Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Mar 25; 10(T7):164-169.
https://doi.org/10.3889/oamjms.2022.9275
eISSN: 1857-9655
Category: *T7 - "Neuroscience, Neurology, Psychiatry and Other Health Sciences - 2022"*
Section: *Pulmonology*

Concordance of Epidermal Growth Factor Receptor Mutation from Tissue Biopsy and Plasma Circulating Tumor DNA in Treatment-Naïve Lung Adenocarcinoma Patients

Noni Novisari Soeroso[1]*, Hendra Taufik[1], Setia Putra Tarigan[1], Erna Mutiara[2]

[1]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Sumatera Utara, Universitas Sumatera Utara Hospital, Medan, Indonesia; [2]Department of Biostatistic, Faculty of Public Health, Universitas Sumatera Utara, Medan, Indonesia

Edited by: Ksenija Bogoeva-Kostovska
Citation: Soeroso NN, Taufik H, Tarigan SP, Mutiara E. Concordance of Epidermal Growth Factor Receptor Mutation from Tissue Biopsy and Plasma Circulating Tumor DNA in Treatment-Naïve Lung Adenocarcinoma Patients. Open Access Maced J Med Sci. 2022 Mar 25; 10(T7):164-169.https://doi.org/10.3889/oamjms.2022.9275
Keywords: Plasma circulating tumor DNA; Epidermal growth factor receptor mutation; Liquid biopsy; Lung cancer; Lung adenocarcinoma
***Correspondence:** Noni Novisari Soeroso, Department of Pulmonology and Respiratory Medicine, Faculty of Medicine Universitas Sumatera Utara, Universitas Sumatera Utara Hospital, Jl. Dr. Mansyur No. 66 Medan 20154, Sumatera Utara, Medan, Indonesia. E-mail: noni@usu.ac.id
Received: 07-Mar-2022
Revised: 12-Mar-2022
Accepted: 15-Mar-2022

Funding: Part of the funding for this study was provided by AstraZeneca Indonesia (AZI) and Roche Indonesia (RI). Patients' test results stayed anonymous and inaccessible by AZI and RI.
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Prevalence of Epidermal Growth Factor Receptor (EGFR) mutation in circulating tumor DNA (ctDNA) in treatment-naïve individuals is not well established in Indonesia. In recent years, ctDNA as a specific and sensitive blood-based biomarker had been developed to detect the mutation.

AIM: The study was done to understand the concordance and acceptance levels of ctDNA in detecting the gene mutation in lung adenocarcinoma patients.

METHODS: This study used cross-sectional approach with purposive sampling design in 100 treatment-naïve non-small cell lung cancer and adenocarcinoma patients. Samples were obtained from bronchoscopy and blood, which were examined to detect the mutation in formalin-fixed paraffin-embedded specimens or plasma samples using QIAampDNA Micro Kit. Mutation was calculated by droplet digital polymer chain reaction.

RESULTS: A 100 subjects with primary tumor tissue samples were compared with the plasma samples and mutation was detected in 20 patients (20.0%), 12 (12.0%) on exon 19, 7 (7.0%) on exon 21, and 1 (1.0%) on both exon 19 and 21. Within the plasma samples, mutation was found in 15 patients (15%) with mutation on exon 19 and 21 in 12 (12.0%) and 3 (3.0%) patients, respectively. Within the two samples, concordance of EGFR mutation was 83.0% (83/100, $p < 0.001$; correlation index: 0.42). Assuming presence of mutation as the benchmark, the accuracy of mutation presence in plasma DNA was 60.0% (9/15). Kappa test showed a weak agreement between the mutation in tissues and plasma, with a coefficient of 0.414 (95% confidence interval).

CONCLUSION: Tissue biopsy was still considered as the main option to detect EGFR mutation in lung cancer. More researches on ctDNA as the standardized tools to detect the mutation are required.

