RESEARCH ARTICLE

Editorial Process: Submission:09/03/2023 Acceptance:11/07/2024

Poor Prognostic Effects of Lymphocytopenia Induced by Preoperative Chemoradiotherapy in Rectal Cancer

Hyung Joo Baik[1], Min Sung An[1], Ji Sun Park[2], Yunseon Choi[3]*

Abstract

Background: We evaluated the prognostic effect of lymphocytopenia caused by preoperative radiation therapy and chemotherapy in patients with rectal cancer. **Methods:** A total of 147 patients with rectal cancer who underwent preoperative chemoradiotherapy and surgical resection between 2008 to 2021 participated. Lymphocyte nadir less than 500/μl were defined as lymphocytopenia in this study. The relationship between lymphocyte nadir after chemoradiotherapy and disease-free survival (DFS) was evaluated. **Results:** Median follow-up was 60.7 months. A total of 21 patients (14.3%) showed lymphocytopenia related to preoperative chemoradiotherapy. Out of the original 147 patients, 29 (19.7%) patients had a diagnosis of diabetes mellitus, and 66 (44.9%) patients were overweight (body mass index exceeding 23). Lymphocytopenia occurred frequently in non-diabetic patients ($p = 0.006$) and non-overweight patients ($p = 0.001$). The pathologic complete response (pCR, $n = 19$) rate after chemoradiotherapy tended to be positively correlated with body mass index ($p = 0.09$). Lymphocytopenia was associated with lower DFS ($p = 0.009$). However, overall survival and intra-pelvic relapse-free survival were not associated with lymphocytopenia ($p = 0.124$ and $p = 0.156$). **Conclusions:** Lymphocytopenia induced by preoperative chemoradiotherapy is associated with lower DFS in patients with rectal cancer. Efforts to avoid lymphocytopenia may help prevent cancer recurrence.

Keywords: Lymphocytopenia- Rectal cancer- Chemotherapy- Radiation therapy- Disease-free survival

Asian Pac J Cancer Prev, **25 (11)**, 3799-3805

Introduction

Preoperative chemoradiotherapy (CRT) has been actively used in recent years to obtain better cancer treatment results in locally advanced rectal cancer. Currently, preoperative CRT is considered the standard treatment for locally-advanced rectal cancer. In particular, stage T3-4 and pelvic lymph node metastases are indicators of CRT. One well-known factor that can influence prognosis after cancer treatment is the patient's immune status, which can be indicated by peripheral blood lymphocyte count. Blood lymphocyte count is closely correlated with the number of T cells, and low levels of blood lymphocyte count are known to interrupt the efficacy of cancer treatment. Lymphocytopenia is especially known to be associated with immune suppression. However, conventional chemotherapy or radiation therapy (RT) is also known to cause lymphocytopenia [1, 2]. Large volume irradiation, such as pelvic nodal irradiation, can lead to RT induced lymphocytopenia [3, 4].

Lymphocytopenia is a potential prognostic factor in various cancers [5-11]; there have been reports that lymphocytopenia is associated with prognosis in lung cancer [5-7, 12] breast cancer [13, 9], esophageal cancer [8, 14, 15], oropharyngeal cancer [16], and rectal cancer [10]. However, the association between lymphocytopenia and prognosis in rectal cancer after preoperative CRT is not as established. Liu et al. [10] recently showed that lymphocytopenia nadir after preoperative CRT is related to tumor response and survival.

The main purpose of this study was to analyze the relationship between lymphocytopenia and disease-free survival (DFS) after surgery for preoperative chemoradiation in rectal cancer. In addition, clinical factors such as metabolic syndromes also were examined to find the potential relationships with lymphocytopenia.

