RESEARCH ARTICLE

Circulating Lymphocytes as Predictors of Sensitivity to Preoperative Chemoradiotherapy in Rectal Cancer Cases

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Abstract

Objective: The objective of this study was to identify clinical predictive factors for tumor response after neoadjuvant chemoradiotherapy (nCRT) in locally advanced rectal cancer (LARC). Methods: All factors were evaluated in 88 patients with LARC treated with nCRT. After a long period of 4-8 weeks of chemoradiotherapy, 3 patients achieved clinical complete response (cCR) and thus aggressive surgery was avoided, and the remaining 85 patients underwent a curative-intent operation. The response to nCRT was evaluated by tumor regression grade (TRG) system. Results: There were 32 patients (36.4%) with good tumor regression (TRG 3-4) and 56 (63.6%) with poor tumor regression (TRG 0-2). Lymphocyte counts and ratios were higher in good response cases (*P*=0.01, 0.03, respectively) while neutrophil ratios and N/L ratios were higher in poor response cases (*P*=0.04, 0.02, respectively). High lymphocyte ratios before nCRT and good tumor regression (TRG3-4) were significantly associated with improved 5-year disease-free survival (*P*<0.05). Pretreatment nodal status was also significantly associated with 5-year disease-free survival and 5-year overall survival (*P*<0.05). Multivariate analysis confirmed that the pretreatment lymphocyte ratio and lymph nodal status were independent prognostic factors. Conclusion: Our study suggested that LARC patients with high lymphocyte ratios before nCRT would have good tumor response and high 5-year DFS and OS.

Keywords: Rectal cancer - lymphocytes - neoadjuvant chemoradiotherapy - tumor regression grade

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Introduction

Neoadjuvant chemoradiotherapy followed by radical resection has been the standard treatment for patients with LARC, showing to reduce postoperative locoregional recurrence and improve postoperative survival. However, the response of individual tumor to nCRT is not uniform and the complete pathologic response rate varies from 8% to 31% (Kim et al., 2002; Sauer et al., 2004; Bosset et al., 2006). It means some patients could benefit from the current therapeutic regimen whereas others could not. How to identify the patients who show poor pathological response to nCRT is very important owning nCRT is time-consuming, costly, and potentially harmful, especially for patients in whom nCRT has little therapeutic effectiveness. Recently, some authors have found that the occurrence and development of tumor is not only related to the biological characteristics of tumor cells, but also to the tumor microenvironment (Coussens and Werb, 2002), which participates in the neoplastic process, fostering proliferation, survival and migration of the tumor cells. The blood cell counts in peripheral blood, which partially reflect the immune function in rectal cancer patients, are considered part of the internal environment. Many investigators have indicated that certain immune cells play an important role in the progression of carcinoma, which may serve as potential factors of prognosis. It has been reported that patients with lymphocytes infiltration around the tumor might have a good prognosis owning to the lymphokine-activated tumor cell killing caused by efficient natural killer cells or lymphocytes (Aaltomaa et al., 1992; Kawata et al., 1992; Slootweg et al., 1994). Based on these concepts, we would explore the relationship between the blood cell levels and tumor regression grade of patients with rectal cancer. In addition, the other clinical factors were also determined.

Materials and Methods

Patients

Between June 2004 and December 2007, 88 patients with clinical T3-T4 stage low rectal cancer (lying below the peritoneal reflection) were treated in our hospital. All patients were diagnosed with primary rectal adenocarcinoma and no evidence of metastasis was found. The flexible endoscopy with rectal biopsy was used and the serum CEA and CA199 level were tested. Chest X-ray, MRI and/or EUS were performed to determine TNM Stage

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II and III tumors. If necessary, PET-CT was also used.

Among the 88 patients, seven patients (8.0%) achieved pathological complete response (pCR), which means there was no evidence of tumor cells in either the primary site or regional lymph nodes in the pathological examination. Three patients (3.4%) showed clinical complete response (cCR) thus aggressive surgery was avoided, and they were included in the good response group.