Introduction

In Indonesia, the highest incidence rate from lung cancer in males reached 19.4/100,000 population, with an average death rate of 10.9/100,000 population, then liver cancer with an incidence rate of 12.4/100,000 population and average death rate of 7.6/100,000 population. On the other hand, breast cancer caused the highest incidence rate in female with 42.1/100,000 population with an average death rate of 17/100,000 population, followed by cervical cancer, 23.4/100,000 population with an average death rate of 13.9/100,000 population [1].

Lung cancer has the highest incidence and death rate annually, compared to other cancer types, and has been a main health concern in the world, although early-stage lung cancer patients had a higher survival rate, 50%, with 5 years survival post-surgery or stereotactic body radiation therapy [2]. In advanced-stage lung cancer, approximately 69% non-small cell lung cancer (NSCLC) patients could be improved through targeted therapy [3]. Lung cancer is known to be very heterogeneous and more than 85% of the patients were diagnosed with NSCLC [4].

The advancement in lung cancer therapy, especially NSCLC, has shifted the algorithms toward targeted therapies and identification from oncogenic drivers. Molecular tests such as Epidermal Growth Factor Receptor (EGFR), ALK, and ROS1 are the current routine screenings for lung cancer in daily practices [5]. A study in Indonesia by Syahruddin *et al.* reported that from 1874 respondents, EGFR mutations were observed in about 44.4% respondents, with common EGFR mutations (exon 19 ins/dels, L858R) and uncommon mutations (G719X, T790M, and L861Q) of about 57.1% and 29%, respectively. Higher frequency in EGFR mutation was seen in female (52.9%) than male (39.1%) [6]. On the other hand, KRAS molecular testing did not find any mutations within North Sumatera population [7]. Tissue

genotyping has been considered as the gold standard in detecting genetic alterations in tumor. However, only 20–30% of these were detected in NSCLC patients during molecular diagnostic tests [8], [9]. Furthermore, repeated biopsy from tissue sources was performed and only minimum amount of tumor cells were observed, thus, molecular testing could not be carried out on NSCLC patients due to disease progression in EGFR Tyrosine Kinase Inhibitors (TKI) treatment [10]. Plasma might contain part of tumor-derived and extracellular DNA (circulating tumor DNA [ctDNA]) and plasma genotyping is one of the tools that could help in analyzing mutation when only a small amount of tumor cells present. However, the low fraction of ctDNA in blood and limited sensitivity in plasma genotyping remained challenges although the advancement in this ctDNA test has been improving to allow for a more accurate diagnosis [11].

In addition to pre-analytical and analytical factors, sensitivity of plasma genotyping also depended on the rate of ctDNA release from tumor (ctDNA shed). False negative result might be obtained if there is no ctDNA shed in plasma, as tumor would have targeted mutations. ctDNA release to plasma was predicted to occur during necrosis or apoptosis of the tumor cells. ctDNA release was also characterized by the tumor size, necrosis, and tumor vascularization [12], [13]. However, comprehensive histopathological assessment from the tumor shedding in NSCLC was not assessed. Most studies in liquid biopsy have been done in advanced-stage NSCLC patients. At this stage, only a small part of biopsy specimens was taken, and sampling bias might occur when evaluating the histopathological features [14]. Thus, this study was done to analyze the concordance and acceptance level of plasma ctDNA in detecting EGFR mutations in lung adenocarcinoma.

Methods

Patients

Patient selection and sampling criteria

Inclusion criteria for standard group were: 1. Patients diagnosed with early-stage or advanced-stage lung cancer; 2. treatment-naïve patients; 3. blood samples collected before or after acquiring tumor tissues within 5 days; and 4. tumor tissue samples obtained cytologically or histopathologically. 8–10 ml of each of patients' blood was taken through venipuncture and stored in EDTA tube. Samples were sent to Kalgen INNOLAB laboratorium under constant room temperature. Sampling and processing time was < 48 h.