Materials and Methods

Patients and treatment

We conducted a retrospective study of patients with rectal cancer who underwent preoperative CRT before surgical resection. This retrospective study was examined and approved by the Institutional Review Board of Busan Paik Hospital (IRB FILE No: 2021-12-057). We analyzed data from patients who had lymphocytopenia after preoperative chemoradiation in stage II-III rectal adenocarcinoma between January 2008 and August

*[1]Department of Surgery, Busan Paik Hospital, Inje University College of Medicine, Busan, Korea. [2]Department of Nuclear Medicine, Busan Paik Hospital, Inje University College of Medicine, Busan, Korea. [3]Department of Radiation Oncology, Busan Paik Hospital, Inje University College of Medicine, Busan, Korea. *For Correspondence: rtyoon@gmail.com*

2021. As for preoperative RT, consisted of 50.4 Gy/28 fractions of radiation (daily dose 1.8 Gy). Preoperative chemotherapy consisted of capecitabine, doxifluridine, and four cycles of fluorouracil chemotherapy, a similar protocol to a previous German rectal cancer trial [17]. The 147 patients included in this study underwent CRT before curative surgery at Inje University Busan Paik Hospital. We analyzed DFS as a primary endpoint. DFS, intra-pelvic relapse free survival, and overall survival (OS) were defined as the periods from rectal cancer diagnosis to relapse, death, or last follow-up.

Lymphocytopenia definition

Lymphocyte counts were evaluated three weeks after beginning of RT to one week before surgical resection. If multiple blood samples were collected, the lowest points of lymphocyte counts (nadir) were selected for evaluation. The cut-off point for lymphocytopenia was defined as less than 500/μl, equivalent to a grade II hematologic adverse event according to Common Terminology Criteria for Adverse Events criteria (version 5, product of the United States National Cancer Institute). The relationship between metabolic syndrome and lymphocytopenia was also evaluated.

Inclusion and exclusion criteria

Patients with rectal cancer adenocarcinoma who received curative aimed preoperative chemoradiation therapy and surgical resection at Inje University Busan Paik Hospital from January 2008 to August 2021 were included in this study.

Exclusion criteria for this study were as follows: 1) patients with distance metastatic cancer at the time of diagnosis, 2) patients who were diagnosed with other co-occurring malignancies and not cured before treatment, and 3) patients who were not able to follow-up at least three months after surgical resection.

Statistical method

This study used MedCalc (MedCalc Software version 19.2.0 bv, Ostend, Belgium) for statistical analysis. An independent t-test was used to determine clinical factors related to lymphocytopenia or treatment response. A correlation coefficient was used for detecting the relationship between lymphocyte count and body mass index (BMI). Survival analysis was performed using the Kaplan-Meyer method. Log rank test was used to compare the survival curves. A Cox regression method was used for the multivariate analysis.

Results

Patient characteristics

Median follow-up period was 60.7 months (range 7.1–163.9 months). Table 1 shows the clinical and treatment characteristics of the participants in this study. In this study, 93 patients (63.3%) were male. The median age of patients was 65 (range = 45–83). Clinically, T3 (n = 99, 67.3%) was the dominant stage in imaging study. In addition, 103 patients (70.1%) had pelvic lymph node metastases at diagnosis. All patients underwent neoadjuvant chemotherapy, consisting of a 5-fluorouracil based regimen (n = 29), capecitabine based regimen (n = 113), and doxifluridine (n = 5). Most patients (n = 124, 84.4%) received low anterior resection (LAR). The pathologic T stage was reported according to tumor stage after neoadjuvant therapy (ypT); ypT0 in 22 patients (15.0%), ypT1 in 10 (6.8%), ypT2 in 31 (21.1%), ypT3 in 80 (54.4%), and ypT4 in four (2.7%). The lymph node status after neoadjuvant therapy (ypN) was as follows; ypN0 in 108 patients (73.5%), ypN1 in 27 (18.4%), and ypN2 in 12 (8.2%). According to the pathology report, circumferential resection margin (CRM) was involved (0 mm) in two patients (1.4%) and 0.1–2.0 mm in 10 (6.8%). In addition, 13 cases (8.8%) of lymphovascular invasion and 18 cases (12.2%) of perineural invasion were reported. According to previous medical history, 29 (19.7%) patients with diabetes mellitus (DM) and 45 (30.6 %) patients with hypertension were included. Additionally, 66 (44.9%) of patients had BMIs exceeding 23, which is considered

