Technology in Cancer Research & Treatment
Volume 21: 1-10

DOI: 10.1177/15330338221116494
journals.sagepub.com/home/tct
SAGE

Peripheral Lymphocyte Counts and Lymphocyte-Related Inflammation Indicators During Radiotherapy for Pelvic Malignancies: Temporal Characterization and Dosimetric Predictors

Xiaoyong Xiang, MM[1,2], Ning Li, MD[1,2], Zhen Ding, PhD[1,2], Zhitao Dai, PhD[1,2], and Jing Jin, MD[1,2]

Abstract

Purpose: To identify the dosimetric predictors of lymphocytopenia and retrospectively analyze the changing trend of peripheral lymphocyte counts and lymphocyte-related inflammatory indicators in patients with simple pelvic radiotherapy. **Methods and Materials:** We retrospectively reviewed the clinical data of 188 patients with pelvic malignancies undergoing pelvic radiotherapy. The absolute count of neutrophils, lymphocytes, monocytes, and platelets at each time point was collected, and lymphocyte-related inflammation indicators were obtained, including neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), and the systemic immune-inflammation index (SII). The total pelvic bone (TPB) and the body within the 5 Gy coverage were retrospectively delineated for each patient. Dose-volume histograms corresponding to the delivered volumetric arc therapy plan were used to assess the dose volumes received by the TPB and body. A paired-samples *t*-test or Wilcoxon signed-rank test for matched pairs was applied for pairwise comparisons. We also established a stepwise multiple linear regression model for the peripheral lymphocyte count (PLC) value at the end of radiotherapy. **Results:** The PLC and lymphocyte-related inflammatory indicators changed significantly after the start of radiotherapy and persisted for 3-6 months after radiotherapy. The nadirs of PLC occurred at RT-End, and the PLC was still significantly lower than the baseline value at RT-3 months and RT-6 months. NLR, PLR, and SII at RT-End are about 3.5 times the value at RT-Baseline, while LMR is one-fourth of the basal value. In a further multiple stepwise linear regression analysis, the basal PLC ($\beta = 0.156$, $p \leq .001$), gender ($\beta = 0.096$, $p = .005$), and TPB-V5 ($\beta = -0.016$, $p \leq .001$) turned out to be the predictor of the absolute value of lymphocytes at the end of radiotherapy. **Conclusions:** The impact of pelvic radiotherapy on PLC and lymphocyte-related inflammatory indicators is considerable and long-lasting. Minimizing pelvic bone radiation exposure dose (5 Gy) may help to avoid severe cases of lymphocytopenia.

Keywords

peripheral lymphocyte counts, lymphocyte-related inflammation indicators, pelvic malignancies, radiotherapy

Abbreviations

AMC, absolute monocyte count; ANC, absolute neutrophil count; CTCAE, Common Terminology Criteria for Adverse Events; GTV, gross tumor volume; IGRT, image-guided RT technique; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-

[1] Department of Radiation Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital & Shenzhen Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Shenzhen, China
[2] Department of Radiation Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Corresponding Author:
Jing Jin, Department of Radiation Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital & Shenzhen Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Shenzhen, 518116, China.
Email: jingjin1025@163.com

lymphocyte ratio; PLC, peripheral lymphocyte count; PLR, platelet-to-lymphocyte ratio; Q1, 25th percentile; Q3, 75th percentile; RT, radiotherapy; SD, standard deviation; SII, systemic immune-inflammation index; TPB, total pelvic bone; VMAT, volumetric arc therapy; WPRT, whole pelvic RT.

Received: March 16, 2022; Revised: July 4, 2022; Accepted: July 8, 2022.

Introduction

The innate immune reaction and systemic inflammatory response play a critical role in tumor initiation, progression, metastasis, unlimited proliferation, and prognosis of various tumors.[1,2] The peripheral lymphocyte count (PLC) is primarily composed of T cells and B cells, which are one of the central effector cells of innate immunity with antitumor activity.[3] Several studies have revealed that the lymphocytes have the effect of high-efficiency induction of apoptosis of tumor cells. It can inhibit tumor metastasis and progression in antitumor immune surveillance.[4,5] The patients with significant lymphocytopenia have a poor prognosis, and lymphocytopenia is an independent predictor of patient outcomes.[6,7] Therefore, the PLC can be an essential indicator to reflect the body's immune status.

Meanwhile, many studies have confirmed that inflammatory indicators related to lymphocytes are robustly associated with the prognosis of various types of cancers, including pituitary adenomas,[8] neuroendocrine carcinomas of the lung,[9] colorectal cancer,[10] and nasopharyngeal carcinoma,[11] etc. These lymphocyte-related inflammation indicators can predict the risk of tumor recurrence and metastasis after surgery and show potential applications in evaluating the efficacy of treatments for cancer. Typically, these inflammation indicators include the neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII). In general, an elevated NLR, PLR, and SII and decreased LMR are correlated with tumor progression and poor clinical outcomes in various human tumors.[6–11]

However, approximately over half of the multipotent hematopoietic stem cells (HSCs) in adults typically reside in the bone marrow of the pelvis bones, lumbar vertebrae, and sacral vertebrae.[12] When bone marrow, lymphoid tissue, or blood circulation are exposed to radiation, it will significantly reduce lymphocytes, platelets, monocyte, and neutrophils. Especially, lymphocytes are susceptible to radiation, and exposure to doses of radiation as low as 2 Gy can lead to a significant decrease in the number of PLC.[13] Therefore, pelvic radiotherapy (RT) will inevitably inhibit the immune and hematopoietic function, which will further affect lymphocyte and lymphocyte-related inflammation indicators, and ultimately may also affect the long-term outcomes of patients.

Although external pelvic RT is one of the most critical strategies for treating pelvic cancers, its effect on the inflammatory and immune states of the body should not be overlooked. In this study, we retrospectively analyzed the changing trend of PLC and lymphocyte-related inflammatory indicators. We explored the dosimetric predictors of lymphocytopenia in the patients who underwent pelvic RT.

