ISSN: 3032-3134 (ONLINE)
InaJBCS Indonesian Journal of Biomedicine and Clinical Sciences

EGFR mutation based on lung laterality in adenocarcinoma type of non-small cell lung cancer

Ryan Feraldy Haroen[1], Paranita Ferronika[1], Rita Cempaka[1], Indrawati[1], Bening Rahimi Titisari[1], Vincent Lau[1], Andrew Nobiantoro Gunawan[1], Brigitta Natasya Halim[1], Vincent Laiman[2], Lina Choridah[2], Didik Setyo Heriyanto[1]*

[1]Department of Anatomical Pathology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada/Dr Sardjito General Hospital, Yogyakarta, Indonesia, [2]Department of Radiology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada/Dr Sardjito General Hospital, Yogyakarta, Indonesia

https://doi.org/10.22146/inajbcs.v56i3.15943

Submitted: 2023-10-22
Accepted : 2024-06-06

ABSTRACT

Targeted therapies have shown promise in improving survival rates for lung adenocarcinoma, a common and deadly malignancy. *EGFR*-targeting tyrosine kinase inhibitors (TKIs) are particularly effective among these therapies in cases with *EGFR* mutations. Detecting these mutations before TKI treatment is essential. Various radiological features have been linked to *EGFR* mutations. However, the relationship between tumor location and mutation types in Indonesian lung adenocarcinoma patients remains unexplored. This study aimed to identify the frequency of *EGFR* mutation in local lung adenocarcinoma cases based on the tumor location. Clinical data of lung adenocarcinoma patients (n = 272) diagnosed between 2018 and 2022 were retrospectively taken from the Department of Anatomical Pathology, Dr. Sardjito General Hospital, Yogyakarta. The qRT-PCR data of *EGFR* mutation status was obtained from the Department of Anatomical Pathology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta. Descriptive analysis was performed using STATA version 14.0. *EGFR* mutations were found in 60.7% of patients, with 58.2% having exon 19 mutations and 21.2% exhibiting exon 21 L858R mutations. Mutation status was found to be significantly different based on the patient's gender (p = 0.022) and age (p = 0.029) but not with lung laterality (p = 0.093). The proportion of exon 19, exon 21 L858R, and uncommon mutations in the right and left lung adenocarcinoma was similar across all samples. This study found no difference between specific *EGFR* mutation types and tumor location in lung adenocarcinoma.

ABSTRAK

Terapi berbasis target telah menunjukkan hasil yang menjanjikan dalam meningkatkan kesintasan untuk pasien dengan adenokarsinoma paru, suatu penyakit ganas yang sering terjadi dan mematikan. Inhibitor tirosin kinase (TKI) yang menargetkan *EGFR* telah diketahui efektif di antara terapi-terapi yang ada pada kasus dengan mutasi *EGFR*. Mendeteksi mutasi-mutasi ini sebelum pengobatan TKI adalah hal esensial. Berbagai ciri radiologis telah dikaitkan dengan mutasi *EGFR*, namun perbedaan antara lokasi tumor dan tipe mutasi pada pasien adenokarsinoma paru di Indonesia masih belum diketahui. Penelitian ini bertujuan untuk mengidentifikasi frekuensi mutasi *EGFR* pada kasus adenokarsinoma paru lokal berdasarkan lokasi tumor. Data klinis pasien adenokarsinoma paru (n = 272) yang didiagnosis antara tahun 2018 dan 2022 diambil secara retrospektif dari Departemen Patologi Anatomi, Rumah Sakit Umum Pusat Dr. Sardjito, Yogyakarta. Data qRT-PCR dari status mutasi *EGFR* diperoleh dari Departemen Patologi Anatomi, Fakultas Kedokteran, Kesehatan Masyarakat, dan Keperawatan, Universitas Gadjah Mada, Yogyakarta. Analisis deskriptif dilakukan menggunakan STATA versi 14.0. Mutasi *EGFR* ditemukan pada 60,7% pasien, dengan 58,2% memiliki mutasi ekson 19 dan 21,2% menunjukkan mutasi ekson 21 L858R. Status mutasi ditemukan memiliki perbedaan signifikan pada perbedaan jenis kelamin pasien (p = 0,022) dan usia (p = 0,029) tetapi tidak dengan lateralitas paru (p = 0,093). Proporsi mutasi ekson 19, ekson 21 L858R, dan mutasi tidak umum memiliki proporsi yang serupa antara paru kanan dan kiri pada keseluruhan sampel. Penelitian ini tidak menemukan perbedaan signifikan antara tipe mutasi *EGFR* spesifik dan lokasi tumor pada adenokarsinoma paru.