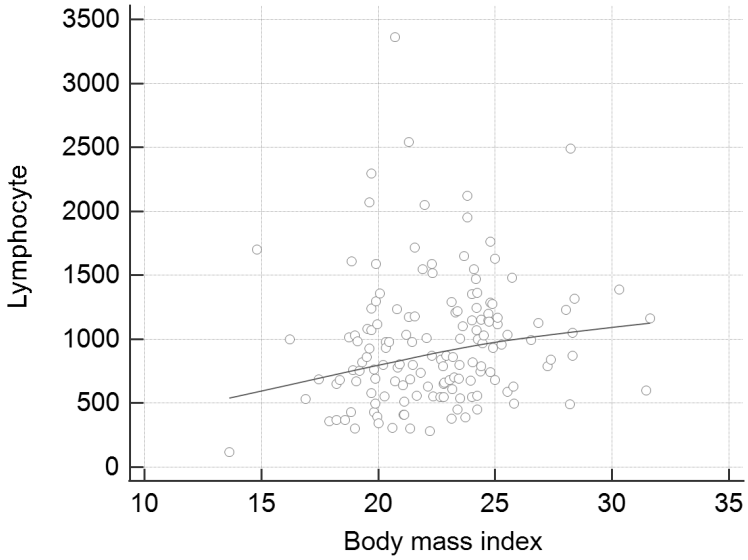

Figure 1. Correlation between Lymphocyte Counts and Body Mass Index

Table 1. Patient Characteristics

Characteristics		No. of Pts	(%)
Gender	Male	93	-63.3
	Female	54	-36.7
Age		median 65 (range 45–83)	
	<60	47	-32
	≥60	100	-68
Clinical T stage			
	cT2	21	-14.3
	cT3	99	-67.3
	cT4	27	-18.4
Clinical N stage			
	cN0	44	-29.9
	cN1	54	-36.7
	cN2	49	-33.3
Pathologic T stage			
	pT0	22	-15
	pT1	10	-6.8
	pT2	31	-21.1
	pT3	80	-54.4
	pT4	4	-2.7
Pathologic N stage			
	pN0	108	-73.5
	pN1	27	-18.4
	pN2	12	-8.2
DM			
	Yes	29	-19.7
	No	118	-80.3
Hypertension			
	Yes	45	-30.6
	No	102	-69.4
BMI			
	<23	81	-55.1
	≥23	66	-44.9
Circumferential resection margin			
	0 mm	2	-1.4
	0.1–2 mm	10	-6.8
	>2 mm	135	-91.8
Lymphovascular invasion			
	Yes	13	-8.8
	No	134	-91.2
Perineural invasion			
	Yes	18	-12.2
	No	129	-87.8
Operation			
	LAR	124	-84.4
	APR	23	-15.6
Neoadjuvant chemotherapy			
	5-fluorouracil	29	-19.7
	Capecitabine	113	-76.9
	Doxifluridine	5	-3.4
Lymphocyte nadir (/μl)			
	<500	21	-14.3
	≥500	126	-85.7
Total		147	-100

DM, diabetes mellitus; BMI, body mass index; LAR, lower anterior resection; APR, abdominal perineal resection

Table 2. Clinical Factors Related to Lymphocytopenia (< 500/μl).

		No. of Pts	p-value
Age			0.776
	<60	7/47	
	≥60	14/100	
DM			0.006
	Yes	2/29	
	No	19/118	
Hypertension			0.661
	Yes	6/45	
	No	15/102	
BMI			0.001
	<23	15/81	
	≥23	6/66	

DM, diabetes mellitus; BMI, body mass index.

Table 3. Clinical Factors Associated Pathological Complete Response.

		No. of pts	p-value
Lymphocyte nadir			1
	<500	02/ 21	
	≥500	17/126	
clinical T stage			0.152
	cT2	05/ 21	
	cT3-4	14/126	
clinical N stage			0.592
	cN0	7/44	
	cN+	12/103	
Age			0.793
	<60	5/47	
	≥60	14/100	
DM			1
	Yes	04/ 29	
	No	15/118	
Hypertension			0.184
	Yes	3/45	
	No	16/102	
BMI			0.09
	<23	14/81	
	≥23	5/66	

