Neutrophil-to-lymphocyte ratio reflects lung injury in thoracic radiotherapy and immune checkpoint inhibitors combination therapy with different sequences

ANNA TONG[1*], ZEWEN WANG[2*], SINIAN WANG[3], XIAOXUE LI[4], QISHENG JIANG[3], FENGSHENG LI[3] and PENG YAN[2]

[1]Radiation Oncology Department, 960 Hospital of the PLA Joint Logistics Support Force, Jinan, Shandong 250031; [2]Oncology Department, Central Hospital Affiliated to Shandong First Medical University, Jinan, Shandong 250013; [3]Department of Nuclear Radiation Injury and Monitoring, The PLA Rocket Force Characteristic Medical Center, Beijing 100032; [4]Pathology Department, Central Hospital Affiliated to Shandong First Medical University, Jinan, Shandong 250013, P.R. China

Received June 24, 2023; Accepted January 3, 2024

DOI: 10.3892/mco.2024.2718

Abstract. The combination of thoracic radiotherapy and immune checkpoint inhibitors (ICIs) has emerged as a novel treatment approach for malignant tumors. However, it is important to consider the potential exacerbation of lung injury associated with this treatment modality. The neutrophil-to-lymphocyte ratio (NLR), an inflammatory marker, holds promise as a non-invasive indicator for assessing the toxicity of this combination therapy. To investigate this further, a study involving 80 patients who underwent thoracic radiotherapy in conjunction with ICIs was conducted. These patients were divided into two groups: The concurrent therapy group and the sequential therapy group. A logistic regression analysis was conducted to ascertain risk factors for grade ≥2 pneumonitis. Following propensity score matching, the NLR values were examined between the concurrent group and the sequential group to evaluate any disparity. A mouse model of radiation pneumonitis was established, and ICIs were administered at varying time points. The morphological evaluation of lung injury was conducted using H&E staining, while the NLR values of peripheral blood were detected through flow cytometry. Logistic regression analysis revealed that radiation dosimetric parameters (mean lung dose, total dose and V20), the inflammatory index NLR at the onset of pneumonitis, and treatment sequences (concurrent or sequential) were identified as independent predictors of grade ≥2 treatment-related pneumonitis. The results of the morphological evaluation indicated that the severity of lung tissue injury was greater in cases where programmed cell death protein 1 (PD-1) blockade was administered during thoracic radiotherapy, compared with cases where PD-1 blockade was administered 14 days after radiotherapy. Moreover, the present study demonstrated that the non-invasive indicator known as the NLR has the potential to accurately reflect the aforementioned injury.

Correspondence to: Dr Peng Yan, Oncology Department, Central Hospital Affiliated to Shandong First Medical University, 105 Jiefang Road, Jinan, Shandong 250013, P.R. China
E-mail: yanpeng5325126@126.com

*Contributed equally

Key words: thoracic radiotherapy, immune checkpoint inhibitors, neutrophil-to-lymphocyte ratio, pneumonitis

Introduction

The advent of immune checkpoint inhibitors (ICIs) represents a groundbreaking development in cancer treatment. ICIs have found extensive application in various forms of cancer therapy, including palliative treatment, neoadjuvant therapy and adjuvant therapy. Nevertheless, the efficacy of ICIs as a monotherapy is limited, as evidenced by a response rate of 20% or lower in patients with cancer (1-3). It has been reported in several preclinical studies that the combination of ICIs with radiotherapy or chemotherapy is increasingly being employed since it can lead to the release of tumor antigens and result in a therapeutic synergistic effect (4,5). As of November 2023, more than 600 clinical trials of radiotherapy in combination with ICIs had been registered in the National Institutes of Health clinical trials (clinicaltrials.gov). However, due to the potential overlap in pulmonary toxicity induced by thoracic radiotherapy and ICIs, studies have also reported an elevated incidence of pneumonitis (6,7). In a comprehensive examination of 1,113 patients diagnosed with non-small cell lung cancer across 11 clinical studies, it was observed that concurrent treatment exhibited a higher occurrence of adverse pneumonitis at all grades (25.8 vs. 21.3%) compared with sequential therapy. Therefore, modifying the timing of medication administration has emerged as a potential strategy for mitigating lung injury.

The neutrophil-to-lymphocyte ratio (NLR) serves as a marker of systemic inflammation. It has previously been used as a robust indicator to assess the severity of community-acquired

pneumonia (8), chronic obstructive pulmonary disease (9) and COVID-19 (10). Moreover, a previous study has revealed that during thoracic radiotherapy, NLR levels were elevated in patients who developed pneumonitis following radiotherapy (11). Additionally, increased NLR levels during ICIs treatment also served as a biomarker for early diagnosis of checkpoint inhibitor-related pneumonitis (CIP) in a recent study (12).

As the combination of thoracic radiotherapy and ICIs becomes more widely used, it is imperative to address two noteworthy aspects: The optimal timing for administering these treatments and the non-invasive methodology for assessing lung injury. To that end, patients that underwent thoracic radiotherapy plus immunotherapy were retrospectively analyzed and animal models were established to evaluate the effect of timing of combination therapy on the occurrence of pneumonitis, and it was observed that NLR is a promising predictor of lung inflammation caused by ICIs combined with radiotherapy.