Sampling technique used was purposive sampling where all samples that were obtained and met the inclusion criteria were used in the research until the required number of samples were achieved. Data were collected from 2018 to 2020 and analyzed for both samples. The main purpose of this research was to determine the concordance of EGFR mutation status between samples that were obtained from tumor tissues and that obtained from blood.

Tumor tissues and plasma samples collection and mutation analysis

Samples were collected from lung adenocarcinoma patients, without prior EGFR-TKI treatment.

Methods used

Qiagen QIAamp® DNA Micro Kit was used for DNA extraction. For mutation analysis, HRM polymer chain reaction (PCR), fragment analysis, direct sequencing, and Idylla platform with 100% specificity were used. Mutant alleles could be detected in at least 10% of the tumor cells. The analysis was confirmed through negative and positive controls.

8 ml of plasma sample from each patient was analyzed by droplet digital PCR (ddPCR), and ctDNA isolation was done as per the standard protocol. ctDNA extracted from plasma and gene mutation was analyzed by ddPCR using QX200 AutoDG ddPCR System (BioRad), which allowed the detection on EGFR mutation in exon 19, 20, and 21. The minimum limit of detection for mutant alleles were 0.2%, 0.3%, and 0.3% on T790M, Exon 19 deletion, and L858R, respectively.

This research has received an approval from the Ethics Committee, Faculty of Medicine of Universitas Sumatera Utara. This study was done in accordance with the Declaration of Helsinki 1964 with amendments. Identity of the patients was collected anonymously. The approving committee accepted the consent exemption of the information as this research was based on the available administration and clinical data.

Statistical analysis

Concordance level, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were determined for tissues/cytology and plasma samples with two-tailed confidence intervals (95% CI). EGFR mutations on tissue samples were considered as benchmark for sensitivity and specificity measures. Level of acceptance was confirmed by Kappa test.

Results

A hundred pairs of ctDNA tumor samples from standard group were collected from several

big hospitals in Medan between 2018 and 2020. Characteristics of the patients are shown in Table 1. Staging of patients' sample was collected based on the classification criteria of International Association for the Study of Lung Cancer version 7. Tumor tissue samples were collected from primary tumor. Samples were consisted of lung adenocarcinoma patients diagnosed cytologically or histopathologically. Smoking status was grouped into ex-smokers and never smokers. There were 71 male (71%) and 29 female (29%) participated in this research, with advanced stage cancer of about 81% and performance status 0–1 of approximately 86%.

Table 1: Baseline characteristic's subjects

Age (years)	n
Mean ± SD	59.15 ± 8.95
Median (min – max)	32–80
< 40	3 (3.0)
40–60	48 (48.0)
> 60	49 (49.0)
Sex	
Male	71 (71.0)
Female	29 (29.0)
Smoking Status	
Never Smoker	28 (28.0)
Ex-smoker	72 (72.0)
Performance status (WHO)	
0–1	86
≥ 2	14
IASLC stage	
Early stage	
I–IIIa	19
Advance stage	
IIIb–IVb	81

A hundred subjects with primary tumor tissue samples were matched with the plasma samples and EGFR mutation was detected in 20 patients (20%), 12 (12.0%) on exon 19, 7 (7.0%) on exon 21, and 1 (1.0%) on both exon 19 and 21. Within the plasma samples, mutation was seen in 15 patients (15%) with mutation on exon 19 in 12 patients (12.0%) and exon 21 in 3 patients (3.0%) (Figure 1). Concordance of the mutation between the two samples was 83.0% (83/100, $p < 0.001$; correlation index 0.42, Table 2). If the presence of mutation within tissues was the benchmark, the accuracy of mutation within the plasma DNA would be 60.0% (9/15). The testing for EGFR mutation within tissues and plasma had a weak acceptance level by kappa test, which showed coefficient of 0.414 with 95% CI.