Keywords:
adenocarcinoma;
EGFR;
laterality;
lung;
tumor locationp

*corresponding author: didik_setyoheriyanto@mail.ugm.ac.id

INTRODUCTION

Lung carcinoma, a malignant neoplasm originating from the rapid proliferation of epithelial cells within the pulmonary system,[1] is a considerable health concern. In Southeast Asia, approximately 20% of cancer diagnoses are lung carcinoma.[2] This disease not only stands as a global primary mortality contributor but also has the highest fatality rate (14.1%) including in Indonesia.[3,4] A previously study has found that the five-year survival probability for individuals diagnosed with lung carcinoma between 2010 and 2014 hovered at a mere 10-20%.[5] This worrisome statistic can be attributed to the lack of early-stage lung carcinoma screening methodologies and the suboptimal efficacy of systemic therapeutic approaches, both of which substantially impact survival rates, particularly among cases in advanced stages.[6,7] Notably, an alarming 90% of Indonesian lung carcinoma patients receive their diagnosis only after the disease has progressed significantly.[8]

Recent advancements have given insights into the realm of genetic mutations as an innovative strategy for managing non-small cell lung cancer (NSCLC), particularly the subtype of adenocarcinoma. At present, the identification and treatment stratification for adenocarcinoma hinge upon the molecular characterization of tumors to enhance patient prognoses through more personalized therapies.[6] According to guidelines stipulated by the National Comprehensive Cancer Network (NCCN), among the pivotal molecular assessments is the examination of genetic alterations within the *EGFR* gene.[9] Across Asian populations, *EGFR* mutations manifest in approximately 40-60% of lung adenocarcinoma cases, contrasting with figures of around 12-15% in Caucasian counterparts.[10] The existence of *EGFR* gene mutations gave path for targeted therapy, which are the tyrosine kinase inhibitors (TKIs).

Correct utilization of the first, second or third generation of *EGFR*-TKI therapy in NSCLC significantly improves response rates and enhanced survival outcomes.[11] The effectiveness and resistance of different *EGFR*-TKI generations depend on the subtype of *EGFR* mutation that occurs, which are classified as the common mutations (deletion in exon 19 or L858R mutation in exon 21) and the uncommon mutations (other mutations along exon 18 to exon 21).[10,12,13] This necessitates *EGFR* mutation analysis before initiating therapy. Nonetheless, Indonesia's molecular testing infrastructure could be improved, to reduce treatment delays. As such, investigations are imperative to predict the *EGFR* mutation status.

Research has been undertaken to assess EGFR mutation status in adenocarcinoma based on tumor anatomical location via radiological methodologies. A 2016 study in Taiwan by Tseng *et al.*[14] revealed a higher prevalence of EGFR mutations (71%) in women with upper lobe adenocarcinoma compared to men harboring lower lobe adenocarcinoma (47%). They also reported[14] that the L858R mutation within exon 21 exhibited more significant predominance in upper lobe tumors relative to the exon 19 deletion and wild-type variants. In 2017, Shi *et al.*[15] published a study which assessed both common *EGFR* mutations in comparison to the wild type, and found similar tendency regarding the lobar occurrence, but it was not reported to be statistically different. Nevertheless, similar studies in terms of predicting *EGFR* mutation subtypes based on lung adenocarcinoma tumor localization are still lacking in Indonesia. Thus, additional research in this domain remains essential.