Materials and methods

Patients. Patients who underwent thoracic radiotherapy combined with ICIs treatments at the Central Hospital Affiliated to Shandong First Medical University (Jinan, China) between January 2019 and May 2022 were reviewed. The sites targeted for radiotherapy encompassed intrapulmonary or mediastinal tumors, such as primary lung cancer, metastatic lung cancer and esophageal cancer, among others. All patients were treated with intensity-modulated radiotherapy using a linear accelerator (Synergy/Infinity, Elekta, Sweden). The purpose of radiotherapy included radical or palliative therapy. The present study was approved (approval no. R202303060092) by the Ethical Committee of Central Hospital Affiliated to Shandong First Medical University (Jinan, China). Oral and written informed consents were obtained from patients and their surrogates in person.

Herein, ICIs agents included antibodies targeting programmed cell death protein 1 (PD-1) or programmed death-ligand 1. There exist two distinct classifications of sequential approach. The first category bore resemblance to the study of Antonia *et al* (13), wherein patients were administered ICIs subsequent to the completion of a radiotherapy course. The second category entailed the suspension of ICIs during the initiation of radiotherapy. Meanwhile, the concurrent treatment approach followed that of the ETOP NICOLAS trial (14) and Keynote-799 study (15), with ICIs being used during radiotherapy.

Data collection and outcome assessment. Patients and treatment characteristics were retrospectively collected from each patient's medical records, including patient demographics, smoking history, tumor types and Eastern Cooperative Oncology Group Performance Status (ECOG PS). Lung V20 (the percentage of lung volume received >20 Gy), the mean lung dose (MLD) and total dose were evaluated on the treatment planning workstation.

Diagnosis of treatment-related pneumonitis (TRP). The patients underwent evaluation one month following radiotherapy, with subsequent evaluations conducted concurrently with tumor assessment. Additional examinations were conducted in patients exhibiting respiratory disease-related symptoms. TRP was diagnosed by experienced pulmonologists and radiologists. TRP was defined as new-onset infiltrates on thoracic imaging and/or clinical symptoms such as cough, shortness of breath, or wheezing, while excluding other etiologies (disease progression, infection, or heart failure). Pneumonitis severity was graded using the Common Toxicity Criteria for Adverse Events version 5.0 (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_5x7.pdf). NLR was collected from a patient's blood routine at the time of diagnosis of TRP.

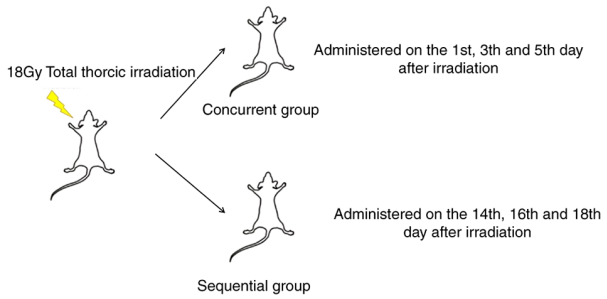

Figure 1. Establishing the model of different timing of administration after thoracic irradiation.

Establishment of the acute radiation lung injury mice model. A total of 16 male C57BL/6 mice (6-8 weeks old, weighing ~18-22 g) were purchased from SiPeiFu Biotechnology Co., Ltd (Beijing, China). Mice were housed under standard light-dark cycle (12/12-h light/dark cycle) and temperature (22±2˚C) conditions with sterilized food and water provided *ad libitum*, following institutional and office of laboratory animal welfare guidelines. All animal experiments were approved (approval no. JNCHIACUC2021-26) by the Ethical Committee of Central Hospital Affiliated to Shandong First Medical University (Jinan, China).

Irradiation. Mice were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg). The acute radiation pneumonitis model was established following established protocols (16). Mice were anesthetized and exposed to whole thorax radiation by X-ray at a dose rate of 600.00 cGy/min and a cumulative radiation dose of 18 Gy from a linear accelerator (Elekta Synergy, Sweden) at our institution. Mice were randomly assigned to two groups prior to irradiation initiation: The concurrent group and the sequential group (n=8 in each group).

PD-1 blockade is administered at different intervals after irradiation. The two dosing strategies used in the present study are demonstrated in Fig. 1. In the concurrent group, the anti-PD-1 antibody (200 μg/mouse; Bio X Cell, cat. no. BE0146; 1:12.5 resuspended with fresh PBS) was administered intraperitoneally on days 1, 3 and 5 after irradiation. In the sequential group, the anti-PD-1 antibody was injected intraperitoneally on days 14, 16, and 18 after irradiation.

Sample collection. Mice were sacrificed by cervical dislocation on the 28th day after irradiation. The lungs were then removed and immersed in 4% paraformaldehyde for 48 h

before being embedded in paraffin. Peripheral blood samples were collected from the mice after euthanasia via the orbital sinus using ethylenediaminetetraacetic acid tubes. All mice were euthanized by dislocating cervical vertebra under general anesthesia at the end of experiments or if a humane endpoint was reached. Humane endpoint was defined as the occurrence of severe dyspnea, vomiting, inability to ambulate or rise for food and water, or a loss of >15% of body weight. However, none of the animals reached these humane endpoints.

Histopathological analysis. Histopathological changes were evaluated following H&E staining. Alveolar congestion, hemorrhage, aggregation of inflammatory cells in airspaces or vessel walls and the thickness of the alveolar walls were assessed using a 0-4-point semi-quantitative histological analysis method (17) (4: Extremely serious; 3: Serious; 2: Middle; 1: Slight; 0: Normal). In total, five fields of view were randomly selected, and the histology score of each sample was determined using an average of all the scores.

