

Pretreatment Lipoprotein(a) as a Biomarker for EGFR Mutation and Prognosis in Lung Adenocarcinoma

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Purpose: This study aims to investigate the correlation between pretreatment serum lipoprotein(a) [Lp(a)] and epidermal growth factor receptor (EGFR) gene mutations, as well as its predictive value for progression-free survival (PFS) in advanced lung adenocarcinoma patients receiving epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) therapy.

Patients and Methods: We determined the optimal cutoff value for Lp(a) by receiver operating characteristic (ROC) curves and Youden's index to categorize Lp(a) into high and low groups. Logistic regression was used to analyze the EGFR mutation rate in different groups. Additionally, the relationship between pretreatment Lp(a) levels and prognostic PFS in patients with advanced (TNM stage IIIB-IV) lung adenocarcinoma treated with EGFR-TKIs was retrospectively analyzed by Cox regression, survival and stratified analysis methods.

Results: We included 338 advanced lung adenocarcinoma patients, with median age of 64 years, and slightly more female patients (51.8%), most of whom had no smoking history (70.7%), no history of chronic lung disease (87.9%), and stage IV (81.1%) patients. The EGFR gene mutation rate was 55.3% and 123 patients were included in the prognostic evaluation through screening. The optimal cutoff value for Lp(a) was 20.48 mg/L. The mutation rate in the high Lp(a) group was significantly lower than the low Lp(a) group (48.0% vs 65.5%, $p = 0.001$). Multivariate logistic regression analysis indicated that Lp(a) is an independent predictor of EGFR mutations (OR = 0.41, 95% CI: 0.25–0.66, $p < 0.001$). Survival analysis showed that the median PFS was significantly longer in the high Lp(a) level group compared to the low level group (16.1 months, 95% CI: 11.9–23.8 months vs 9.6 months, 95% CI: 8.9–13.3 months, $p = 0.015$). Multivariate analysis confirmed that Lp(a) is an independent predictor of PFS in advanced lung adenocarcinoma patients receiving EGFR-TKIs treatment (HR = 0.42, 95% CI: 0.26–0.68, $p < 0.001$).

Conclusion: Pretreatment Lp(a) may be a biomarker for EGFR mutations and the PFS in advanced lung adenocarcinoma patients undergoing EGFR-TKIs treatment.

Keywords: advanced lung adenocarcinoma, lipoprotein (a), epidermal growth factor receptor, progression-free survival, Biomarker

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide. Lung adenocarcinoma is the most common histological subtype of lung cancer, with an average 5-year survival rate of 15%.¹ In recent years, the advent of nucleic acid analysis technology has revolutionized lung cancer treatment,² and molecular targeted therapy has become a research hotspot, replacing traditional chemotherapy and radiotherapy due to its longer survival benefits and fewer side effects. EGFR, as the most common driver gene in lung cancer and the most frequent target gene for targeted therapy in patients with lung adenocarcinoma, has a mutation rate as high as 51.4% in Asian countries.³ Compared to patients with wild-type EGFR, those with EGFR mutations generally have a shorter overall survival and are more prone to distant

metastasis.^{4,5} Clinically, the deletion of E746_A750 in exon 19 (19Del) and the point mutation of leucine to arginine at position 858 in exon 21 (21L858R) are the two most common EGFR mutations.⁶ These mutations may increase the kinase activity of EGFR, leading to excessive activation of its downstream signaling and conferring oncogenic properties to EGFR-mutant cells.⁷ Therefore, EGFR-TKIs have become the first-line treatment for patients with advanced lung adenocarcinoma with EGFR mutations. However, there is currently no effective indicator to predict the prognosis of these patients. The 2023 edition of the National Comprehensive Cancer Network (NCCN) guidelines recommends that all patients with advanced lung adenocarcinoma should undergo testing for lung cancer driver genes. Currently, EGFR mutation testing in the clinic is primarily conducted using Sanger sequencing, quantitative real time polymerase chain reaction (qRT-PCR), or next-generation sequencing (NGS). These methods have specific requirements for laboratory conditions and tissue quantity, making EGFR mutation testing challenging in general primary hospitals. Therefore, there is a need to find effective and convenient assessment methods.

In recent years, the relationship between lipid metabolism and lung cancer has attracted attention.^{8,9} It is well known that lipids, as a class of hydrophobic or amphipathic organic molecules, play a key role in cellular structure, function, and signaling. In NSCLC, dysregulation of EGFR signaling may be associated with EGFR overexpression or EGFR mutations. Studies have found that EGFR signaling can regulate lipid metabolism, inducing lipogenesis, while lipids can activate EGFR signaling by modulating the stability and fluidity of the cell membrane.⁸ And research have found that EGFR signaling events depend on the cholesterol content in lipid rafts. Depletion of cholesterol can promote ligand-independent activation of EGFR,¹⁰ which can lead to uncontrolled cell growth, enhanced proliferation and metastasis of tumor cells, and ultimately contribute to tumor development.¹¹ Additionally, clinical studies have found that serum cholesterol, apolipoprotein A1 (APOA1), and other blood lipids are associated with the prognosis of NSCLC.^{12–15} For example, Zhang et al found that in EGFR-mutant NSCLC patients, those with high TC levels had a 63% lower risk of death compared to those with low TC levels ($p=0.001$).¹⁵ Therefore, we supposed that blood lipids may be related to EGFR mutations, signaling, and prognosis in lung cancer.