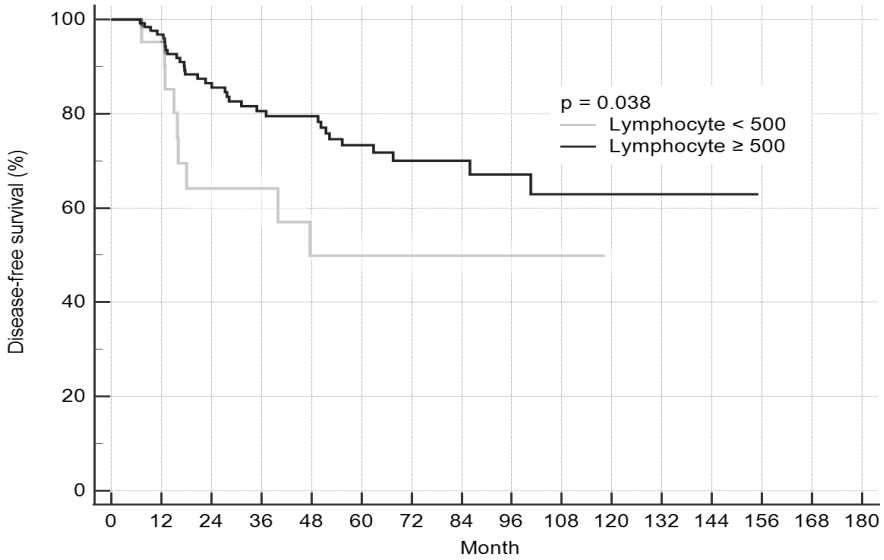

Figure 2. Disease-free Survival by Lymphocyte Counts

overweight according to Asian criteria [18].

Factors related to lymphocytopenia

Table 2 identifies the factors related to occurrence of lymphocytopenia induced by preoperative CRT. Non-DM patients had higher rates of lymphocytopenia compared to DM patients (p = 0.006). Normal-weight or underweight patients had higher risk of lymphocytopenia compared to overweight patients (p = 0.001). Figure 1 shows the correlation between lymphocyte nadir and BMI (p = 0.076). Neither old age (≥ 60, p = 0.776) nor hypertension (p = 0.661) were related to occurrence of lymphocytopenia.

Factors related to complete response

After preoperative CRT, 19 cases (12.9%) of pathological complete response (pCR) were observed in surgical resection. Table 3 shows the factors related to pCR. Lymphocytopenia induced by preoperative CRT did not influence pCR rate. However, being overweight was negatively correlated to pCR rate (p = 0.09).

Treatment outcomes and survival analysis

Overall, 5-year DFS was 70.2%. A total of 41 patients showed disease progression during the follow-up periods. Specifically, 13 patients experienced locoregional (intra-pelvic) failure and 30 patients experienced distant failure (two patients showed both locoregional and distant failure). The lungs (n = 17) were the most frequent site of distant metastases. Additionally, 5-year OS was 94.2%.

Table 4 summarizes the results of univariate and multivariate analysis for DFS. Lymphocytopenia and pathologic T stage were significant factors for determining DFS in univariate analysis. Prognostic effects on DFS of lymphovascular invasion and perineural invasion were not statistically significant in univariate analysis. Pathologic T stage was the only independent prognostic factor for DFS in multivariate analysis.

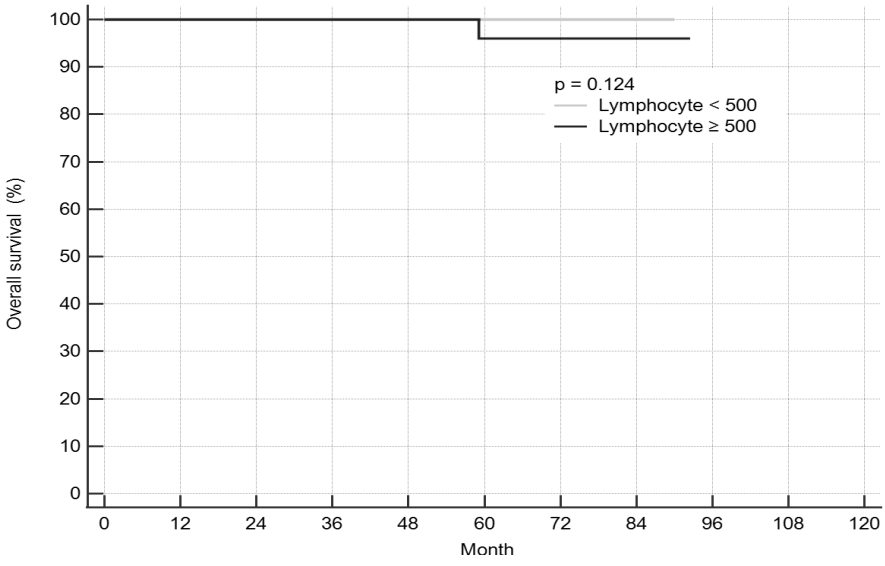

Figure 3. Overall Survival by Lymphocyte Counts.

