lymphocyte ratio (PLR) were subsequently calculated.[10]

Postoperative histopathological details were reviewed and TRG was determined according to the College of American Pathologists guidelines (Fig. 1). Grades 0 and 1 were defined as good response, and grades 2 and 3 were defined as poor response.

Statistical analysis

Kaplan-Meier survival curves were plotted and compared using log-rank tests. The chi-squared test and 2-tailed Fisher's exact test were used to compare TRG according to histopathological features. Numerical values were compared using Student's *t*-test. Data were expressed as means ± standard deviation and statistical significance was defined as $p < 0.05$. Statistical analyses were performed using SPSS for Windows (version 16.0).

Result

We enrolled 222 patients with locally advanced rectal adenocarcinoma treated with preoperative chemoradiotherapy followed by TME. There were 151 men (67.7%) and 71 women (31.8%); the average age was 62.26 years. The most common clinical stage was T3 (N = 149, 66.4%), followed by T4 (N = 43, 13.9%) and T2 (N = 28, 12.6%). There were 172 patients (76.3%) with clinical N stage, and 50 patients (22%) with N0 disease. The ypT0 pathological results showed 57 patients (25.7%) with complete response, 5 patients (2%) with ypT1 stage, 48 patients 90 (21.6%) with ypT2 stage, 102 (45.9%) patients with ypT3 stage, and 10 patients (4.5%) with ypT4 stage. There were 156 patients (70.3%) with pN0 stage, and 66 patients (29.7%) with positively mph node status. The TRG was as follows: 59 patients (26.5%) with grade 0, 113 patients (50.7%) with grade 1, 48 patients (21.5%) with grade 2, and 2 patients (0.9%) with grade 3 (Table 1).

We defined TRGs0 and 1 as good response (N = 174, 78.4%) and TRGs2 and 3 as poor response (N =48, 21.6%). There was a significance difference in overall survival between these two groups. The overall survival for patients with good response was 120.1

No viable cancer cells	0 (Complete response)
Single cells or small groups of cancer cells	1 (Moderate Response)
Residual cancer outgrown by fibrosis	2 (Minimal response)
Minimal or no tumor kill; extensive residual cancer	3 (Poor response)

Fig. 1. Tumor regression grade (according to the College of American Pathologists guidelines).

months and for patients with poor response was 76.5 months (Fig. 2).

Table 2 shows the hematologic characteristics of all patients. The overall distribution showed no difference in most parameters except albumin level. The TRG good response group had higher albumin levels (4.03) than theTRG poor response group (3.90) (p = 0.041). Meanwhile, the preoperative NLR showed a slight difference between TRG good response patients (2.51) and TRG poor response patients (3.01). However, the difference did not reach statistical significance (p = 0.057).

We used a cutoff value of 3 for NLR, 150 for PLR, 200 for LDH level, 3.5 for albumin level, and 10 for pre-chemoradiotherapy Hb level to define the difference in TRG response.[10-12] The results of our study did not show any difference in these markers (Table 3).

Table 1. Patient characteristics

Variable	Category	N (%)
Age	< 65	124 (55.9)
	> 65	98 (44.1)
Sex	Male	151 (67.7)
	Female	71 (31.8)
Clinical T stage	T2	28 (12.6)
	T3	149 (66.4)
	T4	43 (19.3)
Clinical N stage	N0	50 (22)
	N1-3	172 (76.3)
ypT stage	T0	57 (25.7)
	T1	5 (2.2)
	T2	48 (21.6)
	T3	102 (45.9)
	T4	10 (4.5)
ypN stage	N0	156 (70.3)
	N1-3	66 (29.7)
TRG	0	57 (25.7)
	1	115 (51.9)
	2	48 (21.5)
	3	2 (0.9)

TRG = tumor regression grading.