Methods and Materials

Patients

In this study, we retrospectively analyzed the clinical data of patients who received pelvic RT at our institution between October 2018 and June 2021. The inclusion criteria were as follows: (1) all eligible patients were pathologically confirmed with the diagnosis of pelvic malignancies, including cervical cancer, endometrial cancer, vulvar cancer, vaginal cancer, or prostate cancer; (2) all patients received pelvic RT by volumetric arc therapy (VMAT); (3) the patients did not receive any chemotherapy during or prior RT; (4) the patients were evaluated by routine peripheral blood tests every week during RT, and at least once within one week before and after RT. The exclusion criteria included the following: (1) patients who underwent pelvic RT by 3-dimensional conformal RT or intensity-modulated RT; (2) patients with incomplete routine peripheral blood tests data or clinical data; (3) patients with apparent immune system diseases, concomitant inflammatory diseases, or blood system diseases; (4) the hematotoxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Patients with ≥2-grade neutropenia, thrombocytopenia, or lymphocytopenia within one week before pelvic RT were also excluded. Finally, 188 patients with pelvic malignancies met the inclusion and exclusion criteria, including 31 cases of 1-grade neutropenia, 4 cases of 1-grade thrombocytopenia, and 5 cases of 1-grade lymphocytopenia. A formal ethics approval was not required according to our institutional rules for this retrospective observational analysis. The requirement for written informed consent from the patients was waived owing to the retrospective study design. We have de-identified all patient details such that the identity of any person may not be ascertained in any way.

Radiation Planning and Treatment

All patients received pelvic lymphatic drainage areas by VMAT and image-guided RT technique (IGRT; once a day in the first week and once a week from week 2 to 5). For definitive radiation therapy, we used contoured primary tumor and metastatic lymph nodes as the gross tumor volume (GTV) on CT images based on all available clinical information, and GTV includes primary tumor and metastatic lymph nodes. The pelvic lymphatic drainage area typically includes bilateral

total iliac lymphatic areas, external/internal iliac lymphatic areas, lymphatic areas surrounding obturator arteries, and presacral lymphatic areas.

For gynecological malignancies, the lymphatic drainage areas may also need to include the inguinal lymphatic drainage area, para-uterine lymphatic drainage area, and the vagina (entirely or partially). For patients with prostate cancer, except the pelvic lymphatic drainage area, it may also need to include the prostate, tumor bed, or seminal vesicle gland. The planning optimization process was delineated for normal critical structures, bladder, spinal cord, femoral heads, rectum, small intestine, sigmoid colon, and bowel bag. For high-risk prostate cancer, the whole pelvic RT (WPRT) dose was 50 Gy in 2.0 Gy daily fractions, and the primary tumor dose was 67.5 Gy in 2.7 Gy daily fractions. If there is lymph node metastasis confirmed by imaging or puncture pathology, local residual lymph nodes can be replenished with 16-20 Gy after WPRT. For patients with gynecologic malignancies, the total dose to the pelvic lymphatic drainage areas was 45, 47.5, 50, or 50.4 Gy with a daily fraction of 1.8-2.0 Gy. Vaginal brachytherapy was dependent on the specific circumstances of the patients, and the dose to pelvic bone marrow from brachytherapy was considered insignificant.

The Total Pelvic Bones and Body Delineation

All the external contours of the total pelvic bones (TPBs; including lumbosacral vertebrae, ilium, ischium, pubis, bilateral femoral head, and upper femur) and body within 5 Gy coverage were retrospectively delineated by a single radiation oncologist and subsequently reviewed by another senior radiation oncologist.

Dose-volume histograms corresponding to the delivered VMAT plan were used to assess the dose volumes (V5 Gy, V10 Gy, V15 Gy, V20 Gy, V25 Gy, V30 Gy, V35 Gy, V40 Gy, V45 Gy, V50 Gy, respectively) received by the TPBs and body.

Due to the automatic sketching of all the outer contours of the body, we calculated the absolute volume of the body receiving 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 Gy (defined as Body-CV5, Body-CV10, Body-CV15, Body-CV20 Body-CV25, Body-CV30, Body-CV35, Body-CV40, Body-CV45, and Body-CV50).

Peripheral Lymphocyte Count and Lymphocyte-Related Inflammation Indicators

All patients underwent a blood routine examination within one week before pelvic RT. During pelvic RT and within one week following RT completion, the blood routine examination was planned for at least once a week. Generally, patients were followed up every 3 months within the first 2 years after the end of pelvic RT. The frequency of blood routine examinations could be increased if necessary.

The date of the first blood routine test before the start of RT was defined as RT-Baseline, and at the end of RT is defined as RT-End. The first blood routine test date after the beginning of RT was defined as RT-Mid-point, within 3 months after RT was defined as RT-3 months, and within 6 months was defined as RT-6 months. The absolute neutrophil count (ANC), PLC, absolute monocyte count (AMC), and PLT at each time point were collected retrospectively, and their ratios (NLR, PLR, LMR, and SII) were obtained. The SII is defined as ANC×PLT/PLC.

Statistical Analysis

Data analysis was performed using SPSS (IBM SPSS 23.0, SPSS Inc.). Descriptive statistics were generated for relevant clinical and dosimetric parameters. The Shapiro-Wilk test checked the normality of data distribution. According to the normality test results, a paired-sample *t*-test or Wilcoxon signed-rank test was performed to compare the differences between the two groups. We build stepwise multivariate linear regression models for the PLC value at the end of RT. The entry and exit criteria were set to 0.05 and 0.1 according to the default *F*-statistical *P*-value of SPSS software. The test for multicollinearity among the tested variables (including patient characteristics and dosimetric parameters of TPB and body) was performed. The variance inflation factor <5 is considered no multicollinearity. A $p < .05$ was considered statistically significant.

Results

Patient Characteristics

We retrospectively reviewed the clinical data of 188 patients with pelvic malignancies undergoing pelvic RT in our institution between October 2018 and June 2021. Of these, 122 (64.9%) were cervical cancer, 43 (22.9%) were endometrial cancer, 19 (10.1%) were prostate cancer, 3 (1.6%) were vaginal cancer, and 1 (0.5%) was vulvar cancer. All patients received pelvic lymphatic drainage areas by VMAT and IGRT (once a day in the first week and once a week from week 2 to 5). The basic clinical characteristics of the patients are available in Table 1.

Dosimetric Parameters of the Pelvic Bones and Body

The variables related to dosimetric parameters of the pelvic bones (TPB) and body were descriptively analyzed, and the mean values, median values, standard deviations, minimum values, maximum values, 25th percentile, and 75th percentile were recorded (Tables 2 and 3).

The Changing Trend of Blood Cell Counts

A complete blood sample was available for all patients during RT (at RT-Baseline, RT-End, and RT-Mid-point), and for

109 (57.98%), and 92 (48.94%) patients at 3 and 6 months from RT end, respectively.

The PLC value over different time intervals (at RT-Mid-point, RT-End, 3 months, and 6 months) has a significantly decreasing trend compared to the baseline ($p < .0001$), and the mean values at each time point were 1.50 ± 0.46 (at RT-Baseline), 0.87 ± 0.31 (at RT-Mid-point), 0.37 ± 0.16 (at RT-End), 0.75 ± 0.36 (at RT-3 months), and 0.84 ± 0.31 (at RT-6 months), respectively.

