Relationship of Serum Vitamin D Levels and Response Therapy in Patient Lung Adenocarcinoma Advanced with Targeted Therapy

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Abstract

Introduction: Lung cancer is one of the most common malignancies worldwide and adenocarcinoma is the most commonly. Vitamin D is secosteroid hormone and there is evidence supporting the hypothesis of an anticancer effect of vitamin D. Vitamin D is converted to its active form locally in the lung, suggesting that it may play an important role in lung health. The metabolite active of vitamin D (calcitriol) and tyrosine kinase inhibitors (TKIs) are synergistic inhibit EGFR mutation and modulate extracellular signal-regulated kinase (ERK) and Akt pathways. This study aimed to analysis the associated of serum vitamin D levels and response therapy in advanced lung adenocarcinoma patient after used TKIs.
Methods: This study was an observational analytic study. The subjects where patient with advanced lung adenocarcinoma who received tyrosine kinase inhibitors (TKIs) for 3 months at Dr. Soetomo general hospital Surabaya from July 2020 to March 2021 who met the inclusion and exclusion criterias. The independent variable in this study are the serum levels of vitamin D and response therapy objective (RECIST criteria) as the dependent variable.
Results: The results of stastitical analysis showed that there was no significant associated of serum vitamin D levels and response therapy ($p>0.05$). Patient insufficiency with partial response had a greater number than patient sufficiency with partial response. The mean of vitamin D levels in patient with progressive disease was the highest.
Conclusion: Although the serum levels of vitamin D in lung cancer was lower but this study showed that there was no significant associated of serum vitamin D levels and response therapy in patient advanced lung adenocarcinoma with tyrosine kinase inhibitors.

Keywords: Lung adenocarcinoma; vitamin D; response therapy; tyrosine kinase inhibitors

1. INTRODUCTION

Lung cancer is one of the most common malignancies worldwide and adenocarcinoma is the most commonly.[1] Currently, the therapeutic modalities in the field of oncology are very advanced, but the prognosis for lung cancer remains very poor, most of the lung cancers found are at an advanced stage.[2] The working potential of vitamin D in the prevention and treatment of cancer has now been developed through various studies. Optimal vitamin D plays an important role in the prevention of lung cancer, in addition to the kidney, vitamin D is converted into an active metabolite (calcitriol) in other organs, one of which is the lung.[2,3] Calcitriol which binds to the vitamin D receptor on cancer cells (VDRE) and targeted therapy Tyrosine Kinase Inhibitors (TKIs) plays a role in inhibiting the growth of cancer cells (proliferation, DNA replication,

angiogenesis, metastasis and invasion) through a pathway formed by the tyrosine kinase receptor. (RTKs) especially EGFR.

Vitamin D Receptor directly inhibits EGFR transcription activity and inhibits EGFR overexpression by damaging the EGFR-DNA binding and transactivation of the cyclin D_1 promoter. Calcitriol $1,25(OH)_2D_3$ modulates mediators and blocks the RAS and Akt pathways.[3] Findings from in vitro studies suggest that vitamin D can decrease cell proliferation and can induce apoptosis of small cell lung carcinoma (SCLC).[4,5] Kewei et al described that vitamin D deficiency is associated with a poor prognosis in NSCLC lung cancer patients receiving platinum-based chemotherapy.[1,4]

2. RESEARCH METHOD

The design of this study is a prospective longitudinal observational analytic study. The research subjects were patients with advanced adenocarcinoma non-small cell carcinoma lung cancer who were treated at Poli Onkologi Satu Atap (POSA) Dr. Soetomo General Hospital Surabaya from July 2020 to March 2021. This study was approved by Medical Ethics Committee of Dr. Soetomo General Hospital Surabaya. This study sample was all patients with advanced adenocarcinoma with EGFR mutations who received targeted therapy and met the inclusion and exclusion criteria. The Inclusion criteria were patients diagnosed with non-small cell carcinoma lung cancer, stage IIIB, IIIC, and IV adenocarcinoma types with EGFR mutations based on histopathological or cytological results, patients who had never been targeted for therapy, patients who had initial CT scan data with a distance of approximately 1 month before giving targeted therapy, good liver function (AST and ALT <3x normal value), good kidney function (serum creatinine <1.5x normal value), and willing to sign an informed consent. Exclusion criteria were patients who had previously received chemotherapy / radiotherapy, or received a combination with other therapies such as resection surgery, radiotherapy, immunotherapy and patients who did not continue targeted therapy until prior to CT scan evaluation after 3 months of targeted therapy. The minimal size of the sample was 30 people for this study. Each patient who has received EGFR therapy for 3 months will be evaluated to determine the therapeutic response criteria and vitamin d levels. Blood specimens were collected to determine vitamin D level by using CMIA method. Clinical improvement was evaluated with CT Scan. All numerical data were analyzed by a descriptive statistical and cross tabulation method. The descriptive data were analyzed by Chi-square test, the results with p values < 0.05 were considered to be statistically significant.