Previous studies have shown that pretreatment HDL may be a useful marker for predicting EGFR mutations and prognosis in advanced lung adenocarcinoma patients,¹³ but we know that lipid levels, such as HDL, can be affected by factors such as patient lifestyle, diet, medication and age, therefore will inevitably introduce varying degrees of error. Lipoprotein(a), one of the most enigmatic lipoprotein particles in humans, differs from other lipids in that its plasma concentration is mainly genetically determined and usually reaches the adult level at the age of 5 years, independent of external factors such as lifestyle, diet, medication and age.¹⁶ Lp(a) not only serves as a regulator of immune and inflammatory responses,¹⁷ but due to the high homology between apolipoprotein(a)[Apo(a)] and plasminogen,¹⁸ it has long been hypothesized to possess anti-tumor properties. Lp(a) is mainly composed of LDL-like particles and Apo(a), and the LDL-like particles contain apolipoprotein B100 (ApoB 100), which is linked to Apo(a) by a disulfide bond, and also serves as a carrier of oxidized phospholipids (OxPL).¹⁹ It has been confirmed that Lp(a) is an independent risk factor for atherosclerotic cardiovascular diseases.²⁰ In recent years, researchers have found that Lp(a) has an inhibitory effect on angiogenesis and the growth of angiogenesis-dependent tumors.^{21,22} And angiogenesis is a critical step in tumor expansion and metastasis.²³ Therefore, the role of Lp(a) in tumors has garnered increasing attention. However, in real-world studies, reports on the relationship between Lp(a) and cancer are scarce and controversial, and research on the relationship between Lp(a) and EGFR mutations and prognosis in advanced lung adenocarcinoma patients has not been reported. Therefore, this study focuses on advanced lung adenocarcinoma patients and aims to explore the correlation between Lp(a) and EGFR mutations and its predictive role in the clinical prognosis of these patients.

Methods

Patients

This study included 338 patients with advanced lung adenocarcinoma treated at the Department of Respiratory Medicine, Second Affiliated Hospital of Nanchang University, from August 12, 2017, to December 11, 2023. All included patients met the following criteria: (1) Age ≥ 18 years and ECOG PS (Eastern Cooperative Oncology Group Performance Status) score of 0–2; (2) Pathologically confirmed lung adenocarcinoma; (3) EGFR gene mutation status defined; (4) All patients

were identified as stage III B to IV according to the AJCC eighth edition lung cancer TNM stage; (5) Comprehensive imaging assessment at diagnosis; (6) No treatment prior to serum collection; (7) Complete follow-up data were available, and at least one measurable lesion that met Response Evaluation Criteria in Solid Tumors (RECIST). The exclusion criteria for patients were (1) Any relevant disease that may affect lipid metabolism (eg, diabetes and metabolic syndrome, etc.); (2) Patients with a combination of serious cardiac, pulmonary, hepatic, renal, and hematologic diseases or complications; (3) Combined primary malignant tumors elsewhere; (4) History of blood transfusion within the last 4 months prior to admission. This study was approved by the Biomedical Research Ethics Committee of the Second Affiliated Hospital of Nanchang University.

All patients underwent EGFR gene mutation testing. A total of 187 patients exhibited EGFR mutations, including 6 patients with non-EGFR 19Del or 21L858R mutation, and 26 patients were not treated with EGFR-TKIs at our hospital. A total of 155 patients with EGFR-sensitive mutation (19Del or 21L858R) were treated with EGFR-TKIs, of which 26 patients did not have regular follow-up or had incomplete follow-up data, and 6 patients had primary resistance to EGFR-TKIs. Ultimately, 123 advanced lung adenocarcinoma patients were enrolled in the prognostic evaluation (Figure 1).

Data Collection and Patient Follow-Up

We collected clinical and pathological data from 338 included patients, including gender, age, smoking history, chronic pulmonary disease history, EGFR genotype, type of EGFR-TKIs (first-generation EGFR-TKIs include gefitinib, erlotinib, and icotinib; second-generation EGFR-TKIs include afatinib and dacomitinib; and third-generation EGFR-TKIs include osimertinib, almonertinib, and furmonertinib), TNM stage, sites of metastasis, metastasis number, time of first targeted therapy, time to disease progression, and lipoprotein(a) levels within one month prior to treatment (Table S1).

Patients were treated with EGFR-TKIs and followed regularly until disease progression, visiting the hospital after 2 cycles (21 days per cycle) of initial treatment and every 2 months thereafter. Each visit required a chest computed tomography (CT) scan or imaging examination of distant metastatic lesions. The last follow-up was on June 30, 2024.

Evaluation methods

The endpoint of this study was PFS, which was defined as the time from the start of randomization (or the start of treatment in a single-arm trial) to tumor progression or death from any cause (according to RECIST, version 1.1),²⁴ ie, as the time from the date of receipt of EGFR-TKIs to disease progression or death from any cause. Disease evaluation is performed by computed tomography (CT) scan of the neck chest and abdomen, Cranial Magnetic Resonance Imaging (MRI), whole-body bone imaging, or Positron Emission Tomography- Computed Tomography (PET-CT). All patients

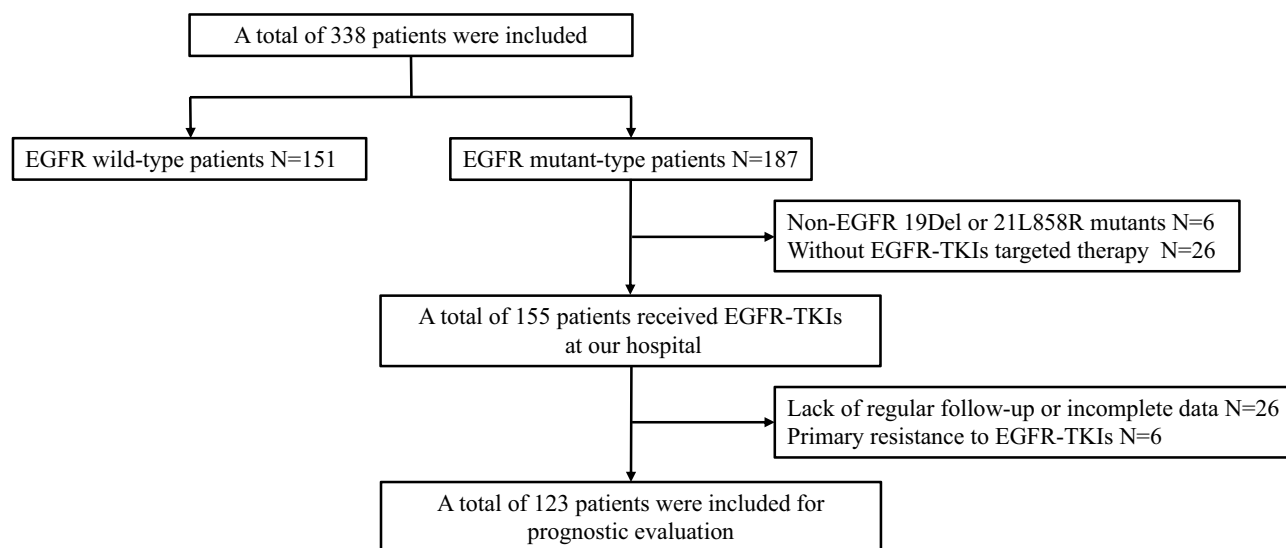


Figure 1 Patient screening flowchart.

were examined after 2 cycles of treatment to ensure the effectiveness of treatment with EGFR-TKIs and to exclude patients with primary resistance. Disease progression was judged according to RECIST version 1.1.

