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ORIGINAL RESEARCH

Elevated Pretreatment Fibrinogen-to-Lymphocyte Percentage Ratio Predict Tumor Staging and Poor Survival in Non-Small Cell Lung Cancer Patients with Chemotherapy or Surgery Combined with Chemotherapy

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Purpose: The objective of our study was to assess the association between lymphocyte percentage (LY%), fibrinogen (FIB), fibrinogen-to-lymphocyte percentage ratio (FLR) and the tumor staging and the clinical outcome role in non-small cell lung cancer (NSCLC) patients with chemotherapy or surgery combined with chemotherapy.

Patients and Methods: Between August 2013 and October 2020, 375 patients initially diagnosed with NSCLC and 201 healthy subjects were enrolled in the retrospective study. The concentrations of LY%, FIB, and FLR were compared between the case group and the control group by using the Mann–Whitney *U*-test or Kruskal–Wallis test, and then these biomarkers were compared in terms of the tumor category and PTNM stage of the test group, etc. The cutoffs of LY%, FIB, and FLR were determined using X-tile software. The prognostic roles of LY%, FIB, and FLR were identified by the Kaplan–Meier curve and Cox regression model. The biological markers on overall survival (OS) were analyzed.

Results: The study showed that the concentration levels of LY%, FIB, and FLR in the stage III–IV group were significant difference from those in the stage I–II group ($P<0.001$), indicating that three biomarkers (LY%, FIB, and FLR) were significantly correlated with tumor staging. Pretreatment high FIB and FLR and low LY% indicated an increased risk of death in NSCLC patients. Also, it was found that the clinical outcome of low FLR patients with chemotherapy or chemotherapy combined with surgery was superior to high FLR patients.

Conclusion: Our findings demonstrated that FLR could be used to predict NSCLC staging and was an independent prognosis factor within NSCLC patients receiving chemotherapy or chemotherapy combined with surgery.

Keywords: fibrinogen-to-lymphocyte percentage ratio, lymphocyte percentage, fibrinogen, non-small-cell lung cancer, overall survival

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Introduction

Lung cancer is one of the most common malignant tumors and poses a great threat to human health.[1] It is worth noting that non-small cell lung cancer (NSCLC) accounts for up to 80% of lung cancer. Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) are common types of NSCLC.[2,3] In recent years,

despite improved treatment options, the five-year survival rate remains about 19%.[4] Currently, TNM staging system (Tumor- node- metastasis system), tissue typing, and genetic markers are commonly used to predict prognosis.[5] However, the prognosis of NSCLC is influenced by various factors such as blood clotting status and immune system status, and the high cost and time-consuming of genetic testing limit the clinical application. Therefore, an economical, simple, and efficient biomarker is needed to accurately predict the prognosis of the NSCLC.

TNM staging is widely used to evaluate the prognosis of NSCLC, mainly to interpret imaging results and describe tumors from a macro perspective. However, because it ignores the influence of factors such as systemic inflammation and hypercoagulability in vivo,[6–8] its application in clinical practice is limited. Cruise et al[9] revealed that the occurrence of malignant tumors, including NSCLC, was closely related to systemic inflammation, which was involved in the resistance of chemotherapy drugs and the initiation of tumor metastasis. Through a variety of ways, tumor cells can activate systemic coagulation, and cause abnormal hemostasis and fibrinolysis, thereby resulting in cancer angiogenesis and metastasis.[10] Circulating neutrophils, lymphocytes, and inflammatory proteins have been found to be candidate biomarkers for evaluating disease prognosis in chronic inflammatory states.[11–13] However, conflicting results of monocytes/lymphocytes (MLR), platelets/lymphocytes (PLR), circulating neutrophils/lymphocytes (NLR) and prognosis of lung cancer have been reported.[14–16] Hou et al[17] indicated that fibrinogen (FIB) and D-dimer levels could be used as candidate biomarkers for the prognosis of NSCLC. It can be inferred that the relationship between FIB and LY% can be used to determine the prognosis of NSCLC. However, few studies have investigated the relationship between the FIB-LY% ratio (FLR) and clinical outcomes in NSCLC. Therefore, the present study hypothesized that FLR is a potent biomarker for predicting NSCLC survival.

The occurrence of malignant tumors is often accompanied by the imbalance of the coagulation system and anticoagulation system and systemic inflammation.[9,18] FIB reflects the degree of hypercoagulability in the body, LY % reflects the degree of inflammation in the body, and they can be detected in blood samples. FLR is defined as the ratio of FIB to LY%. In this study, we evaluated the predict tumor staging and the prognostic roles of LY%, FIB, and FLR in NSCLC patients, and analyzed the effect of FLR on overall survival (OS).

Materials and Methods

Study Population

This study was approved by the First Affiliated Hospital of Nanchang University (Jiangxi Province, China). Patients with histologically or cytologically confirmed NSCLC were enrolled for screening. We collected 375 eligible NSCLC patients from the First Affiliated Hospital of Nanchang University (Jiangxi Province, China) between August 2013 and October 2020. The process of data collection was non-selective and continuous. In addition, the control group comprised 201 healthy volunteers.

Data Collection and Laboratory Detection

The TNM system of the 8th version of the International Association for Lung Cancer Research (IASLC) on lung Cancer was used for newly diagnosing and classifying. The clinical data collected included sex, age, smoking history, education experience, patients' physical status, pathologic types, lymph node metastasis, organ metastasis, etc. It should be noted that smokers were defined as individuals who have smoked continuously or cumulatively for six months or more, or individuals who quit smoking within six months before the diagnosis. Patients who smoked no more than 100 cigarettes in their lifetime were regarded as non-smokers. The patient's physical condition was scored using the performance status of the Eastern Cooperative Oncology Group (ECOG-PS).