Circulating leukocytes were analyzed using flow cytometry. Neutrophil counts were determined by quantifying $Ly6G^+$ $CD11b^+$ cells in peripheral blood using flow cytometric analysis, following established methodologies (18). For the analysis of lymphocyte populations in peripheral blood, a lymphoid gate (low-side scatter) was applied to exclude cells of monocytic origin (19). The following antibodies were utilized: APC-Cy7 anti-mouse CD45 (cat. no. 557659; BD Biosciences), PE anti-mouse CD11b (cat. no. 24965) and PerCP-cy5.5 anti-mouse ly6G (cat. no. 63460) both from Cell Signaling Technology, Inc.). Cell counts were analyzed using a BD Canto II flow cytometer, and the analysis was performed with FACS Diva software (version 6.1.2; BD Biosciences).

Statistical analysis. Univariate and multivariate logistic regression analyses were conducted to identify the independent risk factors for TRP. In addition, the proportional hazard ratio (HR) and 95% confidence intervals (CIs) were also calculated. Receiver operating characteristic (ROC) curves were employed to assess the effects of lung V20, total dose, MLD and NLR on TRP. Propensity score matching (PSM) was adopted to match subjects in the concurrent and sequential groups. A paired Student's t-test was performed after matching. All data represent the mean ± standard error of Mean (SEM) from at least three independent experiments. $P<0.05$ was considered to indicate a statistically significant difference. All statistical analyses were conducted using SPSS 27.0 software (IBM Corp.) and/or Prism GraphPad 8.0 (Dotmatics).

Results

Patient characteristics. The clinicopathological characteristics of the 80 patients included in the present study are listed in Table I. Among them, 31 patients were <70 years old, while the remaining were >70 years old. There were 21 (26.25%) patients with an ECOG PS of 0, 42 (52.5%) patients with a PS of 1, and 17 (21.25%) patients with a PS of 2. In terms of tumor categories, a total of 31 instances of intrapulmonary tumors were observed, constituting 38.75% of the cases, predominantly manifesting as lung metastases. Additionally, there were 29 occurrences of mediastinal tumors, encompassing esophageal cancer, gastroesophageal junction tumors and mediastinal lymph node metastases. A total of 20 cases involved both intrapulmonary and mediastinal lymph node metastases, accounting for 25.00% of the total. In total, three dose-volumetric parameters were selected: Whole lung V20, total dose and MLD. Of the total, 59 patients had V20 <20% (73.75%), while 21 patients had V20 ≥20% (26.25%). For MLD, 63 patients (78.75%) had MLD <10 Gy, and 17 patients (21.25%) had MLD ≥10 Gy. Regarding treatment approach, 14 patients (17.50%) received concurrent ICIs and radiotherapy, while 66 patients did not undergo concurrent administration of thoracic radiotherapy and ICIs. On peripheral blood testing onset of pneumonitis (Mindary Blood Cell Analyzer), 44 (55.00%) had NLR <5 and 36 (45.00%) had NLR ≥5. During follow-up period, a total of 14 patients (17.50%) developed grade ≥2 pneumonitis.

Table I. Clinicopathological characteristics of patients.

Clinicopathological characteristics	Total number (n=80)	Percentage (%)
Age		
<70	31	38.75
≥70	49	61.25
Sex		
Male	52	65.00
Female	28	35.00
Smoking History		
None	38	47.50
Yes	42	52.50
ECOG		
0	21	26.25
1	42	52.50
2	17	21.25
Tumor types		
Intrapulmonary	31	38.75
Mediastinal	29	36.25
Both	20	25.00
V20		
<20%	59	73.75
≥20%	21	26.25
Therapeutic modalities		
Concurrent	14	17.50
Sequential	66	82.50
Neutrophil-to-lymphocyte ratio		
<5	44	55.00
≥5	36	45.00
Mean lung dose		
<10 Gy	63	78.75
≥10 Gy	17	21.25
Grade ≥2 pneumonitis		
Yes	14	17.50
No	66	82.50