The nadirs of PLC (mean values = 24.7% of baseline) occurred at RT-End, and the PLC was still significantly lower than the baseline value at RT-3 months ($p < .0001$, mean values = 50.0% of baseline) and RT-6 months ($p < .0001$, mean values = 56% of baseline).

Table 1. Basic Clinical Characteristics of the Patients.

Patients (n)		188
Age (years)	Median, mean (range, SD)	56, 56.2 (29-87, 13.3)
Duration of RT (days)	Median, mean (range, SD)	35, 35.5 (27-62, 4.2)
Gender (n, %)	Male	19 (10.1)
	Female	169 (89.9)
Postoperative RT (n, %)	Yes	152 (80.9)
	No	36 (19.1)
Tumor type (n, %)	Cervical	122 (64.9%)
	Endometrial	43 (22.9%)
	Prostate	19 (10.1%)
	Vaginal	3 (1.6%)
	Vulvar	1 (0.5%)
Clinical stage* (n, %)	I-IB3	60 (31.9)
	II-IIB	83 (44.1)
	IIIA-IIIC	39 (20.7)
	IVA-IVB	6 (3.2)
Histology, n (%)	Squamous carcinoma	115 (61.2)
	Adenocarcinoma	22 (11.7)
	Endometrioid adenocarcinomas	43 (22.9)
	Others	8 (4.3)

Abbreviations: SD, standard deviation; RT, radiotherapy; Clinical stage*, clinical stages were according to the AJCC eighth staging system and the FIGO 2018 staging system.

The overall trend of ANC, AMC, and PLT values with respect to baseline at different time intervals was not a significant difference ($p > .05$) (Figure 1).

Temporal Characterization of Lymphocyte-Related Inflammation Indicators

The NLR, PLR, and SII values over different time intervals (at RT-Mid-point, RT-End, 3 months, and 6 months) have a significantly increasing trend compared to the baseline ($p < .0001$). The highest values of NLR, PLR, and SII appeared at RT-End, the corresponding values (mean ± SD) were 9.22 ± 6.59 (mean values = 382.4% of baseline), $598.23 \pm 316.46\%$ (mean values = 368.6% of baseline), and $1810.08 \pm 1703.17\%$ (mean values = 335.0% of baseline), respectively.

The LMR value over different time intervals (at RT-Mid-point, RT-End, RT-3 months, and RT-6 months) has a significantly decreasing trend compared to the baseline ($p < .0001$). The nadirs of LMR (mean values = 27.5% of baseline) occurred at RT-End, and the LMR was still significantly lower than the baseline value at RT-3 months ($p < .0001$, mean values = 72.3% of baseline) and RT-6 months ($p < .0001$, mean values = 72.7% of baseline).

Overall, these lymphocyte-related inflammatory indicators changed significantly after the start of RT and persisted for 3-6 months after RT. NLR, PLR, and SII at RT-End are about 3.5 times the value at RT-Baseline, while LMR is one-fourth of the basal value (Figure 2).

Lymphocytopenia

The lymphocytopenia was graded according to the CTCAE v5.0. Grading is specifically defined as: Grade 1, <LLN to 0.8×10^9/L; Grade 2, <0.8-0.5 $\times 10^9$ /L; Grade 3, <0.5-0.2 $\times 10^9$/L; Grade 4, <0.2 $\times 10^9$ /L. At RT-Mid-point, RT-End, RT-3 months, and RT-6 months, Grades 3 and 4 lymphocytopenia were observed in 10 (5.32%), 153 (81.39%), 29 (26.61%), and 11 (11.96%) cases of the patients, respectively. Detailed data are given in Table 4.

Table 2. Descriptive Statistics of Dosimetric Parameters of TPB.

Parameter		Mean	Median	Min	Max	Q1	Q3	SD
TPB	Volume (%)							
	TPB-V5	96.36	96.48	82.84	100	95.05	98.00	2.36
	TPB-V10	89.86	90.00	69.36	99.67	86.78	92.59	4.30
	TPB-V15	81.82	81.99	54.96	97.31	78.86	84.46	5.10
	TPB-V20	70.93	71.23	36.2	91.46	67.73	73.35	5.97
	TPB-V25	58.11	58.14	20.14	79.59	54.26	61.87	6.88
	TPB-V30	44.80	44.40	8.95	67.92	39.79	49.98	7.87
	TPB-V35	32.93	32.53	3.88	57.44	27.73	38.10	7.59
	TPB-V40	23.32	22.76	1.84	47.34	18.98	27.22	6.38
	TPB-V45	15.17	14.81	0	36.37	11.43	18.93	5.64
	TPB-V50	6.50	7.31	0	20.85	0.34	10.02	4.86

Abbreviations: TPB, total pelvic bone; Max, maximum; Min, minimum; Q1, 25th percentile; Q3, 75th percentile; SD, standard deviation.

Table 3. Descriptive Statistics of Dosimetric Parameters of Body.

Parameter		Mean	Median	Min	Max	Q1	Q3	SD
Body	Volume (%)							
	Body-V5	45.47	44.22	26.59	76.38	40.48	49.50	7.38
	Body-V10	38.10	37.21	5.24	65.16	34.24	42.31	7.04
	Body-V15	29.93	29.20	1.10	54.51	27.16	33.49	6.01
	Body-V20	21.62	21.13	2.05	41.95	18.88	24.17	4.79
	Body-V25	15.28	14.85	1.79	31.48	12.75	17.58	3.78
	Body-V30	11.07	10.80	1.07	23.85	9.15	12.77	2.89
	Body-V35	8.35	8.09	0.54	18.59	6.89	9.63	2.29
	Body-V40	6.57	6.39	0.19	14.96	5.51	7.54	1.79
	Body-V45	5.11	4.99	0.00	12.19	4.25	5.93	1.52
	Body-V50	2.88	3.41	0.00	9.04	0.48	4.08	1.94
Body	Volume (cm^3)	28 058.27	27 746.80	16 364.30	48 281.80	24 158.80	31 286.85	5699.79
	Body-CV5	12 609.84	12 462.52	6409.98	23 730.62	10 852.42	14 066.23	2619.26
	Body-CV10	10 530.33	10 534.44	1865.75	20 245.14	9271.83	11 758.47	2280.50
	Body-CV15	8243.39	8254.56	335.01	16 934.66	7212.17	9172.39	1782.66
	Body-CV20	5930.55	5847.36	623.85	13 033.63	5171.56	6505.32	1291.02
	Body-CV25	4175.87	4037.17	635.44	9782.30	3639.10	4613.34	965.55
	Body-CV30	3022.27	2923.60	325.88	7409.74	2620.36	3373.53	717.12
	Body-CV35	2275.50	2214.01	163.66	5775.91	1977.98	2499.48	563.95
	Body-CV40	1790.75	1752.23	65.97	4646.61	1556.50	1951.79	440.35
	Body-CV45	1397.48	1378.31	0.69	3788.90	1218.48	1538.35	382.10
	Body-CV50	798.91	987.66	0.00	2807.49	119.13	1111.26	528.42

Abbreviations: CV5, CV10, CV15, CV20, CV25, CV30, CV35, CV40, CV45, CV50, absolute volumes receiving 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 Gy; Body, defined as within the 5 Gy coverage; Max, maximum; Min, minimum; Q1, 25th percentile; Q3, 75th percentile; SD, standard deviation.