In addition, the population collected in this study met the following inclusion and exclusion criteria. The patients had no history of chemotherapy, radiotherapy, or other treatment. Patients with abnormal liver function, tuberculosis, inflammation-related diseases, autoimmune diseases, other malignancies, secondary lung cancer, or patients without complete clinic pathological data were excluded.

Before any treatment, 2 mL pretreated circulating blood and plasma samples were collected from 6:30 a.m. to 8:30 a.m. to detect LY%, plasma FIB on Sysmex XE-2100 machine (Sysmex, Tokyo, Japan) and Sysmex CS5100 machine (Sysmex, Tokyo, Japan), respectively, within two hours. The process was strictly in accordance with the instructions of the kit.

Follow-Up Procedure

OS was defined as the time from the date of diagnosis to death or the deadline. The 3 years' OS was the determined endpoint in our study, which was defined as the time from the first treatment to death or the deadline. The second author was in charge of the following work by telephone to record the survival data. All enrolled patients were followed up until death or 31 January, 2021. Three hundred and seventy-five patients were followed up, but follow-up data were available for only 209 patients, and the remaining patients were loss of contact.

Statistical Analysis

The statistics were analyzed using SPSS software 25.0 (SPSS Inc, Chicago, IL, USA), the graphs were showed by using Graph Pad Prism 8.0. Continuous variables with normal distribution were presented as the mean ±SD. Continuous variables with non-normal distribution were performed as the median (IQR), and categorical variables were expressed as frequencies or percentages. Chi-square test, Mann–Whitney *U*-test, Kruskal–Wallis test, Student's *t*-test, and analysis of variance (ANOVA) were selected to compare the differences in qualitative and continuous variables, respectively. The survival of the best cut-off point for each candidate biomarker was predicted using X-tile software. Univariate and multivariate risk regressions were performed using Cox proportional-hazards regression, and the strength between them was measured using hazard ratios (HR) and 95% confidence intervals (CI). Cumulative OS was estimated using Kaplan–Meier curves and compared using Log rank tests. Also, $P<0.05$ (bilateral) indicated statistical significance.

Results

Study Population's Characteristics

Ultimately, 375 NSCLC patients (243 males and 132 females, aged 59.38±7.75 years) and 201 control subjects (126 males and 59 females, aged 60.62±7.87 years) were enrolled in our research. Baseline characteristics of the total subjects and subgroups were performed in Table 1. When NSCLC patients were compared with control subjects, significant differences were found in distributions of tobacco history and education experience; while age and gender were demonstrated little significance ($P>0.05$). The circulating median concentrations of LY%, FIB, and FLR were 32.90, 2.60 g/L, and 7.89, respectively, in the control subjects, and 21.30, 3.41 g/L, and 16.34, respectively, in the NSCLC patients. Significant differences in LY%, FIB, and FLR were observed in the two subgroups.

The TNM stages in the NSCLC group included 77 cases in stage I, 104 cases in stage II, 90 cases in stage III, and 104 cases in stage IV. There were 342 cases with ECOG equal to zero and 33 cases with ECOG greater than zero. About half of the patients (52.3%) received chemotherapy without surgery regimen and 47.2% patients received surgical with adjuvant chemotherapy resection. All these NSCLC patients were histologically diagnosed as LUSC in 177 cases (47.2%) and LUAD in 198 cases (52.8%). Among the lymph metastatic patients, 141 and 234 patients showed no lymph node metastasis and one or more lymph nodes metastasized, respectively. The number of the organ metastatic patients was 104, with 73 cases (70.2%) one organ metastatic and 31 cases two or more organs metastatic (29.8%).

Comparison of the Relationship Between LY%, FIB, and FLR Levels with Tumor Staging

To analyze the differential value of LY%, FIB, and FLR in tumor staging, patients were stratified according to the TNM stage, and the results were showed in Table 1 and Figure 1.

The circulating median concentrations of LY%, FIB, and FLR in the stage II patients were 25.20 (22.03, 29.45), 2.82 (2.48, 3.41), and 11.06 (8.89,14.25), respectively. For the stage III patients, the circulating median concentrations of LY%, FIB, and FLR were 15.45 (11.76, 19.75), 3.89 (3.19, 4.93), and 24.85 (19.07, 37.81), respectively. The circulating median concentrations of LY%, FIB, and FLR were not statistically different between the stage I and II patients, as well as the situation between the stage III and IV patients. It was worth noting that compared with the stage I and II patients, LY% of stage III and IV patients were noticeably reduced, while FIB and FLR dramatically increased. It indicated a significant association between circulating LY%, FIB, FLR and TNM stage (Figure 1A–C). In particular, it had good reliability in distinguishing between the stage II and III patients. So we combined stage I and stage II to form a new group stage I–II, and combined stage III and stage IV to form the new group stage III–IV (Table 1). The circulating median concentrations of LY%, FIB, and FLR were statistically different between the two new groups (Figure 1D–F). The three biomarkers (LY%, FIB, and FLR) were related to tumor staging.

