

Tumor Immune-Infiltrate Landscape After Chemo-Radiotherapy in a Case Series of Patients with Non-small Cell Lung Cancer: Pretreatment Predictors and Correlation With Outcome

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Abstract

Introduction: Data on tumor immune-milieu after chemo-radiation (CT-RT) are scarce. Noninvasive tools are needed to improve the treatment of non-small cell lung cancer (NSCLC), especially in the locally advanced (LA) setting.

Methods: We collected a series of superior-sulcus (SS)- patients with NSCLC referred to our Institute (2015-2019), eligible for a preoperative CT-RT. We characterized tumor-infiltrating immune cells (TIICs), determined PD-L1-TPS and the residual viable tumor cells (RVTC). Radiological and metabolic responses were reviewed. We calculated pre-surgery neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR).

Results: Eight patients were included. Radiological responses were 6 disease stabilities (SD) and 2 partial responses (PR). Metabolic responses were 4 SD and 4 PR. CD68+TIICs were correlated with metabolic response and lower RVTC. CD68+TIICs were associated with higher PLR. Higher PLR values seemed linked with lower RVTC.

Conclusions: These preliminary results could be useful for consolidation treatment selection for patients with LA-NSCLC without evaluable baseline PD-L1 and higher PLR values.

Key words: locally advanced NSCLC; platelet-to-lymphocyte ratio; tumor immune microenvironment; chemo-radiotherapy; immunotherapy.

Introduction

Tumor immune-infiltrate characterization of non-small cell lung cancer (NSCLC) has risen the interest in the latest years, for its prognostic role in patients stratification and as a predictor of treatment outcome with immune-checkpoint inhibitors (ICIs).

ICIs have changed lung cancer treatment, first in the advanced/metastatic setting, more recently for the locally advanced (LA) unresectable stage, as described in the PACIFIC trial.¹

This trial brought to the fore the unresolved issue of predictive biomarkers in the decision-making of lung cancer care. In fact, in the PACIFIC trial, patients were enrolled regardless of tumor PD-L1 expression. European Medicines Agency (EMA) requested post hoc exploratory analysis of survival rates, using PD-L1 expression-level cutoff of 1%. Given the stronger benefit experienced by patients with

PD-L1 positive, EMA approved durvalumab after chemo-radiotherapy (CT-RT) only for this subset of patients,² firing several controversies. The analysis was not preplanned, therefore not powered enough to produce statistically strong results. PD-L1 expression was not available in over 37% of cases and patients with an unknown PD-L1 status also seem to benefit from durvalumab. Finally, CT-RT could enhance immunogenicity even in “immune-cold” tumors,^{3,4} leaving no biological basis to recommend durvalumab consolidation to selected cases. Theoretically, tumor re-biopsy during or after CT-RT might help redefine the sensitivity to ICI. However, a new biopsy is not always practically feasible, nor tolerable for patients.

Few data on immune-infiltrate and checkpoint expression after CT-RT are available. The primary aim of our exploratory analysis was to describe histological features of tumor specimens after CT-RT, to correlate them with the

response, and to find new tools for the prediction of tumor immune-milieu, which may overlook tissue re-biopsy in LA-NSCLC.

We decided to focus on Superior-Sulcus(SS)-NSCLCs: this way we had the chance to analyze samples of patients treated with radical-intent CT-RT,⁵ without the risk of tissue inequity, as it was obtained from surgical specimens.

Materials and Methods

We retrospectively collected a series of patients with SS-NSCLC referred to our Center from 2015 to 2019 and deemed eligible for a preoperative CT-RT. The backbone of chemotherapy was a carboplatin-based regimen plus a third-generation agent. Concurrent radiotherapy was administered 5 fractions per week, 200 cGy per fraction. Surgery was planned after 3 weeks since the conclusion of radiotherapy.

Pre- and post-treatment whole-body CT scans and FDG-PET-CT were performed. Radiologic and metabolic responses were evaluated according to RECIST 1.1 and EORTC criteria, respectively.

Tissue serial sections were stained using monoclonal antibodies anti-CD8, anti-CD3, anti-CD4, anti-CD68, and anti-PD-L1. Immunoreactivity for CD3-, CD4-, and CD8-lymphocytes, and for CD68-macrophages was expressed as a score 0-3.⁶ The rate of residual viable tumor cells (RVTC), fibrosis, and necrosis were also determined. The pathological response was assessed according to the IASLC recommendations.⁷

Finally, we calculated the pre-surgery neutrophil-to-lymphocyte ratio (NLR, absolute neutrophil count/absolute lymphocyte count) and platelet-to-lymphocyte ratio (PLR, platelet count/lymphocyte count), using pre-surgery blood counts data.