Stepwise Multivariate Linear Regression Analysis for PLC at RT-End

We build stepwise multivariate linear regression models for the PLC value at the end of RT. The entry and exit criteria were set to 0.05 and 0.1 according to the default F-statistical P-value of SPSS software. The test for multicollinearity among the tested variables (including patient characteristics and dosimetric parameters of TPB and body) was performed. The test showed that there is no multicollinearity among these tested variables (variance inflation factor <5). The final model consists of 3 variables that explain the PLC value at the end of RT (Table 5). Specifically, basal PLC, TPB-V5, and gender were selected, represented in the following equation:

$$\text{PLC (at RT-End)} = 1.716 + 0.156 \times \text{basal PLC} - 0.016 \times \text{TPB-V5} + 0.096 \times \text{Gender}$$

As can be seen in the equation, the basal PLC ($\beta = 0.156$, $p \leq .001$) and gender ($\beta = 0.096$, $p = .005$) were positively correlated with PLC (at RT-End), while TPB-V5 ($\beta = -0.016$, $p \leq .001$) was negatively correlated with PLC (at RT-End). The standardized regression coefficient of basal PLC was 0.456, which was higher than that of TPB-V5 (-0.248) and gender (0.185). Therefore, compared with gender, the basal PLC has a stronger effect on the PLC value (at RT-End), followed by TBP-V5. The R^2 value for the equation is 0.297, which means that 29.7% of the variability in the dependent variable can be explained or accounted for by the basal PLC, TPB-V5, and gender. Detailed information is shown in Table 6.

Discussion

The occurrence and development of tumors are closely related to the human body's inflammation and immune function status. Peripheral blood lymphocyte counts (PLC) and lymphocyte-related inflammation indicators can be used as important indicators to reflect the immune and inflammatory status of the body, respectively. Recent studies have revealed a significant relationship between PLC and RT in many solid tumors, which in turn can affect the changes in lymphocyte-related inflammation indicators.[6,13–15] This study was to explore the effects of pelvic RT on lymphocytes and lymphocyte-related inflammation indicators. In this present study, we found that PLC and lymphocyte-related inflammatory indicators changed significantly during pelvic RT and persisted for 3-6 months after RT. The changes were most obvious at the end of RT (RT-End) compared with the baseline level. The value of NLR, PLR, and SII at RT-End was about 3.5 times the value at RT-Baseline, while LMR and PLC were one-fourth of the basal value. The lymphocytopenia was graded according to the CTCAE v5.0, and Grades 3 and 4 lymphocytopenia were observed in 81.4% of patients (RT-End). In addition, the result of our study has provided evidence of an association between dosimetric predictors (TPB-V5) and PLC (RT-End).

Several studies have revealed that the lymphocytes have the effect of high-efficiency induction of apoptosis of tumor cells. It