Statistical Analysis

We used the receiver operating characteristic (ROC) curve and Youden's index to determine the optimal cutoff value for Lp(a), which was categorized into high and low level groups. Pearson's Chi-squared test was used to compare the clinical characteristics of the EGFR mutated and non-mutated groups. Logistic regression analysis was used to test the effects of clinical characteristics such as sex, age, smoking history and Lp(a) level on EGFR mutations, and to determine the independent variables associated with EGFR mutations. The median follow-up time of 123 patients was calculated using the reversed Kaplan–Meier method, the Kaplan–Meier method was used to plot the survival curves of patients, and the Log rank test was used to compare the progression-free survival probability of patients with high and low levels of Lp(a). The effect of Lp(a) levels on survival of patients with advanced lung adenocarcinoma in different populations was assessed by gender and other variables using Kaplan–Meier stratified analysis methods. The effects of gender, age, smoking history, chronic pulmonary disease history, EGFR genotype, TNM stage, intrapulmonary metastasis, brain metastasis, liver metastasis, bone metastasis, pleural metastasis, metastases number, types of EGFR-TKIs and the level of Lp(a) on PFS in advanced lung adenocarcinoma patients were examined using COX regression analysis method. Variables that were considered clinically relevant or statistically significant (p -value <0.05) in the univariate analysis were included in the multivariate regression model, and the variables included were carefully selected to ensure parsimony of the final model, taking into account the number of available events.²⁵ All reported confidence intervals were two-tailed, and a p -value <0.05 was considered statistically significant. All of the analyses were performed with the statistical software package R (<http://www.R-project.org>, The R Foundation) as well as using MSTATA version 0.93 (<https://www.MSTATA.com/>) and Empower Stats version 4.2 (<http://www.empowerstats.com>, X&Y Solutions, Inc., Boston, MA) software.

Results

Cutoff Value for Lipoprotein(a)

We used mutation or non-mutation of the EGFR gene as a binary variable in the ROC curve,¹³ and Youden's index (specificity + sensitivity-1) was maximal when the cutoff value of Lp(a) was 20.48 mg/L (Figure 2). We defined Lp(a) > 20.48 mg/L as the high level group and Lp(a) ≤ 20.48 mg/L as the low level group.

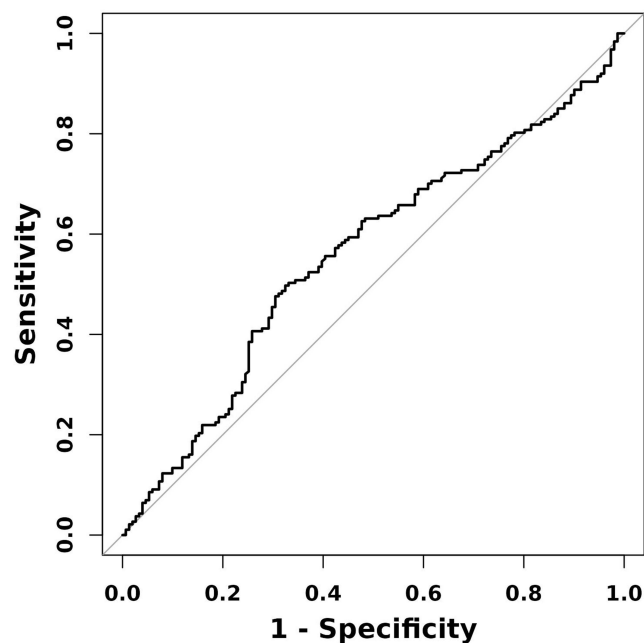


Figure 2 Receiver-operating characteristic curves analysis for Lp(a).

Patient Clinical Characteristics

The correlation between patient demographics and clinical characteristics with EGFR mutations was shown in Table 1. The median age of the 338 patients was 64 years (Range: 26–89 years). The majority of patients were female (51.8%), no smoking history (70.7%), no history of chronic lung disease (87.9%), stage IV (81.1%), no intrapulmonary metastases (72.8%), no brain metastases (71.0%), no liver metastases (90.5%), no bone metastases (69.5%), no pleural metastases (62.7%), and high levels of lipoprotein(a) (58.0%). The incidence of EGFR mutations was 55.3% (N = 187), of which 123 patients with EGFR-sensitive mutations received EGFR-TKIs treatment, the demographic characteristics of the patients are

Table 1 The Relationship Between the Clinical Characteristic and EGFR Mutation

Characteristic	EGFR mutations			p value
	Overall N = 338	Mutant N = 187	Wild N = 151	
Gender				<0.001***
Male	163	70	93	
Female	175	117	58	
Age (years)				0.633
<64	163	88	75	
≥64	175	99	76	
Smoking history				<0.001***
Ever smokers	99	39	60	
Never smokers	239	148	91	
Chronic pulmonary disease				0.117
No	297	169	128	
Yes	41	18	23	
TNM stage				0.074
IIIB	64	29	35	
IV	274	158	116	
Intrapulmonary metastasis				0.476
No	246	139	107	
Yes	92	48	44	
Brain metastasis				0.009**
No	240	122	118	
Yes	98	65	33	
Hepatic metastasis				0.218
No	306	166	140	
Yes	32	21	11	
Bone metastasis				0.017*
No	235	120	115	
Yes	103	67	36	
Pleural metastasis				0.872
No	212	118	94	
Yes	126	69	57	
Metastasis number				0.059
<2	205	105	100	
≥2	133	82	51	
Lp(a) (mg/L)				0.001**
≤20.48	142	93	49	
>20.48	196	94	102	

Notes: *Indicates p value less than 0.05 level, **Indicates p value less than 0.01 level, ***Indicates p value less than 0.001 level.