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Table 1 Clinical and Pathological Characteristics in 375 Eligible NSCLC Patients and 201 Control Subjects

		Total Subjects (n = 576) No. of Subjects (%)								
					NSCLC (n = 375)					
Variables	**Categories**	**Control Subjects**	**NSCLC Patients**	***P***	**Stage I**	**Stage II**	**Stage III**	**Stage IV**	**Stage I–II**	**Stage III–IV**
Number		201	375		77	104	90	104	181	194
Gender										
	Male	126 (62.7%)	243 (64.8%)	0.61	40 (51.9%)	64 (61.5%)	70 (77.8%)	69 (66.3%)	104 (57.5%)	139 (71.6%)
	Female	59 (37.3%)	132 (35.2%)		37 (48.1%)	40 (38.5%)	20 (22.2%)	35 (33.7%)	77 (42.5%)	55 (28.4%)
Age	Year	60.62±7.87	59.38±7.75	0.077	57.81±6.15	59.04±7.50	60.54±7.83	59.89±8.68	58.51±6.97	60.20±8.28
Tobacco										
	Never	140 (69.6%)	191 (50.9%)	<0.001	44 (57.1%)	56 (53.8%)	33 (36.7%)	58 (55.8%)	100 (55.2%)	91 (46.9%)
	Former	15 (7.5%)	19 (5.10%)		1 (1.3%)	8 (7.7%)	5 (5.6%)	5 (4.8%)	9 (5.0%)	10 (5.2%)
	Current	46 (22.9%)	165 (44.0%)		32 (41.6%)	40 (38.5%)	52 (57.8%)	41 (39.4%)	72 (39.8%)	93 (47.9%)
Education										
	≤12 years/completed high school	123 (61.2%)	343 (91.5%)	<0.001	71 (92.2%)	97 (88.5%)	80 (88.9%)	95 (91.3%)	168 (92.8%)	175 (90.2%)
	>12 years	78 (38.8%)	32 (8.5%)		6 (7.8%)	7 (15.5%)	10 (11.1%)	9 (8.7%)	13 (7.2%)	19 (9.8%)
ECOG										
	0	N/A	342 (91.2%)		73 (94.8%)	99 (95.2%)	82 (91.1%)	88 (84.6%)	172 (95.0%)	170 (87.6%)
	>0	N/A	33 (8.8%)		4 (5.2%)	5 (4.8%)	8 (8.9%)	16 (15.4%)	9 (5.0%)	24 (12.4%)
Therapy										
	SC	N/A	179 (47.7%)		63 (81.8%)	73 (70.2%)	39 (43.3%)	4 (3.8%)	136 (75.1%)	43 (22.2%)
	C	N/A	196 (52.3%)		14 (18.2%)	31 (29.8%)	51 (56.7%)	100 (96.2%)	45 (24.9%)	151 (77.8%)
Tumor types										
	LUSC	N/A	177 (47.2%)		34 (44.2%)	49 (47.1%)	61 (67.8%)	33 (31.7%)	83 (45.9%)	94 (48.5%)

	LUAD	N/A	198 (52.8%)		43 (55.8%)	55 (52.9%)	29 (32.2%)	71 (68.3%)	98 (54.1%)	100 (51.5%)
Lymph node										
	N0	N/A	141 (35.0%)		76 (98.7%)	54 (51.9%)	5 (5.6%)	6 (5.8%)	130 (71.8%)	11 (5.7%)
	N1–N3	N/A	234 (65.0%)		1 (1.3%)	50 (48.1%)	85 (94.4%)	98 (94.2%)	51 (28.2%)	183 (94.3%)
Metastasis										
	One organ	N/A	73 (70.2%)		N/A	N/A	N/A	73 (70.2%)	N/A	73 (37.6%)
	Two organs or more organs	N/A	31 (29.8%)		N/A	N/A	N/A	31 (29.8%)	N/A	31 (16.0%)
LY% Median (IQR)	%	32.90 (28.75, 37.90)	21.30 (14.40, 27.00)	<0.001	27.50 (23.25, 31.25)	25.20 (22.03, 29.45)	15.45 (11.76, 19.75)	15.15 (9.78, 19.63)	25.90 (22.80, 30.05)	15.35 (10.80, 19.70)
FIB Median (IQR)	g/L	2.60 (2.31, 2.97)	3.41 (2.77, 4.32)	<0.001	2.86 (2.49, 3.25)	2.82 (2.48, 3.41)	3.89 (3.19, 4.93)	4.23 (3.59, 4.97)	2.85 (2.49, 3.33)	4.11 (3.50, 4.97)
FLR Median (IQR)	%	7.89 (6.61, 9.30)	16.34 (10.53, 29.61)	<0.001	10.54 (8.20, 13.11)	11.06 (8.89, 14.25)	24.85 (19.07, 37.81)	29.74 (19.91, 45.27)	10.78 (8.71, 13.79)	27.80 (19.62, 42.13)

Abbreviations: N/A, not available; ECOG score, eastern cooperative oncology group score; FIB, fibrinogen; LY%, lymphocyte percentage; FLR, fibrinogenlactate -to- lymphocyte percentage ratio; IQR, interquartile range; SC, surgical resection with adjuvant chemotherapy; C, chemotherapy without surgery; LUSC, squamous cell lung cancer; LUSD, lung adenocarcinoma.