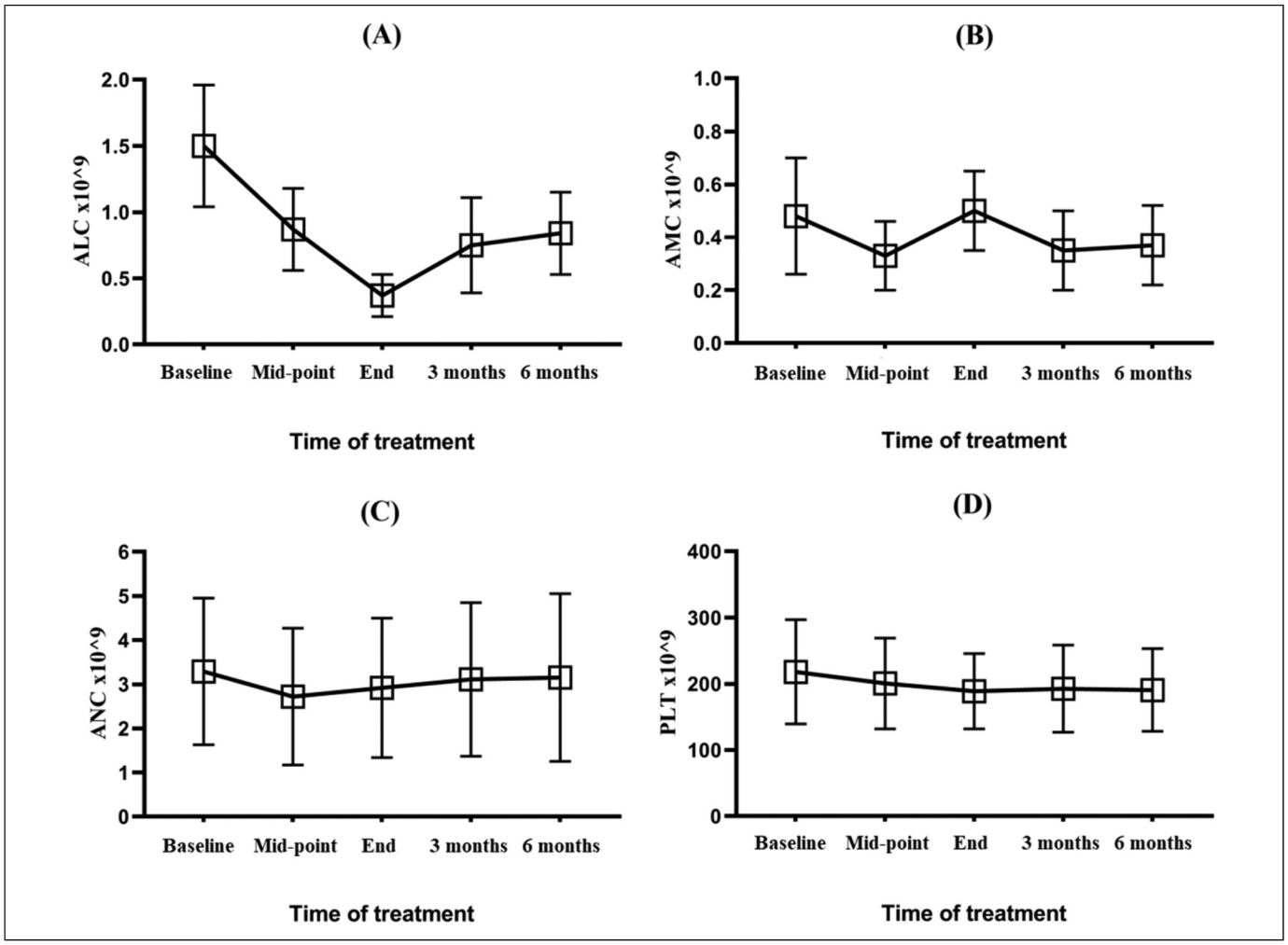

Figure 1. The overall trend of PLC, ANC, AMC, and PLT values with respect to baseline at different time intervals (mean and standard deviation plotted).
Abbreviations: PLC, peripheral blood lymphocyte counts; ANC, absolute neutrophil count; AMC, absolute monocyte count; PLT, platelet.

can inhibit tumor metastasis and progression in antitumor immune surveillance.[4,5] The patients with significant lymphocytopenia have a poor prognosis, and lymphocytopenia is an independent predictor of patient outcomes. For instance, a meta-analytic review demonstrated that 2 months after initiating chemoradiation, 43% of patients with solid tumors experienced persistent and severe lymphopenia, and grades 3 and 4 lymphocytopenia are associated with a greater risk of early death (HR, 2.1; 95% CI, 1.54-2.78; $p < .0001$).[14] Also, in another study of 133 patients with locally advanced pancreatic cancer undergoing stereotactic body radiation therapy or conventional chemoradiation therapy, results of this study showed that higher posttreatment PLC was associated with a relatively good prognosis regardless of RT technique (HR for death, 2.059; 95% CI, 1.310-3.237; $p = .002$).[16] The impact of radiation-induced lymphopenia on survival outcomes in solid tumors was also reported in a systemic review.[17] Moreover, some studies showed that circulating lymphocytes and immature lymphoblast precursor cells are exquisitely sensitive to ionizing radiation, even to low scattered doses. For example, Zhao et al[18] analyzed 115 patients with unresectable stage III NSCLC who received definitive chemoradiation therapy and found that 54.8% of the patients developed grades 3 and 4 lymphocytopenias at the fifth week following RT, and lymphocytopenia persisted to 2 months after RT completion. Another study on patients with esophageal cancer showed that severe lymphocytopenia was rare (0.6%) during the induction chemotherapy phase, but nearly 90% of patients developed grades 3 and 4 lymphopenias during neoadjuvant chemoradiation.[15] Therefore, lymphopenia should be given more attention by clinicians.

In cancer patients, the majority of the active bone marrow is located in the pelvic bones,[12] so the pelvic bones is a potential organ at risk during RT. Several studies have analyzed the relationship between pelvic dosimetry parameters and acute hematotoxicity in patients receiving pelvic RT combined with chemotherapy.[19–21] These acute hematotoxicity mainly include acute neutropenia, leukopenia, anemia, and thrombocytopenia. Their results revealed that the exposure volume of the TPBs (V10 and V20) is closely related to acute

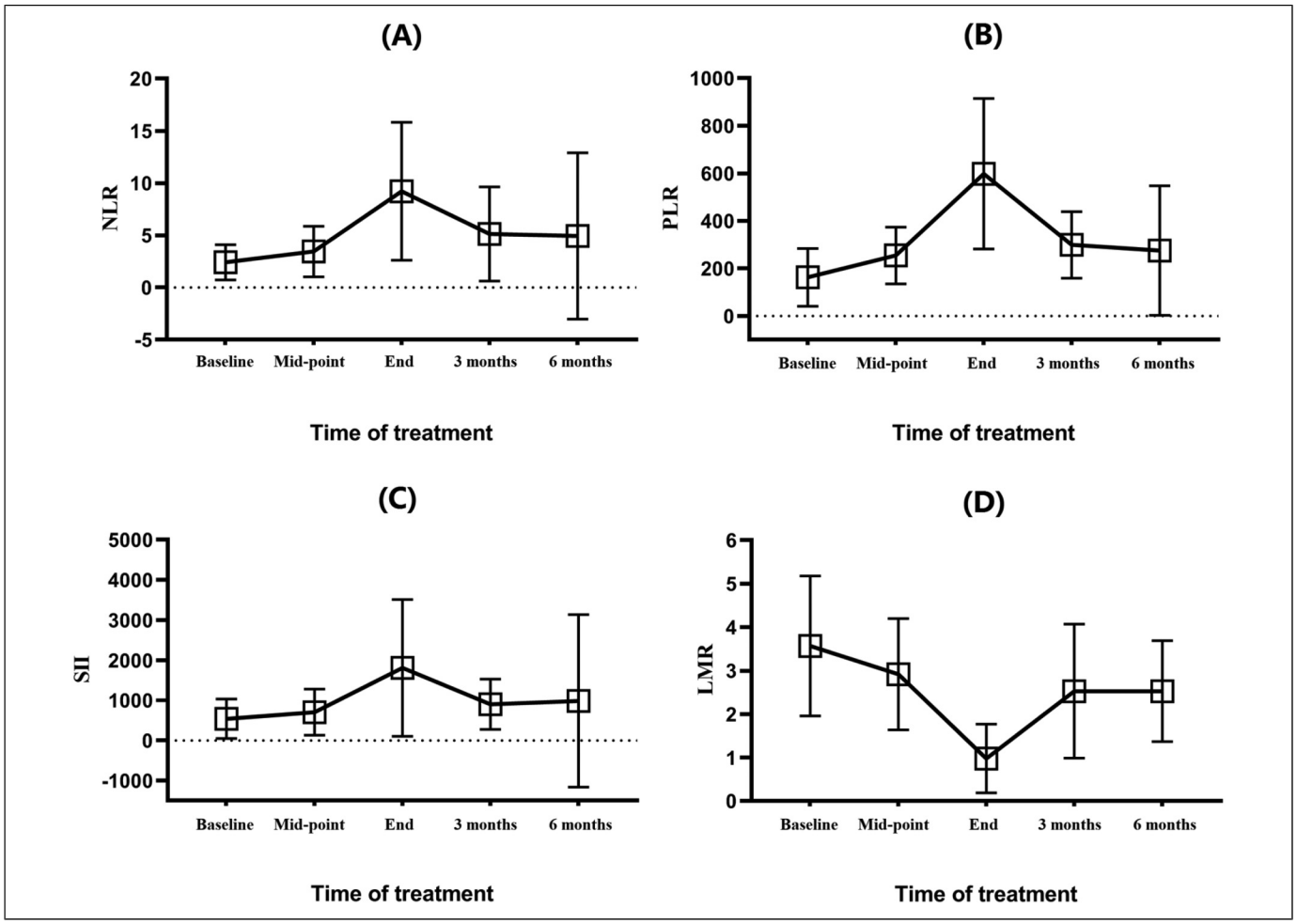

Figure 2. The overall trend of NLR, PLR, LMR, and the SII values with respect to baseline at different time intervals (mean and standard deviation plotted).
Abbreviations: NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; SII, systemic immune-inflammation index.

