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Prognostic value of neutrophil to lymphocyte ratio and lymphocyte counts before durvalumab consolidation after radio-chemotherapy in locally advanced non-small cell lung cancer

Arnaud Colomb[1*], Benoit Allignet[1,2], Mehdi Lamkhioued[3], Aurelie Swalduz[4], Lionel Falchero[5], Aurélie Kienlen[6], Michaël Duruisseaux[7,8,9] and Coralie Moncharmont[1]

Abstract

Background Durvalumab, an anti-PD-L1 immune checkpoint inhibitor, after radio-chemotherapy (RCT) has changed the management of locally advanced non-small cell lung cancer (LA NSCLC). A series of retrospective studies have investigated different cut-off of lymphocyte count (LyC) and neutrophil-to-lymphocyte ratio (NLR) to predict survival in LA NSCLC. None of these studies has validated their threshold in an independent group of patients. We wanted to assess the OS prognostic value of NLR and LyC in patients with LA NSCLC treated by RCT and durvalumab, with threshold determination and their validation in an external cohort.

Methods Patients were enrolled in four institutions between Oct. 2017 and Jan. 2022. Pre durvalumab LyC, neutrophils count (NC) and NLR were collected. To define NLR and LyC cut-off value predicting survival event, time dependent Receiver Operating Characteristics (ROC) curves was performed. Survival outcomes were estimated by the Kaplan-Meier method and differences were compared using univariate and multivariate Cox proportional hazard models.

Results We included 76 patients in the training set and 85 in the test set. The best cut off were 2,94 for NLR and 0,61 G/l for LyC to predict OS in the training set. For patients with NLR > 2,94, univariate analysis showed no significant deterioration in OS in either the training set ($p = 0{,}066$) or the test set ($p = 0{,}12$). Patients with LyC > 0,61 G/L, in univariate analysis, had longer OS in training set ($p = 0{,}030$) and in test set ($p = 0{,}0062$). This OS increase was not found in multivariate analysis ($p = 0{,}057$) in training set but was confirmed in test set (0,039).

Conclusion LyC > 0,61 G/l is associated with longer OS for LA NSCLC patient's treated with RCT and durvalumab in univariate analysis. In this context, a particular expectation for organs at risk sparing during RT to avoid lymphopenia seems important.

*Correspondence:
Arnaud Colomb
arnaud.colomb425@gmail.com

Full list of author information is available at the end of the article

Trial registration Retrospectively registered.

Keywords Locally advanced non-small cell lung cancer, Durvalumab consolidation, Radio-chemotherapy, Overall survival, Neutrophil to lymphocytes, Lymphocytes count

Background

With more than 1,7 million deaths per year worldwide, lung cancer remains the leading cause of cancer-related death [1]. Because the disease is long asymptomatic, most patients develop locally advanced or metastatic disease. Standard treatment of unresectable locally advanced non-small-cell lung cancer (LA NSCLC) has long remained the combination of radiotherapy and concomitant chemotherapy (RCT) [2]. Recently, the addition of durvalumab consolidation was reported to increase overall survival (OS) in this population [3]. Durvalumab is an anti-PD-L1 immune checkpoint inhibitor (ICI) acting on CD8+T lymphoctyes and stimulating anti-tumor immune response. The tumoral PD-L1 expression is known to influence treatment efficacy [4]. Nevertheless progress is still needed to predict patients' prognosis.

Tumor cells are known to promote inflammation of their microenvironment and to evade the immune system [5]. ICI combat tumor escape from the immune system. Neutrophil count (NC) and lymphocyte count (LyC) provide information on the patient's inflammatory and immune status. The neutrophil-to-lymphocyte ratio (NLR) combines these two components. Its prognostic value has been reported in several retrospective studies in various neoplasms [6, 7], in unresectable LA NSCLC prior to the immunotherapy (IO) era [8] and in metastatic NSCLC patients receiving nivolumab [9]. On the other hand, correlation between LyC, progression-free survival (PFS) and OS was reported in several retrospective studies in inoperable LA NSCLC [10–13]. However, the cut-offs were variable, evaluated by various methods, and none was validated on an external cohort.

We sought to retrospectively assess the prognostic value of NLR and LyC in LA NSCLC patients treated with durvalumab consolidation, with threshold determination and their validation in an external cohort.

Method

Study design and patients

We retrospectively and systematically collected data of LA NSCLC patients treated with RCT and at least one infusion of consolidation durvalumab between October 2017 and January 2022 of four French institutions. Chemotherapy could be delivered concomitantly or sequentially. To reflect real world practice, all these patients were included without exclusion factor. To be eligible for durvalumab, patients must have at least stable disease after RCT. Two cohorts were defined to separately establish (training set) and validate (test set) the NLR and LyC thresholds. The training set included patients treated in the Leon Berard comprehensive cancer center (CLB) and the General Hospital of Villefranche-Sur-Saône. The test set included patients treated in Lyon University Hospital cancer center and in Saint-Pierre University Hospital.