Table 4. Univariate and Multivariate Analyses for DFS.

		Univariate analysis		Multivariate analysis	
		5-year (%)	p-value	95% CI (HR)	p-value
Lymphocyte nadir (/μl)			0.038		0.078
	<500	49.9		0.511 (0.242–1.077)	
	≥500	73.3			
clinical T stage			0.061		
	cT2	84.6			
	cT3-4	67.2			
clinical N stage			0.957		
	cN0	68			
	cN+	71.1			
pathologic T stage			0.022		0.038
	pT0-2	78.8		2.086 (1.040–4.183)	
	pT3-4	63.7			
pathologic N stage			0.401		
	pN0	70.4			
	pN+	69.5			
Age			0.178		
	<60	82.8			
	≥60	63.5			
Circumferential resection margin			0.166		
	0-2mm	46.3			
	≥2 mm	70.9			
Lymphovascular invasion			0.208		
	Yes	48.9			
	No	72			
Perineural invasion			0.996		
	Yes	66.5			
	No	70.7			
DM			0.928		
	Yes	67.1			
	No	70.5			
Hypertension			0.896		
	Yes	69.2			
	No	70.6			
BMI			0.319		
	<23	67.7			
	≥23	73.2			

DM, diabetes mellitus; BMI, body mass index; CI, confidential interval; HR, hazard ratio.

Figure 2 shows the relationship between lymphocytopenia and DFS. Lymphocytopenia was associated with reduced DFS (p = 0.038). Figure 3 shows the relationship between OS and lymphocytopenia. OS was not significantly correlated with lymphocytopenia (p = 0.124).

Discussion

This study showed that lymphocytopenia <500 /μl after preoperative CRT in patients with rectal cancer decreased DFS in patients. Lymphocytopenia itself can be a strong indicator of patient immunity and can be a determining factor of the effectiveness of cancer therapy. Lymphocytopenia is related to prognosis after chemoradiation in solid tumors. In reference to lung cancer, lymphocytopenia was associated with PFS and OS reduction in a 2021 meta-analysis. Moreover, lymphocytopenia after CRT has been associated with poor prognosis in patients with pancreatic cancer. Additionally, lymphocytopenia after CRT has been found to be a factor inhibiting therapeutic outcomes in esophageal cancer.

This study's results support the results of the aforementioned studies and show that lymphocytopenia

is a factor determining DFS even when tumors have been surgically removed.

Lymphocytopenia after CRT has substantially long-lasting effects on treatment outcomes

Shortening the duration of RT [19] or bone marrow sparing [20] using intensity-modulated radiation therapy or proton therapy [21] can help prevent lymphocytopenia. Interleukin-7 may be effective for RT induced lymphocytopenia according to a recent study Byun et al. [22]. However, further research is needed to determine whether this approach definitively improves cancer treatment outcomes.

Overweight patients in this study as classified by BMI showed lower risk of lymphocytopenia compared to that of normal weight patients. In contrast, pCR rates of overweight patients were lower than those of normal weight patients. Moreover, neither survival gain nor better disease control were detected in overweight patients. Overweight patients may have a higher number of baseline lymphocytes in their blood during CRT compared to normal weight patients. These patients may also have and additional temporary reservoir of lymphocytes. However, being overweight has not been correlated with treatment response or long-term cancer treatment outcome in rectal cancer. Obesity itself is not beneficial for health or immunity. Moreover, a previous study by Sun et al. [23] showed that obese patients who underwent preoperative CRT had unfavorable survival outcomes in rectal cancer. Therefore, we can conclude that maintaining appropriate BMI is still important for patients with rectal cancer.