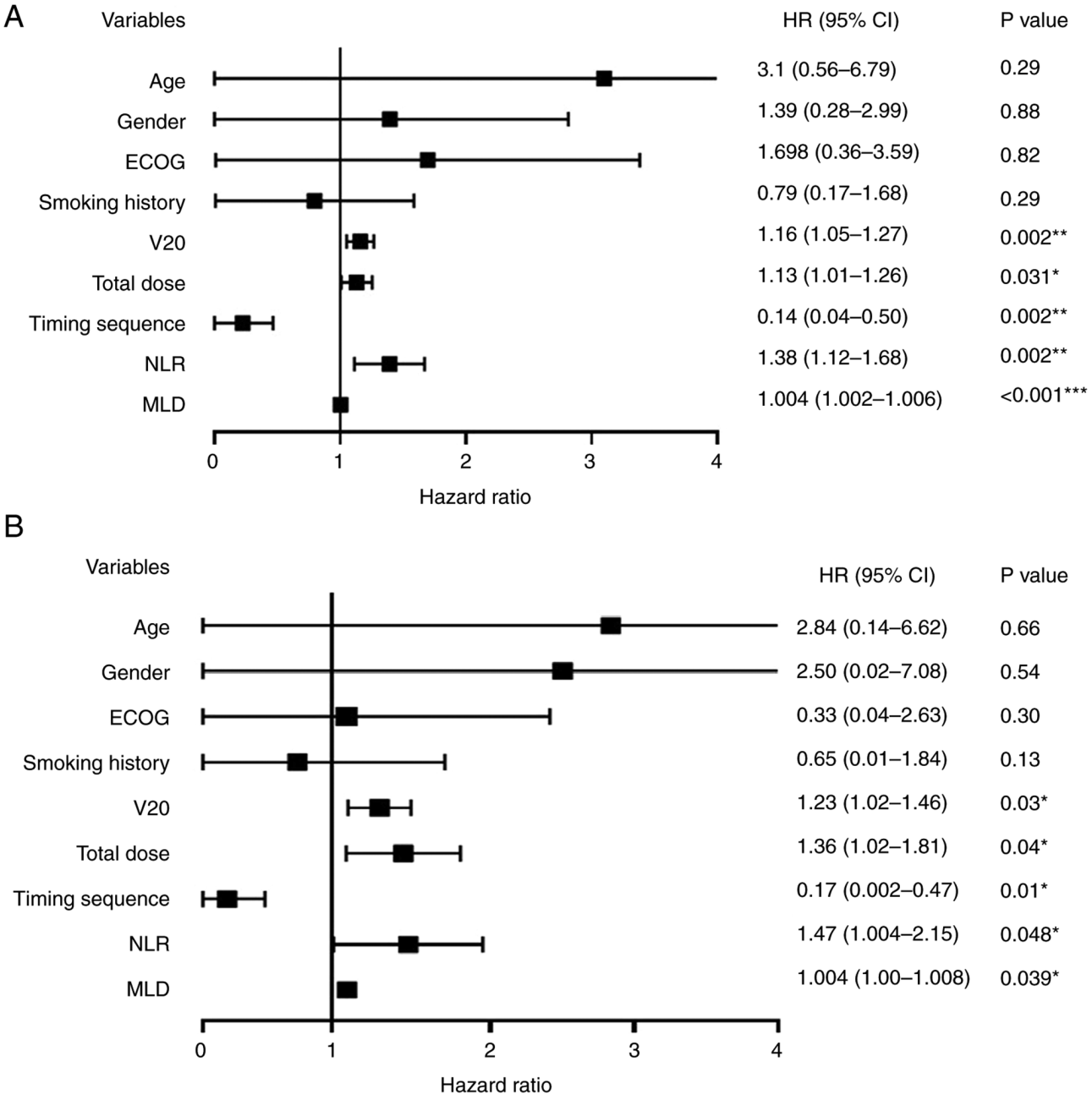

Figure 2. Results of (A) univariate and multivariate (B) logistic regression analyses. *P<0.05, **P<0.01 and ***P<0.001.

Univariate and multivariate analysis of TRP. The relationships between TRP and clinical characteristics are revealed in Fig. 2. In the univariate analysis, V20, total dose, MLD, sequence of administration and NLR were found to be independent predictive factors for TRP. Univariate logistic regression identified that the HR value of whole lung V20 was 1.16 (95% CI, 1.05-1.27; P=0.002), the HR value of MLD was 1.004 (95% CI, 1.002-1.006; P<0.001), the HR value of total dose was 1.13 (95% CI, 1.01-1.26; P=0.031), the HR value for administration sequence was 0.14 (95% CI, 0.04-0.5; P=0.002) and the HR value of NLR was 1.38 (95% CI, 1.12-1.68; P=0.002).

In the multivariate analysis, V20, MLD, total dose, administered sequence and NLR remained independent predictive factors for TRP. Multivariate logistic regression revealed that the HR value of whole lung V20 was 1.23 (95% CI, 1.02-1.46; P=0.03), the HR value of MLD was 1.004 (95% CI, 1.002-1.008; P=0.039), the HR value of total dose was 1.36 (95% CI, 1.02-1.81; P=0.04), the HR value of administration sequence was 0.17 (95% CI, 0.002-0.47; P=0.01) and the HR value of NLR was 1.47 (95% CI, 1.004-2.15; P=0.048).

ROC curve analyses were constructed to determine the area under the ROC curve (AUC) for each variable (Fig. 3). Based on the results of the ROC curve test, the AUC for V20 was 0.769 (22.87% was used as the cutoff value). The AUC for total dose was 0.683, with 54 Gy used as the cutoff value. The AUC for MLD was 0.786, with 10.47 Gy used as the cutoff value. The AUC for NLR was 0.796, with 6.08 used as the cutoff value.

Effect of combination therapy on lung injury with different time intervals. To evaluate pathological changes, H&E

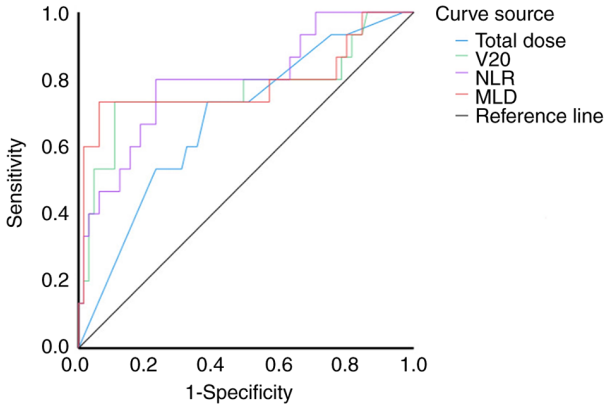

Figure 3. Receiver operating characteristic curve for treatment-related pneumonitis. NLR, neutrophil-to-lymphocyte ratio; MLD, mean lung dose.

staining was utilized in mouse models (Fig. 4). The staining was quantified by pathologists to assess lung injury. Notably, the concurrent treatment group exhibited more severe lung tissue injury compared with the sequential treatment group, with a score of 3.2±0.2 in the concurrent treatment group vs. 2.2±0.3 in the sequential treatment group (P<0.05).