MATERIAL AND METHODS

Study design

This study constitutes a retrospective observational-analytical study employing a cross-sectional

methodology. The primary objective is to assess the difference between tumor location based on radiological discoveries and the *EGFR* mutation status within lung adenocarcinoma cases documented at the Department of Anatomical Pathology, Dr. Sardjito General Hospital, Yogyakarta, between 2018 and 2022. Slides from patients diagnosed with adenocarcinoma type non-small cell lung carcinoma by cytopathology were included. These slides were stored in enclosed drawers at room temperature. The radiological data were accessed via the hospital's electronic medical records system. Initially, 438 lung adenocarcinoma cases were collected. However, cases with inadequate radiological data or specimens unsuitable for PCR examination were excluded. This resulted in a dataset of 272 patients, including 213 who had CT-guided transthoracic needle aspiration (TTNA) and 59 who underwent pleural puncture. PCR examination for *EGFR* mutation was performed at the Department of Anatomical Pathology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada. The study adhered to the principles outlined in the Declaration of Helsinki. Specimen and data collection was approved by the Medical and Health Research Ethics Committee (MHREC), Universitas Gadjah Mada (reference number: KE-FK-0291-EC-2023).

DNA extraction

DNA extraction was performed from cytologic slides or cell blocks using GeneAll® Exgene™ Clinic SV mini 108-101 (Gene All Biotechnology Co., Ltd., Seoul, Korea) in adherence with the manufacturer's protocol.

Quantitative real-time polymerase chain reaction (qRT-PCR)

The DNA assay was done using The Human *EGFR* Gene Mutations Fluorescence Polymerase Chain Reaction (PCR) Diagnostic Kit by Amoy Diagnostics Co., Ltd. (Xiamen, China), which covers G719A, G719S, G719C, T790M, S768I, L858R, L861Q mutations, three insertions in exon 20, and 19 base changes in exon 19. Bioneer Exicycler™ 96 Real-Time Quantitative Thermal Block was utilized for quantitative PCR examination. The application of real-time PCR in this study was preferred as it is more efficient than conventional PCR, while also being in accordance with previous study.[15] The diagnostic kit and the PCR system were per the manufacturer's guidelines.

The DNA region of interest was amplified using PCR with specific primers and probes for mutant gene detection. Amplification continued until double-stranded DNA separation occurred. The mutation detection was based on the cycle threshold (Ct) values and would be determined as positive (present) if there were any signals exceeding the kit background fluorescence. For every cycle we conduct, we consistently utilize both positive and negative controls of the kit to ensure the accuracy of mutation detection. The real-time PCR included an initial denaturation cycle at 95 °C for 5 min, followed by 15 cycles of annealing at 95 °C for 25 sec and 64 °C for 20 sec, and then 31 cycles of extension at 93 °C for 25 sec, 60 °C for 35 sec, and 72 °C for 20 sec.

Data analysis

The PCR results for *EGFR* mutations were classified according to the previous literatures,[12,15] as wild-type, common mutation, and uncommon mutation. Negative *EGFR* mutation PCR result was considered as wild-type. Deletion in exon 19 and L858R mutation in exon 21 were both classified as the common mutations, while the other detected mutations were classified as the uncommon mutations.

Statistical analysis was conducted through STATA software version 14, whereby data consisting of age, gender, tumor location and *EGFR* mutation status was analyzed with chi-square test. In

cases where the data did not align with the prerequisites for the chi-square test, Fisher's exact test was implemented. The proportional difference would be considered statistically different if the p-value is less than 0.05.

RESULTS

Among the study cohort, 153 individuals (56.3%) were female, while 119 (43.7%) were male. The mean age of the participants was 60 yr, predominantly comprising those aged >50 (78.7%), with the oldest participant being 92 y.o. and the youngest being 17 y.o. The prevalence of adenocarcinoma was notably higher in the right lung, accounting for 59.2% of cases, in contrast to the left lung (39.3%), and a minority manifested in both lungs simultaneously (1.5%). Most adenocarcinoma patients (60.7%) exhibited a positive *EGFR* mutation status. The most prevalent EGFR mutation was identified in exon 19, manifesting in 96 individuals (58.2%), followed by the L858R mutation within exon 21, observed in 35 patients (21.2%). A comprehensive overview of the subjects' attributes is provided in TABLE 1.

