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ORIGINAL ARTICLE

Pretreatment neutrophil-lymphocyte ratio is not a significant prognostic factor in epidermal growth factor receptor-mutant non-small cell lung cancer patients treated with tyrosine kinase inhibitors

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Abstract

Background: The neutrophil-lymphocyte ratio (NLR) is a marker of poor prognosis in lung cancer patients. However, previous data have been based on an heterogeneous population of lung cancer patients and various treatments. In this study, we evaluate the prognostic value of NLR in an homogeneous population of epidermal growth factor receptor (EGFR)-mutant non-small cell lung cancer (NSCLC) patients.

Methods: We restrospectively evaluated the data of 250 NSCLC patients with EGFR mutations. All data are based on first-line treatment.

Results: All tumors harbored in-frame deletions in exon 19 or an L858R point mutation. Eighty-five patients were treated with tyrosine kinase inhibitors (TKIs), while 165 received cytotoxic chemotherapy as first-line treatment. Multivariate survival analysis revealed that the NLR was a significant prognostic factor for first-line progression-free survival (PFS) in the chemotherapy group (hazard ratio [HR] 1.882, 95% confidence interval [CI] 1.319–2.686, $P = 0.001$), but was not significant in the TKI group (HR 1.239, 95% CI 0.693–2.215, $P = 0.469$). The response rate (RR) to first-line treatment was 76.5% in the TKI group and 29.5% in the chemotherapy group; however, the RR, according to the NLR (≤3 vs. > 3), was the same for both groups.

Conclusions: The NLR was a significant prognostic factor in the chemotherapy group, but it did not affect either RR or PFS in EGFR-mutant NSCLC patients treated with TKIs.

Introduction

Inflammation and cancer are related diseases; inflammation can not only promote carcinogenesis but tumor cells can also stimulate systemic inflammation, which is reflected in changes in various inflammatory markers.[1,2] However, most inflammatory markers are not routinely assessed before cancer treatments.[3–5]

For clinical use, prognostic factors should be simple, inexpensive, and reproducible.[5] One attractive marker of cancer-related inflammation is the neutrophil-lymphocyte ratio (NLR), which is readily available in complete blood cell (CBC) counts and is associated with disease prognosis in various types of cancer.[6–8]

However, the utility of this marker in epidermal growth factor receptor (EGFR)-mutant non-small cell lung cancer (NSCLC) is not known. Recent discovery of EGFR mutation and use of tyrosine kinase inhibitors (TKIs) has substantially improved survival outcome.[9,10] However, it is not known whether the NLR can reflect disease prognosis. Herein, we investigated whether the NLR is a prognostic factor of progression-free survival (PFS) in EGFR-mutant NSCLC patients treated with first-line cytotoxic chemotherapy or TKI.

Methods

Patients and study design

We retrospectively analyzed the EGFR-TKI registry database of the Seoul National University Hospital (Seoul, Korea). Patients who met the following inclusion criteria were enrolled in the study: (i) pathological diagnosis of NSCLC between January 2005 and December 2011; (ii) presence of a gefitinib-sensitive EGFR mutation, specifically, either an in-frame deletion in exon 19 (del19) or an L858R point mutation in exon 21; (iii) clinical stage IIIB or IV disease, as determined by imaging; and (iv) a CBC count within 31 days of commencing first-line treatment. The exclusion criteria were: presence of an active infection, or treatment with steroids or antimicrobial drugs before the CBC count was performed. Objective tumor responses to chemotherapy were assessed by Response Evaluation Criteria in Solid Tumors (version 1.0).[11]

This study was approved by the institutional review board of the Seoul National University Hospital and was performed in accordance with the Declaration of Helsinki.

Statistical analyses

Categorical variables were analyzed using Pearson's $\chi 2$ or Fisher's exact tests. Survival analyses were performed using the Kaplan–Meier method or Cox proportional hazard model. All groups were compared with the log-rank test. Receiver operating characteristic curve analysis was performed to determine the NLR cut-off, which produced the best sensitivity and specificity for median PFS. Specifically, NLRs >3.0 were considered to be high, while those ≤3.0 were considered to be low.