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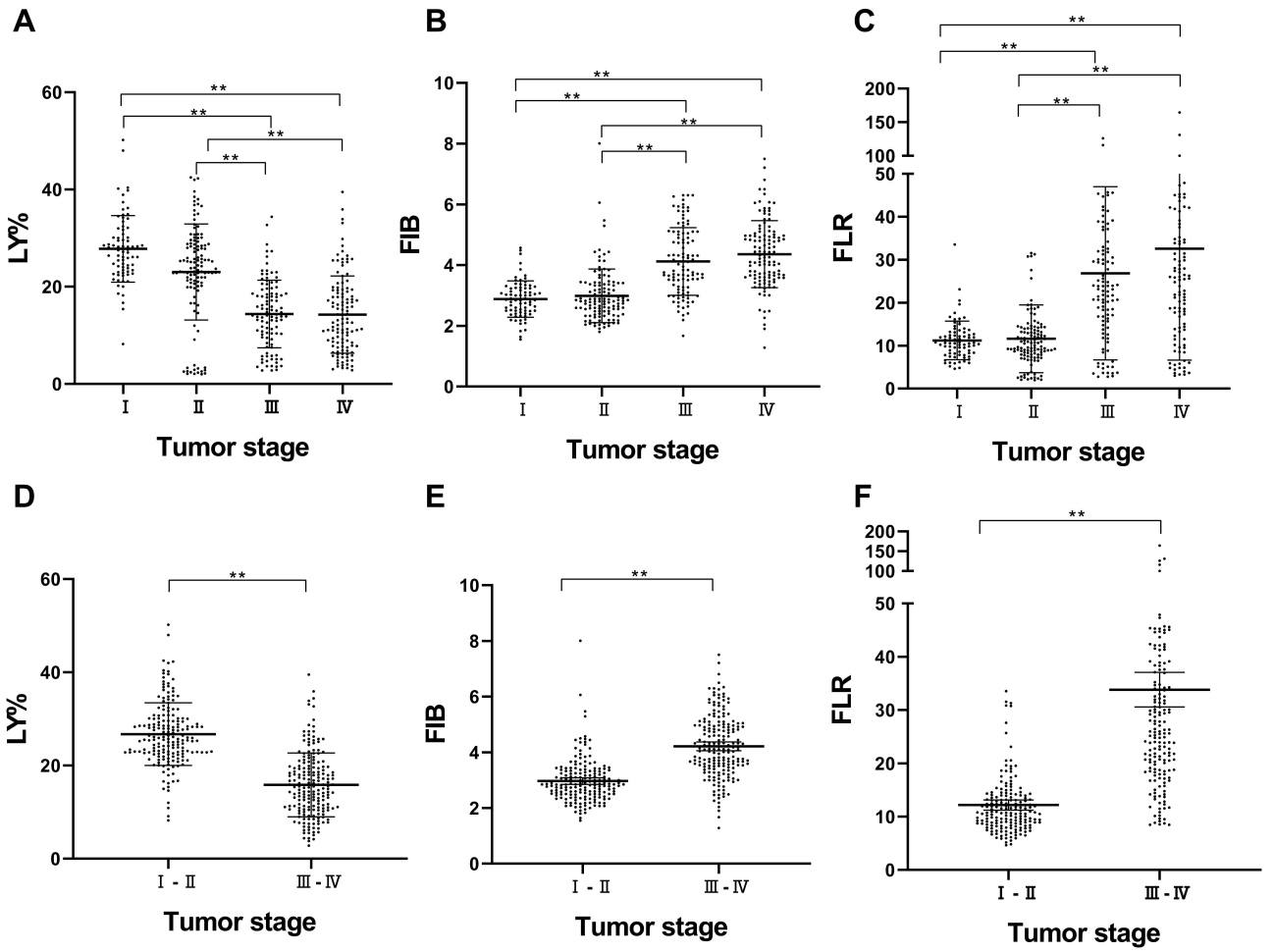

Figure 1 The correlation between target parameters and tumor stage in NSCLC patients. These graphs show the relationship between LY% and tumor stage in NSCLC (**A** and **D**). These graphs show the relationship between FIB and tumor stage in NSCLC (**B** and **E**). These graphs show the relationship between FLR and tumor stage in NSCLC (**C** and **F**). **P < 0.001.

To further verify the ability of the three biomarkers to identify tumor staging, we analyzed the NSCLC population in this study, and the results were shown in Table 2. The circulating median concentrations of LY%, FIB, and FLR in male case-patients were 20.10 (13.40, 25.50), 3.60 (2.86, 4.70), and 19.55 (11.68, 32.54), respectively. The circulating median concentrations of LY%, FIB, and FLR in female case-patients were 23.00 (18.03, 28.65), 3.10 (2.59, 3.75), and 13.18 (9.16, 20.68), respectively. Furthermore, given the statistical differences in circulating median concentrations of the three biomarkers in the stage I–II patients, as well as the stage III–IV patients, were not observed, patients with stage I–II and stage III–IV were used as sample groups for further statistical analysis, respectively. The circulating median concentrations of LY%, FIB, and FLR were 25.9 (22.80, 30.05), 2.85 (2.49, 3.33), and 10.78 (8.71, 13.79) in the stage I–II patients, respectively, which were 15.35 (10.80, 19.70), 4.11 (3.50, 4.97), and 27.80 (19.62, 42.13) in the stage III–IV patients, respectively.

In no lymph node metastasis patients, the circulating median concentrations of LY%, FIB, and FLR were 25.50 (21.90, 29.30), 2.89 (2.50, 3.40), and 11.55 (8.89, 14.42), respectively, while the concentrations of LY%, FIB, and FLR were 17.85 (11.78, 23.40), 3.76 (3.04, 4.80), and 22.76 (13.68, 37.79), respectively, among one or more lymph nodes metastasized patients. Among zero organ metastasis patients, the circulating median concentrations of LY%, FIB, and FLR were 23.00 (17.90, 28.30), 3.07 (2.60, 3.80), and 13.10 (9.42, 21.51), respectively, which were 16.70 (11.05, 21.70), 4.14 (3.58, 4.93), and 17.20 (11.16, 22.36) among one organ metastasis patients, respectively, and 11.30 (7.80, 17.40), 4.26 (3.72, 5.12), and 42.12 (25.98, 56.18) among two or more organ

Table 2 Comparisons of Pretreatment Circulating LY%, FIB, and FLR in Different Variables Among NSCLC Patients