Table 2: The summary of EGFR mutation status, concordance, sensitivity, specificity, positive, and negative predictive values of tumor samples versus ctDNA for EGFR mutation status (All the screened patients were evaluated in both procedure, n = 100)

ctDNA (%)	Tissue (%)		Total
	Mutation (+)	Mutation (-)	
Mutation (+)	9 (45.0)	6 (7.0)	15 (15.0)
Mutation (-)	11 (55.0)	74 (87.0)	85 (85.0)
Total	20 (100)	80 (100)	100
	n	%	
Sensitivity	(9/20)	45	
Specificity	(74/80)	92.5	
Positive predictive values	(9/15)	60	
Negative predictive values	(74/85)	87	
Concordance	100	83	

Correlation index 0.42; $p < 0.001$. EGFR: Epidermal growth factor receptor.

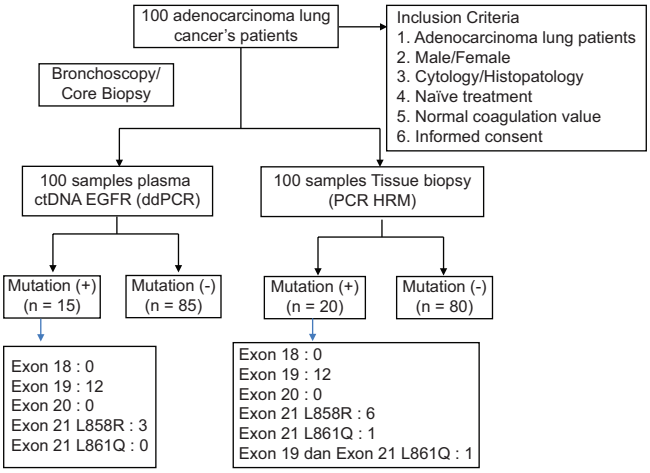

Figure 1: The flow chart of all patients who were screened. It was used to determine the status of epidermal growth factor receptor mutations between tumor DNA and circulating tumor DNA of the circulating free tumor from plasma

Discussion

This study was focused on lung adenocarcinoma which had been increasing in numbers in recent years and research in Indonesia had focused on the advanced stage [15], [16], [17], or those had spread to other organs (metastatic) [18]. Study showed that smoking is independent risk factor for lung cancer, particularly in males, in which clove cigarettes are preferred choice [19].

Methods for ctDNA detection could be categorized into two groups. First, PCR-based method, that is able to detect a single DNA distortion per reaction at a very high sensitivity, which might include Amplification Refractory Mutation System (ARMS), ddPCR, or beads, emulsion, amplification, and magnetics. Second, sequencing-based method, which could detect many distortions at the same time, including Whole Genome Sequencing, amplicon, and targeted capture sequencing [3].

The use of peripheral blood, plasma or serum, for instance, as a replacement for tumor tissues had been investigated comprehensively. This research found a high concordance (83.0%) in EGFR mutation (exon 19 or 21) between matched plasma ctDNA and primary tumor tissue in NSCLC patients, adenocarcinoma type, although a study by Huang *et al.* [20] only reported a concordance of 77.0%. Furthermore, we had described that status of the detected EGFR mutation in plasma before EGFR-TKI treatment might anticipate the effectivity and the survival rate post-EGFR-TKI treatment. The overall frequency of EGFR mutation in the tumor tissues samples was 20.0%. As the population in this study was only limited to adenocarcinoma patients, the number was smaller than the earlier reported data. This finding might be due to the ability to detect mutation only in exon 19

and 21, and both mutations contributed to about 90% of the EGFR mutation in the population as reported from the previous study [21], which lowered the overall frequency of EFGR mutation.