Abbreviations: EGFR, epidermal growth factor receptor; Lp(a), lipoprotein(a).

shown in [Table S2](#). 61 (49.6%) patients were treated with first-generation EGFR-TKIs, 10 (8.1%) patients were treated with second-generation EGFR-TKIs, and 52 (42.3%) patients were treated with third-generation EGFR-TKIs.

The Correlation Between Baseline Levels of Lipoprotein(a) and EGFR Mutations

Analysis of the relationship between EGFR mutations and clinical characteristics revealed that lipoprotein(a), gender, smoking history, brain metastases, and bone metastases are associated factors for EGFR mutations ([Table 1](#)). Lp(a) high-level group had a significantly lower rate of EGFR mutations compared to the low-level group (48.0% vs 65.5%, $p = 0.001$). Variables that were considered clinically relevant or statistically significant (p -value < 0.05) in the univariate analysis were included in the multivariate logistic regression analysis (The variables are independent of each other, with no multicollinearity, as shown in [Table S3](#)), which showed that females (OR = 2.24, 95% CI:1.26~4.02, $p = 0.006$), brain metastases (OR = 1.88, 95% CI:1.10~3.26, $p = 0.022$) and bone metastasis (OR = 1.70, 95% CI:1.01~2.91, $p = 0.049$) were independent risk factors for EGFR gene mutation, while high level of Lp(a) was an independent protective factor against EGFR mutations (OR = 0.41, 95% CI:0.25~0.66, $p < 0.001$) ([Table 2](#)).

The number of people in the Lp(a) high level group was 1.38 times higher than that in the low level group and based on the EGFR mutation rates in both groups, power was estimated by using Empower Stats version 4.2 (<http://www.empowerstats.com>, X&Y Solutions, Inc., Boston, MA) software, setting a significance of 0.05 and a two-sided test, the sample size of 338 cases was calculated to yield approximately 88% of the test power.

Baseline Levels of Lipoprotein(a) and Progression-Free Survival

Based on follow-up data, 123 advanced lung adenocarcinoma patients who had EGFR sensitive mutations (19Del or 21L858R) underwent long-term, regular treatment with EGFR-TKIs until disease progression, the median follow-up time was 31.8 months (95% CI: 30.7-NA, [Figure S1](#)). The median progression-free survival (PFS) for 123 patients with

Table 2 Multivariate Logistic Analysis of Factors Associated with EGFR Mutation

Characteristic	N	Event N	OR	95% CI	p value
Gender					
Male	163	70	—	—	
Female	175	117	2.24	1.26, 4.02	0.006**
Age					
<64	163	88	—	—	
≥64	175	99	1.25	0.78, 2.01	0.361
Smoking history					
Ever smokers	99	39	—	—	
Never smokers	239	148	1.41	0.76, 2.62	0.273
Chronic pulmonary disease					
No	297	169	—	—	
Yes	41	18	0.88	0.43, 1.80	0.727
Brain metastasis					
No	240	122	—	—	
Yes	98	65	1.88	1.10, 3.26	0.022*
Bone metastasis					
No	235	120	—	—	
Yes	103	67	1.70	1.01, 2.91	0.049*
Lp(a)					
≤20.48	142	93	—	—	
>20.48	196	94	0.41	0.25, 0.66	<0.001***

Notes: *Indicates p value less than 0.05 level, **Indicates p value less than 0.01 level, ***Indicates p value less than 0.001 level.

Abbreviations: EGFR, epidermal growth factor receptor; Lp(a), lipoprotein(a); OR, odds ratio; CI, confidence interval.

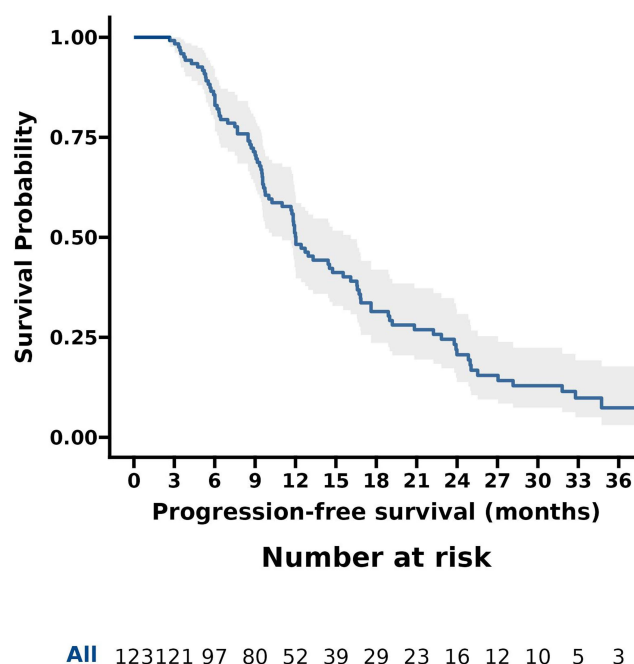


Figure 3 Progression-free survival (PFS) Kaplan–Meier curve for all patients.

advanced lung adenocarcinoma was 12.0 months (95% CI:11.0–16.1, [Figure 3](#)). Median PFS was significantly longer in advanced lung adenocarcinoma patients in the high-level Lp(a) group than in the low-level group (16.1 months, 95% CI:11.9–23.8 vs 9.6 months, 95% CI: 8.9–13.3 $p = 0.015$, [Figure 4](#)). And the progression-free survival probability of advanced lung adenocarcinoma patients in the high-level Lp(a) group were higher than those in the low-level group and all advanced lung adenocarcinoma patients at 6, 12, 18, and 24 months ([Table 3](#)). In stratified survival analyses by gender, age, smoking history, EGFR genotype, TNM stage, and presence of bone metastases, advanced lung adenocarcinoma patients with high levels of Lp(a) had longer PFS than those with low levels ([Figure 5](#)).