Table 4. Lymphocytopenia Graded According to the CTCAE v5.0 at Different Time Intervals.

Lymphocytopenia	Grade 1 (n, %)	Grade 2 (n, %)	Grade 3 (n, %)	Grade 4 (n, %)
RT-Mid-point	102 (54.26%)	76 (40.43%)	10 (5.32%)	0 (0%)
RT-End	4 (2.13%)	31 (16.49%)	139 (73.94%)	14 (7.45%)
RT-3 months (n = 109)	42 (38.53%)	38 (34.86%)	28 (25.69%)	1 (0.92%)
RT-6 months (n = 92)	50 (54.35%)	31 (33.70%)	10 (10.87%)	1 (1.09%)

Abbreviations: RT-End, at the end of radiotherapy; RT-Mid-point, the date of the first blood routine test after the beginning of radiotherapy; RT-3 months, within 3 months after radiotherapy; RT-6 months, within 3 to 6 months after radiotherapy; CTCAE, Common Terminology Criteria for Adverse Events.

Table 5. Stepwise Multivariate Linear Regression: Model Summary.

Model	R	R^2	Adjusted R^2	R^2 change	Durbin-Watson test
1	0.472[a]	0.223	0.218	0.13904	
2	0.516[b]	0.266	0.258	0.13546	
3	0.545[c]	0.297	0.286	0.1329	2.170

Abbreviations: PLC, peripheral lymphocyte count; TPB, total pelvic bone.
[a]Predictors: constant, basal PLC
[b]Predictors: constant, basal PLC, TPB-V5
[c]Predictors: constant, basal PLC, TPB-V5, gender (male = 1, female = 0).

hematotoxicity. In our study, patients with pelvic RT alone were enrolled and showed that at RT-Mid-point, RT-End, RT-3 months, and RT-6 months, grades 3 and 4 lymphocytopenia were observed in 10 (5.32%), 153 (81.4%), 29 (26.61%), and 11 (11.96%) of the patients, respectively. Except for lymphocytopenia, no other grade 3/4 hematotoxicities were noted, such as leukopenia, neutropenia, and thrombocytopenia. Our study suggested that pelvic RT alone mainly affects peripheral blood lymphocytes but has little effect on neutrophils, leukocytes, and platelets.

Table 6. Independent Predictors of PLC (at RT-End) in a Stepwise Multiple Linear Regression Analysis.

Model		Unstandardized β		Standardized β	T	*P*-value	95.0% CI: β		VIF
		β	SD				Lower limit	Upper limit	
1	Constant	0.128	0.035		3.696	0	0.06	0.197	
	Basal PLC	0.161	0.022	0.472	7.297	0	0.118	0.205	1
2	Constant	1.463	0.404		3.616	0	0.665	2.261	
	Basal PLC	0.168	0.022	0.491	7.767	0	0.125	0.211	1.009
	TPB-V5	−0.014	0.004	−0.209	−3.31	0.001	−0.022	−0.006	1.009
3	Constant	1.716	0.407		4.221	0	0.914	2.518	
	Basal PLC	0.156	0.022	0.456	7.198	0	0.113	0.198	1.049
	TPB-V5	−0.016	0.004	−0.248	−3.9	0	−0.025	−0.008	1.055
	Gender	0.096	0.034	0.185	2.865	0.005	0.03	0.163	1.095

Abbreviations: β, regression coefficients; SD, standard deviation; CI, confidence interval; VIF, variance inflation factor; PLC, peripheral lymphocyte count; TPB, total pelvic bone.

In a previous study concerning pelvic RT for prostate cancer, 2 models were developed to predict lymphocytopenia. The results showed that baseline PLC, TPB-V40, ilium-V40, and smoking (AUC = 0.904) were associated with acute/late lymphopenia.[22] We build stepwise multivariate linear regression models for the PLC value at the end of RT and showed that the basal PLC ($\beta = 0.156$, $p \leq .001$) was identified as a significant factor, which was consistent with the results of the previously reported literature.[22] In our study, male patients were all with high-risk prostate cancer. Because the clinical target volume range of prostate cancer irradiated pelvic cavity is significantly smaller than that of gynecological tumor patients, the absolute volume of the pelvic bones receiving irradiation in female patients is higher than that in male patients. In addition, males may have more advantages than females in the recovery of bone marrow function and general physical condition. Therefore, our results show that females were more likely to develop lymphocytopenia at the end of RT than males.

A previous study revealed that higher body dose-volume parameters (V5 / V10) might predict severe lymphocytopenia after palliative RT, while bone marrow dose-volume parameters are not.[23] Nevertheless, compared with this study, contrary results were concluded in our study, which found that TPB-V5 was negatively correlated with PLC (at RT-End). Still, no correlation was found between the absolute volume of the body receiving irradiation and lymphocytopenia. The possible reason for this may be as follows: Firstly, only pelvic malignancies were included in our study, and the majority of the patients received postoperative adjuvant RT rather than palliative RT at other sites. Secondly, no chemotherapy was administered to our patients, but whether the patients had undergone chemotherapy may be a major confounder. Thirdly, since lymphocytes are formed by pluripotent stem cells in the bone marrow and distributed to immune systems throughout the body through lymph and blood circulation, the pelvic bone is more likely to predict radiation-related lymphopenia than body volume when patients receive pelvic RT. Finally, the pelvis radiation exposure dose must be minimized (TPB-V5), consistent with the clinical phenomenon whereby lymphocytes are susceptible to radiation.[13] These situations suggest that minimizing severe lymphopenia is particularly important when introducing RT regimens for pelvic malignancies, and research on the influencing factors of lymphopenia should be strengthened.