Seventy-nine subjects in a study by Huang *et al.* in 2012 had positive EGFR mutation in plasma samples although the tumor tissue samples appeared negative [20]. False positive rate was reported to be 30.0%, greater than that stated by Goto *et al.* (2012) with concordance of 66.3% in their analysis on the significance in EGFR clinical status which was detected in free circulating DNA extracted from serum on IPASS study (0%). The possible explanation for these findings might be due to the extraction of negative cancer or normal tumor cells, which caused the negative mutation status in the tumor tissue samples and the presence of tumor tissues heterogeneity through the EFGR mutation analysis from each tumor in focus through microdissection. Overall, concordance was maintained at moderate levels, 70–80%, and the consistency in the past and present data was reproducible and stable in detecting EFGR mutation in plasma DNA by Denaturing High Performance Liquid Chromatography (DHPLC) method [22]. Our findings found 15 samples with positive mutation in plasma; however, six negative samples were seen in the tumor tissues with a false positive rate of about 7.5% by ddPCR method.

The concordance level in this study was 83%, which was similar to the study by Reck *et al.* (2016), 89%, using ctDNA detection tools on the concordance analysis between plasma and tissues on 1162 samples [23]. Wang *et al.* (2014) found only 17 (12.6%) samples with ctDNA mutation within 134 samples by ARMS method, with 59% concordance [24]. Using the same method, Douillard *et al.* (2014) reported ctDNA mutation in 105 (16.1%) samples from 652 samples with concordance of 94.3% [25]. Huang *et al.* (2012) used DHPLC method and reported that ctDNA mutation was observed in 270/822 (32.1%) samples with concordance of 77% [21] and concordance of 74% was observed in the study by Bai *et al.* with the similar method [26].

A study by Zhao *et al.* 2011 found weak agreement level for mutation of EGFR in tissue and plasma samples by kappa test with coefficient of 0.342 (95% CI) [27]. Similar trend was seen in our study, with coefficient of 0.414 (95% CI).

The difference in ctDNA diagnostic tools highly affected the concordance, also sensitivity and specificity produced, from pre-analytical such as DNA extraction and analytical methods, such as RT-PCR, next generation sequencing (NGS), and digital PCR, since each method and tool had different approach in detecting EGFR mutation in ctDNA [28]. Moreover, some methods were only able to detect known mutation and were not able to detect rare mutation [29].

A diagnostic study called ASSESS done in Japan and Europe showed the use of ctDNA samples from plasma with excellent concordance (89%) in 1162 matched tissues/cytological samples (sensitivity 46%, specificity 97%, PPV 78%, and NPV 90%) [30]. In our analysis, the sensitivity was found to be low despite the high concordance observed. Our study detected mutation in NSCLC subjects from tumor samples (15.1%) and ctDNA plasma samples (11.0%).

Conversely, a study by Zhang *et al.* in China showed that 35 subjects had plasma and tissue samples with 68.6% (24/35) concordance [31]. IGNITE study found that the concordance of mutation status in 2581 matched samples was 80.5% and 77.7% worldwide and in China, respectively [32]. A relatively smaller concordance in our study could be due to the limited number of matched samples and the difference in sampling time.

According to the study in South Korea by Park *et al.*, a concordance between tissue and plasma NGS was observed in 198 samples (77.6%) from cohort 1 [33]. Within the conflicted cases, additional genomic alteration in 11 patients (4.2%) was discovered through plasma testing. From 50 patients without tissue-based NGS results in cohort 3, ctDNA-based analysis observed genomic alterations in 20 samples (40.0%). The median of the mutation of allele frequency (AF) detected through ctDNA-based NGS (0.74%) was smaller than that through tissue-based NGS test (13.90%). Despite the ctDNA AF result, the clinical response toward the targeted therapy was observed. An upfront ctDNA-based analysis was able to diagnose 60.4% patients with genomic alterations. Furthermore, ctDNA-based testing found 12% additional alterations that could be responded when done after tissue-based NGS. This study showed that ctDNA-based testing could identify more subjects with responded genomic alterations and thus could be adopted accordingly with the conventional tissue-based analysis in NSCLC patients [33].