NLR in patients and animal models with different treatment timing sequence. In the patients reviewed, baseline characteristics were not initially balanced. To address this, PSM was performed using SPSS software (version 27.0; IBM Corp.). Ultimately, 26 patients were successfully matched. A paired Student's t-test revealed that the NLR in the concurrent treatment group was significantly higher compared with the sequential group (T=2.27, P=0.043) (Fig. 5A). In the animal model of combination treatment, the NLR was 0.58±0.08 in the concurrent treatment group and 0.26±0.06 in the sequential treatment group (P<0.01) (Fig. 5B).

Discussion

The treatment approach of ICIs combined with radiotherapy in several types of cancer has demonstrated significant clinical benefits. Studies have reported that irradiation may increase non-synonymous mutation burden and trigger neoantigen production in cancer cells, possibly favoring *in situ* vaccine development and tumor microenvironment (TME) reprogramming (20). The combination of ICIs with radiotherapy has been revealed to reverse the suppressive TME, potentially making a significant difference in cancer treatment. Currently, two combination therapy strategies are widely employed clinically, namely sequential utilization of radiotherapy and ICIs, and concurrent implementation of both modalities. Previously, the therapeutic strategy of radiotherapy combined with ICIs for thoracic cancer was centered on understanding the influence of radiotherapy on the immune response in patients (21,22). Nevertheless, it is essential to acknowledge the deleterious effects induced by combination therapy on patients' quality of life. Pneumonitis is a common and potentially lethal complication in the treatment of patients with thoracic tumors using radiotherapy or ICIs. Radiation-induced pneumonitis stands out as a significant toxicity in thoracic radiotherapy, occurring in ~5-15% of patients (23,24). Although CIP is not frequently observed in patients treated with ICIs, it remains the leading cause of ICI-related death, with a fatality rate ranging from 10-17% (25). Due to the overlapping toxicity profiles of the two treatment modalities in the lungs, interest has been drawn to the approach of reducing lung toxicity when combining these two treatments.

In the present study, an escalation was observed in the severity of pulmonary inflammation when thoracic irradiation and ICIs were administered concurrently. The collected data revealed that among the examined cases, 50% (7/14) of patients in the concurrent treatment group experienced grade 2 or higher pneumonitis. By contrast, only 10.6% (7/66) of patients in the sequential treatment group exhibited the same level of pneumonitis. Furthermore, in mouse models of acute radiation pneumonitis, the group receiving PD-1 within 14 days exhibited more severe lung injury compared with the group receiving PD-1 after 14 days.

As the mechanism of pneumonitis in combination therapy remains unclear, it is known that radiation induces inflammatory cell infiltration (26), DNA damage and reactive oxygen species generation, which further leads to the release of various cytokines to promote inflammation (27,28). It was reasoned that ICIs administered in this inflammatory environment can lead to an immune-boosting effect through a series of processes involving autoreactive lymphocytes, autoantibodies and cytokines, such as IL-3, -6, -10 and -17, TNF-α and TGF-β (29). It was concluded that the possible crosstalk among signaling pathways was inflammatory cell infiltration and numerous cytokines released. Besides, the administration of ICIs could also amplify the inflammatory response in irradiated healthy tissues. After a certain period of time, the local inflammatory response was reduced, using of ICIs might not lead to the aforementioned inflammatory cascade. This could potentially account for the relatively low prevalence of pneumonitis observed in patients undergoing sequential treatment.

In addition, the current investigation also delved into the potential of NLR as a non-invasive measure for detecting treatment-related lung injury. The present findings revealed that V20, total dose, MLD and NLR were significant independent predictors of treatment-associated pneumonitis. Based on the ROC curves for V20, total dose, MLD and NLR, the optimal cut-off values in the present study were determined to be 22.87%, 54 Gy, 10.47 Gy and 6.08, respectively. Notably, a previous study reported consistent cut-off values of 24% for V20 and 12.26 Gy for MLD in relation to grade ≥2 radiation pneumonitis (30). Alongside dosimetric parameters, the present study revealed that the NLR is an independent prognostic factor for the development of treatment-associated pneumonitis in combination therapies. Importantly, this was the inaugural identification of such associations.

Previous studies have established that the NLR can partially reflect the systemic inflammation status (31-33). Given its demonstrated ability to reflect the severity of radiation pneumonitis and predict the occurrence of CIP, it was hypothesized that NLR could serve as a highly efficient indicator for pneumonitis in patients receiving combination therapy. Herein, the NLR value ≥6.08 was used as a reference to reflect lung injury in patients treated with thoracic radiotherapy and ICIs. Furthermore, *in vivo* models were used to

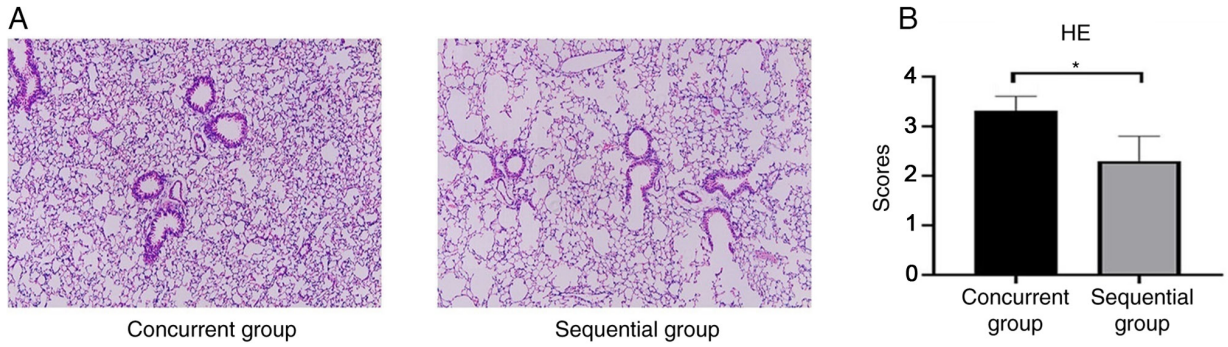

Figure 4. H&E staining reveals lung tissue injury. (A) Representative images of H&E staining in the lungs of the concurrent group and the sequential group. (B) The statistical analyses of lung tissue damage scores of the two groups. *P<0.05.