Variables	LY%		FIB		FLR	
	Median (IQR)	*P*	Median (IQR)	*P*	Median (IQR)	*P*
Gender		<0.001		<0.001		<0.001
Male	20.10 (13.40, 25.50)		3.60 (2.86, 4.70)		19.55 (11.68, 32.54)	
Female	23.00 (18.03, 28.65)		3.10 (2.59, 3.75)		13.18 (9.16, 20.68)	
Education		0.379		0.356		0.283
≤12 years/completed high school	21.50 (14.70,27.00)		3.41 (2.74, 4.32)		15.47 (10.49, 29.54)	
>12 years	18.45 (11.93, 27.20)		3.52 (2.91, 4.73)		20.49 (10.98, 33.14)	
Tobacco		0.067		0.61		0.231
Never	22.40 (15.40, 28.40)		3.20 (2.65, 4.17)		14.58 (9.25, 29.20)	
Former	21.80 (15.40, 26.60)		3.33 (2.80, 4.34)		19.55 (11.74, 21.82)	
Current	20.60 (13.85, 25.45)		3.60 (2.87, 4.40)		18.58 (11.56, 31.71)	
ECOG		0.101		0.107		0.082
0	21.85 (14.68, 27.30)		3.38 (2.76, 4.26)		15.51 (10.45, 29.43)	
>0	18.40 (13.25, 25.40)		3.80 (2.82, 5.01)		20.71 (12.80, 34.57)	
Tissue types		0.22		0.018		0.066
LUAD	21.90 (15.08, 28.2)		3.27 (2.66, 4.11)		15.20 (9.81, 27.82)	
LUSC	21.00 (14.15, 25.85)		3.52 (2.87, 4.68)		18.54 (11.42, 31.27)	
TNM stage		<0.001		<0.001		<0.001
I–II	25.90 (22.80, 30.05)		2.85 (2.49, 3.33)		10.78 (8.71, 13.79)	
III–IV	15.35 (10.80, 19.70)		4.11 (3.50, 4.97)		27.80 (19.62, 42.13)	
Lymph node		<0.001		<0.001		<0.001
N0	25.50 (21.90, 29.30)		2.89 (2.50, 3.40)		11.55 (8.89, 14.42)	
N1–N3	17.85 (11.78, 23.40)		3.76 (3.04, 4.80)		22.76 (13.68, 37.79)	
Metastasis		<0.001		<0.001		<0.001
Zero organ	23.00 (17.90, 28.30)		3.07 (2.60, 3.80)		13.10 (9.42, 21.51)	
One organ	16.70 (11.05, 21.70)		4.14 (3.58, 4.93)		26.45 (18.25, 42.08)	
Two organs or more organs	11.30 (7.80, 17.40)		4.26 (3.72, 5.12)		42.12 (25.98, 56.18)	

Note: Mann–Whitney *U*-test or Kruskal–Wallis test (two or multigroup comparison).

Abbreviations: ECOG score, eastern cooperative oncology group score; LUSC, squamous cell lung cancer; LUSD, lung adenocarcinoma; FIB, fibrinogen; LY%, lymphocyte percentage; FLR, fibrinogen-to-lymphocyte percentage ratio; IQR, interquartile range.

metastasis patients, respectively. There was no significant difference in circulating LY%, FIB, and FLR in terms of educational experience, tobacco, and ECOG among all patients (P>0.05). However, these three biological markers were significantly related to gender, lymph node metastasis, organ metastasis, and TNM stage. It was worth pointing out that FIB was a significant difference in tissue types (P=0.018) (see Table 2).

Clinical Value of FLR in Overall Survival of NSCLC Patients

To analyze the prognostic values of LY%, FIB, and FLR in NSCLC patients, X-tile software was used to calculate the optimal thresholds of the three biological markers among 209 cases-patients followed up, which were 17.8, 3.4, and 16.8, respectively, for survival prediction in NSCLC patients (Figure 2). Subsequently, 209 case patients were divided into low or high groups following the best cut-off values of these biomarkers. LY %, FIB, and FLR were then examined in univariate and multivariate analysis to identify prognostic markers in patients with NSCLC. In univariate analysis, gender, age, pT category, pTNM stage, Lymph node, organ metastasis, LY%, FIB, and FLR were identified as significant prognostic factors, while tobacco, education experience, ECOG, and tissue type were not associated with 3 years' OS. In multivariate analysis, we found that p TNM stage (p=0.028), organ metastasis (p≤ 0.001), FIB (p=0.032), and FLR (p=0.001) were associated with 3 years' OS, while gender, age, pT category, lymph node, and LY% were not related to 3 years' OS. FIB (p=0.032) and FLR (p=0.001)