Statistical correlations and distributions among clinical and pathological variables were evaluated using Pearson's correlation coefficient (PCC), chi-square test, and Mann-Whitney test as appropriate. The statistical significance level was set at $P < .05$ for all tests.

Results

Eleven patients diagnosed with SS-NSCLC were treated with preoperative CT-RT followed by surgery. Three patients were not included, due to incomplete radiological data needed to perform the analyses, so a total of 8 cases were tested. Data about gender, age, smoking status, tumor biopsy, histology, pre-surgery median NLR and PLR, radiologic and metabolic responses are depicted in Table 1.

The inflammatory cell characterization was performed on surgical samples, as it was not representative of the overall microenvironment in the diagnostic biopsy samples. PD-L1 status could be assessed in 5 tumor biopsies: 2 were PD-L1 negative and 3 were PD-L1 positive. Three biopsy samples were insufficient for molecular testing. PD-L1 determination was possible only in 6 surgical samples and resulted negative in all evaluable cases.

Median RVTC value was 10% (0%-70%), with 4 patients achieving a major pathological response.⁷ Median fibrosis and necrosis rates reached 58% (20%-95%) and 20% (5%-50%), respectively.

Table 1. Patients' characteristics.

Sulcus superior patients with NSCLC		Nº of patients = 8	
Gender	Female	1 (12.5%)	
	Male	7 (87.5%)	
Age	Median (years, range)	60.5 (37.2-77.1)	
Smoking status	Former/current	8 (100.0%)	
Tumor biopsy route	CT-guided fine needle biopsy	6 (75.0%)	
	Bronchoscopy	2 (25.0%)	
Histology	Adenocarcinoma	6 (75.0%)	
	Squamous cell carcinoma	1 (12.5%)	
	NOS	1 (12.5%)	
Chemotherapy (carboplatin based)	8 cycles qw	6 (75.0%)	
	3 cycles q3w	2 (25.0%)	
Radiotherapy	Median (range; gray)	50 (44-64)	
Radiological response	Stable disease	6 (75.0%)	
	Partial response	2 (25.0%)	
Metabolic response	Stable disease	4 (50.0%)	
	Partial response	4 (50.0%)	
Pre-surgery NLR	Median (range)	3.5 (1.7-4.6)	
Pre-surgery PLR	Median (range)	199 (132-264)	
RVTC	<10%	4 (50.0%)	
	10%-20%	2 (25.0%)	
	20%-50%	0 (0.0%)	
	>50%	2 (25.0%)	
Tumor immune infiltrate	CD3+	1+	2 (25.0%)
		2+	4 (50.0%)
		3+	2 (25.0%)
	CD4+	1+	7 (87.5%)
		2+	1 (12.5%)
	CD8+	1+	1 (12.5%)
		2+	4 (50.0%)
		3+	3 (37.5%)
	CD68+	1+	1 (12.5%)
		2+	3 (37.5%)
		3+	4 (50.0%)
	Fibrosis	Median (% , range)	58 (20-95)
Necrosis	Median (% , range)	20 (5-50)	

NLR, neutrophil-to-lymphocyte ratio; NSCLC, non-small cell lung cancer; PLR, platelet-to-lymphocyte ratio; RVTC, residual viable tumor cells.

The presence of CD3-lymphocytes was associated with stronger CD8-infiltrate (PCC of 0.8, $P = .017$). No correlations were found among other subsets of immune infiltrate. A stronger presence of CD68-macrophages was linked to lower RVTC (PCC = -0.708, $P = .049$; Fig. 1A).

Immune infiltrate did not correlate with radiologic response to treatment. A correlation between CD68-infiltrate and better metabolic response was observed ($P = .025$, chi-square test) (Fig. 1B).

Pre-surgery NLR and PLR values were not linked to radiologic or metabolic responses. PLR values were associated both to stronger CD68-infiltrate (PCC = 0.8, $P = .016$; Supplementary Fig. 1A) and to lower RVTC values (PCC = -0.7, $P = .047$; Supplementary Fig. 1B).