More researches on the appropriate methods and analysis are required before being used as the alternative for diagnostic testing or screening. If there were no false positive results and improvements in reporting of results observed in most patients, or the capability to lower false negative results, serum testing might be a convenient option for patients with no available tumor samples [22].

Conclusion

Mutation in EGFR on tumor tissue and ctDNA samples was mainly single mutation and observed in exon 19. Concordance was 83.0% with kappa coefficient of 0.414. Tissue samples were still considered as the

main option to detect EGFR mutation in lung cancer. The use of ctDNA samples requires further research before it could be used as the standard testing for EGFR mutation diagnosis and is very dependent on the tools being used.

Acknowledgments

We would like to thank University Sumatera Utara Hospital, H. Adam Malik General Hospital and Santa Elisabeth Hospital for the permission to provide the research subjects.

References

1. Kemenkes RI, Pedoman Nasional Pelayanan Kanker 2014. Jakarta. Available from: http://www.kanker.kemkes.go.id/guidelines_read.php?id=1&cancer=4 [Last accessed on 2017 Oct 12].
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65:87-108. https://doi.org/10.3322/caac.21262
   PMid:25651787
3. Hirsch FR, Scagliotti GV, Mulshine JL, Kwon R, Curran WJ, Wu Y, *et al.* Lung cancer: Current therapies and new targeted treatments. Lancet. 2016;6736:1-13. https://doi.org/10.1016/S0140-6736(16)30958-8
   PMid:27574741
4. Chen Z, Fillmore CM, Hammerman PS, Kim CF, Wong KK. Non-small-cell lungcancers: A heterogeneous set of diseases. Nat Rev Cancer. 2014;14:535-46. https://doi.org/10.1038/nrc3775
   PMid:25056707
5. Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH, *et al.* Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: Guideline from the college of american pathologists, the international association for the study of lung cancer, and the association for molecular pathology. Arch Pathol Lab Med. 2018;142:3:321-46. https://doi.org/10.5858/arpa.2017-0388-CP
   PMid:29355391
6. Syahruddin E, Wulandari L, Muktiati NS, Rima A, Soeroso N, Ermayanti S, Levi M, *et al.* Uncommon EGFR mutations in cytological specimens of 1,874 newly diagnosed indonesian lung cancer patients. Lung Cancer. 2018;9:25-34. https://doi.org/10.2147/LCTT.S154116
   PMid:29615847
7. Soeroso NN, Ananda FR, Pradana A, Tarigan SP, Syahruddin E, Noor DR. The Absence of mutations in the Exon 2 KRAS Gene in Several Ethnic Groups in North Sumatra may not the main factor for lung cancer. Acta Inform Med. 2021;29(2):108-12. https://doi.org/10.5455/aim.2021.29.108-112
   PMid:34584333
8. Herbreteau G, Vallee A, Charpentier S, Normanno N, Hofman P, Denis MG. Circulating free tumor DNA in non-small cell lung cancer (NSCLC): Clinical application and future perspectives. J Thorac Dis. 2019;11(1):S113-26. https://doi.org/10.21037/jtd.2018.12.18
   PMid:30775034.