Radiotherapy

A planning CT-scanner was performed in the treatment position with head shoulder mask for apical lung tumor. 4D CT-scan image acquisition was performed to delineate an internal target tumor volume to passively manage tumor motion. Normofractionated RT delivered a dose of 60 to 70 Gy (Gy), five daily fractions per week on primary tumor and invaded lymph nodes. Dose was prescribed to the 95% isodose of planning target volume (PTV). Few patients could receive stereotactic body radiotherapy (SBRT) on the primary tumor and normofractionated RT on lymph nodes. These patients were not excluded. Image guided RT was performed with daily tomographic image.

Systemic treatments

Chemotherapy was platinum-based and associated with gemcitabine, vinorelbine, taxane or pemetrexed. It was performed concurrently with RT whenever safely possible. In case of gemcitabine doublet or in patients with poor general condition, chemotherapy and RT were delivered sequentially.

Intravenous durvalumab dose was either 10 mg/kg every two weeks or 1500 mg every month, for 12 months, except in cases of severe toxicity or tumoral progression.

Monitoring

Clinical and biological evaluation were performed at each durvalumab infusion. Cerebral, thoracic and abdomino-pelvic computed tomography (CT) were performed every 2 months during the durvalumab consolidation. Patients could also be evaluated with cerebral magnetic resonance imaging (MRI) or 18-fluorodeoxyglucose positron-emission tomography (PET/CT). After treatment, clinical and imaging evaluation were performed every 3 months for 2 years, and every 6 months afterwards.

NLR and lymphocyte count

The NLR is defined as the ratio of NC (G/L) to LyC (G/L). Blood sample results were recorded on the blood test after the end of RT and before the first infusion of durvalumab.

Statistical analyses

Categorical variables were reported as counts (percentages) and continuous variables as median (interquartile range), respectively. We dichotomized pre-durvalumab NLR to make the results more understandable and clinically useful. To define NLR and LyC cut-off value predicting survival event, time dependent Receiver Operating Characteristics (ROC) curves was performed. The NLR and LyC selected threshold were the values that maximized the sum of the sensitivity and specificity of OS event. OS was defined as the time from last day of radiotherapy until death from any cause. PFS was defined as the time from last day of radiotherapy until local or distant relapse according to imaging follow-up. The data cut-off time was December 2023. Median follow-up was calculated using the reverse Kaplan Meier method. Survival outcomes were estimated by the Kaplan-Meier method from the first cycle of durvalumab and differences were compared using univariate and multivariate Cox proportional hazard models. Univariate analysis tested Age > 70, female gender, histology, BMI < 18 and >/= 30, PS 0–1 vs. 2, PDLI status, tumoral stage, NLR and LyC. Variables with a univariate $p < 0.05$ were included in the multivariate model, with adjustment systematically planned for the age, PS and histology. OS event was death of any cause, and PFS event was local, local regional or distant relapse, or death from any cause.

We evaluated the predictive value of NLR and LyC, of various categorical (histology, expression of PD-L1, tobacco, and tumor stage) and quantitative variables (BMI) using Fisher's exact test and Pearson's correlation test, respectively. Two-sided p-value < 0,05 was considered significant. Statistical analyses were performed using R software version 4.2.3 (R Foundation for Statistical Computing, Vienna, Austria).

Ethics

The study protocol was reviewed and approved by the Institutional Review Board of CLB and was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The requirement for written consent was waived and processing of personal data was performed according to French reference methodology n°004 of the Informatic and Liberties National Commission.

Results

Patients

Between October 2017 and January 2022, 76 and 85 LA NSCLC patients who received durvalumab were included in the training and test sets, respectively (Table 1). Median age was 62 and 65 years, respectively, and 74% of patients were male. Most patients were active or wean smokers, and presented adenocarcinoma (55,2% and 44,7% in training and test set, respectively) and stage IIIB disease (51,3% and 56,4%). In training set PD-L1 positivity was mainly upper than 49% while the PD-L1 status was mainly between 1 and 49% in the test set. The mean time between the last RT session and the first course of durvalumab was 29 days (3–67) and 35 days (3–88). Many adenocarcinomas (61,9% and 47,3%) presented with a driver mutation, including 4,8% and 7,9% of EGFR mutation.

In training set, 8 patients (10,5%) received 50 to 54 Gy in 3 to 5 fractions using an exclusive stereotactic technique on the tumor volume and normofractionated radiotherapy (RT) on the lymph node volume. In test set, 21 patients (24,4%) received 70 Gy on tumor and lymph node volumes. The most common dose received was 66 Gy (60,5% and 68,6%).

The median NLR was nearby between training set (3,87 [2,36−5,61]) and test set (4,1 [2,76−5,41]), as was the median LyC (0,850 [0,613-1,10] and 0,885 [0,590-1,09]).

Median (interquartile) follow up was 35,39 (19,97, 49,23) months in training set and 35,74 (24,47, 45,87) months in test set.

In overall population, median OS was 59,4 months (95% CI: 42,4-NR) (Fig. 1A) and median PFS was 32,6 months (95%CI: 20-NR) (Fig. 1B).