Univariate and multivariate analyses were performed using the Kaplan–Meier method and Cox regression. A two-sided *P* value <0.05 was considered statistically significant.

Results

Patients

We identified 270 patients in the NSCLC database who had EGFR mutations and received TKIs. Of these, 20 were excluded (7 received steroids or antimicrobials before treatment and 13 did not have a CBC count within 31 days of the first treatment), which left 250 patients in the study (Fig 1). Patients' median age was 64 years (range: 31–85). Most patients had lung adenocarcinoma tumors (91.6%). Eastern Cooperative Oncology Group performance status (PS) was 0 or 1 in 214 patients (85.6%) and ≥2 in 36 patients (1.4%). First-line treatment was cytotoxic chemotherapy in 165 patients (66%) and TKIs in 85 patients (34%). In the first-line cytotoxic chemotherapy group, 155 out of 165 patients

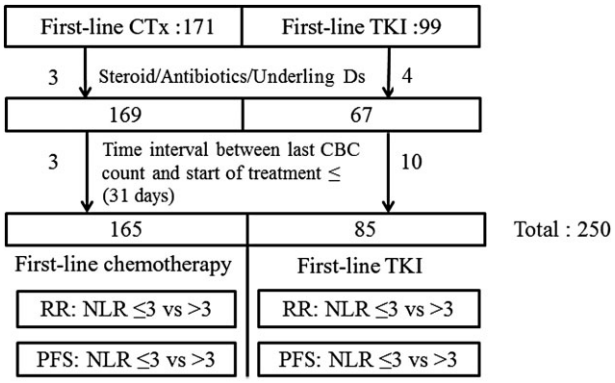

Figure 1 Patient number and analysis flow. CBC, complete blood cell; NLR, neutrophil-lymphocyte ratio; PFS, progression-free survival; RR, response rate; TKI, tyrosine kinase inhibitors.

(93.9%) received EGFR-TKI treatment as second-line treatment, while the remaining patients received EGFR-TKI as third-line or further. In the first-line EGFR-TKI group, 23 out of 33 patients (69%) who were eligible for subsequent treatment received cytotoxic chemotherapy as second-line treatment. Age, gender, PS, and the number of metastatic sites differed significantly between the cytotoxic chemotherapy and TKI groups ($P = 0.004$, 0.044, 0.001, and 0.030, respectively) (Tables 1, S3).

Relationship between the neutrophil-lymphocyte ratio and response rate

Complete blood cell counts and differential count tests were performed for routine pretreatment assessment (Table S1). The median time between the CBC count and the first treatment was seven days.

The response rates (RRs) were 29.5% (95% confidence interval [CI] 22.9–37.1) in the cytotoxic chemotherapy group and 76.5% (95% CI 66.2–84.4) in the TKI group (Table S2). Thirteen patients were not included as a result of loss to follow-up or early withdrawal from treatment. There was no difference in RR between high and low NLR patients in both the cytotoxic chemotherapy and TKI groups ($P = 0.595$ and 0.173, respectively) (Table 2).

Factors associated with progression-free survival

Median PFS was 4.9 months (95% CI 4.266–5.534) in the cytotoxic chemotherapy group and 10.8 months (95% CI 9.167–12.433) in the TKI group (Fig 2).