9. Douillard JY, Ostoros G, Cobo M, Ciuleanu T, McCormack R, Webster A, *et al.* First-line gefitinib in Cau-casian EGFR mutation-positive NSCLC patients: A phase-IV, open-label, single-arm study. Br J Cancer. 2014;110(1):55-62. https://doi.org/10.1038/bjc.2013.721
   PMid:24263064
10. Redig AJ, Costa DB, Taibi M, Boucher D, Johnson BE, Janne PA, *et al.* Prospective study of repeated biopsy feasibility and acquired resistance at disease progression in patients with advanced EGFR mutant lung cancer treated with erlotinib in a phase 2 trial. JAMA Oncol. 2016;2(9):1240-2. https://doi.org/10.1001/jamaoncol.2016.1304
    PMid:27387964.
11. Sacher AG, Komatsubara KM, Oxnard GR. Application of plasma genotyping technologies in non-small cell lung cancer: A practical review. J Thorac Oncol. 2017;12(9):1344-56. https://doi.org/10.1016/j.jtho.2017.05.022
    PMid:28611011
12. Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, *et al.* DNA fragments in the blood plasma of cancer patients: Quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res. 2001;61(4):1659-65.
    PMid:11245480
13. Stroun M, Lyautey J, Lederrey C, Olson-Sand A, Anker P. About the possible origin and mechanism of circulating DNA apoptosis and active DNA release. Clin Chim Acta. 2001;313(1-2):139-42.
    PMid:11694251
14. Cho MS, Park CH, Lee S, Park HS. Clinicopathological parameters for circulating tumor DNA shedding in surgically resected non-small cell lung cancer with EGFR or KRAS mutation. PLoS One. 2020;15(3):e0230622. https://doi.org/10.1371/journal.pone.0230622
    PMid:32196518
15. Soeroso NN, Zain-Hamid R, Sinaga BY, Sadewa AH, Syafiuddin T, Syahruddin E, *et al.* Genetic polymorphism of CYP2A6 and its relationship with nicotine metabolism in male bataknese smokers suffered from lung cancer in Indonesia. Open Access Maced J Med Sci. 2018;6(7):1199-205. https://doi.org/10.3889/oamjms.2018.259
    PMid:30087722
16. Soeroso NN, Zain-Hamid R, Sinaga BY, Sadewa AH, Syafiuddin T, Syahruddin E, *et al.* The role of CYP2A6 genetic polymorphism in nicotine dependence and tobacco consumption among bataknese male smokers. Open Access Maced J Med Sci. 2018;6(5):864-6. https://doi.org/10.3889/oamjms.2018.224
    PMid:29875862
17. Tarigan SP, Soeroso NN, Tumanggor CA, Gani S, Pradana A. Clinical profile of male patients with non-small cell lung cancer in Adam Malik General Hospital, Medan, Indonesia. Open Access Maced J Med Sci. 2019;7(16):2612-4. https://doi.org/10.3889/oamjms.2019.404
    PMid:31777616
18. Soeroso NN, Tarigan SP, Saragih W, Sari ND, Lubis N, Lubis H. Lung adenocarcinoma presenting with an orbital metastasis. Respir Med Case Rep. 2018;25:116-8. https://doi.org/10.1016/j.rmcr.2018.08.005
    PMid:30112271
19. Soeroso NN, Tanjung MF, Afiani D, Pradana A, Tarigan SP, Wahyuni AS. Procalcitonin level in non-small cell lung cancer patients among Indonesia population. Open Access Maced J Med Sci. 2018;6(11):2123-7. https://doi.org/10.3889.2018.421
    PMid:30559873