As for the limitations of this study, its retrospective nature meant that blood sampling timing of patients was irregular, it was not always at the end of RT. In addition, since the number of anti-cancer or regulatory T cells [24] were not classified or measured specifically for this study, further analysis is needed to determine whether the therapeutic effect was reduced by a smaller number of cytotoxic T cells.

In summary, we found that lymphocytopenia after preoperative CRT plays an important role in determining DFS in patients with rectal cancer. Lymphocytopenia after preoperative CRT was related to lower DFS in rectal cancer. Efforts to avoid lymphocytopenia may help prevent cancer recurrence after preoperative CRT. The relationship between a patient's immune status and cancer treatment outcome is an advancing topic of interest, and it is expected that a number of related studies will be conducted in the future.

Author Contribution Statement

Y.C. designed the research; collected, summarized, and analyzed clinical data; and wrote the paper. Y.C. is the corresponding author; H.J.B., M.S.A., and J.S.P. collected the data, wrote and approved the final version for publication, and gave critique. All authors read and approved the final manuscript.

Acknowledgements

Funding statement

This research was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HR21C1003)

Conflict of interest

The authors have no conflict of interest to declare.

References

1. Campian JL, Ye X, Sarai G, Herman J, Grossman SA. Severe treatment-related lymphopenia in patients with newly diagnosed rectal cancer. Cancer Invest. 2018;36(6):356-61. https://doi.org/10.1080/07357907.2018.1499028.
2. Patil VM, Singh GK, Noronha V, Joshi A, Menon N, Lashkar SG, et al. Lymphopenia during chemoradiation-foe or friend. Ecancermedicalscience. 2020;14:1109. https://doi.org/10.3332/ecancer.2020.1109.
3. Iorio GC, Spieler BO, Ricardi U, Dal Pra A. The impact of pelvic nodal radiotherapy on hematologic toxicity: A systematic review with focus on leukopenia, lymphopenia and future perspectives in prostate cancer treatment. Crit Rev Oncol Hematol. 2021;168:103497. https://doi.org/10.1016/j.critrevonc.2021.103497.
4. Lissoni P, Meregalli S, Bonetto E, Mancuso M, Brivio F, Colciago M, et al. Radiotherapy-induced lymphocytopenia: Changes in total lymphocyte count and in lymphocyte subpopulations under pelvic irradiation in gynecologic neoplasms. J Biol Regul Homeost Agents. 2005;19(3-4):153-8.
5. Abravan A, Eide HA, Helland Å, Malinen E. Radiotherapy-related lymphopenia in patients with advanced non-small cell lung cancer receiving palliative radiotherapy. Clin Transl Radiat Oncol. 2020;22:15-21. https://doi.org/10.1016/j.ctro.2020.02.005.
6. Abravan A, Faivre-Finn C, Kennedy J, McWilliam A, van Herk M. Radiotherapy-related lymphopenia affects overall survival in patients with lung cancer. J Thorac Oncol. 2020;15(10):1624-35. https://doi.org/10.1016/j.jtho.2020.06.008.
7. Joseph N, McWilliam A, Kennedy J, Haslett K, Mahil J, Gavarraju A, et al. Post-treatment lymphocytopaenia, integral body dose and overall survival in lung cancer patients treated with radical radiotherapy. Radiother Oncol. 2019;135:115-9. https://doi.org/10.1016/j.radonc.2019.03.008.
8. Feng JF, Liu JS, Huang Y. Lymphopenia predicts poor prognosis in patients with esophageal squamous cell carcinoma. Medicine. 2014;93(27):e257. https://doi.org/10.1097/md.0000000000000257.
9. Cho O, Chun M, Kim SW, Jung YS, Yim H. Lymphopenia as a potential predictor of ipsilateral breast tumor recurrence in early breast cancer. Anticancer Res. 2019;39(8):4467-74. https://doi.org/10.21873/anticanres.13620.
10. Liu H, Wang H, Wu J, Wang Y, Zhao L, Li G, et al. Lymphocyte nadir predicts tumor response and survival in locally advanced rectal cancer after neoadjuvant chemoradiotherapy: Immunologic relevance. Radiother Oncol. 2019;131:52-9. https://doi.org/10.1016/j.radonc.2018.12.001.
11. Venkatesulu BP, Mallick S, Lin SH, Krishnan S. A systematic review of the influence of radiation-induced lymphopenia on survival outcomes in solid tumors. Crit Rev Oncol Hematol. 2018;123:42-51. https://doi.org/10.1016/j.