3. RESEACRH RESULT

A total of 31 male and female patients with advanced pulmonary adenocarcinoma who underwent targeted therapy for 3 months met the inclusion and exclusion criteria. The data obtained included gender, age, occupation, cigarette exposure, time, duration and frequency of sun exposure, EGFR mutations, and EGFR-TKIs. The characteristics of the sample are presented in Table 1.

Table 1. Sample characteristics

Subject Category	Description	N	Percent
Gender	Male	17	54.8%
	Female	14	45.2%

Age	36-45 yo	2	6.5%
	46-55 yo	18	58.1%
	56-65 yo	8	25.8%
	66-75 yo	2	6.5%
	76-85 yo	1	3.2%
Occupation	Indoor	24	77.4%
	Outdoor	7	22.6%
Cigarette exposure	Exposed	19	61.3%
	Not exposed	12	38.7%
Time of sun exposure	07.00-09.00 a.m	24	77.4%
	09.01-11.00 a.m	6	19.4%
	Never	1	3.2%
Duration of sun exposure (daily)	≥ 15 minutes	11	35.5%
	16-30 minutes	10	33.2%
	≥ 31 minutes	9	29.0%
	Never	1	3.2%
Frequency of sun exposure (of the week)	1-2 day	6	19.4%
	3-4 day	6	19.4%
	5-7 day	18	58.1%
	Never	1	3.2%
EGFR mutations (Exon)	18 G719A, 21 L858R	1	3.2%
	18 G719D, 19 Del, 21 L861Q	1	3.2%
	18 G719V, 20 S768I	1	3.2%
	19 Del	21	67.7%
	19 Del, 21 L816Q	1	3.2%
	21 L858R	4	12.9%
	21 L861Q	1	3.2%
	21 L858R, 21 L861Q	1	3.2%
EGFR-TKIs	Afatinib	11	35.5%
	Gefitinib	19	61.3%
	Erlotinib	1	3.2%

In addition to the data contained in the characteristics table above, metastases were also found in the study patients. The metastases that arise vary widely, there are some patients who experience metastases in 2 organs and even in 5 organs. Most metastases were 2 organs and pleural effusion metastases are the most as shown in Table 2.

Table 2. Metastases in organs

Organ	Frekuensi	Percent
Pleural effusion	2	6.5
Pleural effusion, Pericard effusion, Lung	1	3.2
Pleural effusion, Pericard effusion, Lung, Bone, Cervix	1	3.2
Pleural effusion, Pericard effusion, Bone, Hepar	1	3.2
Pleural effusion, Kidney	1	3.2
Pleural effusion, Brain	1	3.2
Pleural effusion, Lung	4	12.9
Pleural effusion, Lung, Bone	4	12.9
Pleural effusion, Bone	4	12.9
Pleural effusion, Bone, Hepar	1	3.2
Pleural effusion, Bone, Hepar, Kidney	1	3.2
Pleural effusion, Bone, Hepar, Brain	1	3.2
Pleural effusion, Pericard effusion	1	3.2
Hepar	1	3.2
Brain	1	3.2
Brain, Bone	1	3.2
Lung	1	3.2
Lung, Bone	2	6.5
Lung, Bone, Hepar	1	3.2
Bone	1	3.2
Total	31	100.0

Serum vitamin D levels in lung cancer patients are grouped into 4 categories, namely deficiency, insufficiency, sufficiency and toxicity. However, in this study only insufficiency and sufficiency groups were found as shown in Table 3.

Table 3. Category of vitamin D

Category	Frekuensi	Percent
Insufficiency	25	80.6
Sufficiency	6	19.4
Total	31	100.0

Differences in therapy (EGFR-TKIs) obtained by patients also resulted in different therapeutic responses, although there is a significant relationship between the two as shown in table 4 ($P < 0.05$).