20. Won YW, Han JY, Lee GK, Park SY, Lim KY, Yoon KA, *et al.* Comparison of clinical outcome of patients with non-small-cell lung cancer harbouring epidermal growth factor receptor exon 19 or exon 21 mutation. J Clin Pathol. 2011;64(11):947-52. https://doi.org/10.1136/jclinpath-2011-200169
PMid:21725039

21. Huang Z, Wang Z, Bai H, Wu M, An T, Zhao J, *et al.* The detection of EGFR mutation status in plasma is reproducible and can dynamically predict the efficacy of EGFR-TKI. Thoracic Cancer. 2012;3(4):334-40. https://doi.org/10.1111/j.1759-7714.2012.00133.x
PMid:28920271

22. Goto K, Ichinose Y, Ohe Y, Yamamoto N, Negoro S, Nishio K, *et al.* Epidermal growth factor receptor mutation in circulating free DNA in serum: From IPASS, a phase III study of gefitinib or carboplatin/paclitaxel in non-small cell lung cancer. J Thorac Oncol, 2012;7(1):115-21. https://doi.org/10.1097/JTO.0b013e3182307f98
PMid:21900837

23. Reck M, Hagiwara K, Han B, Tjulandin S, Grohé C, Yokoi T, *et al.* ctDNA determination of EGFR mutation status in European and Japanese patients with advanced NSCLC: The ASSESS study. J Thorac Oncol. 2016;11:1682-9. https://doi.org/10.1016/j.jtho.2016.05.036
PMid:27468938

24. Wang S, Han X, Hu X, Wang X, Zhao L, Tang L, *et al.* Clinical significance of pretreatment plasma biomarkers in advanced non-small cell lung cancer patients. Clin Chim Acta. 2014;430:63-70. https://doi.org/10.1016/j.cca.2013.12.026
PMid:24378285

25. Douillard JY, Ostoros G, Cobo M, Ciuleanu T, Cole R, McWalter G, *et al.* Gefitinib treatment in EGFR mutated caucasian NSCLC: Circulating-free tumor DNA as a surrogate for determination of EGFR status. J Thorac Oncol. 2014;9(9):1345-53. https://doi.org/10.1097/JTO.0000000000000263
PMid:25122430

26. Bai H, Mao L, Wang HS, Zhao J, Yang L, An TT, *et al.* Epidermal growth factor receptor mutations in plasma DNA samples predict tumor response in Chinese patients with stages IIIB to IV non-small-cell lung cancer. J Clin Oncol. 2009;27(16):2653-9. https://doi.org/10.1200/JCO.2008.17.3930
PMid:19414683

27. Zhao X, Han RB, Zhao J, Wang J, Yang F, Zhong W, *et al.* Comparison of epidermal growth factor receptor mutation statuses in tissue and plasma in stage I-IV non-small cell lung cancer patients. Respiration. 2013;85(2):119-25. https://doi.org/10.1159/000338790
PMid:22797485

28. Diaz LA Jr., Bardelli A. Liquid biopsies: Genotyping circulating tumor DNA. J Clin Oncol. 2014;32(6):579-86. https://doi.org/10.1200/JCO.2012.45.2011
PMid:24449238

29. Marchetti A, Normanno N, Pinto C, Taddei GL, Adamo V, Ardizzoni A, *et al.* Recommendations for mutational analysis of EGFR in lung carcinoma. Pathologica. 2010;102(3):119-26.
PMid:21171518

30. Arriola E, Paredes-Lario A, García-Gomez R, Diz-Tain P, Constenla M, García-Girón C, *et al.* Comparison of plasma ctDNA and tissue/cytology-based techniques for the detection of EGFR mutation status in advanced NSCLC: Spanish data subset from ASSESS. Clin Transl Oncol. 2018;20(10):1261-7. https://doi.org/10.1007/s12094-018-1855-y
PMid:29623586

31. Zhang S, Zhu L, Chen X, Zhang X, Chen E, Fang H, *et al.* ctDNA assessment of EGFR mutation status in Chinese patients with advanced non-small cell lung cancer in real-world setting. J Thorac Dis. 2018;10(7):4169-77. https://doi.org/10.21037/jtd.2018.06.166
PMid:30174861

32. Han B, Tjulandin S, Hagiwara K, Normanno N, Wulandari L, Laktionov K, *et al.* EGFR mutation prevalence in Asia-Pacific and Russian patients with advanced NSCLC of adenocarcinoma and non-adenocarcinoma histology: The IGNITE study. Lung Cancer. 2017;113:37-44. https://doi.org/10.1016/j.lungcan.2017.08.021
PMid:29110846

33. Park S, Olsen S, Ku BM, Lee MS, Jung HA, Sun JM, *et al.* High concordance of actionable genomic alterations identified between circulating tumor DNA-based and tissue-based next-generation sequencing testing in advanced non-small cell lung cancer: The Korean lung liquid versus invasive biopsy program. Cancer. 2021;127(16):3019-28. https://doi.org/10.1002/cncr.33571
PMid:33826761