Samples and criteria

Pretreatment blood data was obtained from samples collected 0-7 days before the start of nCRT. Patients with WBC greater than 10×10^9/L or lower than 4×10^9/L were not enrolled for the potential impact on the results. We determined the median ratios of 24.6% as the cut-off point of lymphocyte ratios.

Treatment

All patients received preoperative radiotherapy with a dose of 45 Gy in 25 fractions, followed by a boost of 5.4 Gy in 3 fractions to the primary tumor over a period of 5.5 weeks (1.8 Gy/fraction). All patients underwent CT simulation for three-dimensional conformal radiotherapy planning. Clinical target volume included the gross tumor volume, anus, and regional lymph nodes. The regional lymph nodes included nodes around the inferior mesenteric, internal iliac, middle rectal vessels, presacral nodes, and the nodes around the obturator foramen. The chemotherapeutic regimens were various: 8 patients received 5-day continuous infusion of 5-FU, 35 patients received 3-day bolus injection of 5-FU and leucovorin, 12 patients received capecitabine and oxaliplatin, 27 patients received capecitabine, and 6 patients received capecitabine and irinotecan. After completion of nCRT, all except 3 cCR cases underwent a curative-intent operation and the standard TME techniques were used. The interval between the completion of nCRT and surgery was 4-8 weeks.

Pathologic assessment

Response to nCRT was evaluated using the tumor regression grade (TRG) system proposed by Dworak et al. (1997) through analyzing the samples of surgical specimens. TRG definitions were as follows: grade 0, no regression; grade 1, minor regression (dominant tumor mass with obvious fibrosis in 25% or less of the tumor mass); grade 2, moderate regression (dominant tumor mass with obvious fibrosis in 26 to 50% of the tumor mass); grade 3, good regression (dominant fibrosis outgrowing the tumor mass; i.e., more than 50% tumor regression); and grade 4, total regression (no viable tumor cells, only fibrotic mass). TRG 3 and 4 were defined as "good response" and TRG 0, 1 and 2 were defined as "poor response."

Follow-up

Regularly follow-up occurred every 3 months for the first year, 6 months for the second year, and yearly thereafter. Examinations include clinical history, physical examination and blood tests including blood tests and CEA measurements. In addition, colonoscopy, X-ray, abdominopelvic CT or MRI were also used. Recurrence and metastasis were defined as a combination of radiological examinations or histological confirmation.

Table 1. Clinicopathologic Factors and Pre-CRT WBC and Its Subsets Data in 88 Patients with Rectal Cancer

Variables	TRG0-2 (n=56)	TRG3-4 (n=32)	*p* value
Age			
<50	33	17	0.60
≥50	23	15	
Gender			
Male	34	19	0.90
Female	22	13	
Histology			
Differentiated	52	30	0.87
Undifferentiated	4	2	
cT stage			
3	25	18	0.30
4	31	14	
cN stage			
Negative	39	29	0.02
Positive	17	3	
Tumor size(cm)			
<5	35	17	0.39
≥5	21	15	
Distance from anal verge (cm)			
<4	29	19	0.49
≥4	27	13	
WBC (×109/L)	7.66 ±2.71	8.09 ±3.24	0.72
Lymphocyte count(×109/L)	1.41 ± 0.42	2.43 ± 0.91	0.01
Lymphocyte ratio	20.54 ± 7.43	30.15 ± 8.33	0.03
Neutrophil count(×109/L)	5.14 ± 2.98	4.88 ± 2.14	0.81
Neutrophil ratio	68.75 ± 8.23	59.63 ± 6.89	0.04
Monocyte count(×109/L)	0.59 ±0.78	0.53 ±0.31	0.53
Monocyte ratio	7.82 ± 2.30	6.92 ± 1.70	0.28
Eosinophil count(×109/L)	0.21 ± 0.18	0.19 ± 0.12	0.67
Eosinophil ratio	3.13 ± 2.67	2.64 ± 1.16	0.63
Basophil count(×109/L)	0.04 ± 0.02	0.05 ± 0.02	0.09
Basophil ratio	0.49 ± 0.26	0.67 ± 0.22	0.23
N/L ratio[1]	4.07 ± 2.32	2.25 ± 1.15	0.02

Data were analyzed using the Wilcoxon test, Pearson's chi-square or Fisher's extract test; [1]N/L ratio Neutrophil ratio/ Lymphocyte ratio

Statistical Analysis

To identify potential predictors of response to nCRT, we explored the following parameters: age, gender, histologic differentiation, cT classification, cN status, tumor size, and distance from the anal verge.