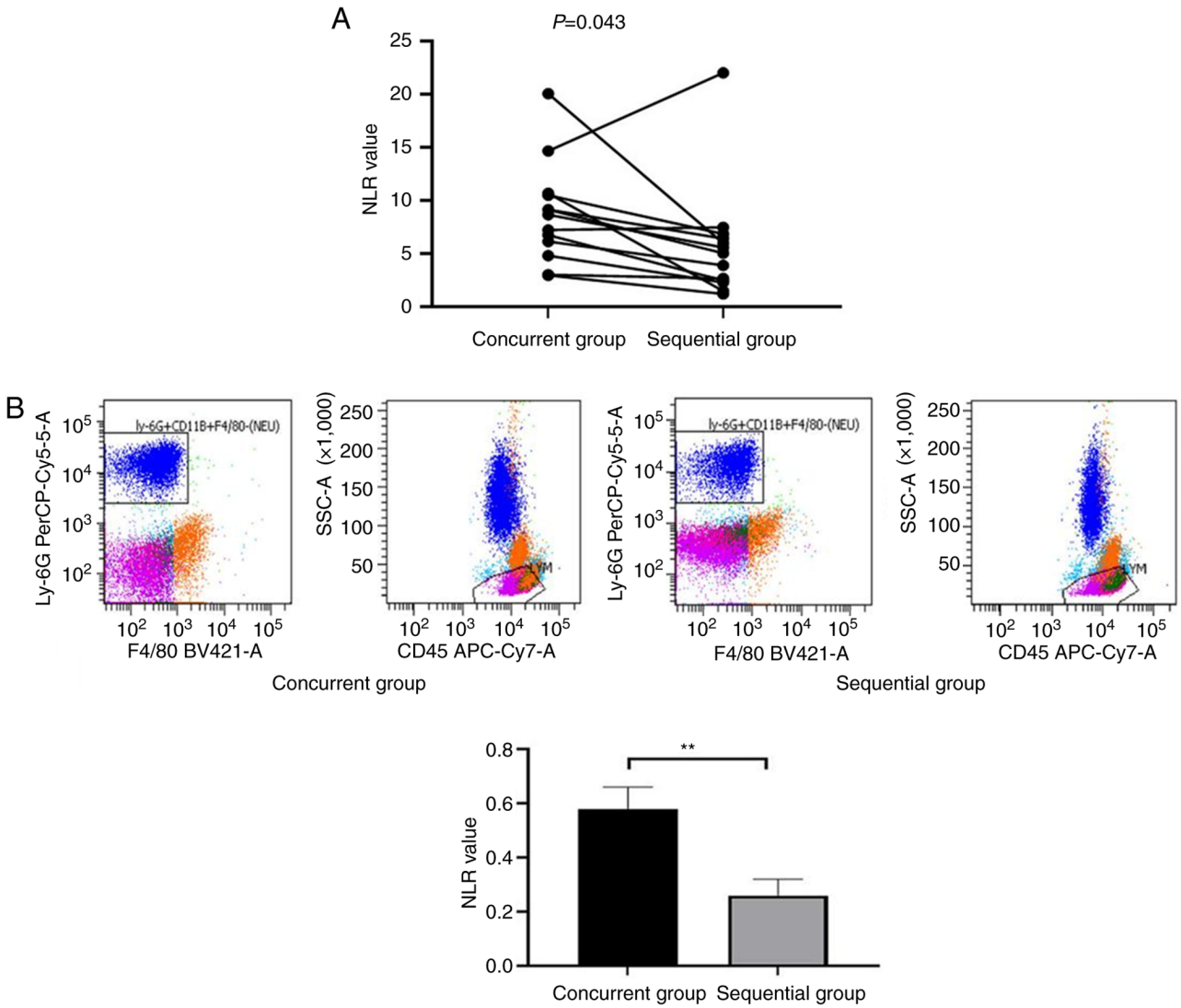

Figure 5. NLR values in peripheral blood. (A) NLR values in peripheral blood were analyzed by automated blood cell analyzer in patients with two different treatment models. (B) NLR values in peripheral blood were analyzed by flow cytometer in mice with two different treatment models. **P<0.01. NLR, neutrophil-to-lymphocyte ratio.

further demonstrate that NLR could effectively serve as an indicator of pulmonary damage resulting from the administration of combination therapy.

Nonetheless, the present study has certain limitations. Firstly, the study sample size was small, and patients were not randomized. Following the matching process, the concurrent and sequential groups consisted of only 26 patients each. Additionally, the investigation did not explore the optimal timing for administering ICIs in patients undergoing thoracic radiotherapy. The study also lacked a dynamic observation of

NLR, preventing the establishment of a correlation between pre- and post-treatment NLR values and pneumonitis occurrence. Lastly, while it has been documented in numerous studies that NLR can serve as an independent indicator for evaluating inflammatory status (34,35), a more precise determination of inflammatory states can potentially be achieved by combining NLR with comprehensive markers of inflammation, including C-reactive protein, IL-6, IL-10, IL-17 and other inflammatory cytokines.