Within the scope of this investigation, a significant difference was identified between *EGFR* mutations and gender (p=0.022), as well as age (p=0.029). Regarding the tumor's distribution, the *EGFR* mutation status demonstrated no statistically significant link to lung laterality (p=0.226). Nevertheless, an observation emerged indicating a marginally elevated frequency of *EGFR* mutations within the right lung relative to the left lung. The specific difference between *EGFR* mutation status and gender, age, and lung laterality are outlined in TABLE 2.

TABLE 1. Characteristics of study subjects

Characteristics	Frequency
Gender	
Male	119 (43.7)
Female	153 (56.3)
Age [median (min. – max.) yr]	59 (17-92)
≤ 50 [n (%) yr]	58 (21.3)
> 50 yr [n (%) yr]	214 (78.7)
Lung laterality [n (%)]	
Right	161 (59.2)
Left	107 (39.3)
Bilateral	4 (1.5)
EGFR mutation [n (%)]	
Mutation (+)	165 (60.7)
Exon 19	96 (58.2)
Exon 21 (L858R)	35 (21.2)
Uncommon	34 (20.6)
Mutation (-)	107 (39.3)
Total [n (%)]	272 (100)

EGFR: epidermal growth factor receptor

TABLE 2. *EGFR* mutation status based on gender, age and lung laterality

Parameter	Mutation status [n (%)]		p
	Negative	Positive	
Gender			
Male	56 (47.1)	63 (52.9)	0.022*
Female	51 (33.3)	102 (66.7)	
Age			
≤ 50 yr	30 (51.7)	28 (48.3)	0.029*
> 50 yr	77 (36.0)	137 (64.0)	
Laterality			
Right	66 (41.0)	95 (59.0	
Left	38 (35.5)	69 (64.5)	0.226+
Bilateral	3 (75.0)	1 (25.0)	

EGFR: epidermal growth factor receptor; *Analyzed using chi-square test; +Analyzed using Fischer's exact test.

TABLE 3. Frequency of mutation in exon 19, 21 (L858R), and others based on lung laterality

Lung laterality	Exon mutation [n (%)]			p*
	19	21 (L858R)	Uncommon	
Right	55 (57.9)	24 (25.3)	16 (16.8)	
Left	41 (59.4)	10 (14.5)	18 (26.1)	0.093
Bilateral	0 (0.0)	1 (100)	0 (0.0)	

*Analyzed using Fisher's exact test.

The prevailing mutations identified within the right and left lungs during this study predominated on exon 19. Furthermore, it was observed that the proportion of exon 19, exon 21 L858R, and uncommon mutations in the right and left lung adenocarcinoma was similar across all samples. TABLE 3 shows the frequency distribution of common mutations within exon 19, exon 21 (L858R), and the uncommon mutations categorized by lung laterality.

TABLE 4 demonstrates the frequency distribution of exon 19 and exon 21 (L858R) mutations alongside the uncommon mutations, focusing on gender and age aspects within each side of lung. Notably, across all age groups and genders in both lungs, the most prevalent mutations occurred within exon 19. The right lung exhibited the highest prevalence of exon 21 (L858R) mutation among older female patients diagnosed with lung adenocarcinoma. In contrast, it was observed that the uncommon mutations were primarily localized in the left lung of younger female patients. Nevertheless, no differences were identified.