The Relationship Between Baseline Levels of Lipoprotein(a) and the Prognosis of Patients with Advanced Lung Adenocarcinoma

Univariate COX regression analysis showed that smoking history, TNM stage, bone metastasis, type of EGFR-TKIs, and lipoprotein(a) were influential factors for prognostic PFS ([Table 4](#)). We combined variables that were statistically significant (p -value < 0.05) in the univariate analysis with gender, age, and EGFR genotype (clinical experience) in the multivariate COX regression analysis (The variables are independent of each other, with no multicollinearity, as shown in [Table S4](#)). The results showed that smoking history (HR = 0.27, 95% CI: 0.11–0.64, $p = 0.003$), EGFR genotype (HR = 1.90, 95% CI: 1.22–2.96, $p=0.004$), TNM stage (HR = 2.20, 95% CI: 1.00–4.81, $p=0.049$), bone metastasis (HR = 2.27, 95% CI: 1.37–3.76, $p = 0.001$) and Lp(a) (HR = 0.42, 95% CI: 0.26–0.68, $p < 0.001$) were independent factors affecting prognostic PFS in advanced lung adenocarcinoma patients treated with EGFR-TKIs ([Figure 6](#)).

Discussion

Previous studies had indicated that circulating HDL-C and other lipid indicators were useful biomarkers for predicting EGFR gene mutations and prognosis in patients with NSCLC.^{12–15} In contrast to HDL and other lipid markers, Lp(a) levels are largely genetically dependent, reaching adult levels by age 5, and are less susceptible to interference from external factors.¹⁶ Thus Lp(a) may be a naturally good biomarker with greater stability compared to other lipid indicators. To our knowledge, this is the first study to explore the relationship between Lp(a) and EGFR mutations as well as prognosis in patients with advanced lung adenocarcinoma. Using stratified and regression models, we found that

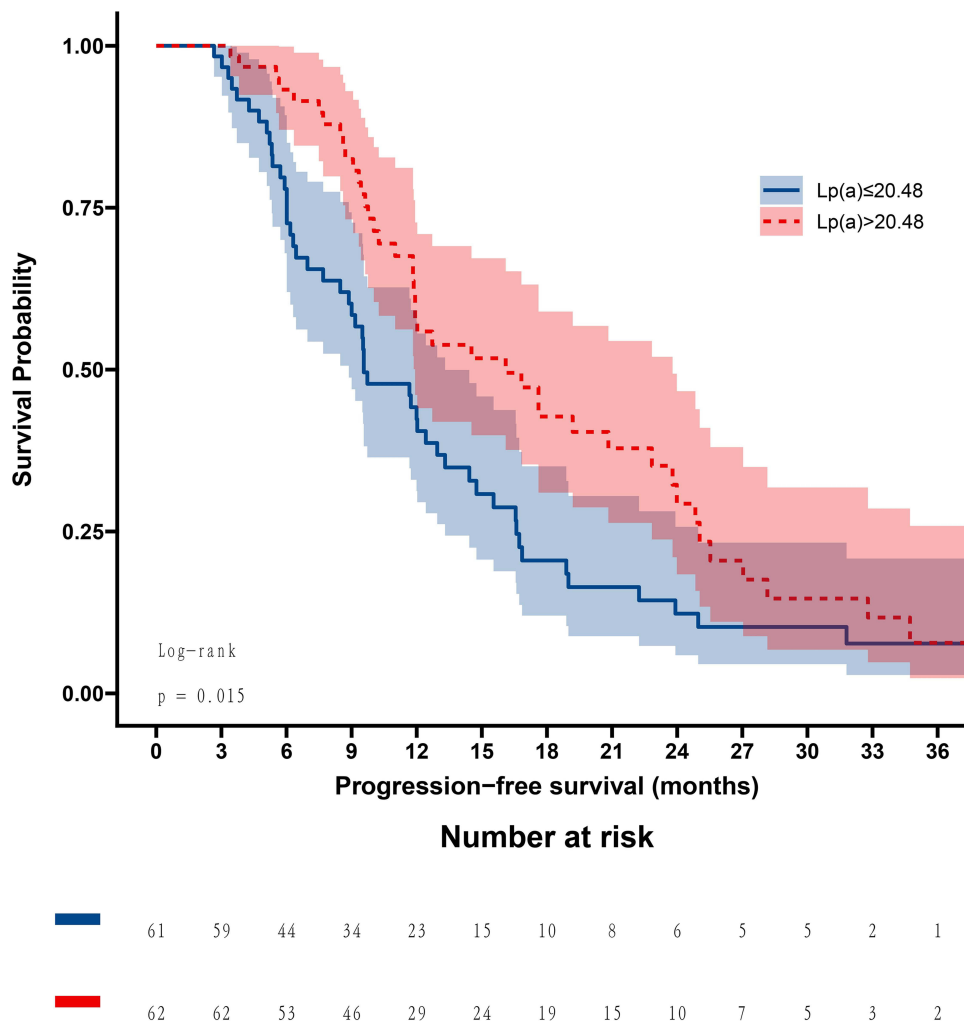


Figure 4 Kaplan–Meier curve of progression-free survival (PFS) according to Lp(a).

pretreatment Lp(a) was an independent predictor of EGFR mutations and PFS in patients with advanced lung adenocarcinoma, after adjusting for confounding factors such as gender, age, and smoking history. Patients with high levels of Lp(a) have lower EGFR mutation rates and better survival prognosis. This discovery will guide the selection of treatment

Table 3 6, 12, 18, 24-Month Progression-Free Survival Rate

strata	Time (months)	Number at Risk	Number of Events	Survival rate (%)	95% CI Lower	95% CI Upper
All patients	6	97	17	85.62	79.51	92.20
Lp(a) ≤ 20.48	6	44	13	77.90	67.97	89.28
Lp(a) > 20.48	6	53	4	93.23	87.03	99.87
All patients	12	52	39	50.16	41.65	60.41
Lp(a) ≤ 20.48	12	23	20	42.35	31.25	57.39
Lp(a) > 20.48	12	29	19	57.88	46.08	72.71
All patients	18	29	18	31.44	23.59	41.91
Lp(a) ≤ 20.48	18	10	11	20.52	12.01	35.06
Lp(a) > 20.48	18	19	7	42.75	31.00	58.95
All patients	24	16	9	20.64	13.82	30.81
Lp(a) ≤ 20.48	24	6	4	12.31	5.89	25.73
Lp(a) > 20.48	24	10	5	29.29	18.38	46.67

Abbreviations: Lp(a), lipoprotein(a); CI, confidence interval.