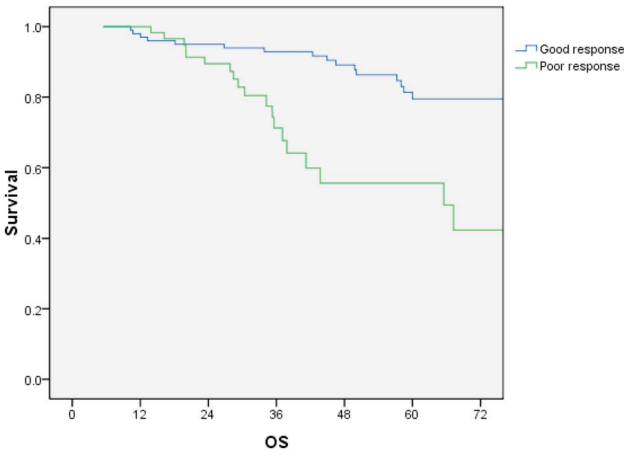

Fig. 2. Overall survival of patients.

Table 2. Hematologic characteristics

Parameter	Pathologic response		p value
	Good	Poor	
	Mean (SD)	Mean (SD)	
White blood cell	8666 (15333)	7298 (2210)	0.267
Neutrophil	5731 (5731)	4651 (4651)	0.302
Lymphocyte	2039 (1794)	1794 (630)	0.186
Platelet	262174 (101545)	244220 (78094)	0.195
Pre-CRT NLR	2.51 (1.26)	3.01 (2.30)	0.057
Pre-CRT PLR	155.00 (74.18)	151.27 (77.79)	0.762
LDH	185.73 (51.43)	194.32 (75.99)	0.384
Albumin	4.03 (0.35)	3.90 (0.42)	0.041
Pre-CRT Hb	12.61 (2.28)	12.77 (2.12)	0.654

CRT = Chemoradiotherapy; NLR = neutrophil/lymphocyte ratio; SD = standard deviation.

Table 3. Predictors of pathologic tumor response

	TRG		p value
	Good (%)	Poor (%)	
Pre-CRT NLR			0.244
< 3	121 (59.1)	32 (15.3)	
3	40 (19.1)	16 (7.7)	
Pre-CRT PLR			0.653
< 150	88 (42.1)	28 (13.4)	
150	73 (34.9)	20 (9.6)	
LDH			0.268
< 200	111 (55.5)	35 (17.5)	
200	45 (22.5)	9 (4.5)	
Albumin			0.490
> 3.5	139 (69.8)	39 (19.6)	
3.5	15 (7.5)	6 (3.0)	
Pre-CRT Hb			0.992
> 10.0	147 (68.4)	42 (19.5)	
10.0	20 (9.3)	6 (2.8)	

CRT = Chemoradiotherapy; Hb = hemoglobin; LDH = lactate dehydrogenase; NLR = neutrophil/lymphocyte ratio; PLR = platelet/lymphocyte ratio.

Discussion

Preoperative chemoradiotherapy followed by TME is currently recognized as the standard strategy for locally advanced rectal cancer.[1] Patients with pathologic complete response after chemoradiation have better long-term outcomes than those without pathologic complete response. TRG has predictive value in rectal cancer patients treated with preoperative radiotherapy. In our study, patients with a good response had better overall survival than patients with poor response. These results confirm the hypothesis that patients with better TRG have improved survival. However, several side effects have been associated with radiotherapy including increased risk of surgical morbidity, wound infection, and anastomosis leakage.[5] Radiotherapy also effects sexual, anorectal, and urinary function and may delay the initiation of adjuvant chemotherapy. A more selective approach to radiotherapy could avoid unnecessary radiation and improve quality of life.

The role of inflammationin malignancies has been widely discussed in recent years, both as a predictor of prognosis and as a therapeutic target. Macrophages, mast cells, and neutrophilsplay a role in tumor progression and metastatic capacity.[13] Neutrophils might enhance angiogenesis, promote tumor invasion, and stimulate growth. During animmune response, neutrophils are highly bactericidal. Therefore, they may be considered as potential antitumor cells. However, they did not respond as expected in tumor cells. Lymphocytes, in many tumor types, are often densely infiltrated in the areas adjacent to basement membrane breakdown during tumor invasion. This suggests that lymphocytes may play a role in tumor immune response against tumor. Lymphocytes are thought to be responsible for mediating anti-tumor response and tumor-infiltrating lymphocytes have been shown to play an anti-tumor role in colorectal cancer.[14-17]