Intergroup comparisons were analyzed using Wilcoxon's test, chi-squared test or Fisher's exact test, which were depending on the data. To determine the association between response and various clinical parameters, multivariate stepwise logistic regression analysis was used in order to determine the independence of all variables identified as possibly significant. The end-points were disease-free survival (DFS) and overall survival (OS) and they were measured from the time of diagnosis. To determine the differences in DFS and OS, Kaplan–Meier method were used, and the comparisons between potentical prognostic factors were performed with the log-rank test. Then, a Cox proportional hazards model was used for the multivariate survival analysis. All analyses were performed with Statistics 17.0, and P values less than 0.05 or 95% was considered statistically significant differences.

Results

Patient characteristics

The clinical or pathological data of the 32 patients (36.4%) with good response and other 56 cases (63.6%) with poor response was shown in Table 1. Lymph node status is significantly different in two groups (*P*=0.02) and none of other factors were significantly associated with the tumor regression grade.

The count and ratio of lymphocyte and neutrophil were inversely in two groups

The blood cell counts taken before nCRT were compared between good and poor response cases (Table 1). Samples derived from patients of good response group tended to contain more lymphocytes than those from the poor response one (*P*=0.01), while the neutrophil counts in good response cases were lower, but the differences were not statistically significant (*P*=0.81). If the ratio of lymphocytes in total WBC population was considered, good response cases had significantly higher ratios than those in poor response ones (*P*=0.03). Conversely, the ratios of neutrophils were lower in good response cases (*P*=0.04). The results also revealed the Neutrophil ratio/ Lymphocyte ratio (N/L ratio) in poor cases were higher than in good cases (*P*=0.02). The levels of monocytes, eosinophils, and basophils did not show significant difference between two groups.

High lymphocyte ratio showed correlation with good tumor regression and good outcomes

In multivariate analysis, the lymphocyte ratio showed significant correlation with good tumor regression (OR=3.99; 95% CI 1.37-8.28, P<0.01), and lymph nodal status was also kept in the model as an independent predictive factor for tumor response to nCRT (OR=1.19; 95% CI 0.03-3.02, *P*=0.03). There were no significant correlations between good tumor response and the other factor such as lymphocyte count, neutrophil ratio, and N/L ratio (Table 2).

By univariate analysis, lymph nodal status and lymphocyte ratio were found to be correlated significantly with 5-year DFS and OS (Table 3). As seen in Table 3, patients with poor response (TRG0-2) had lower 5-year disease-free survival of 63.7% compared with 80.2% (*P*<0.01). In multivariate analysis, we found lymphocyte ratio was important prognostic factor for 5-year DFS and OS (Table 4, Figure 1). At the same time, as listed in Table 4, lymph nodes status was also a significant prognostic factor for two end points.

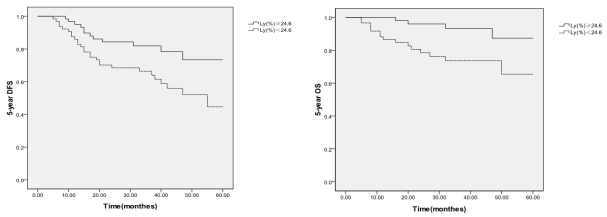

Figure 1. Impact of Pretherapeutic Lymphocyte Ratio on 5-year DFS and OS

Table 2. Multivariate Analysis for Good Tumor Response

Variables	Odds ratio	95% CI	*p* value
cN stage	1.19	0.03-3.02	0.03
Lymphocyte count(×109/L)	2.16	1.07-5.57	0.42
Lymphocyte ratio			
≤24.6% vs >24.6%	3.99	1.37-8.28	<0.01
Neutrophil ratio	1.17	0.29-3.25	0.25
N/L ratio[1]	3.58	1.77-6.23	0.54