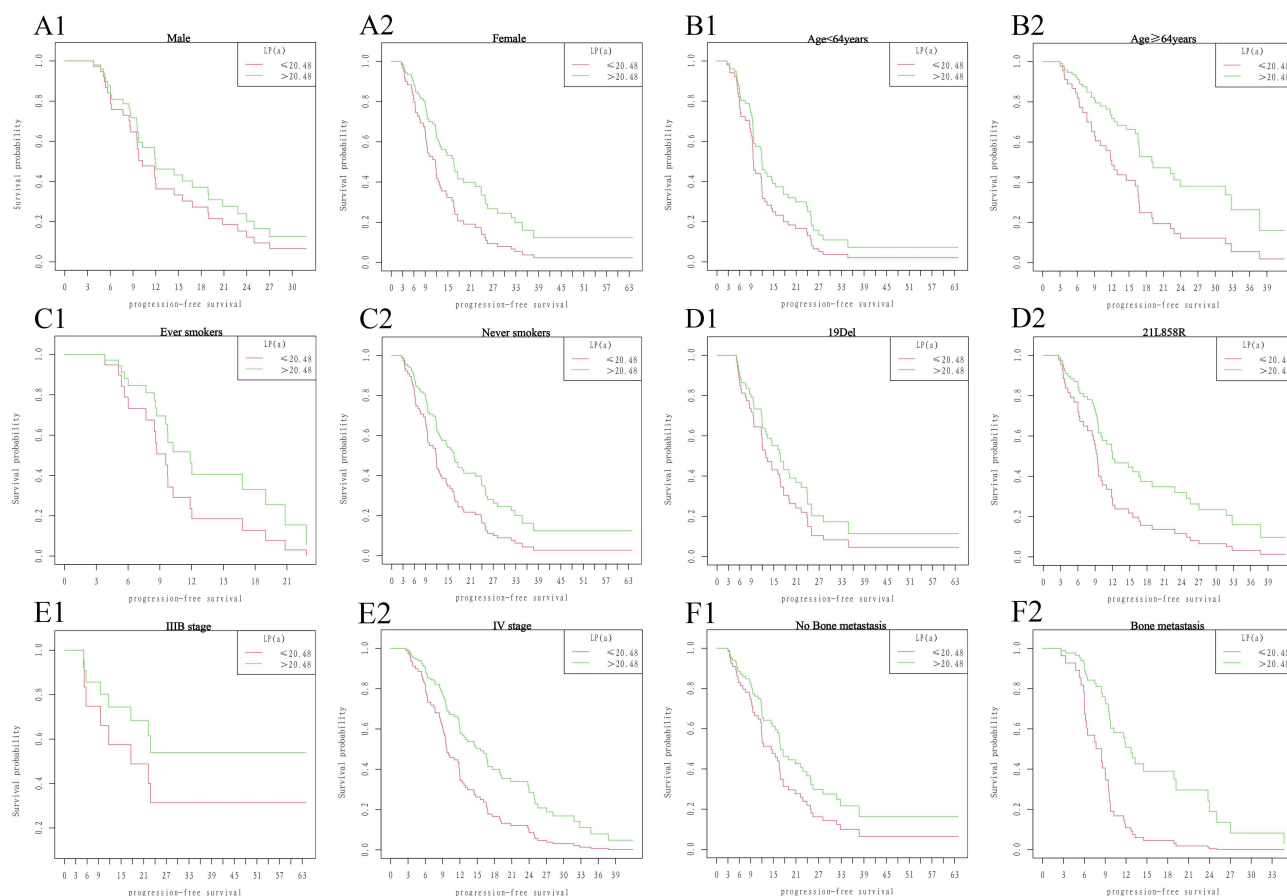


Figure 5 Progression-free survival (PFS) stratified by Lp(a) level among Patients with advanced lung adenocarcinoma in different stratifications. (A1–A2) Stratified by gender; (B1–B2) stratified by age; (C1–C2) stratified by Smoking history; (D1–D2) stratified by EGFR genotype; (E1–E2) stratified by TNM stage; (F1–F2) stratified by Bone metastasis.

options for hospitals and patients who are temporarily unable to carry out EGFR mutation testing, and further screen the population suitable for EGFR-TKIs targeted therapy, thus achieving the goals of precise treatment, effective treatment, and avoiding the waste of medical resources.

Table 4 Univariate Cox Regression Analysis of Prognostic Factors for PFS

Characteristic	N	Event N	HR	95% CI	p value
Gender					
Male	44	33	—	—	
Female	79	60	0.79	0.52, 1.22	0.289
Age (years)					
<64	62	54	—	—	
≥64	61	39	0.74	0.49, 1.11	0.145
Smoking history					
Ever smokers	26	19	—	—	
Never smokers	97	74	0.56	0.33, 0.95	0.031*
Chronic pulmonary disease					
No	111	86	—	—	
Yes	12	7	0.87	0.40, 1.88	0.717
EGFR genotype					
19DEL	68	47	—	—	
21L858R	55	46	1.39	0.92, 2.10	0.113

(Continued)

Table 4 (Continued).