TABLE 4. Frequency of mutation in exon 19, 21 (L858R), and others based on lung laterality, stratified by gender and age

Gender	Age (yr)	Lung laterality	*EGFR* mutation [n (%)]			p*
			19	21(L858R)	Uncommon	
Male	≤ 50	Right	4 (66.7)	1 (16.7)	1 (16.7)	-
		Left	0 (-)	0 (-)	0 (-)	
		Bilateral	0 (-)	0 (-)	0 (-)	
	> 50	Right	19 (65.5)	5 (17.2)	5 (17.2)	1.000
		Left	18 (64.3)	4 (14.3)	6 (21.4)	
		Bilateral	0 (-)	0 (-)	0 (-)	
Female	≤ 50	Right	8 (80)	2 (20)	0 (0)	0.207
		Left	6 (50)	2 (16.7)	4 (33.3)	
		Bilateral	0 (-)	0 (-)	0 (-)	
	> 50	Right	24 (48)	16 (32)	10 (20)	0.156
		Left	17 (58.6)	4 (13.8)	8 (27.6)	
		Bilateral	0 (0)	1 (100)	0 (0)	

EGFR: epidermal growth factor receptor; *Analyzed using Fisher's exact test.

DISCUSSION

Adenocarcinoma of the lung stands as the most documented subtype within non-small cell lung cancer (NSCLC), as indicated by multiple studies[16,17] and is associated with a notably high global mortality rate, as reported by Sung *et al.*[4] in 2021. Notably, *EGFR* mutations exhibit a higher incidence among patients with lung adenocarcinoma in Asia than in other continents. At the same time, Europe showcases the lowest prevalence, as noted in studies by Malapelle *et al.*[10] in 2021 and Melosky *et al.*[18] in 2022. Identifying *EGFR* mutations in adenocarcinoma of the lung opens avenues for targeted therapeutic interventions, potentially yielding improvements in overall prognosis.[11]

In this study, lung adenocarcinoma predominantly affected females (56.3% of all cases), as previously reported by Barta *et al.*,[19] with a more pronounced gender disparity in Asia due to indoor cooking smoke exposure.[20] Individuals over 50 years accounted for 78.7% of diagnoses, consistent with a study by Zhou *et al.*,[21] which is likely to be related to age-associated oncogenic mutation accumulation.[22] Adenocarcinoma was more prevalent in the right lung, attributed to its anatomical features.[23,24] *EGFR* mutations were identified in 60.7% of cases, primarily exon 19 (58.2%) and exon 21 L858R mutations (21.2%), in line with previous research findings.[25,26] The detection of exon 19 deletions and L858R mutations in this study was noteworthy as lung adenocarcinoma with these common mutations are sensitive with the more widely available first-line therapy of EGFR-TKIs, such as gefitinib, erlotinib and afatinib.[13,15] This phenomenon was thought to be related with the specific protein alteration by common mutations that lower the affinity of adenosine triphosphate (ATP) in contrast to the wild-type receptor, and making it more responsive to EGFR-TKI instead.[13]

This study underscores a notable difference in the prevalence of positive *EGFR* mutations between genders, with 66.7% of cases occurring in females. This observation is consistent with earlier research, which reported a heightened

occurrence of *EGFR* mutations in females, with frequencies ranging from 54.9 to 71.5%. This trend is particularly pronounced in Asian populations, including Indonesia, as documented in a Syahruddin *et al*.,[27] study in 2018. One hypothesis was that Asian women may exhibit elevated estrogen levels compared to the Caucasian population.[28] Also, a correlation between estrogen receptors and *EGFR* mutations in lung adenocarcinoma has been suggested.[29]

The high prevalence of lung adenocarcinoma exhibiting positive *EGFR* mutations among the elderly population (64%) in this study aligns with the earlier investigation.[22] *EGFR* mutations, besides being linked to the genetic changes that accumulate with age, are also characterized by their dormant nature, rendering them more challenging to detect at a younger age, as stated by Herceg and Hainaut.[30] In contrast, younger individuals diagnosed with adenocarcinoma of the lung tend to manifest a more aggressive disease profile and a rarer subtype of *EGFR* mutation, culminating in a less favorable prognosis and response to EGFR-TKI therapy, a phenomenon elucidated by Hsu *et al*.[31]

The prevalence of *EGFR*-mutant tumors in the right lung was found to be lower at 59% compared to 64.5% in the left lung, this is in contrast with the non-mutated *EGFR* group. It's important to note that while this observation did not reach statistical significance, it is in contrast with previous study conducted in Asian populations.[24] Furthermore, this study's findings align with those of Rizzo *et al*.,[32] in Wisconsin, United States, involving a sample of 280 patients, which similarly reported no difference between tumor location and *EGFR* mutation status in lung adenocarcinoma.