Cut off determination

For OS, with an area under the curve (AUC) of 0,603 (95%CI 0,4674-0,7385) the best NLR cut off was 2,94 (appendix A. 1). For Lyc the more significant cut off was 0,61 G/L with an AUC of 0,582 (95%IC 0,4366-0,727) (appendix A. 2).

Overall survival according to NLR

In training set, in univariate analysis, patients with NLR > 2,94 did not present a worse OS (median 36,7 months vs. not reached (NR); HR: 2,42, 95% CI: 0,94−6,2, $p = 0,066$) (Fig. 2A, Table 2A). In the test set, NLR > 2,94 was also not associated with a significant reduction in OS (HR: 1,86, 95% CI: 0,77 ; 3,76, $p = 0,12$) (Fig. 2B, Table 2C).

Overall survival according to lymphocytes count

In training set, in univariate analysis, patients with LyC > 0,61 G/L had better OS (median NR vs. 28 months; HR: 0,39, 95% CI: 0,17, 0,91, $p = 0,030$) (Fig. 3A, Table 2A). In multivariate analysis, this OS improvement for patients with LyC > 0,61 G/L was not found (HR: 0,41, 95% CI: 0,17, 1,00, $p = 0,057$) while PS 2 was significantly associated with shorter OS (HR: 4,02, IC95%: 1,60, 10,1, $p = 0,002$) (Table 2B). In test set patients with LyC > 0,61 G/L had higher OS in univariate (HR: 0,68, IC95% : 0,18 ; 0,78, $p = 0,0062$) (Fig. 3B, Table 2C) and multivariate analysis (HR: 0,46, IC95%: 0,22, 0,96, $p = 0,039$) (Table 2D).

Table 1 Baseline clinicopathologic characteristics

Variables	Training set	Test set
Gender		
Male	56 (73,6%)	63 (74,1%)
Female	20 (26,3%)	22 (25,8%)
Age		
Median (Q1-Q3)	62,0 (58,3–67,0)	65,0 (57,0–70,0)
BMI		
Median (Q1-Q3)	24,0 (20,3–27,3)	24,0 (22,0–27,8)
Smoker		
No	3 (3,9%)	2 (2,3%)
Wean	47 (61,8%)	33 (38,8%)
Active	26 (34,2%)	26 (30,5%)
Missing	0 (0%)	24 (28,2%)
Performans status (PS)		
0	42 (55%)	35 (41,2%)
1	30 (39,4%)	46 (54,1%)
2	4 (5,2%)	4 (4,7%)
Radiation dose		
60 Gy	30 (39,5%)	5 (5,8%)
66 Gy	46 (60,5%)	59 (68,6%)
70 Gy	0 (0%)	21 (24,4%)
Of which SBRT on primary tumor[1]	8 (10,5%)	0 (0%)
Chemotherapy		
Paclitaxel	18 (23,6%)	29 (34,1%)
Pemetrexed	20 (26,3%)	9 (10,5%)
Vinorelbine	36 (47,3%)	47 (55,2%)
Gemcitabine	2 (2,6%)	0 (0%)
Sequential	2 (2,6%)	0 (0%)
Concomitant	71 (93,4%)	66 (77,6%)
Missing	3 (3,9%)	19 (22,3%)
Time between last radiotherapy session and first DURVALUMAB treatment		
Mean (days)	29 (3–67)	35 (3–88)
Stage		
< 3	2 (2,6%)	0 (0%)
3 A	28 (36,7%)	22 (25,8%)
3B	39 (51,3%)	48 (56,4%)
3 C	7 (9,2%)	15 (17,6%)
Histology		
Adenocarcinoma	42 (55,2%)	38 (44,7%)
Squamous cell carcinoma	26 (34,2%)	36 (42,3%)
Sarcomatoid	2 (2,6%)	1 (1,2%)
Undifferenciated	6 (7,9%)	10 (11,7%)
PDL1		
< 1%	13 (17,1%)	16 (18,8%)
1–49%	28 (36,7%)	39 (45,8%)
>=50%	31 (40,7%)	27 (31,7%)
Missing	4 (5,2%)	3 (3,5%)
ALK/ROS1 mutation (adenocarcinoma only)		
ALK	1 (1,3%)	1 (1,2%)
ROS1	1 (1,3%)	1 (1,2%)
Oncogenic addiction (adenocarcinoma only)		
No mutation	16 (38,1%)	18 (47,3%)
Mutation	26 (61,9%)	18 (47,3%)
Missing	0 (0%)	2 (5,3%)

Table 1 (continued)

Variables	Training set	Test set
Of which EGFR mutation	2 (4,8%)	3 (7,9%)
Neutrophil count (G/L) on assessment biology before first Durvalumab infusion		
Median (Q1-Q3)	3,05 (2,17 – 4,20)	3,42 (2,44 – 4,66)
Lymphocytes count (G/L) on assessment biology before first Durvalumab infusion		
Median (Q1-Q3)	0,850 (0,613-1,10)	0,885 (0,590-1,09)
NLR on assessment biology before first Durvalumab infusion		
Median (Q1-Q3)	3,87 (2,36 – 5,61)	4,10 (2,76 – 5,41)