critrevonc.2018.01.003.
12. Li H, Yu H, Lan S, Zhao D, Liu Y, Cheng Y. Aberrant alteration of circulating lymphocyte subsets in small cell lung cancer patients treated with radiotherapy. Technol Cancer Res Treat. 2021;20:15330338211039948. https://doi.org/10.1177/15330338211039948.
13. Chen F, Yu H, Zhang H, Nong Y, Wang Q, Jing H, et al. Risk factors for radiation induced lymphopenia in patients with breast cancer receiving adjuvant radiotherapy. Ann Transl Med. 2021;9(16):1288. https://doi.org/10.21037/atm-21-2150.
14. Wang X, Zhao Z, Wang P, Geng X, Zhu L, Li M. Low lymphocyte count is associated with radiotherapy parameters and affects the outcomes of esophageal squamous cell carcinoma patients. Front Oncol. 2020;10:997. https://doi.org/10.3389/fonc.2020.00997.
15. Xu C, Jin JY, Zhang M, Liu A, Wang J, Mohan R, et al. The impact of the effective dose to immune cells on lymphopenia and survival of esophageal cancer after chemoradiotherapy. Radiother Oncol. 2020;146:180-6. https://doi.org/10.1016/j.radonc.2020.02.015.
16. Ng SP, Bahig H, Jethanandani A, Pollard C, Berends J, Sturgis EM, et al. Lymphopenia during radiotherapy in patients with oropharyngeal cancer. Radiother Oncol. 2020;145:95-100. https://doi.org/10.1016/j.radonc.2019.12.023.
17. Sauer R, Liersch T, Merkel S, Fietkau R, Hohenberger W, Hess C, et al. Preoperative versus postoperative chemoradiotherapy for locally advanced rectal cancer: Results of the german cao/aro/aio-94 randomized phase iii trial after a median follow-up of 11 years. J Clin Oncol. 2012;30(16):1926-33. https://doi.org/10.1200/jco.2011.40.1836.
18. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. The Lancet. 2004;363(9403):157-63. https://doi.org/10.1016/s0140-6736(03)15268-3
19. Zhao Q, Chen G, Ye L, Shi S, Du S, Zeng Z, et al. Treatment-duration is related to changes in peripheral lymphocyte counts during definitive radiotherapy for unresectable stage iii nsclc. Radiat Oncol. 2019;14(1):86. https://doi.org/10.1186/s13014-019-1287-z.
20. Liu M, Li X, Cheng H, Wang Y, Tian Y. The impact of lymphopenia and dosimetric parameters on overall survival of esophageal cancer patients treated with definitive radiotherapy. Cancer Manag Res. 2021;13:2917-24. https://doi.org/10.2147/cmar.S297010.
21. Mohan R, Liu AY, Brown PD, Mahajan A, Dinh J, Chung C, et al. Proton therapy reduces the likelihood of high-grade radiation-induced lymphopenia in glioblastoma patients: Phase ii randomized study of protons vs photons. Neuro Oncol. 2021;23(2):284-94. https://doi.org/10.1093/neuonc/noaa182.
22. Byun HK, Kim KJ, Han SC, Seong J. Effect of interleukin-7 on radiation-induced lymphopenia and its antitumor effects in a mouse model. Int J Radiat Oncol Biol Phys. 2021;109(5):1559-69. https://doi.org/10.1016/j.ijrobp.2020.12.004.
23. Sun Y, Xu Z, Lin H, Lu X, Huang Y, Huang S, et al. Impact of body mass index on treatment outcome of neoadjuvant chemoradiotherapy in locally advanced rectal cancer. Eur J Surg Oncol. 2017;43(10):1828-34. https://doi.org/10.1016/j.ejso.2017.07.022.
24. McCoy MJ, Hemmings C, Miller TJ, Austin SJ, Bulsara MK, Zeps N, et al. Low stromal foxp3+ regulatory t-cell density is associated with complete response to neoadjuvant chemoradiotherapy in rectal cancer. Br J Cancer. 2015;113(12):1677-86. https://doi.org/10.1038/bjc.2015.427.