In this investigation, no difference was observed between the affected lung in adenocarcinoma and the specific subtypes of *EGFR* mutations, a finding consistent with prior research conducted by Rizzo *et al*.[32] However, there were variations in the distribution of *EGFR* mutation frequencies. While the occurrence of exon 19 mutations was comparable in both lungs, the L858R mutation in exon 21 manifested more frequently in the right lung, in contrast to uncommon mutations, which displayed a higher prevalence in the left lung.

A previous study by Shi *et al.*[15] involving 179 EGFR-mutant adenocarcinoma patients, indicated that both exon 19 and L858R mutations tended to occur more often in primary tumors of the right lung, at 59% and 57%, respectively. Similarly, Isaka *et al*.[33] presented a higher frequency of both exon 19 (53.4%) and exon 21 (59.7%) of *EGFR*-mutated tumors in the right lung among 212 Japanese lung adenocarcinoma patients, although statistical significance was not reached. The inconsistencies in these findings compared to our study may stem from differences in the proportional distribution of *EGFR* mutation subtypes. Both previous studies observed a higher frequency of exon 21 mutations relative to exon 19 mutations in their respective populations, hinting at potential racial variations in their prevalence.

The current study provides a comprehensive profile of *EGFR* mutations prevalent in Indonesian patients, revealing that 58.2% have exon 19 mutations and 21.2% exhibit exon 21 L858R mutations. This information is crucial for clinicians to make informed decisions regarding targeted therapies. By analyzing the relationship between tumor location and *EGFR* mutation types, the study concludes that there is no difference between specific *EGFR* mutation types and tumor location. This finding challenges previous assumptions and underscores the need for further investigation in this area. The study focuses on a specific population in Yogyakarta, Indonesia. Future research

should include a more diverse geographic and sample representation to validate these findings and understand regional variations in *EGFR* mutation prevalence. Additionally, the current study did not examine exon 20 mutations of the *EGFR*. Further research is required as new targeted therapies, such as amivantamab, have been developed to target *EGFR* exon 20 mutations previously resistant to tyrosine kinase inhibitors.

CONCLUSION

The prevalence of *EGFR*-mutant lung adenocarcinoma at Dr. Sardjito General Hospital is 60.7%. *EGFR* mutations are more prevalent in females and individuals aged over 50 years. The study did not observe any differences in the proportions of *EGFR* mutation subtypes with respect to lung laterality.

ACKNOWLEDGEMENTS

We would like to thank Nur Eka Wiraditya for generously providing the essential data required for this study.

REFERENCES

1. Borczuk AC. WHO classification of tumours: thoracic tumours. International Agency for Research on Cancer; 2021.
2. Sharma R. Mapping of global, regional and national incidence, mortality and mortality-to-incidence ratio of lung cancer in 2020 and 2050. Int J Clin Oncol 2022; 27(4):665-75. https://doi.org/10.1007/s10147-021-02108-2
3. Ferlay J, Ervik M, Lam F, Laversanne M, Colombet M, Mery L, Piñeros M, *et al.* Global cancer observatory: cancer today. Lyon, France: International Agency for Research on Cancer 2024. https://gco.iarc.who.int/media/globocan/factsheets/populations/360-indonesia-fact-sheet.pdf
4. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71(3):209-49. https://doi.org/10.3322/caac.21660
5. Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Nikšić M, *et al*. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet 2018; 391(10125):1023-75. https://doi.org/10.1016/S0140-6736(17)33326-3
6. Brainard J, Farver C. The diagnosis of non-small cell lung cancer in the molecular era. Mod Pathol 2019; 32(Suppl 1):16-26. https://doi.org/10.1038/s41379-018-0156-x
7. Socinski MA, Evans T, Gettinger S, Hensing TA, Sequist LV, Ireland B, *et al*. Treatment of stage IV non-small cell lung cancer: Diagnosis and management of lung cancer: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013; 143(5):e341S-68S. https://doi.org/10.1378/chest.12-2361
8. Harsal A, Suratman E, Tambunan T. P1-038: Overview of lung cancer in Dharmais National Cancer Hospital, Jakarta, Indonesia. J Thor Oncol 2007; 2(8):S564. https://doi.org/10.1097/01.JTO.0000283652.11456.81
9. Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman JR, Bharat A, *et al*. Non–small cell lung cancer, version 3.2022, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 2022; 20(5):497-530. https://doi.org/10.6004/jnccn.2022.0025