[1]stereotactic body radiation therapy

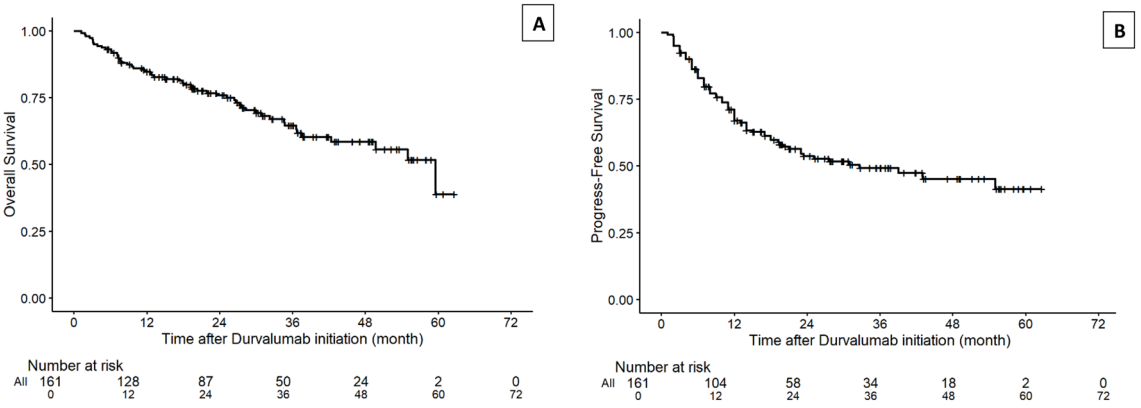

Fig. 1 Survival of the entire population. (**A**) OS. (**B**) PFS

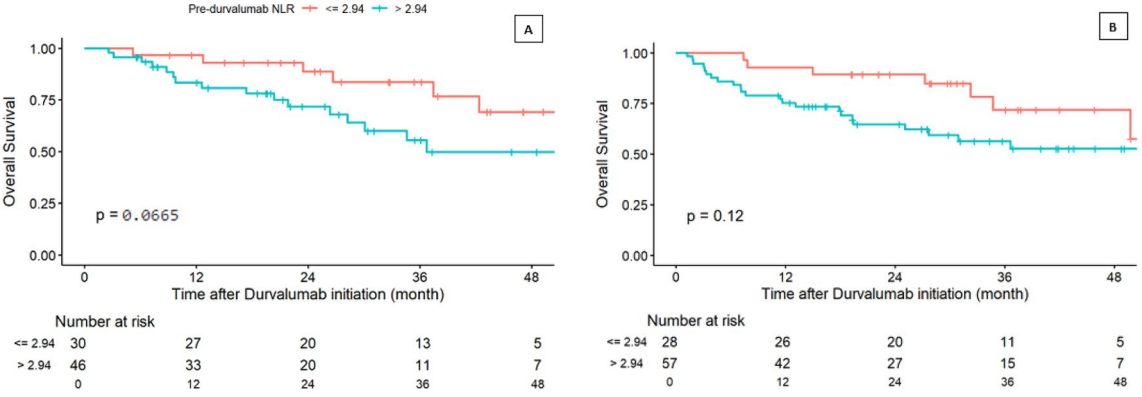

Fig. 2 OS according to pre durvalumab NLR. (**A**) Training set. (**B**) Test set

Progression free survival according to NLR

In training set, patients with NLR>2,94 did not have shorter PFS (HR=1,68, p=0,15) with median of 18 months (95% CI: 12, NR) vs. not reached (95% CI: 23, NR) (Fig. 4A).

The same results was observed in test set (HR: 1,25, p=0,51) with a median PFS of 25,1 months (95% CI: 17, NR) vs. 39 months (95% CI: 19, NR), (Fig. 4B).

Progression free survival according to lymphocytes count and type of recurrence

In training set, patients with LyC>0,61 G/L did not have longer PFS (HR 0,66, p=0,28) with a median PFS NR (95% CI: 18, NR) vs. 18 months (95% CI: 8, NR), (Fig. 5A).

In test set patients with LyC>0,61 had better PFS (HR: 0,5 p=0,042), with a median PFS of 43 months (95% CI: 25,NR) vs. 12 months (95% CI: 10, NR), (Fig. 5B).

In training set, 21 patients (27,6%) had local relapse, mainly in lymph nodes. Twenty-two patients (28,9%) had distant metastasis, 11 extracranial (14,5%), 13 intracranial (17,1%) including 2 (2,6%) patients with synchrone intra and extracranial recurrence (appendix B). In comparison in test set, 43 patients (50,5%) had local relapse, mainly in lymph nodes. Thirty-one (36,4%) had distant metastasis, 17 extracranial (20%), 19 intracranial (22,4%) including 2 (2.4%) patients with synchrone intra and extracranial recurrence (appendix B).