Systemic inflammation has been proposed as predictor of response in colorectal cancer patients. Although the details of how systemic inflammation works in cancer patients is not yet clear, it has been demonstrated that NLR is an independent predictor in a variety of cancers, including lung cancer, gastric cancer, pancreatic cancer, colorectal cancer, and ovarian cancer.[8,12] In our study, the NLR was higher in patients with favorable TRG (3.01) than those with poor TRG (2.51), although this difference was not statistically significant ($p = 0.057$). Additionally, when we used a cutoff valve of NLR = 3 to evaluate tumor response, the results did not appear to be helpful in predicting response. Different cutoff values for NLR have been reported in previous studies.[8,11,12] A higher cut-off point (NLR = 5) leads to higher specificity and accuracy but the sensitivity is lower. Conversely, a lower cut-off values (NLR = 3) has higher sensitivity, but lower specificity. More precise cut off points, e.g., determined by receiver operating characteristic (ROC) curve analysis, should be determined for predicting TRG.

Other inflammation factors, such as platelets, LDH, albumin and Hb, have been previously evaluated in other studies.[13-17] Serum albumin is a known parameter for systemic nutritional status and inflammatory condition.[6] It has been shown that the Glasgow prognostic score, constructed using both C-reactive protein and serum albumin levels, has a predictive value for survival in colorectal cancer.[8,10] LDH, as a marker of hypoxia, is a product of aerobic glycolysis. High serum LDH has been demonstrated to be a poor prognostic factor in many cancers. In our study, we did not identify any relationships between these inflammation markers and TRG.

The main limitation of this study is that it was retrospective with a relatively small sample size. Limited power may have resulted in statistical insignificance as a result; therefore, a larger prospective study is needed to confirm these results. Larger prospective studies might be able to clarify the relationship between NLR and TRG. Furthermore, immunohistochemitry may be useful to evaluate markers of radiotherapy response such as hypoxia-inducible factor 1α (HIF-1α), carbonic anhydrase 9 (CA-IX), vascular endothelial growth factor (VEGF), and glucose transporter 1 (GLUT-1).

In conclusion, our study showed that lower preoperative NLR may lead to better TRG. However, the cut-off point will require validation in a prospective, stratified study before it can be accepted into clinical practice.

Sources of Financial Support

Nil.

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原　著

血液生物標記應用於預測直腸癌電化療之反應

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目的　手術前合併電療及化療可以將直腸癌降階。我們希望以治療前的血色素，白血球與淋巴球比例，血小板與淋巴球比例，血液中白蛋白，及乳酸脫氫酵素的值來預測病人對於術前電化療的反應。

方法　總共有 222 位術前接受電化療治療的直腸癌病人被納入統計。我們回溯性的檢視治療前血液中的血色素，白血球與淋巴球比例，血小板與淋巴球比例，血液中白蛋白，及乳酸脫氫酵素的數值與直腸癌對於電化療的反應。

結果　根據美國病理協會的定義去做分類，總共 48 位病人被定義為對電化療反應不佳。我們用高的白血球與淋巴球比值 (> 3)，血液中低的白蛋白質 (< 3.5 g/dL)，高的乳酸脫氫酵素 (> 200 U/L)，高的血小板與淋巴球比值 (> 150)，以及貧血 (< 10 g/dL)做為預測值。在我們的檢驗中以上的預測值並沒有辦法預測病人直腸腫瘤對於電化療的反應。對於電化療反應良好的病人，其治療前的白血球與淋巴球比值 (2.51) 略低於對於電化療反應不好的病人 (3.01)，但在統計上並沒有達到有意義的差距 ($p = 0.057$)。

結論　治療前較低的白血球與淋巴球比值可能會造成比較良好的腫瘤電化療反應，然而其預測值的切點還需要更大的前瞻性研究，才有辦法將其應用在臨床上。

關鍵詞　直腸癌、電化療、血液生化指數。