10. Malapelle U, Pilotto S, Passiglia F, Pepe F, Pisapia P, Righi L, *et al*. Dealing with NSCLC EGFR mutation testing and treatment: A comprehensive review with an Italian real-world perspective. Crit Rev Oncol Hematol 2021; 160:103300. https://doi.org/10.1016/j.critrevonc.2021.103300
11. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsuratani J, *et al*. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010; 11(2):121-8. https://doi.org/10.1016/S1470-2045(09)70364-X
12. Gristina V, Malapelle U, Galvano A, Pisapia P, Pepe F, Rolfo C, *et al*. The significance of epidermal growth factor receptor uncommon mutations in non-small cell lung cancer: A systematic review and critical appraisal. Cancer Treat Rev 2020; 85:101994. https://doi.org/10.1016/j.ctrv.2020.101994
13. Jorge SEDC, Kobayashi SS, Costa DB. Epidermal growth factor receptor (EGFR) mutations in lung cancer: preclinical and clinical data. Braz J Med Biol Res 2014; 47(11):929-39. https://doi.org/10.1590/1414-431X20144099
14. Tseng CH, Chen KC, Hsu KH, Tseng JS, Ho CC, Hsia TC, *et al*. EGFR mutation and lobar location of lung adenocarcinoma. Carcinogenesis 2016; 37(2):157-62. https://doi.org/10.1093/carcin/bgv168
15. Shi Z, Zheng X, Shi R, Song S, Yang R, Zhang Q, *et al*. Radiological and clinical features associated with epidermal growth factor receptor mutation status of exon 19 and 21 in lung adenocarcinoma. Sci Rep 2017; 7(1):364. https://doi.org/10.1038/s41598-017-00511-2
16. Lamberti G, Andrini E, Sisi M, Rizzo A, Parisi C, Federico AD, *et al*. Beyond EGFR, ALK and ROS1: Current evidence and future perspectives on newly targetable oncogenic drivers in lung adenocarcinoma. Crit Rev Oncol Hematol 2020; 156:103119. https://doi.org/10.1016/j.critrevonc.2020.103119
17. Mulawarman A, Haryana SM, Sadewa AH. Ekspresi Serum miR-148 dan miR-155 sebagai kandidat biomarker prognosis kanker paru jenis karsinoma bukan sel kecil (KPKBSK) stage lanjut di Indonesia menggunakan liquid biopsy [Dissertation]. Yogyakarta: Universitas Gadjah Mada; 2020.
18. Melosky B, Kambartel K, Haentschel M, Bennetts M, Nickens DJ, Brinkmann J, *et al*. Worldwide prevalence of epidermal growth factor receptor mutations in non-small cell lung cancer: a meta-analysis. Mol Diagn Ther 2022; 26(1):7-18. https://doi.org/10.1007/s40291-021-00563-1
19. Barta JA, Powell CA, Wisnivesky JP. Global epidemiology of lung cancer. Ann Glob Health 2019; 85(1):8. https://doi.org/10.5334/aogh.2419
20. Nakamura H, Saji H. A worldwide trend of increasing primary adenocarcinoma of the lung. Surg Today 2014; 44(6):1004-12. https://doi.org/10.1007/s00595-013-0636-z
21. Zhou L, Li H, Yang S. Age does matter in adolescents and young adults vs. older adults with lung adenocarcinoma: A retrospective analysis comparing clinical characteristics and outcomes in response to systematic treatments. Oncol Lett 2022; 24(4):362. https://doi.org/10.3892/ol.2022.13482
22. Ueno T, Toyooka S, Suda K, Soh J, Yatabe Y, Miyoshi S, *et al*. Impact of age on epidermal growth factor