Table 2 Prognostic factors for OS

A. Univariate analysis in training set			
Characteristics	**HR[1]**	**95%CI[2]**	***p*-value**
NLR (2,94 ; 35,1)	2,42	0,94 ; 6,20	0,066
LyC (0,61 ; 3,89)	0,39	0,17 ; 0,91	0,030
Age > 70	1,85	0,62 ; 5,54	0,3
Female gender	1,15	0,49 ; 2,72	0,8
Non adenocarcinoma	1,76	0,76 ; 4,08	0,2
BMI < 18	2,07	0,46 ; 9,29	0,3
BMI >/= 30	0,48	0,11 ; 2,07	0,3
PS 0–1	ref	ref	ref
PS 2	4,60	1,88 ; 11,3	< 0,001
PDL1 >/= 50%	ref	ref	ref
PDL1 1–49%	1,02	0,38 ; 2,75	0,9
PDL1 < 1%	2,55	0,89 ; 7,34	0,082
Stage 3 A	ref	ref	ref
Stage 3B	1,79	0,74 ; 4,36	0,2
Stage 3 C	0,84	0,18 ; 3,98	0,8
B. Multivariate analysis in training set with LyC			
Characteristics	**HR**	**95%CI**	**p-value**
LyC (0,61 ; 3,89)	0,41	0,17 ; 1,00	0,057
Age > 70	2,11	0,67 ; 6,71	0,2
Non adenocarcinoma	1,12	0,47 ; 2,64	0,8
PS 2	4,02	1,60 ; 10,1	0,002
C. Univariate analysis in test set			
Characteristics	**HR[1]**	**95%CI[2]**	**p-value**
NLR (2,94 ; 25,5)	1,86	0,77 ; 3,76	0,12
LyC (0,61 ; 3,21)	0,68	0,18 ; 0,78	0,0062
Age > 70	1,70	0,80 ; 3,50	0,2
Female gender	0,97	0,43 ; 2,17	0,9
Non adenocarcinoma	1,73	0,81 ; 3,68	0,2
BMI >/= 30	1,02	0,35 ; 2,92	0,9
PS 0–1	ref	ref	ref
PS 2	2,49	1,12 ; 5,55	< 0,025
PDL1 >/= 50%	ref	ref	ref
PDL1 1–49%	1,44	0,62 ; 3,30	0,4
PDL1 < 1%	0,85	0,26 ; 2,78	0,8
Stage 3 A	ref	ref	ref
Stage 3B	0,89	0,38 ; 2,08	0,8
Stage 3 C	1,50	0,54 ; 4,15	0,4
D. Multivariate analysis in test set with LyC			
Characteristics	**HR**	**95%CI**	**p-value**
LyC (0,61 ; 3,89)	0,46	0,22 ; 0;96	0,039
Age > 70	1,23	0,56 ; 2,68	0,6
Non adenocarcinoma	1,51	0,70 ; 3,27	0,3
PS 2	2,18	0,94 ; 5,02	0,068

[1]Hazard ratio

[2]95% confidence interval

Discussion

Summary of results

With median OS of 59,4 against 47,5 months and median PFS of 32,6 against 16,9 months, global survival in our entire population is higher than that of the Pacific cohort [4].

Patients with exon 21 L858R mutation or exon 19 deletion of the deletion of exon 19 of the EGFR gene accounted for 4,8% and 7,9% in training and test set compared with 12% in the French thoracic interactive group [14]. Since the results of the LAURA study were

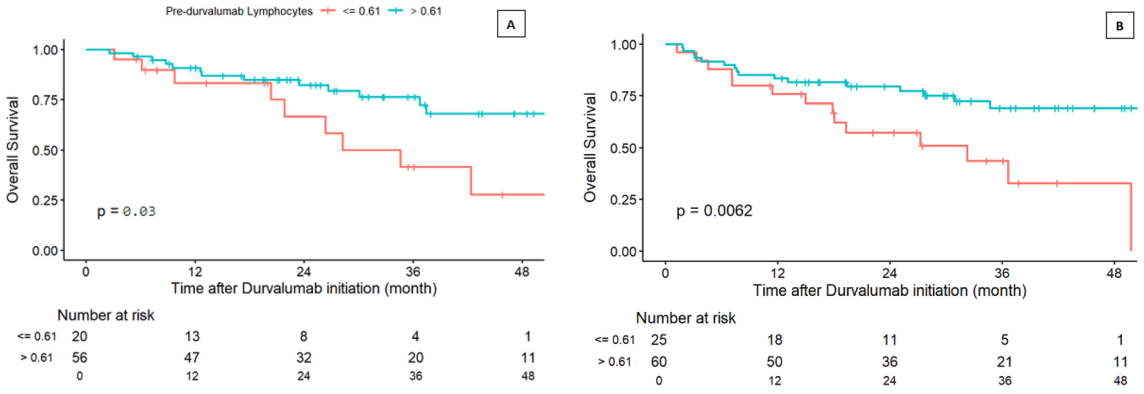

Fig. 3 OS according to pre durvalumab lymphocytes count (G/L). (**A**) Training set. (**B**) Test set