In the cytotoxic chemotherapy group, univariate analysis showed that poor PS, high NLR, and large numbers of metastatic sites were significantly associated with reduced PFS ($P =$

Table 1 Patient characteristics

Variable		Chemotherapy group (n = 165)	TKI group (n = 85)	P value	Total (n = 250)
Age	Median (range)	62 (31–82)	67 (46–85)	0.004	63.6 (31–85)
Gender	Male	73	26	0.044	99 (39.6%)
	Female	92	59		151 (60.4%)
Disease status	Recurred disease	36	20	0.805	56 (22.4%)
	Initial metastatic disease	129	65		194 (77.6%)
Smoking History	Smoker	53	21	0.247	74 (29.6%)
	Never-smoker	112	64		176 (70.4%)
Histologic type	Adenocarcinoma	150	79	0.856	229 (91.6%)
	SqCC	2	1		3 (1.2%)
	NSCLC, not subtyped	13	5		18 (7.2%)
ECOG PS	0, 1	155	59	0.001	214 (85.6%)
	≥2	10	26		36 (1.4.4%)
EGFR mutation type	Del19	101	52	0.988	153 (61.2%)
	L858R	64	33		97 (38.8%)
Number of metastatic sites	1	42	29	0.030	71 (28.4%)
	2	46	31		77 (30.8%)
	≥3	77	25		102 (40.8%)
First-line treatment regimen	Platinum based combination	157	0	NA	157 (62.8%)
	Gemcitabine single	7	0		7 (2.8%)
	Vinorelbine single	1	0		1 (0.4%)
	TKI	0	85		85 (34.0%)
TKI treatment line	First-line	–	85	NA	85 (34.0%)
	Second-line	152	–		152 (60.8%)
	Third-line or further	13	–		13 (5.2%)

ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; PS, performance status; SqCC, squamous cell carcinoma; TKI, tyrosine kinase inhibitor.

0.004, 0.001, and 0.021, respectively; Table 3). However, in the TKI group, a large number of metastatic sites was the only statistically significant association with reduced PFS (P = 0.002). Multivariate analysis revealed that the NLR was significantly associated with PFS in the cytotoxic chemotherapy group (P < 0.001, HR 1.882, 95% CI 1.319–2.686), but not in the TKI group (P = 0.469, HR 1.239, 95% CI 0.693–2.215; Table 4). Age and PS were also significantly associated with PFS in the cytotoxic chemotherapy group (P = 0.038 and 0.008, respectively), but the number of metastatic sites at baseline was the only significant association with PFS in the TKI group (P = 0.003).

Discussion

Our results showed that the NLR had different effects on PFS, according to treatment type. The NLR was a significant prognostic factor in the chemotherapy group, but in EGFR-mutant NSCLC patients treated with TKIs, PFS was not affected. As for treatment response, the NLR did not affect the RR of cytotoxic chemotherapy or TKI treatment in EGFR-mutant NSCLC patients.

Cancer-induced inflammation can elevate the NLR, because both neutrophilia and relative lymphocytopenia occur in various tumors.[12] Several studies have suggested that increased cytokine levels may be responsible for changes in the NLR.[12–15] For example, increases in interleukin 7 (IL-7) may increase innate anti-tumor immunity, whereas increases in IL-17 or IL-4 may be associated with tumor-induced immune suppression.[14,15]

Although the underlying causes of changes in the NLR are not well defined, several studies agree that a high NLR (defined as NLR >3–5) is associated with poor PFS or overall survival (OS) in adjuvant treatment or palliative care

Table 2 Response rate of first-line treatment according to (NLR)

First-line treatment	Response rate (%) NLR ≤ 3	NLR > 3	Total	P value
Cytotoxic chemotherapy	30/101 (29.7)	16/55 (29.1)	46/156 (29.5)	0.595
EGFR-TKI	46/57 (80.7)	16/24 (66.6)	62/81 (76.5)	0.173
Total	76/158 (48.1)	32/79 (40.5)	108/237 (45.6)	

EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; NLR, neutrophil-lymphocyte ratio.