receptor mutation in lung cancer. Lung Cancer 2012; 78(3):207-11. https://doi.org/10.1016/j.lungcan.2012.09.006
23. Arslan SA, Aral İP, Altınışık İnan G, Karabuga H, Acikgoz S, Unal I, *et al*. Right predilection of lung cancer, does it affect oncologic outcome?. Acta Oncol Turc 2019; 52(2):232-7. https://doi.org/10.5505/aot.2019.01488
24. Liu G, Xu Z, Ge Y, Jiang B, Groen H, Vliegenthart R, *et al*. 3D radiomics predicts EGFR mutation, exon-19 deletion and exon-21 L858R mutation in lung adenocarcinoma. Transl Lung Cancer Res 2020; 9(4):1212-24. https://doi.org/10.21037/tlcr-20-122
25. Midha A, Dearden S, McCormack R. EGFR mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: a systematic review and global map by ethnicity (mutMapII). Am J Cancer Res 2015; 5(9):2892-911.
26. Quan X, Gao H, Wang Z, Li J, Zhao W, Liang W, *et al*. Epidermal growth factor receptor somatic mutation analysis in 354 Chinese patients with non-small cell lung cancer. Oncol Lett 2018; 15(2):2131-8. https://doi.org/10.3892/ol.2017.7622
27. Syahruddin E, Wulandari L, Sri Muktiati N, Rima A, Soeroso N, Ermayanti S, *et al*. Uncommon EGFR mutations in cytological specimens of 1,874 newly diagnosed Indonesian lung cancer patients. Lung Cancer (Auckl) 2018; 9:25-34. https://doi.org/10.2147/LCTT.S154116
28. Imyanitov EN, Demidova IA, Gordiev MG, Filipenko ML, Kekeyeva TV, Moliaka YK, *et al*. Distribution of EGFR mutations in 10,607 Russian patients with lung cancer. Mol Diagn Ther 2016; 20(4):401-6. https://doi.org/10.1007/s40291-016-0213-4
29. Tanaka K, Shimizu K, Kakegawa S, Ohtaki Y, Nagasima T, Kaira K, *et al*. Prognostic significance of aromatase and estrogen receptor beta expression in EGFR wild-type lung adenocarcinoma. Am J Transl Res 2016; 8(1):81-97.
30. Herceg Z, Hainaut P. Genetic and epigenetic alterations as biomarkers for cancer detection, diagnosis and prognosis. Mol Oncol 2007; 1(1):26-41. https://doi.org/10.1016/j.molonc.2007.01.004
31. Hsu CL, Chen KY, Shih JY, Ho CC, Yang CH, Yu CJ, *et al*. Advanced non-small cell lung cancer in patients aged 45 years or younger: outcomes and prognostic factors. BMC Cancer 2012; 12:241. https://doi.org/10.1186/1471-2407-12-241
32. Rizzo S, Petrella F, Buscarino V, De Maria F, Raimondi S, Barberis M, *et al*. CT radiogenomic characterization of EGFR, K-RAS, and ALK mutations in non-small cell lung cancer. Eur Radiol 2016; 26(1):32-42. https://doi.org/10.1007/s00330-015-3814-0
33. Isaka T, Yokose T, Ito H, Nagata M, Furumoto H, Nishii T, *et al*. Correlations between the EGFR mutation status and clinicopathological features of clinical stage I lung adenocarcinoma. Medicine (Baltimore) 2015; 94(42):e1784. https://doi.org/10.1097/MD.0000000000001784