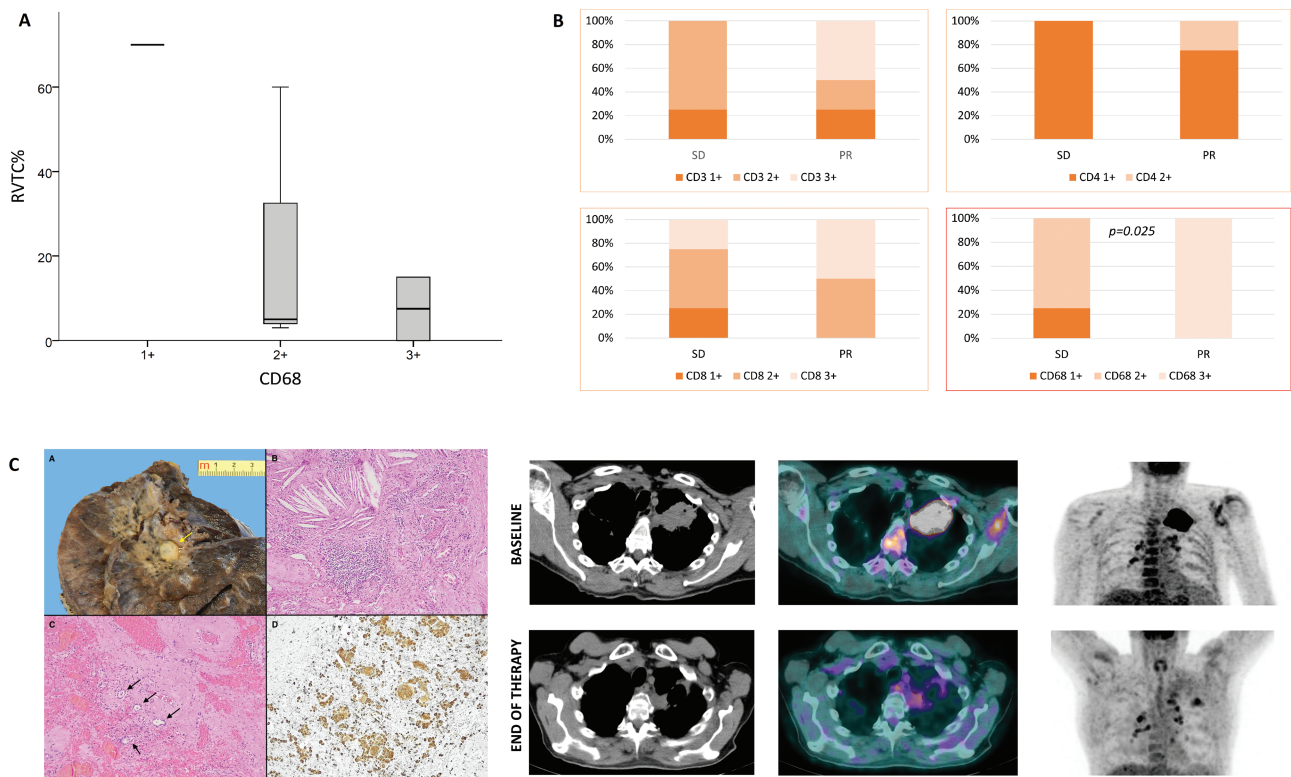


Figure 1. Correlation between stronger presence of CD68+ macrophages and lower RVTC values (A) and between CD68+ infiltrate and a superior metabolic response (B). Emblematic case of a patient with a superior sulcus adenocarcinoma showing strong CD68+ infiltrate, who achieved major pathological response and a partial metabolic response (C). A nodular necrotic area is evident (yellow arrow) (C.A). The tumor bed shows fibrosis and dense lymphoplasmacytic inflammatory infiltrate with cholesterol clefts (C.B; hematoxylin and eosin stain, original magnification $\times 50$). Fibrotic area with neovascularization in which some residual neoplastic glands (black arrows) are present (C.C; hematoxylin and eosin stain, original magnification $\times 50$). The CD68 immunoreaction highlights numerous macrophages infiltrating the tumor bed (C.D.; CD68 immunoperoxidase staining, original magnification $\times 100$). On the right, 18-FDG PET-CT scan images show the metabolic response after treatment.

Discussion

The diagnostic-therapeutic pathway of LA-NSCLC is complex and heterogeneous because of the different staging subgroups included and the need for a multidisciplinary treatment approach. Recently, survival of not resectable cases was improved by the advent of 12 months consolidation with durvalumab in responder patients after CT-RT. In this setting, durvalumab improved progression-free and overall survival (OS) rates, with an astonishing 60-month OS rate of 42.9% over a 33.4% for the placebo arm.¹

As previously mentioned, patients' selection for durvalumab treatment is currently based on pre-treatment evaluation of PD-L1 expression, which does not reflect the tumor-milieu after CT-RT.