In conclusion, the findings of the present study indicated that the simultaneous administration of ICIs and thoracic radiation therapy may elevate the likelihood of grade 2 and higher pneumonitis. Additionally, the NLR exhibited promise as a non-invasive method for monitoring lung damage in real-time during combination therapy.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Youth Project of Natural Science Foundation of Shandong (grant no. ZR2022QH187), the Shandong Medical and Health Technology Development Project (grant no. 202104080454) and the Shandong Traditional Chinese Medicine Science and Technology Project (grant no. Q-2022004).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

AT conducted the data analysis and authored the original draft. ZW was responsible for the collection and visualization of clinical data. SW and QJ assumed oversight and leadership in the research. XL generated pathology slides and interpretation. FL and PY designed the study. PY and ZW performed the animal experiments. PY provided funding. AT and PY confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved (approval no. R202303060092) by the Ethical Committee of Central Hospital Affiliated to Shandong First Medical University (Jinan, China). Oral and written informed consents were obtained from patients and their surrogates in person. The present study followed the guidelines of the Declaration of Helsinki. All animal experiments were approved (approval no. JNCHIACUC2021-26) by the Ethical Committee of Central Hospital Affiliated to Shandong First Medical University (Jinan, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, *et al*: Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 373: 1627-1639, 2015.
2. Shitara K, Özgüroğlu M, Bang YJ, Di Bartolomeo M, Mandalà M, Ryu MH, Fornaro L, Olesiński T, Caglevic C, Chung HC, *et al*: Pembrolizumab versus paclitaxel for previously treated, advanced gastric or gastro-oesophageal junction cancer (KEYNOTE-061): A randomised, open-label, controlled, phase 3 trial. Lancet 392: 123-133, 2018.
3. Ikeda S, Goodman AM, Cohen PR, Jensen TJ, Ellison CK, Frampton G, Miller V, Patel SP and Kurzrock R: Metastatic basal cell carcinoma with amplification of PD-L1: Exceptional response to anti-PD1 therapy. NPJ Genom Med 1: 16037, 2016.
4. Sharabi AB, Nirschl CJ, Kochel CM, Nirschl TR, Francica BJ, Velarde E, Deweese TL and Drake CG: Stereotactic radiation therapy augments antigen-specific PD-1-Mediated antitumor immune responses via cross-presentation of tumor antigen. Cancer Immunol Res 3: 345-355, 2015.
5. Deng L, Liang H, Burnette B, Beckett M, Darga T, Weichselbaum RR and Fu YX: Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. J Clin Invest 124: 687-695, 2014.
6. Shaverdian N, Lisberg AE, Bornazyan K, Veruttipong D, Goldman JW, Formenti SC, Garon EB and Lee P: Previous radiotherapy and the clinical activity and toxicity of pembrolizumab in the treatment of non-small-cell lung cancer: A secondary analysis of the KEYNOTE-001 phase 1 trial. Lancet Oncol 18: 895-903, 2017.
7. Theelen WSME, Peulen HMU, Lalezari F, van der Noort V, de Vries JF, Aerts JGJV, Dumoulin DW, Bahce I, Niemeijer AN, de Langen AJ, *et al*: Effect of pembrolizumab after stereotactic body radiotherapy vs pembrolizumab alone on tumor response in patients with advanced non-small cell lung cancer: Results of the PEMBRO-RT phase 2 randomized clinical trial. JAMA Oncol 5: 1276-1282, 2019.
8. Lee H, Kim I, Kang BH and Um SJ: Prognostic value of serial neutrophil-to-lymphocyte ratio measurements in hospitalized community-acquired pneumonia. PLoS One 16: e0250067, 2021.
9. Liu J, Liu J and Zou Y: Relationship between neutrophil-lymphocyte ratio and short-term prognosis in the chronic obstructive pulmonary patients with acute exacerbation. Biosci Rep 39: BSR20190675, 2019.
10. Liu J, Liu Y, Xiang P, Pu L, Xiong H, Li C, Zhang M, Tan J, Xu Y, Song R, *et al*: Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage. J Transl Med 18: 206, 2020.
11. Lee YH, Choi HS, Jeong H, Kang KM, Song JH, Lee WS, Lee GW, Song HN, Kim HG, Kang MH, *et al*: Neutrophil-lymphocyte ratio and a dosimetric factor for predicting symptomatic radiation pneumonitis in non-small-cell lung cancer patients treated with concurrent chemoradiotherapy. Clin Respir J 12: 1264-1273, 2018.
12. Lin X, Deng H, Yang Y, Wu J, Qiu G, Li S, Xie X, Liu M, Xie Z, Qin Y, *et al*: Peripheral blood biomarkers for early diagnosis, severity, and prognosis of checkpoint inhibitor-related pneumonitis in patients with lung cancer. Front Oncol 11: 698832, 2021.
13. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, Kurata T, Chiappori A, Lee KH, de Wit M, *et al*: Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. N Engl J Med 379: 2342-2350, 2018.
14. Peters S, Felip E, Dafni U, Belka C, Guckenberger M, Irigoyen A, Nadal E, Becker A, Vees H, Pless M, *et al*: Safety evaluation of nivolumab added concurrently to radiotherapy in a standard first line chemo-radiotherapy regimen in stage III non-small cell lung cancer-The ETOP NICOLAS trial. Lung Cancer 133: 83-87, 2019.
15. Jabbour SK, Lee KH, Frost N, Breder V, Kowalski DM, Pollock T, Levchenko E, Reguart N, Martinez-Marti A, Houghton B, *et al*: Pembrolizumab plus concurrent chemoradiation therapy in patients with unresectable, locally advanced, stage III non-small cell lung cancer: The phase 2 KEYNOTE-799 nonrandomized trial. JAMA Oncol 7: 1-9, 2021.