To better establish the clinical utility of lymphocyte-related inflammation indicators, the ratio of common inflammatory cells to lymphocytes was defined as lymphocyte-related inflammation indicators, including NLR, LMR, PLR, and the SII. Currently, the mechanisms of lymphocyte-related inflammation indicators mainly contain the following aspects. Neutrophils initiate the production of reactive oxygen species during the early rolling stage of the inflammatory response, which may be involved in the regulation of inflammatory response by inducing the exfoliation of L-selectin in neutrophils.[24] Lymphocytes can release many kinds of substances such as perforin, granzymes, and granulysin, which have direct and indirect anti-tumor properties.[25] Circulating monocytes can be derived into macrophages (TAMs) and myeloid-derived suppressor cells, which can participate in the tumor progression, invasion, and migration by regulating the epithelial-mesenchymal transition transformation.[26,27] Cancer can induce nonspecific inflammation through the tumor microenvironment, leading to the release of a variety of proinflammatory mediators that can induce an increased platelet count.[28] Activated platelets can promote the distant metastasis and growth of tumors by secreting various cytokines such as platelet-derived growth factor, platelet-activating factor, and vascular endothelial growth factor.[29]

For clinical application, peripheral blood is easily obtained and cost-effective. Accumulating studies indicated that lymphocyte-related inflammation indicators, such as NLR, PLR, LMR, and SII, are associated with tumor prognosis.[8–11,30] However, at present, it is not yet clear what the optimal cut-off value of lymphocyte-related inflammation indicators would be in clinical practice. In general, an elevated NLR, PLR, and SII and decreased LMR are correlated with tumor progression and poor clinical outcomes in various human tumors.[6–11] Our study found that NLR, PLR, and SII values over different time intervals (at RT-Mid-point, RT-End, RT-3 months, and

RT-6 months) had a significantly increasing trend compared to the baseline, while LMR values decreased significantly compared with the baseline. Overall, these lymphocyte-related inflammatory indicators changed significantly after the start of RT and persisted for 3-6 months after RT. NLR, PLR, and SII at RT-End are about 3.5 times the value at RT-Baseline, while LMR is one-fourth of the basal value. Together, our results show that pelvic RT has higher effects on PLC and lymphocyte-related inflammation indicators and persists for a more extended period. In tandem with the current studies,[6–11,30,31] it is reasonable to conclude that the changes in lymphocytes and lymphocyte-related inflammation indicators caused by pelvic RT may cause a poor prognosis. Thus, each patient's prognosis needs to be quantified, estimated, and weighed with the benefits and risks of pelvic RT before treatment.

Nevertheless, we acknowledged that this study has some unavoidable limitations. To begin with, selection bias was inevitable because this was a single-center, retrospective study involving a small number of patients, especially since too few males were included. In addition, in our study, patients with prostate cancer did not undergo surgical treatment. In contrast, the majority of the patients with gynecological tumors received postoperative adjuvant RT, which may impact the final results in the regression models. Finally, we contoured the external contours of the bones as opposed to the actual proliferating active bone. The effect of lymphopenia parameters was not studied on survival or clinical outcome of the patient is also one of the limitations of our study. Despite the above limitations, this study still highlights the predictive value of dosimetric parameters for lymphopenia in pelvic malignancies patients who underwent RT alone. We further investigated trends in PLC and lymphocyte-related inflammatory indicators over time.

Conclusions

Based on the findings of our study, the following conclusions have been drawn. The impact of pelvic RT on PLC and lymphocyte-related inflammatory indicators is considerable and long-lasting. In a further multiple stepwise linear regression analysis, the basal PLC, gender, and TPB-V5 turned out to be the predictor of the absolute value of lymphocytes at the end of RT. Minimizing pelvic bones radiation exposure dose (TPBV5) may help to avoid severe cases of lymphocytopenia. However, the influencing factors of lymphopenia and lymphocyte-related inflammation indicators during RT for pelvic malignancies remain further investigated.

Author Contributions

J.J. was responsible for the primary concept and the design of the study; X.X., N.L., Z.D., and Z.D. performed the data capture and analysis; X.X. drafted the manuscript; all the external contours of the bones and body were delineated by X.X. and subsequently reviewed by N.L.; all authors revised the manuscript. All authors have read and approved the final manuscript.

Data Availability Statement

The datasets used during the current study are available from the corresponding author upon reasonable request.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Statement

The institutional review board and ethics committee of the National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital & Shenzhen Hospital deemed that a formal ethical review was not needed for this retrospective research.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Sanming Project of Medicine in Shenzhen, Shenzhen High-level Hospital Construction Fund, Shenzhen Key Medical Discipline Construction Fund, (grant number SZSM201612063, SZXK013).

ORCID iDs

Xiaoyong Xiang https://orcid.org/0000-0002-4569-9199
Jing Jin https://orcid.org/0000-0003-2158-7392

References

1. Rosenthal R, Cadieux EL, Salgado R, et al. Neoantigen-directed immune escape in lung cancer evolution. *Nature*. 2019;567(7749):479-485. doi:10.1038/s41586-019-1032-7
2. Greten FR, Grivennikov SI. Inflammation and cancer: triggers, mechanisms, and consequences. *Immunity*. 2019;51(1):27-41. doi:10.1016/j.immuni.2019.06.025
3. Ostroumov D, Fekete-Drimusz N, Saborowski M, Kühnel F, Woller N. CD4 And CD8 T lymphocyte interplay in controlling tumor growth. *Cell Mol Life Sci*. 2018;75(4):689-713. doi:10.1007/s00018-017-2686-7
4. Farhood B, Najafi M, Mortezaee K. CD8 + cytotoxic T lymphocytes in cancer immunotherapy: a review. *J Cell Physiol*. 2019;234(6):8509-8521. doi:10.1002/jcp.27782
5. Mohme M, Riethdorf S, Pantel K. Circulating and disseminated tumour cells – mechanisms of immune surveillance and escape. *Nat Rev Clin Oncol*. 2017;14(3):155-167. doi:10.1038/nrclinonc.2016.144
6. Cho Y, Park S, Byun HK, et al. Impact of treatment-related lymphopenia on immunotherapy for advanced non-small cell lung cancer. *Int J Radiat Oncol Biol Phys*. 2019;105(5):1065-1073. doi:10.1016/j.ijrobp.2019.08.047
7. Zidar DA, Al-Kindi SG, Liu Y, et al. Association of lymphopenia with risk of mortality among adults in the US general population. *JAMA Netw Open*. 2019;2(12):e1916526. doi:10.1001/jamanetworkopen.2019.16526.
8. Marques P, de Vries F, Dekkers OM, et al. Pre-operative serum inflammation-based scores in patients with pituitary adenomas. *Pituitary*. 2021;24(3):334-350. doi:10.1007/s11102-020-01112-5