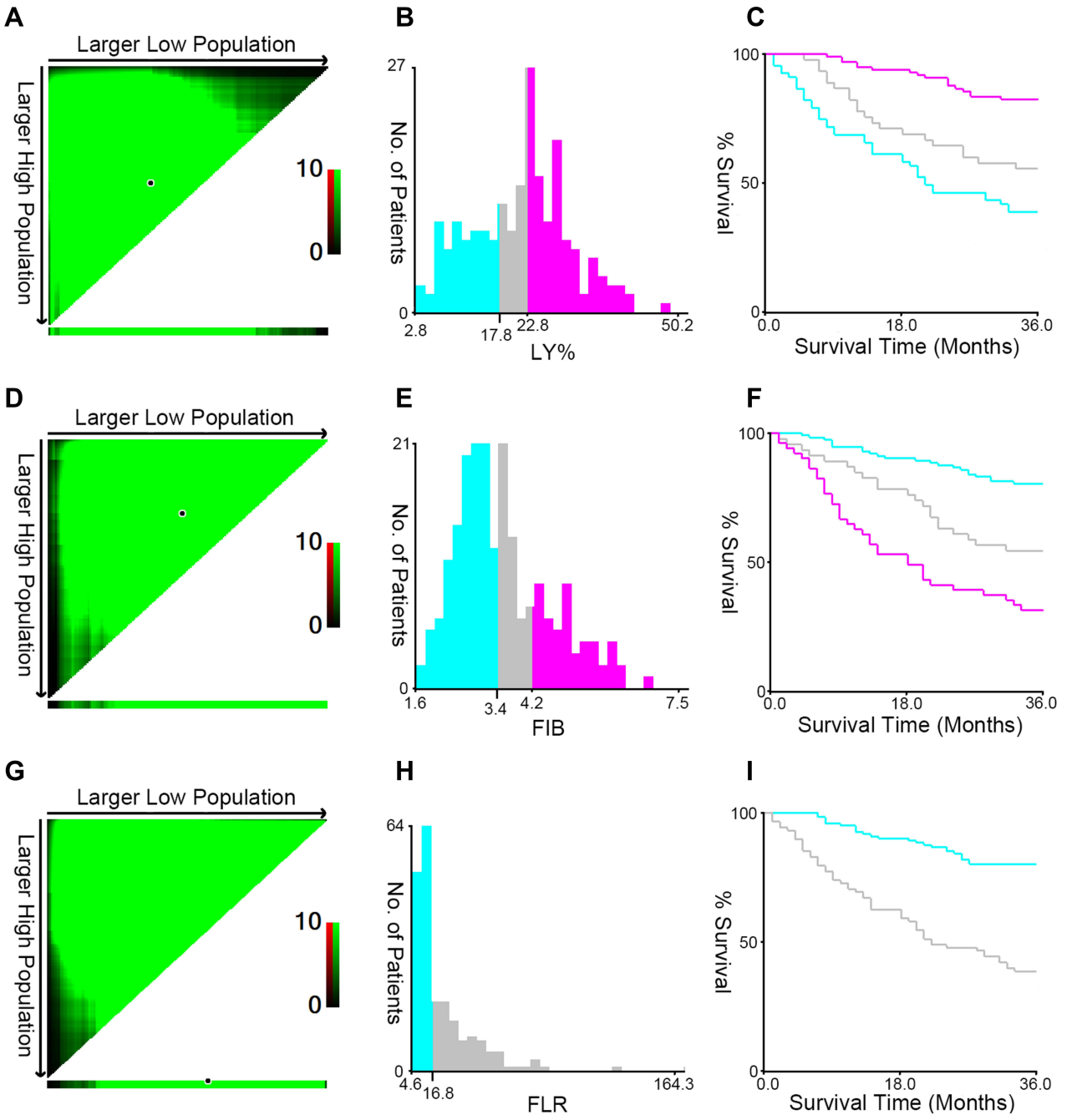

Figure 2 The optimal cutoffs values of LY% (**A–C**), FIB (**D–F**), and FLR (**G–I**) in 375 NSCLC patients by using X-tile software. Data is graphically represented as a right-angled triangle grid, where each point represents data from a given set of partitions. These graphs show the generated $\chi 2$ log-rank values and divide them into three or two groups according to the cutoff points (**A, D** and **G**). Determining the optimal cut points (17.8, 3.4, and 16.8) by locating the brightest pixels on the x-tile map. The number of patients was represented by histogram (**B, E** and **H**). The corresponding population was represented by Kaplan–Meier curve (**C, F** and **I**), respectively.

were independent predictive factors for poor prognosis. However, FLR (adjusted HR = 2.812, 95% CI = 1.519–5.206) was better than FIB (adjusted HR =1.978, 95% CI = 1.060–3.691) in predicting the 3 years' OS (Table 3).

To further analyze the clinical value of FLR, the association between FLR and clinical efficacy of therapeutic tools in NSCLC patients was investigated (see Figure 3). We found that the clinical outcomes of low FLR patients with chemotherapy and chemotherapy combined with surgery were superior to high FLR patients (Figure 3A and B). The OS of surgical patients with chemotherapy combined with surgery was significantly longer than that of

Table 3 Univariate and Multivariate Analysis of Cox Regression Model for Candidate Prognostic Factors for 209 NSCLC Patients

		Univariate Cox Regression			Multivariate Cox Regression		
Variables		**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Gender (male)	77vs132	1.782	1.080–2.939	0.024	1.207	0.631–2.311	0.57
Age (>60 years)	117vs92	1.796	1.149–2.807	0.010	1.566	1.000–2.453	0.051
Tobacco (yes)	103vs106	1.442	0.921–2.256	0.109			
Education (>12 years)	12vs197	0.897	0.328–2.454	0.832			
ECOG (≥1)	13vs196	1.498	0.651–3.447	0.341			
LUAD (LUSC)	109vs100	0.993	0.637–1.548	0.975			
pT category							
II (vs I) III (vs I)	66vs53 45vs53	2.561 4.258	1.016–6.452 1.699–10.673	0.046 0.002	1.665 2.933	0.628–4.410 0.825–10.424	0.305 0.096
pTNM stage							
Stage III–IV vs stage I–II Lymph node (N1–N3)	90vs119 120vs89	4.172 3.034	2.575–6.759 1.791–5.141	<0.001 <0.001	2.223 0.808	1.089–4.536 0.596–1.096	0.028 0.171
Metastasis (vs zero organ)							
One organ Two or more organs LY% (<17.8%) FIB (>3.4g/L) FLR (>16.7)	29vs164 16vs164 66vs143 97vs112 88VS121	6.655 5.662 3.129 3.821 4.339	3.968–11.160 2.963–10.820 2.004–4.885 2.345–6.226 2.678–7.031	<0.001 <0.001 <0.001 <0.001 <0.001	4.241 3.253 1.270 1.978 2.812	2.355–7.638 1.596–6.630 0.703–2.295 1.060–3.691 1.519–5.206	<0.001 0.001 0.429 0.032 0.001

Abbreviations: HR, hazard ratio; CI, confidence interval; ECOG score, eastern cooperative oncology group score; LUSC, Lung squamous cell carcinomasL; LUAD, Lung adenocarcinoma; LY%, lymphocyte percentage; FIB, fibrinogen; FLR, fibrinogen-to-lymphocyte percentage ratio; Multivariate analysis with covariant, such as gender, age, tobacco, ECOG, tumor stage, and metastasis.

patients with only chemotherapy. Also, low FLR patients after receiving the two treatment tools had a greater difference in cumulative survival, and the difference increased with the increase in OS (Figure 3C and D).