Most patients with NSCLC are diagnosed using small biopsy specimens, often inadequate for a complete characterization.⁸ Moreover, concordance of PD-L1 positivity between biopsy and resected tumor specimens varied according to reports, due to spatial heterogeneity and differences in biopsy methods.⁹

Our case series confirmed these data, showing a considerable percentage of cases with NOS histology (12.5%) and without evaluable PD-L1 expression (>30%). Considering the inadequacy of most diagnostic samples for PD-L1 evaluation, it is tempting to speculate that a rebiopsy could be helpful. However, our analysis of surgical specimens showed low RVTC with about half of patients achieving a major pathological response, underlining the difficult feasibility and clinical application of a rebiopsy after CT-RT.

In our exploratory analysis, pre-surgery higher PLR levels seemed to predict lower RVTC and higher CD68-infiltrate after CT-RT.

Additionally, CD68-infiltrate, as described for other malignancies,¹⁰ was associated with a stronger pathological response and with a better metabolic response.

We acknowledge the limitations of our study. Pretreatment samples were not sufficient for a complete immune-infiltrate evaluation and a comparison with surgical specimens. Furthermore, it is a single-institutional, retrospective, and small-sized study; therefore correlations between histological and clinical parameters should be interpreted with caution. However, we think that these data, suggesting PLR as a potential circulating biomarker identifying best responder patients to CT-RT, deserve further prospective investigation in a wider patient population, to explore durvalumab consolidation benefit for those without evaluable baseline PD-L1 and higher PLR values.

Supplementary Material

Supplementary material is available at *The Oncologist* online.

Conflict of Interest

Valentina Guarneri: Novartis, Eli Lilly, MSD, Gilead (SAB), Eli Lilly, GSK, Novartis (H). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board.

Author Contributions

Conception/design: G.P., A.P., F.C., and L.E. Provision of study material or patients: F.C., L.E., F.L., F.P., and F.F. Data analysis and interpretation: G.P., A.P., and A.F. Manuscript writing: G.P., A.P., and A.F., F.C., V.G., and P.C. Final approval of manuscript: All authors.

Data Availability

The data underlying this article will be shared at reasonable request to the corresponding author.

References

1. Spigel DR, Faivre-Finn C, Gray JE, et al Five-year survival outcomes with durvalumab after chemoradiotherapy in unresectable stage III NSCLC: an update from the PACIFIC trial. *J Clin Oncol*. 2021;39:8511-8511.
2. Postmus PE, Kerr KM, Oudkerk M, et al Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2017;28(suppl_4):iv1-iv21. <https://doi.org/10.1093/annonc/mdx222>.
3. Marciscano AE, Haimovitz-Friedman A, Lee P, et al Immunomodulatory effects of stereotactic body radiation therapy: preclinical insights and clinical opportunities. *Int J Radiat Oncol Biol Phys*. 2021;110(1):35-52.
4. Zitvogel L, Kroemer G. Subversion of anticancer immunosurveillance by radiotherapy. *Nat Immunol*. 2015;16(10):1005-1007.
5. Favaretto A, Pasello G, Loreggian L, et al Preoperative concomitant chemo-radiotherapy in superior sulcus tumour: a mono-institutional experience. *Lung Cancer*. 2010;68(2):228-233.
6. Pasello G, Urso L, Mencoboni M, et al MDM2 and HIF1alpha expression levels in different histologic subtypes of malignant pleural mesothelioma: correlation with pathological and clinical data. *Oncotarget*. 2015;6(39):42053-42066.
7. Travis WD, Dacic S, Wistuba I, et al IASLC multidisciplinary recommendations for pathologic assessment of lung cancer resection specimens after neoadjuvant therapy. *J Thorac Oncol*. 2020;15(5):709-740.
8. Vanderlaan PA, Yamaguchi N, Folch E, et al Success and failure rates of tumor genotyping techniques in routine pathological samples with non-small-cell lung cancer. *Lung Cancer*. 2014;84(1):39-44.
9. Yoshimura K, Inoue Y, Karayama M, et al Heterogeneity analysis of PD-L1 expression and copy number status in EBUS-TBNA biopsy specimens of non-small cell lung cancer: Comparative assessment of primary and metastatic sites. *Lung Cancer* 2019;134:202-209. <https://doi.org/10.1016/j.lungcan.2019.06.002>.
10. McLemore LE, Janakiram M, Albanese J, et al An immunoscore using PD-L1, CD68, and tumor-infiltrating lymphocytes (TILs) to predict response to Neoadjuvant chemotherapy in invasive breast cancer. *Appl Immunohistochem Mol Morphol*. 2018;26(9):611-619.