16. Gao J, Peng S, Shan X, Deng G, Shen L, Sun J, Jiang C, Yang X, Chang Z, Sun X, *et al*: Inhibition of AIM2 inflammasome-mediated pyroptosis by Andrographolide contributes to amelioration of radiation-induced lung inflammation and fibrosis. Cell Death Dis 10: 957, 2019.
17. Mikawa K, Nishina K, Takao Y and Obara H: ONO-1714, a nitric oxide synthase inhibitor, attenuates endotoxin-induced acute lung injury in rabbits. Anesth Analg 97: 1751-1755, 2003.
18. Dave MN, Silva JE, Eliçabe RJ, Jeréz MB, Filippa VP, Gorlino CV, Autenrieth S, Autenrieth IB and Di Genaro MS: Yersinia enterocolitica YopH-Deficient strain activates neutrophil recruitment to Peyer's patches and promotes clearance of the virulent strain. Infect Immun 84: 3172-3181, 2016.
19. Loken MR, Brosnan JM, Bach BA and Ault KA: Establishing optimal lymphocyte gates for immunophenotyping by flow cytometry. Cytometry 11: 453-459, 1990.
20. Herrera FG, Bourhis J and Coukos G: Radiotherapy combination opportunities leveraging immunity for the next oncology practice. CA Cancer J Clin 67: 65-85, 2017.
21. Suzuki Y, Mimura K, Yoshimoto Y, Watanabe M, Ohkubo Y, Izawa S, Murata K, Fujii H, Nakano T and Kono K: Immunogenic tumor cell death induced by chemoradiotherapy in patients with esophageal squamous cell carcinoma. Cancer Res 72: 3967-3976, 2012.
22. Ma JL, Jin L, Li YD, He CC, Guo XJ, Liu R, Yang YY and Han SX: The intensity of radiotherapy-elicited immune response is associated with esophageal cancer clearance. J Immunol Res 2014: 794249, 2014.
23. Segawa Y, Takigawa N, Kataoka M, Takata I, Fujimoto N and Ueoka H: Risk factors for development of radiation pneumonitis following radiation therapy with or without chemotherapy for lung cancer. Int J Radiat Oncol Biol Phys 39: 91-98, 1997.
24. Kong FM, Hayman JA, Griffith KA, Kalemkerian GP, Arenberg D, Lyons S, Turrisi A, Lichter A, Fraass B, Eisbruch A, *et al*: Final toxicity results of a radiation-dose escalation study in patients with non-small-cell lung cancer (NSCLC): Predictors for radiation pneumonitis and fibrosis. Int J Radiat Oncol Biol Phys 65: 1075-1086, 2006.
25. Cadranel J, Canellas A, Matton L, Darrason M, Parrot A, Naccache JM, Lavolé A, Ruppert AM and Fallet V: Pulmonary complications of immune checkpoint inhibitors in patients with nonsmall cell lung cancer. Eur Respir Rev 28: 190058, 2019.
26. Klein D, Steens J, Wiesemann A, Schulz F, Kaschani F, Röck K, Yamaguchi M, Wirsdörfer F, Kaiser M, Fischer JW, *et al*: Mesenchymal stem cell therapy protects lungs from radiation-induced endothelial cell loss by restoring superoxide dismutase 1 expression. Antioxid Redox Signal 26: 563-582, 2017.
27. Azzam EI, Jay-Gerin JP and Pain D: Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. Cancer Lett 327: 48-60, 2012.
28. Abratt RP, Morgan GW, Silvestri G and Willcox P: Pulmonary complications of radiation therapy. Clin Chest Med 25: 167-177, 2004.
29. Zhai X, Zhang J, Tian Y, Li J, Jing W, Guo H and Zhu H: The mechanism and risk factors for immune checkpoint inhibitor pneumonitis in non-small cell lung cancer patients. Cancer Biol Med 17: 599-611, 2020.
30. Sheng L, Cui X, Cheng L, Chen Y and Du X: Risk factors of grade ≥ 2 radiation pneumonitis after gemcitabine induction chemotherapy for patients with non-small cell lung cancer. Radiat Oncol 14: 229, 2019.
31. Yuan C, Pan Y and Ning Y: Predictive Value of IL-6 Combined with NLR in inflammation and cancer. Cancer Invest 39: 489-504, 2021.
32. Cheng H, Luo G, Lu Y, Jin K, Guo M, Xu J, Long J, Liu L, Yu X and Liu C: The combination of systemic inflammation-based marker NLR and circulating regulatory T cells predicts the prognosis of resectable pancreatic cancer patients. Pancreatology 16: 1080-1084, 2016.
33. Mundama M, Van Cauter M, Detrembleur C, Cornu O, Dubuc JE and Yombi JC: Neutrophil-to-lymphocyte ratio (NLR) distribution shows an advantage compared to C-reactive protein (CRP) for the early inflammation monitoring after total hip arthroplasty. Acta Orthop Belg 86: 405-411, 2020.
34. Huang Z, Fu Z, Huang W and Huang K: Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: A meta-analysis. Am J Emerg Med 38: 641-647, 2020.
35. Buonacera A, Stancanelli B, Colaci M and Malatino L: Neutrophil to lymphocyte ratio: An emerging marker of the relationships between the immune system and diseases. Int J Mol Sci 23: 3636, 2022.