Discussion

Lung cancer is a malignant tumor with the highest morbidity and mortality and has attracted wide attention.[19] Majority of NSCLC patients present changes in certain genes that drive oncogenesis including KRAS, EGFR, ALK, or HER2. Mutations in these driver genes lead to tumor growth and invasiveness.[20,21] In lung cancer patients, endogenous CD4+ T cells attack cancer cells by recognizing neoantigens induced by recurrent oncogenic KRAS and ERBB2 (Her2) driver mutations.[22] Lung cancer patients with EGFR mutations and ALK rearrangements have been shown to have lower levels of CD8+ T cell infiltration.[23] Tumor infiltrating lymphocytes (TILs) are now widely believed to be associated with better clinical outcomes in cancer.[24] In addition, elevated pretreated lymphocytes are the favorable prognostic factor for OS in patients with NSCLC.[25] It can be seen that lymphocytes cells are closely related to tumor driver mutations. It should be noted that as many as 94% of cancer patients are reported to have one or more coagulation abnormalities.[26] FIB can reflect clotting function. Also, FIB, as an acute phase reaction protein, has a significant increase in concentration when inflammation occurs.[27] Guan et al[28] have reported that EGFR mutation status may be related to Hyperfibrinogenemia in patients with NSCLC, and FIB is an independent prognostic factor for EGFR gene mutation status. The proposed indicator FLR takes into account the combined effect of FIB and LY%, which improves its sensitivity to predicting prognosis. In our study, the concentrations of LY%, FIB, and FLR in different groups were compared by using the Mann–Whitney *U*-test or Kruskal–Wallis test. Compared with the stage I–II group, no lymph node metastasis group,

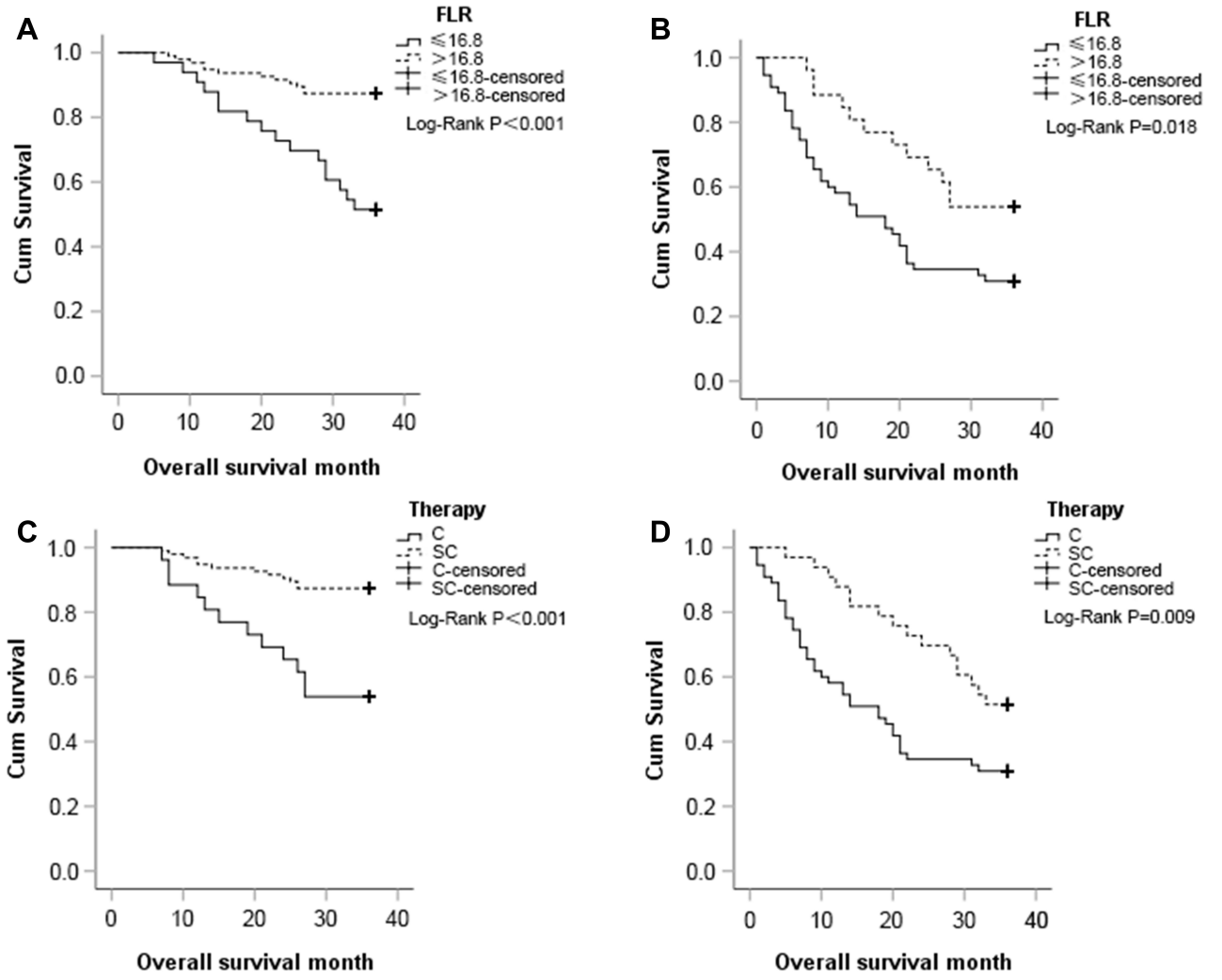

Figure 3 Kaplan–Meier curves of 209 NSCLC patients with treatment of chemotherapy (C therapy) or surgery combined with chemotherapy (SC therapy). (**A**) Kaplan–Meier curve for overall survival probability within NSCLC patients receiving SC therapy according to circulating FLR concentration. (**B**) Kaplan–Meier curve for overall survival probability within NSCLC patients receiving C therapy according to circulating FLR concentration. (**C** and **D**) Kaplan–Meier curve for overall survival probability within NSCLC patients according to two therapy methods in low FLR group and high FLR group, respectively.