Data were determined by stepwise logistic regression analysis; [1]N/L ratio Neutrophil ratio/ Lymphocyte ratio

Discussion

The effect of host immune capability was firstly reported in 1979 by Stone et al. (1979). From then on,

Table 3. Analysis of Prognostic Factors for 5-year Disease-free Survival and 5-year Overall Survival

Variables	Cases	5-Year disease-free Survival (%)	*P* value	5-Year overal Survival (%)	*P* value
Age					
<50	50	62.50%	0.43	70.00%	0.32
≥50	38	65.40%		79.60%	
Gender					
Male	53	68.10%	0.89	73.90%	0.77
Female	35	66.30%		72.60%	
Histology					
Differentiated	82	60.80%	0.31	69.40%	0.89
Undifferentiated	6	43.50%		47.9	
cT stage					
3	43	58.30%	0.57	69.20%	0.42
4	45	45.90%		54.60%	
cN stage					
Negative	68	71.30%	0.02	78.00%	0.03
Positive	20	50.80%		59.10%	
Tumor size(cm)					
<5	52	73.20%	0.73	84.60%	0.54
≥5	36	58.80%		65.50%	
Distance from anal verge (cm)					
<4	48	65.60%	0.26	69.10%	0.65
≥4	40	79.80%		84.40%	
Tumor response					
TRG 0-2	56	63.70%	<0.01	74.40%	0.28
TGR 3-4	32	80.20%		85.60%	
Lymphocyte ratio					
<24.6%	49	55.70%	0.02	72.60%	<0.01
≥24.6%	39	70.10%		83.30%	

Table 4. Multivariate Analysis for 5-year Disease-free Survival and 5-year Overall Survival after Treatment

Variables	Disease-free survival			Overall survival		
	Hazard ratio	95% CI for hazard ratio	P value	Hazard ratio	95% CI for hazard ratio	P value
cN stage	1.71	0.99-3.67	<0.01	2.07	0.53-6.61	0.03
Lymphocyte ratio	1.93	0.45-3.77	0.04	0.78	0.30-1.68	0.02
Tumor response	1.83	1.02-4.61	0.01	4.33	2.05-9.04	0.82

the theory that tumor shrinkage is a combination effect of both direct damage to tumor cells and host immune response has been proposed. Several lines of evidence have indicated that peripheral blood lymphocytes level has a close relationship with the malignancy. Now, it is clear that peripheral blood lymphocytes level or its ratios are not only correlate with survival of patients with cancers such as glioblastoma multiforme, renal cell carcinoma, colorectal, uterine cervix and breast cancers (Riesco et al., 1970; Bambury et al., 2013; Fox et al., 2013; Shimazaki et al., 2013), but also with tumor recurrence in bladder cancer, head and neck squamous cell carcinoma (O'Toole and Unsgaard, 1979; Kuss et al., 2004). Galon et al. (2012) demonstrated that T-cell infiltrates could be considered as a stronger independent prognostic factor than the conventional clinicopathological factors such as tumor size, depth of infiltration, tumor differentiation, or the nodal status.

It has been proven that many factors might predict tumor response to nCRT, but an exact model that could predict pathologic tumor response after nCRT has not been available until now. Recent studies have suggested that the radiosensitivity was not only depend on the biological characteristics of tumor cells, but also being affected by tumor microenvironment (Barcellos-Hoff et al., 2005), which gave us the inspiration that the blood cells, as part of the microenvironment, might play a critically important role for tumor regression after nCRT in patients with LARC. In fact, some results have clearly shown that circulating lymphocyte levels were significantly different in CR patients and non-CR patients (Kitayama et al., 2011; Choi et al., 2012). But to our knowledge, the report that circulating lymphocyte can predict TRG to nCRT in rectal cancer was rare.