Characteristic	N	Event N	HR	95% CI	p value
TNM stage					
IIIB	16	8	—	—	
IV	107	85	2.27	1.09, 4.73	0.028*
Intrapulmonary metastasis					
No	93	68	—	—	
Yes	30	25	1.14	0.72, 1.81	0.571
Brain metastasis					
No	76	54	—	—	
Yes	47	39	1.11	0.73, 1.67	0.626
Hepatic metastasis					
No	108	81	—	—	
Yes	15	12	1.36	0.73, 2.50	0.330
Bone metastasis					
No	80	53	—	—	
Yes	43	40	2.06	1.36, 3.13	<0.001***
Pleural metastasis					
No	77	60	—	—	
Yes	46	33	0.90	0.58, 1.38	0.624
Metastasis number					
<2	71	48	—	—	
≥2	52	45	1.50	1.00, 2.27	0.051
Type of EGFR TKIs					
1st	61	53	—	—	
2nd	10	7	1.34	0.60, 2.98	0.470
3rd	52	33	0.58	0.37, 0.89	0.014*
Lp(a) (mg/L)					
≤20.48	61	50	—	—	
>20.48	62	43	0.60	0.40, 0.91	0.016*

Notes: *Indicates p value less than 0.05 level, ***Indicates p value less than 0.001 level.

Abbreviations: PFS, progression-free survival; EGFR, epidermal growth factor receptor; TKIs, tyrosine kinase inhibitors; 1st, first-generation EGFR-TKIs; 2nd, second-generation EGFR-TKIs; 3rd, third-generation EGFR-TKIs; Lp(a), lipoprotein(a); HR = hazard ratio; CI, confidence interval.

Previous epidemiological studies have found that EGFR mutations in lung cancer are related to factors such as smoking history, race, gender, and histological type. Compared to Western countries, Asian countries have a higher EGFR mutation rate in lung adenocarcinoma patients, reaching as high as 51.4%, especially among women and non-smokers.³ Consistent with previous findings, our study results show that the EGFR mutation rate in lung adenocarcinoma patients is as high as 55.3%, with significantly higher rates in women and non-smokers compared to men and smokers.

In addition, we found that EGFR mutation status was closely related to pretreatment Lp(a) levels, and the EGFR mutation rate was markedly elevated in the cohort with low levels of Lp(a) compared to those with high levels of Lp(a), which was verified to be an independent predictor of EGFR mutation by multivariate analysis. We know that genetic mutations are often caused by external factors such as environmental influences and genetic susceptibility. It is well known that EGFR mutations, such as 19 Del or 21 L858R, lead to persistent activation of EGFR, even in the absence of a ligand.²⁶ This promotes uncontrolled cell growth and survival, thereby facilitating cancer development. Similarly, lipid metabolism can also impact EGFR expression and its signaling pathways.^{27,28} It is well known that lipid rafts, as “signaling platforms” of the cell membrane, provide an environment for the activation of various signaling pathways, including those related to cancer.²⁹ Studies have found that EGFR signaling events depend on the cholesterol content in lipid rafts, depletion of cholesterol in lipid rafts can promote ligand-independent activation of EGFR.¹⁰ Therefore, it is possible that EGFR mutations are somehow associated with lipids. This speculation was further confirmed in clinical

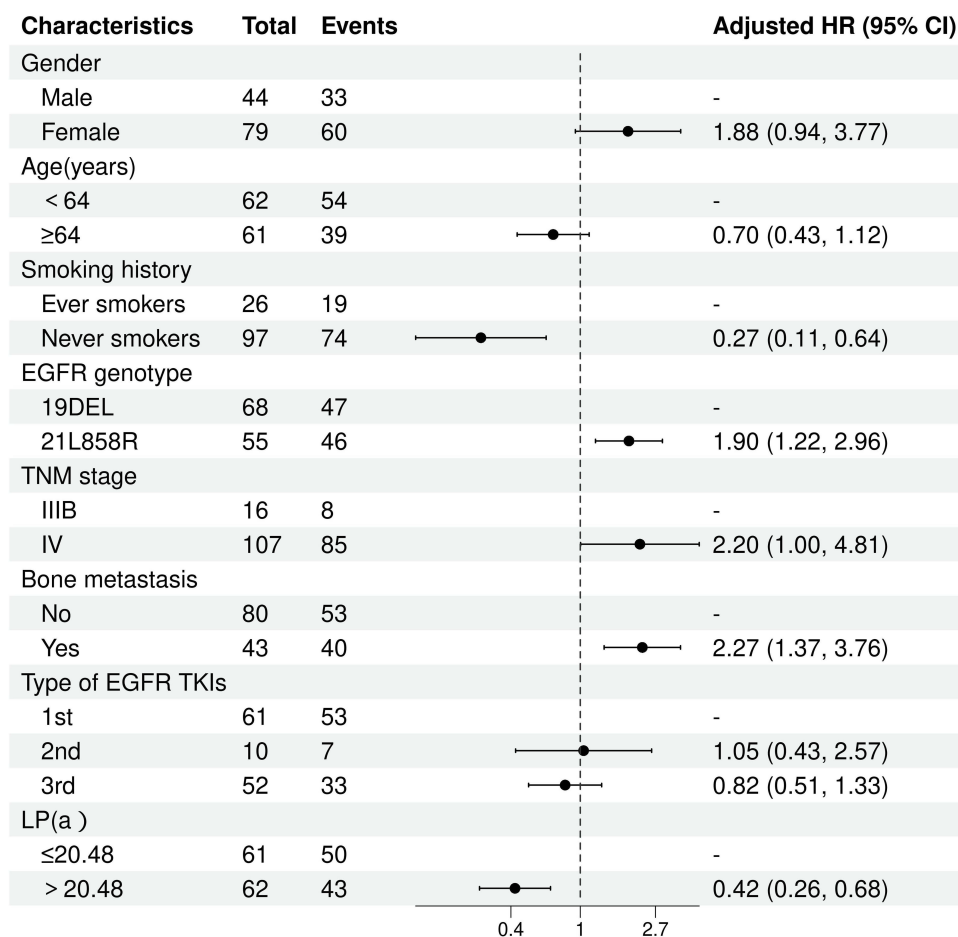


Figure 6 Multivariate Cox regression analysis of prognostic factors for PFS Forest plot.

studies by Lv et al who have shown that high levels of HDL-C are independent predictors of EGFR mutations, with the EGFR mutation rate in the high HDL-C group being significantly higher than that in the low HDL-C group.¹³ In our study, lung adenocarcinoma patients in the low Lp(a) group exhibited a higher EGFR mutation rate, and multivariate analysis confirmed that Lp(a) is an independent predictor of EGFR mutations. In summary, both basic and clinical research suggest that lipids are associated with EGFR mutations. Lp(a) acts as a cholesterol transporter in the circulation, carrying cholesterol to peripheral tissues where it accumulates,³⁰ potentially leading to an increase in cholesterol content within lipid rafts. Therefore, low levels of Lp(a) may affect cholesterol levels in lipid rafts and thus promote EGFR activation. Several other studies support this theory.^{31,32} Cholesterol depletion not only enhances the high affinity of EGFR for EGFR-TKIs, but even reverses the sensitivity of resistant cells to EGFR-TKIs. The above findings suggest that lipid metabolism is closely related to EGFR mutation status.