9. Galvano A, Peri M, Guarini AA, et al. Analysis of systemic inflammatory biomarkers in neuroendocrine carcinomas of the lung: prognostic and predictive significance of NLR, LDH, ALI, and LIPI score. *Ther Adv Med Oncol*. 2020;12:1758835920942378. doi:10.1177/1758835920942378.
10. Park JW, Chang HJ, Yeo HY, et al. The relationships between systemic cytokine profiles and inflammatory markers in colorectal cancer and the prognostic significance of these parameters. *Br J Cancer*. 2020;123(4):610-618. doi:10.1038/s41416-020-0924-5
11. Zhang J, Feng W, Ye Z, Wei Y, Li L, Yang Y. Prognostic significance of platelet-to-lymphocyte ratio in patients with nasopharyngeal carcinoma: a meta-analysis. *Future Oncol*. 2020;16(5):117-127. doi:10.2217/fon-2019-0520
12. Hayman JA, Callahan JW, Herschtal A, et al. Distribution of proliferating bone marrow in adult cancer patients determined using FLT-PET imaging. *Int J Radiat Oncol Biol Phys*. 2011;79(3):847-852. doi:10.1016/j.ijrobp.2009.11.040
13. Wang X, Wang P, Zhao Z, Mao Q, Yu J, Li M. A review of radiation-induced lymphopenia in patients with esophageal cancer: an immunological perspective for radiotherapy. *Ther Adv Med Oncol*. 2020;12:1758835920926822. doi:10.1177/1758835920926822.
14. Grossman SA, Ellsworth S, Campian J, et al. Survival in patients with severe lymphopenia following treatment with radiation and chemotherapy for newly diagnosed solid tumors. *J Natl Compr Canc Netw*. 2015;13(10):1225-1231. doi:10.6004/jnccn.2015.0151
15. Shiraishi Y, Fang P, Xu C, et al. Severe lymphopenia during neoadjuvant chemoradiation for esophageal cancer: a propensity matched analysis of the relative risk of proton versus photon-based radiation therapy. *Radiother Oncol*. 2018;128(1):154-160. doi:10.1016/j.radonc.2017.11.028
16. Wild AT, Herman JM, Dholakia AS, et al. Lymphocyte-Sparing effect of stereotactic body radiation therapy in patients with unresectable pancreatic cancer. *Int J Radiat Oncol Biol Phys*. 2016;94(3):571-579. doi:10.1016/j.ijrobp.2015.11.026
17. 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. doi:10.1016/j.critrevonc.2018.01.003
18. Zhao Q, Chen G, Ye L, 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. doi:10.1186/s13014-019-1287-z.
19. Rose BS, Aydogan B, Liang Y, et al. Normal tissue complication probability modeling of acute hematologic toxicity in cervical cancer patients treated with chemoradiotherapy. *Int J Radiat Oncol Biol Phys*. 2011;79(3):800-807. doi:10.1016/j.ijrobp.2009.11.010
20. Mell LK, Kochanski JD, Roeske JC, et al. Dosimetric predictors of acute hematologic toxicity in cervical cancer patients treated with concurrent cisplatin and intensity-modulated pelvic radiotherapy. *Int J Radiat Oncol Biol Phys*. 2006;66(5):1356-1365. doi:10.1016/j.ijrobp.2006.03.018
21. Albuquerque K, Giangreco D, Morrison C, et al. Radiation-related predictors of hematologic toxicity after concurrent chemoradiation for cervical cancer and implications for bone marrow-sparing pelvic IMRT. *Int J Radiat Oncol Biol Phys*. 2011;79(4):1043-1047. doi:10.1016/j.ijrobp.2009.12.025
22. Sini C, Fiorino C, Perna L, et al. Dose-volume effects for pelvic bone marrow in predicting hematological toxicity in prostate cancer radiotherapy with pelvic node irradiation. *Radiother Oncol*. 2016;118(1):79-84. doi:10.1016/j.radonc.2015.11.020
23. Saito T, Toya R, Matsuyama T, Semba A, Oya N. Dosimetric predictors of treatment-related lymphopenia induced by palliative radiotherapy: predictive ability of dose-volume parameters based on body surface contour. *Radiol Oncol*. 2016;51(2):228-234. doi:10.1515/raon-2016-0050.
24. Domínguez-Luis MJ, Armas-González E, Herrera-García A, et al. L-selectin expression is regulated by CXCL8-induced reactive oxygen species produced during human neutrophil rolling. *Eur J Immunol*. 2019;49(3):386-397. doi:10.1002/eji.201847710
25. Chen W, Qin M, Chen X, Wang Q, Zhang Z, Sun X. Combining photothermal therapy and immunotherapy against melanoma by polydopamine-coated Al2O3 nanoparticles. *Theranostics*. 2018;8(8):2229-2241. doi:10.7150/thno.24073.
26. Katoh H, Watanabe M. Myeloid-Derived suppressor cells and therapeutic strategies in cancer. *Mediators Inflamm*. 2015;2015:159269. doi:10.1155/2015/159269
27. Marvel D, Gabrilovich DI. Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected. *J Clin Invest*. 2015;125(9):3356-3364. doi:10.1172/JCI80005
28. Yun SH, Sim EH, Goh RY, Park JI, Han JY. Platelet activation: the mechanisms and potential biomarkers. *Biomed Res Int*. 2016;2016:9060143. doi:10.1155/2016/9060143
29. Schumacher D, Strilic B, Sivaraj KK, Wettschureck N, Offermanns S. Platelet-derived nucleotides promote tumor-cell transendothelial migration and metastasis via P2Y2 receptor. *Cancer Cell*. 2013;24(1):130-137. doi:10.1016/j.ccr.2013.05.008
30. Huang QT, Zhou L, Zeng WJ, et al. Prognostic significance of neutrophil-to-lymphocyte ratio in ovarian cancer: a systematic review and meta-analysis of observational studies. *Cell Physiol Biochem*. 2017;41(6):2411-2418. doi:10.1159/000475911
31. Mandaliya H, Jones M, Oldmeadow C, Nordman II. Prognostic biomarkers in stage IV non-small cell lung cancer (NSCLC): neutrophil to lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR), platelet to lymphocyte ratio (PLR) and advanced lung cancer inflammation index (ALI). *Transl Lung Cancer Res*. 2019;8(6):886-894. doi:10.21037/tlcr.2019.11.16