and no organ metastasis group, the concentrations of LY% for stage III-IV group, lymph node metastasis group, and organ metastasis group were lower, while the concentrations of FIB and FLR were higher. The concentration differences of the three biomarkers were statistically significant. Therefore, LY%, FIB, and FLR had auxiliary roles in tumor staging. In univariate and multivariate analysis, FIB and FLR were independent predictive factors for poor prognosis. However, the prognostic prediction of FLR was better than that of FIB. High FLR was associated with poor OS in NSCLC patients with chemotherapy or surgery combined with chemotherapy.

The association of pretreatment LY%, FIB, and FLR with tumor staging and the clinical prognosis were retrospectively analyzed in 375 NSCLC patients. Limited literature has reported the coagulation function of NSCLC patients. The relationship between the occurrence of NSCLC and changes in coagulation function can usually be explained from the following aspects: FIB can enhance the adhesion of tumor cells to PLT, which is induced by tumor cells to aggregate and release thrombin, thus promoting FIB to form a dense fibrin layer around tumor cells to protect tumor cells from natural killer cytotoxicity.[29,30] Elevated FIB concentration indicates activation of the hemostasis and fibrinolysis system, which promotes tumor angiogenesis, metastasis, and invasion.[31,32] We found that patients with advanced NSCLC tended to have lower LY % and higher FIB,

which was consistent with the results reported by Iseki et al[33] and Zhang et al.[34] Therefore, LY %, FIB, and FLR were significantly associated with tumor staging, which was mentioned in some previous studies.[27,34,35] However, the research conducted by Li et al[36] showed that there was no statistical difference in the concentration of FIB between different tumor stages, which might be due to the large differences in the number of patients with early, middle, and advanced disease in the selected patients. Circulating FLR, FIB, and LY% were significantly associated with 3-year OS in NSCLC patients. In addition, NSCLC patients with low FLR had significantly better clinical outcomes than those with high FLR, and NSCLC patients with surgically adjuvant chemotherapy had longer survival in the lower FLR subgroup. These results indicated that FLR could predict the clinical efficacy of chemotherapy combined with surgical resection.

Davalos et al revealed that the role of fibrinogen evolved from a marker of vascular rupture to a multifaceted signaling molecule with multiple functions that changed the balance between preventing infection and widespread inflammation. Perisanidis et al[38] suggested that plasma fibrin had pro-inflammatory effects in several types of cancer and might be involved in multiple stages of cancer progression.

This study investigated the influence of FLR on the NSCLC tumor staging and prognosis considering the combined effect of LY% and FIB. FLR could be used as a novel and potent biomarker for tumor staging and prognosis prediction of NSCLC. It is worth explaining that there are many immune cells in the microenvironment of NSCLC that can synthesize and release a variety of inflammatory factors such as fibroblast growth factor 2 (FGF-2), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF).[39,40] These inflammatory factors interact with inflammatory cells through integrin and non-integrin receptors to promote the production of FIB,[41] resulting in an increase in FIB level. Plasma hyperfibrinogenemia causes a hypercoagulable state, thereby promoting the adhesion and survival of tumor cells after perfusion and leading to the metastasis potential of lung cancer models.[42] FLR amplifies immune and inflammatory sensitivity in NSCLC patients and is superior to LY % and FIB in predicting NSCLC survival. Therefore, we recommend the use of FLR level before treatment to predict a 3-year prognosis in NSCLC patients with chemotherapy or surgery combined with chemotherapy. Because LY % and FIB are routinely measured in patients with NSCLC prior to treatment, clinicians may also consider using them in combination with clinical practice.

The limitations of this study were the retrospective analysis of an observational database. One major limitation is that lacking data on molecular tumor driver mutation status (eg, KRAS, EGFR, ALK, or HER2). Secondly, all enrolled patients were from a single institution, and the age distribution of the enrolled patients was limited. The age of the participants ranged from 41 to 85 years, while the actual age of lung cancer patients showed a decreasing trend. Thirdly, this study only included gender, age, smoking status, and educational experience, while other confounding factors such as alcohol, diabetes, and complications were not further studied. Finally, only a preliminary prognostic was established in this study, and further confirmatory studies were needed. Our short follow-up period made it impossible to perform an effective analysis, which reduced our statistical ability to detect differences between groups. We will consider extending the follow-up time and including more cases in further studies.

Conclusions

Our findings showed that circulating pretreatment LY%, FIB, and FLR were helpful in predicting NSCLC stage. FLR was an independent prognostic factor and was better than FIB in predicting the 3 years' OS within NSCLC patients with chemotherapy or surgery combined with chemotherapy.

Ethics Approval and Informed Consent

This study was ethically approved by the institutional review board of First Affiliated Hospital of Nanchang University (Jiangxi Province, China). To protect patient privacy, all data was anonymous, the requirement for informed consent was waived. This study was conducted in accordance with the Declaration of Helsinki.

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Disclosure

The authors declare that there is no conflicts of interest.

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