In our study, when compared good response with poor response cases, higher lymphocyte ratios in good response cases were observed and it is an independent predictive factor of good tumor regression. In addition, a significantly higher 5-year DFS and OS were observed. Our results were consistent with some other scholars. Previous reports have suggested that a high number of tumor infiltrating lymphocytes was strongly associated with good outcomes in the patients with colorectal cancer (Galon et al., 2006; Morris et al., 2008; Laghi et al., 2009; Cañadas-Garre et al., 2012). A recent study conducted by Kitayama et al. (2011) showed the association of a high lymphocyte ratio in peripheral blood before and during preoperative radiotherapy in the patients of rectal cancer with a complete pathological response, suggesting the lymphocyte-mediated immune response plays an important role in the eradication of tumor cells by preoperative radiotherapy. The authors also demonstrated that patients with high lymphocytes level showed significantly better outcomes in overall and disease-free survival. Many scholars believed that the lymphocytes, especially T cells, are thought to play a central role in anti-tumor immunity, thus the count of lymphocytes could be considered the ability of one body to eliminate tumor cells. In addition, infiltration by lymphocytes indicated the generation of an effective anti-tumor cellular immune response (Rabinowich et al., 1987). All above reasons might explain why patients with high lymphocyte ratios would have good tumor regression and high 5-year DFS and OS.

However, in contrast to lymphocytes, the neutrophil ratios in poor response group were higher than those in good response one. Previous studies have demonstrated that a high density of neutrophil was correlated with poor prognosis in patients with various carcinomas such as breast, head and neck, and sarcoma (Barcellos-Hoff et al., 2005), and the existence of high neutrophil counts could promote the growth and metastasis of tumor (Coussens and Werb, 2002). The role of high neutrophil counts to malignant tumors is a combination of T-cell suppression by producing some active substance such as reactive oxygen species (ROS), nitric oxide (NO) and arginase (Müller et al., 2009; Rodriguez et al., 2009) and stimulation of tumor angiogenesis by producing IL-8, vascular endothelial growth factor, elastase, and matrix metalloproteinase (Shamamian et al., 2001; Scapini et al., 2002; Di et al., 2003; Schaider et al., 2003). In rectal cancer, owing to the contact with external environment and thus existed potential bacterial infection, a high neutrophil count might be observed with a complex reason of peritumoural inflammation, necrosis or edema accompanied with tumor, which could caused the suppression of lymphocyte-mediated immunity.

In our study, we considered the blood cell ratio as greater powerful factor than blood cell count. In humans, the absolute counts of blood cells and their subsets were varying from one person to another. Furthermore, existing evidence had demonstrated that granulocytes show an increase in the daytime while T cells, B cells, alphabet T cells, and CD4+ lymphocytes show an increase at night (Suzuki et al., 1997). Thus the blood cell ratio as a relative value can eliminate the influence of these changes and reflect the anti-tumor efficiency of the host immune mechanism more exactly than blood cell count can.

Unfortunately, owning to our study is a retrospective study of a relatively small patient population, further analyses of the predictive implications of the immune cells are needed. Experience came from Gabelova et al. (2008) showed that a control group including cancer-free patients should be considered, for the different results between cervical cancer patients with or without cancer. What's more, the peripheral blood cells count can be affected easily by the total condition of the host, which means there may be many uncertain factors that could influence the results. Although these limitations, the significant association between the circulating lymphocyte number and tumor regression rates raises the hypothesis that peripheral blood lymphocytes may have significant immunologic effects on antitumor response of nCRT. In addition, it is quite easily to get the specimen and measure the level of lymphocyte in the blood at low cost, thus it could be utilized as a potential biomarker in the clinical setting.

In conclusion, our results showed that high lymphocytes ratio was predictive factor of good tumor response to nCRT and good outcomes in patients with LARC. However, future prospective studies including large patient populations are needed to confirm our conclusions.

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