It has been established that angiostatin (proteolytic fragment of plasminogen) can inhibit tumor metastasis and angiogenesis.¹⁷ Based on the high homology (78% to 100%) between the cDNA sequence of Apo(a) carried by Lp(a) and plasminogen,¹⁸ Lp(a) has been considered to have potential antitumor effects. Recombinant kringle fragments derived from human Apo(a) [known as rhLK68, an anti-angiogenic molecule composed of the kringle structural domain KIV9-KIV10-KV of human Apo(a)] can inhibit angiogenesis in vivo and in vitro. Kim et al found that rhLK68 exhibited dose-dependent inhibition of basic fibroblast growth factor-stimulated proliferation and migration of human umbilical vein endothelial cells in vitro and suppressed the growth of human lung (A549) and colon (HCT15) tumors in nude mice in vivo, with tumor immunohistochemical examinations and in situ hybridization analyses showing a significant reduction in vascular density.²¹ Similarly, Lee et al demonstrated that LK68 significantly inhibited the proliferation and migration of human umbilical vein endothelial cells in vitro and suppressed the growth of transplanted hepatocellular

carcinoma tumors in mice in vivo.³³ An 11-amino acid short peptide derived from the kringle V domain was also shown to have anti-tumor activity.³⁴ All these basic studies support that Lp(a) has anti-tumor effects. A large-scale, multicenter, population-based cohort study conducted in Japan found that low Lp(a) concentrations are associated not only with cancer mortality (including various primary malignancies such as lung cancer) but also with all-cause mortality.³⁵ Another study also identified Lp(a) as a protective factor against the occurrence of lung cancer,³⁶ which is consistent with our findings.

Our study indicates that patients with high pretreatment Lp(a) levels have better survival rates and longer PFS, with a 58% reduced risk of disease progression compared to those with low levels. Multivariate analysis confirms that Lp(a) is a predictive marker for PFS in patients with advanced lung adenocarcinoma receiving EGFR-TKIs. This may be related to high Lp(a) levels inhibiting EGFR activation, as well as the potential antitumor effects of Lp(a) itself. However, some clinical studies report conflicting results. For instance, Li et al found that Lp(a) levels were significantly higher in patients with head and neck squamous cell carcinoma compared to healthy controls, with elevated Lp(a) linked to poorer prognosis.³⁷ This suggests that Lp(a) levels may vary depending on tumor type and histological type, as they are largely influenced by genetic factors.¹⁶ And the study included patients from stages I to IV, but did not perform stratified analysis for staging in the survival analysis, so this prognostic conclusion requires further validation. Additionally, Yang et al also observed that male primary lung cancer patients had elevated Lp(a) levels compared to healthy controls, with a significant positive correlation between tumor stage and Lp(a) levels in stages I–III ($R = 0.162$, $p = 0.006$).³⁸ However, this study focused exclusively on male lung cancer patients from stages I to IV, primarily those with squamous cell carcinoma (272 participants), and included only 65 healthy male controls, which differs significantly from our study population. The study found that Lp(a) levels decreased in stage IV patients compared to those in stage III ($p=0.03$), and the association between Lp(a) and lung cancer risk was influenced by smoking variables. When smoking was included in the model, the independent association between Lp(a) and lung cancer risk disappeared. Therefore, the study was affected by various factors, including the study population, the number of controls, and confounding factors such as smoking. Overall, our current finding suggests that pretreatment Lp(a) may be a potential biomarker for EGFR mutations and prognosis in advanced lung adenocarcinoma patients. However, this conclusion still needs to be further validated in a large multi-centre prospective cohort study.

This study faces several limitations: firstly, as a single-center retrospective study, which lacks a validation cohort, and to avoid introducing bias, future related studies need to be carried out in other centres to further validate the findings of this study. Secondly, a small number of patients had received short-term chemotherapy and antiangiogenic therapy along with EGFR TKIs, which may introduce some confounding factors. Thirdly, retrospective studies are not precise in assessing patients' physical condition, which limits the analysis of the impact of this factor on prognosis. Lastly, due to the limited follow-up time and sample size, the lack of correlation analysis between Lp(a) and overall survival (OS) did not allow for a better and comprehensive assessment of the prognostic value of Lp(a); therefore, future follow-up will be continued and the sample size will be further expanded to analyze the correlation between Lp(a) and OS.

Conclusion

In conclusion, this study found for the first time that low levels of Lp(a) were independently associated with high rate of EGFR mutations and poor prognosis in patients with advanced lung adenocarcinoma treated with EGFR-TKIs. Pretreatment Lp(a) may be an inexpensive and practical predictor for predicting EGFR mutations and the PFS of patients with advanced lung adenocarcinoma undergoing EGFR-TKIs treatment. Although the underlying mechanisms need to be further explored, this finding may provide guidance for clinical diagnosis and therapeutic strategies, especially in general primary hospitals.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. Data are not made publicly available due to privacy or ethical restrictions.

Ethics Approval and Consent to Participate

This study was approved by the Biomedical Research Ethics Committee of the Second Affiliated Hospital of Nanchang University. Informed consent was waived because of retrospective design. During the study, patient data confidentiality and compliance with the Declaration of Helsinki were followed.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The author(s) report no conflicts of interest in this work.

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