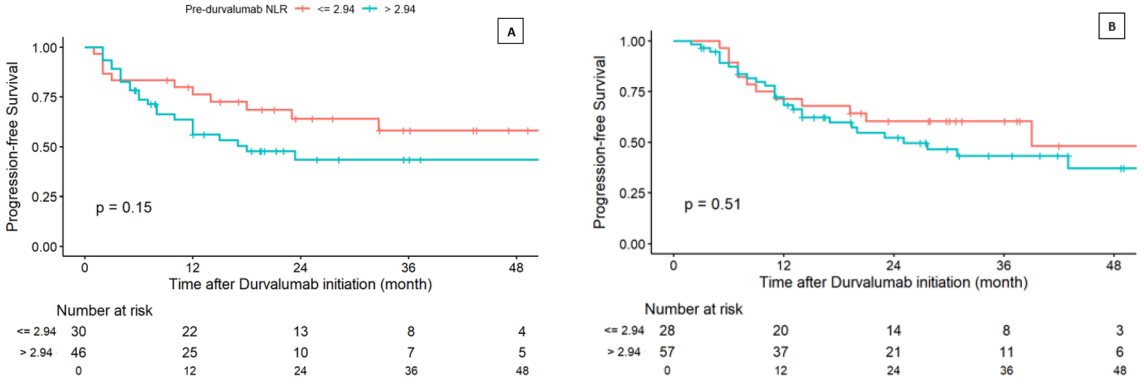

Fig. 4 PFS according to pre durvalumab NLR. (**A**) Training set. (**B**) Test set

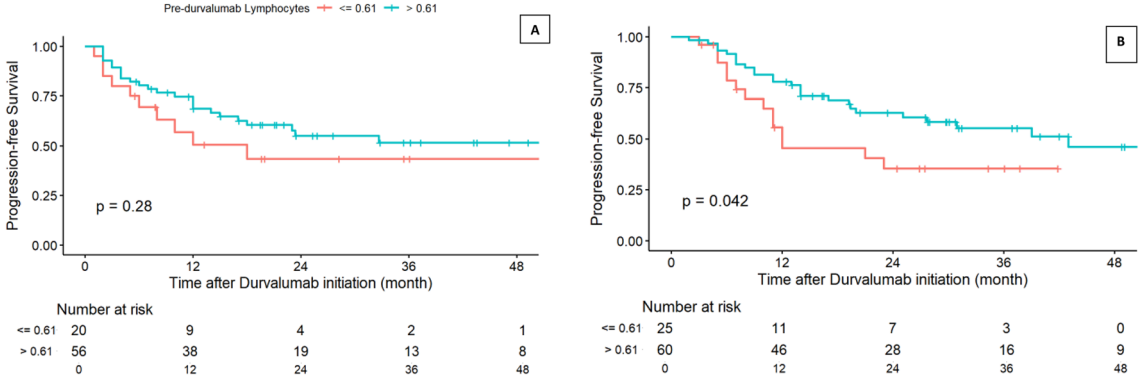

Fig. 5 PFS according to pre durvalumab lymphocytes count (G/L). (**A**) Training set. (**B**) Test set

published, these patients will receive post-RCT maintenance with osimertinib rather than durvalumab [15].

The literature contains a few retrospective single-center studies describing the impact of NLR and lymphopenia on survival in patients treated for LA NSCLC with RCT and durvalumab consolidation. In this context, we report results defined by a robust methodological approach in two independent multicenter cohorts. We determined and validated a LyC cut-off associated with OS in univariate analysis. Multivariate model failed to identify this association. To the best of our knowledge, our work is the first to validate training set results in an independent test set for association between LyC and OS in univariate analysis in this population.

A high NLR was not associated to shorter OS in both training and test set. This suggests that the prognostic impact of NLR described in the literature is more related to lymphopenia than to neutrophil increase.

Indeed, lymphopenia is a sign of the patient's immune deficiency and we know that immunosuppressed patients are more likely to develop neoplasia. For example, HIV patients have a higher incidence of lung cancer [16].

Moroever, immunodeficient transplant patients have a 3-fold increased risk of developing cancer [17]. Patients with pre-transplant malignancy have higher cancer-specific mortality [18].

Comparison to literature for LyC cut off in the same population before and after appearance of durvalumab

Other retrospective studies corroborate the negative impact of lymphopenia on outcomes for LA NSCLC patients treated with RCT and durvalumab.

In a single-center study [12] patients in the upper tertile for baseline NLR and NC and in the lower tertile for LyC had poorer PFS. This association was not found before initiation of durvalumab. In this study, median LyC at baseline and before ICI was 1,6 G/L and 0,7 G/L, respectively, illustrating the lymphopenic impact of concomitant RCT [12]. Our data show a similar 50% decrease in LyC after RCT (data not shown). Jing et al. also reported a shorter PFS and OS in lymphopenic patients before and after durvalumab appearance. In their cohort, among patients with radiation induce lymphopenia (RIL), durvalumab did not improve OS [13]. Another single-center study [10] on 78 patients reported that grade 3 lymphopenia prior to ICI initiation was associated with PFS reduction. After RCT, the median LyC decreased from 1,52 G/L to 0,72 G/L, and 23% of patients developed grade 3 lymphopenia. Our LyC threshold is close of the grade 3 lymphopenia used by Friedes et al. [10]. In comparison, 13% and 14% of patients presented grade 3 RIL in our training set and our test set (data not shown).