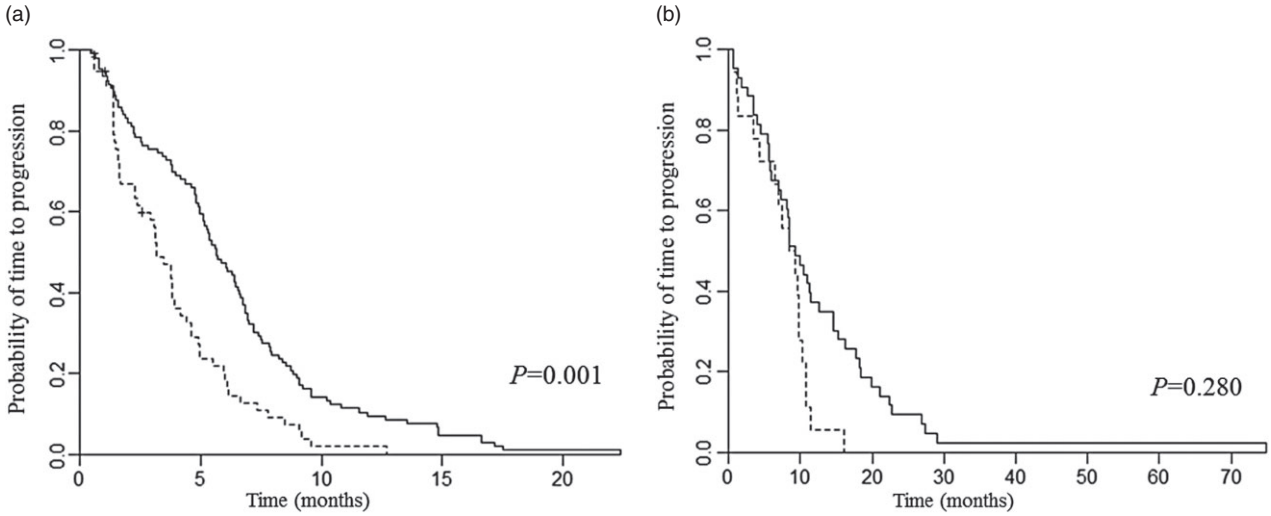

Figure 2 Kaplan–Meier plot for progression-free survival (PFS) according to neutrophil-lymphocyte ratio (NLR). A high NLR showed poor PFS in (**a**) the cytotoxic chemotherapy group but (**b**) did not in the TKI group. ——, NLR ≤ 3.0; ----, NLR > 3.0.

settings.[7,12] However, it is difficult to compare our findings because most previous studies on the prognostic utility of the NLR in NSCLC did not consider EGFR mutation status or treatment with TKIs. This study evaluated the prognostic utility of the NLR in a relatively homogeneous group of NSCLC patients who all had EGFR mutations, unlike previous studies.[16,17]

In patients who received first-line cytotoxic chemotherapy, the NLR was a significant prognostic factor for PFS, consistent with the results of previous reports.[7,18] Although a direct comparison was not possible because of the lack of an EGFR mutation negative study population, this result may suggest that the effect of NLR may have a consistent effect on PFS, regardless of EGFR mutation status, in patients treated with cytotoxic chemotherapy.

There have been few studies investigating the utility of the NLR in NSCLC patients with EGFR mutations treated with TKIs. The post-hoc analysis of the First-SIGNAL study showed that the difference between pretreatment and post-treatment NLRs is a significant prognostic factor, but that pretreatment NLR alone was not significantly associated with either RR or survival.[17] Another study also

Table 3 Univariate analysis of PFS in chemotherapy and TKI treatment groups

		Cytotoxic chemotherapy group				TKI treatment group			
Variable		*n*	HR	95% CI	*P* value	*n*	HR	95% CI	*P* value
Age at diagnosis	≤65	97	0.721	0.530–1.008	0.056	38	0.871	0.523–1.449	0.595
	≥65	68				47			
Gender	M	73	0.863	0.631–1.181	0.358	26	0.681	0.402–1.153	0.152
	F	92				59			
Pathology	Adenocarcinoma	150	1.096	0.640–1.875	0.738	79	1.317	0.521–3.331	0.560
	SqCC or NSCLC not-subtyped	15				6			
ECOG PS	0, 1	155	2.573	1.347–4.917	0.004	59	1.469	0.835–2.584	0.182
	≥2	10				26			
Smoking	Smoker	53	0.989	0.708–1.384	0.950	21	0.737	0.424–1.282	0.280
	Never-smoker	112				64			
Initial disease presentation	Metastatic	129	0.724	0.498–1.051	0.089	65	0.581	0.322–1.049	0.072
	Recurred	36				20			
NLR	≤3	107	2.037	1.454–2.854	0.001	58	1.363	0.777–2.393	0.280
	>3	58				27			
Initial no. of metastatic sites	1,2	88	1.457	1.059–2.003	0.021	60	2.406	1.389–4.166	0.002
	≥3	77				25			

CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; NLR, neutrophil-lymphocyte ratio; NSCLC, non-small cell lung cancer; PS, performance status; SqCC, squamous cell carcinoma; TKI, tyrosine kinase inhibitor.

Table 4 Multivariate analysis of PFS according to first line treatment

		Chemotherapy			TKI		
Variables		HR	95% CI	*P* value	HR	95% CI	*P* value
Age	≤ 65 years	1	0.511–0.981	0.038	1	0.533–1.532	0.707
	>65 years	0.708			0.904		
ECOG PS	0, 1	1	1.276–4.946	0.008	1	0.662–2.157	0.555
	≥2	2.513			1.195		
No. of metastatic sites before treatment	1, 2	1	0.847–1.678	0.313	1	1.319–4.022	0.003
	≥3	1.192			2.303		
Initial disease presentation	Metastatic	1	0.593–1.278	0.480	1	0.346–1.152	0.134
	Recurred	0.871			0.631		
NLR	<3	1	1.319–2.686	<0.001	1	0.693–2.215	0.469
	≥3	1.882			1.239		

CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; NLR, neutrophil-lymphocyte ratio; PS, performance status; TKI, tyrosine kinase inhibitor.

showed that a pretreatment NLR is not associated with TKI RR.[16]

In our study, the pretreatment NLR was not related to the RR of first-line treatment nor was it a prognostic factor for PFS in the TKI group.

This suggests that immune status affects the PFS of NSCLC patients treated with TKIs less than those who are treated with cytotoxic chemotherapy as first-line therapy. Because NSCLC tumors with activating EGFR mutations are dependent on EGFR signal transduction, inhibition of EGFR signaling pathways could counteract immune suppressive effects in the tumor microenvironment.

This study has several limitations. First, because of the retrospective nature of this study, pretreatment CBC counts were not performed at the same time in all patients; however, this might reflect actual clinical settings. Second, post-treatment blood cell counts were not analyzed. However, the post-treatment blood cell count can be affected by various situations, such as hidden infection or treatment-induced hematologic toxicity. Therefore, it may not be a robust factor reflecting survival outcomes. Third, we did not investigate the association between the pretreatment NLR and OS. However, all patients received TKI and more than half of the patients received third-line or further chemotherapy. Subsequent treatments can affect OS, which makes analysis difficult.

Recently, the results of a programmed death-1 (PD-1) or programmed death ligand-1 (PD-L1) antibody agent study shed light on immune treatment for lung cancer.[19] The immunologic process involved in cancer development and progress is gaining attention. In terms of TKI treatment, our study suggested that pretreatment immune status may not affect TKI treatment outcome. The survival outcome of a current trial involving a combination of TKIs and an immune checkpoint agent should soon be available.

In conclusion, our results demonstrated that the NLR was a significant prognostic factor in the chemotherapy group, but did not affect either RR or PFS in EGFR-mutant NSCLC patients treated with TKIs. These findings suggest that pretreatment immune status may not affect the outcome of TKI therapy; however, further research on the role of the NLR in NSCLC patients with EGFR mutations is needed.

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Disclosure

No authors report any conflict of interest.

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Supporting information

Additional Supporting Information can be found in the online version of this article at the publisher's website:

Table S1 Complete blood cell (CBC) count profile according to treatment type.

Table S2 Response rate of first-line treatment.

Table S3 Platinum-based chemotherapy regimen in first-line cytotoxic chemotherapy group.