Cho et al. reported a correlation between recovery from lymphopenia 3 months after RCT on PFS and OS. In their population, 33% of patients had received ICI during RCT and 6% pembrolizumab maintenance [11].

Moreover, in a retrospective cohort of patients treated with RCT for esophageal cancer, those with grade 3 or 4 lymphopenia and low lymphocyte recorvery had lower OS and PFS [19].

Furthermore, it should be noted that prior to PACIFIC publication, retrospective studies had already identified a relationship between NLR, LyC, PFS and OS in this population [20–22]).

Factors influencing RCT-induced lymphopenia

Dosimetric variables and clinical practice influencing them

In this context, limiting RIL seems important. Abravan et al. reported results in 901 localized NSCLC and SCLC patients, and 305 localized esophageal cancer patients. OS was shorter in lung cancer patients with grade 3 lymphopenia during RT, and in esophageal cancer patients with grade 4 lymphopenia. Lymphopenia was significantly associated with higher mean lung dose, mean heart dose and volume of thoracic vertebrae receiving 20 Gy (V20Gy). They confirmed these results on the independent esophageal cohort [23]. Also in a cohort of patients treated with RCT for esophageal cancer, bone marrow V5Gy was associated with a higher risk of insufficient lymphocyte recovery [19]. In a single center cohort of 372 stage II-III NSCLC patients, Li et al. identified a beneficial prognostic impact of nadir NLR < 1,525 on PFS and OS. They identified aortic V10Gy and the addition of chemotherapy before or concomitantly with RT as factors associated with a higher NLR [24]. In a retrospective cohort treated in the 2000s, Tang et al. found a correlation between lung V5Gy and V10Gy and LyC nadir. This correlation was not found for higher doses. The addition of concomitant CT also correlated with a lower nadir LyC. Patients with a nadir LyC > 0,51 G/L had better OS [25]. Before the durvalumab era, Contreras et al. showed an association between an increase in NLR at 4 months after RCT and heart V50Gy > 25%. OS was independently negatively associated with both increased NLR and heart V50Gy > 25% [26].

The estimated dose of radiation to immune cells (EDRIC) model combines some of these dosimetric parameters, taking into account the mean dose to the heart (MHD), lungs (MLD) and whole body (MBD).

Thor et al. reported a correlation between EDRIC and nadir LyC, and with LyC at the start of immunotherapy [12]. Friedes et al. studied the EDRIC model in a cohort of patients treated for some by proton therapy and not all having received ICI consolidation. A EDRIC value > 4,7 Gy was associated with shorter OS and PFS. Predictive factors for EDRIC > 4,7 Gy were photon (vs. proton) therapy, lower lobe tumor localization, N3 disease, primary tumor and nodes volumes. Interestingly the predictive value of a high EDRIC was increased in patients receiving consolidation ICI [27]. To contrast, this prognostic value of EDRIC was not confirmed by Thor et al. [12].

In clinical practice some attitudes are likely to limit the RIL. Limiting irradiation to invaded lymph nodes seems to correlate with a reduction in the EDRIC model. Pasquier et al. showed a correlation between the inclusion of prophylactic mediastino-hilar lymph nodes, EDRIC and RIL, with a negative impact on PFS [28]. In addition, a deleterious impact of prophylactic mediastino-hilar lymph nodes irradiation on two years OS has been demonstrated in a phase 3 trial with a 3D conformational RT (3D-RT) technique [29]. The use of intensity-modulated RT largely lower the dose to non-involved lymph nodes (NILNs) compared to 3D-RT (23,2 Gy vs. 40 Gy) [28, 30]. Thanks to the Bragg peak phenomenon, protons deliver a highly focused dose and thus allow a better organs at risk (OAR) protection [27]. With this technique, the volume of bone marrow receiving more than 10 Gy can be reduced by more than 25% [31]. In esophageal cancer, proton therapy is associated with a significant reduction

in the risk of grade 4 lymphopenia [32]. A Phase 3 trial is currently ongoing to investigate whether these dosimetric gains observed with protons translate into improved PFS and OS in inoperable stage II-IIIB NSCLC patients [33].

Duration of radiotherapy

Moreover the EDRIC model takes into account the number of fractions, which, when high, exposes more lymphocytes to a lethal dose. Indeed T and B lymphocytes show significant DNA fragmentation after exposure to 1 to 5 Gy [34]. In the human body, cardiac output is around 5 L per minute [35]. This means that during a thoracic VMAT session, all circulating lymphocytes pass through the irradiation field several times. The more sessions, the greater the number of grays received by circulating lymphocytes. This suggests that, in current practice, hypofractionation and SBRT could reduce lymphocyte irradiation. In the future, FLASH-RT, which uses a high dose rate (40 Gy/s for FLASH-RT vs. 0,01 Gy/s in conventional RT), will enable extremely rapid irradiation and may enable better sparing of circulating lymphocytes [36].

Radiation induce stimulation of the immune response

On the other hand, if RT have lymphopenic properties, part of its action can be explained by strong immunogenic phenomena. RT stimulates multiple immune response pathways, including the promotion of tumor cell phagocytosis [37]. A large part of its immuno-mediated action relies on enhancing the anti-tumor response of cytotoxic T lymphocytes. Indeed, RT enhances exposure to tumor antigen and dendritic cell activation [38]. This increases T-cell-mediated tumor death [38, 39]. Furthermore, RT-induced interferon release represents an important cytokine pathway of immune activation that sustains the anti-tumor lymphocyte response [40]. Up regulation of Fas and TNFalpha apoptosis pathway receptors increases tumor susceptibility to T-cell-mediated cytolysis [41]. In breast cancer, radiation-induced CXCL16 release by tumoral cells attracts effector T cells [42].

Also RT action on endothelial environment increases the concentration of tumor infiltrating lymphocytes (TILs) [40, 43].

Thus, the immunogenic effects of RT relying on T lymphocytes are likely to play a crucial role in the efficacy of durvalumab [3].

These mechanisms of action merit further study, with potential combinations of immunotherapy and RT synergistically stimulating other pathway of the antitumor immune response.

LyC is an imperfect marker of lymphocyte anti-tumor response

Moreover, LyC is a simple marker that does not sum up patient's entire immune capacity. Anti-tumor effect of durvalumab is based on CD8+T lymphocytes interaction with tumor cells. An increase of TiLs was associated with longer OS and PFS in a prospective cohort of NSCLC patients treated with RCT [44]. Thus most of CD8+T lymphocytes are outside the bloodstream in the case of LA NSCLC cancer. Therefore this lymphocyte population is only partially represented by LyC. Furthermore, LyC also contains other lymphocytes such as CD4+T cells.

Strengths and limits of the study

The retrospective format of the study does not allow standardization of patient management. Variations in measurements between laboratories could limit the comparability of LyC and NC between patients.

Patients were included in 4 centers, of which 2 were university hospitals, one general hospital and including one oversea center, ensuring a representative sample of patients.

The choice to use an independent test set instead of carrying out the tests on a single cohort reduces the power of our sample, which may explain the non-significance of the results in multivariate analyses. However, this enabled us to verify the reproducibility of our cut-off. The association between LyC and OS is still significant between the two cohorts in univariate analysis.

Our work is the first to define and validate an association of an LyC cut off and OS with a training set and a test set in this population.

As our objective was clinical, we opted for an extensive multicenter data collection. Thus, we did not study dosimetric data. We did not assess the impact of CT type on LyC. This could be the subject of future studies.

Conclusion

LyC>0,61 G/l is associated with longer OS for LA NSCLC patient's treated with RCT and durvalumab in univariate analysis. In this context, a particular expectation for OAR sparing during RT to avoid lymphopenia seems important.

Abbreviations

EDRIC	Estimated dose of radiation to immune cells
Gy	Gray
LA NSCLC	Locally advanced non-small-cell lung cancer
LyC	Lymphocyte count
NC	Neutrophil count
NLR	NC/LyC ratio
OS	Overall survival
OAR	Organs at risk
RCT	Radio-chemotherapy
RIL	Radiation-induced lymphopenia
RT	Radiotherapy
PFS	Progression-free survival

TILs	Tumor-infiltrating lymphocytes
VMAT	Volumetric intensity modulation by arc therapy
V(n)Gy	Volume of a structure receiving (n) grays

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions
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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study protocol was reviewed and approved by the Institutional Review Board of centre Léon Bérard and was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The requirement for written consent was waived and processing of personal data was performed according to French reference methodology n°004 of the Informatic and Liberties National Commission.

Consent for publication
The requirement for written consent was waived and processing of personal data was performed according to French reference methodology n°004 of the Informatic and Liberties National Commission.

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author details
[1]Department of Radiation Oncology, Centre Léon Bérard, 28 rue Laennec, Lyon 69673, France
[2]Univ Lyon, INSA-Lyon, Université Claude Bernard Lyon 1, UJM-Saint Etienne, CNRS, Inserm, CREATIS UMR 5220, Lyon U1294, France
[3]Pneumology Departement, Centre Hospitalo-Universitaire de Lyon sud, Hospices Civils de Lyon, Lyon, France
[4]Pneumology Departement, Centre Léon Bérard, 28 rue Laennec, Lyon 69673, France
[5]Pneumology Departement, Centre Hospitalier de Villefranche de Rouergue, Villefranche, France
[6]Pneumology Departement, CHU de Saint-Pierre, Saint-Pierre, France
[7]Respiratory Department and Early Phase, Louis Pradel Hospital, Hospices Civils de Lyon Cancer Institute, Lyon, France
[8]Oncopharmacology Laboratory, Cancer Research Center of Lyon, Unité mixte de recherche (UMR) Institut national de la santé et de la recherche médicale (INSERM) 1052 Centre national de la recherche scientifique (CNRS) 5286, Lyon, France
[9]Université Claude Bernard, Université de Lyon, Lyon, France

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