Table 4. EGFR-TKIs therapy with therapeutic response

	Progressive Disease	Stable Disease	Partial Response	Total	P
Afatinib	0 (0%)	2 (18.2%)	9 (81.8%)	11 (100%)	
Gefitinib	3 (15.8%)	7 (36.8%)	9 (47.4%)	19 (100%)	0.189
Erlotinib	0 (0%)	1 (100%)	0 (0%)	1 (100%)	
Total	3 (9.7%)	10 (32.3%)	18 (58.1%)	31 (100%)	

There are 2 variables in the study, level of vitamin D in serum and response therapy in patients with advanced lung adenocarcinoma to prove whether or not there is a significant relationship between the two variables. Then Chi Square test was carried out with SPSS software and the following results were obtained, according to table 5.

Table 5. Relationship between vitamin D categories and therapeutic response

Vitamin D categories	Partial Response	Stable Disease	Progressive Disease	Total	P
Insufficiency	14 (77,8%)	10 (100%)	1 (33,3%)	25 (80,6%)	
Sufficiency	4 (22,2%)	0 (0%)	2 (66,7%)	6 (19,4%)	1,00
Total	18 (100%)	10(100%)	3 (100%)	31 (100%)	

The results of the chi square test in table 5 show a P value of 1.00 or $P > 0.05$, which means that there is no significant relationship between serum vitamin D levels and the therapeutic response of patients with advanced pulmonary adenocarcinoma. In Table 5 shows their patients with vitamin D insufficiency but showed a partial response by 14 patients (77.8%), whereas there are also patients with progressive disease that shows vitamin D levels sufficiency by 2 patients (66.7%). Mean value of vitamin D levels in progressive disease was the highest compared to other therapeutic responses, as shown in Table 6.

Table 6. Mean vitamin D values based on treatment response

Therapeutic response	Value of vitamin D (mean)
Partial Response	24,011 ± 7,093
Stable Disease	20,120 ± 6,013
Progressive Disease	28,500 ± 9,297

Therapeutic response attributed to vitamin D categories according to Afatinib and Gefitinib is shown in table 7.

Table 7. Therapeutic response and vitamin D levels based on TKI

			Progressive Disease	Stable Disease	Partial Respons	Total
Afatinib	Category Vit D	Insufficiency	0	2(18.2%)	6(54.5%)	8(72.7%)

		Sufficiency		0	3(27.3%)	3(27.3%)
	Total		0	2(18.2%)	9(81.8%)	11(100%)
Gefitinib	Category Vit D	Insufficiency	1(5.3%)	7(36.8%)	8(42.1%)	16(84.2%)
		Sufficiency	2(10.5%)		1(5.3%)	3(15.8%)
	Total		3(15.8%)	7(36.8%)	9(47.4%)	19(100%)

4. DISCUSSION

4.1. Characteristics of Pulmonary Adenocarcinoma Patients with Positive EGFR mutations

There were 31 patients with the highest sex of men (54.8%), in the age group the most occurred in the range 46-55 years (58.1%), and most of the patients were exposed to cigarette smoke (61.3%). According to literature that risk of lung cancer increases at the age above 40 years and occurs more in men due to smoking habits, so that men tend to be more frequent and longer than women.[6,7] Although smoke exposure is not the only risk factor for lung cancer, lung cancer in nonsmokers is more common in women with younger age and histological types of adenocarcinoma, is reported frequently in East Asia, and is often found in an advanced stage.[8] In this study, most of the patients had indoor work (77.4%) because most of them did not work due to their illness.

All patients presented with metastatic or stage IV with varying symptoms, most metastases involved 2 organs, most metastases were pleural effusions and the second most common metastases were bone. Badawy et al explained that about 57% of lung cancer patients had metastases when the first diagnosis was made and 90% of lung cancer deaths were related to metastatic spread. The study also showed that the most metastases were in bone organs 28.6%, then CNS and pleural effusions with almost the same percentage (22.7% vs 22.5%).[9] Riihimäki et al stated that the most metastases in lung cancer are in the cerebrospinal system (CNS) followed by bone metastases in the second place. The study also stated that lung adenocarcinoma patients who experienced the most death metastasize to the CNS then bone with Hazard rates of 1.19.[10] The most EGFR mutations in this study were Exon 19 deletions (77.4%), this is because exon 19 deletions are common mutations. Research conducted by Rahmayani in 2017 stated that the most EGFR mutations were exon 19 and the sex with the most positive EGFR mutations was female.[11] Kobayashi et al in 2016 found the proportion of EGFR mutations as follows: del 19 44.8%, L858R 39.8%, G719X 3.1%, L861Q 0.9%, del 18 0.3% .[12]

4.2. Levels of Vitamin D Serum for Lung Cancer Patients

The study data showed that 80,6% of the patients had insufficient vitamin D levels and only 19,4% had vitamin D suffficiency levels, but none of the patients had deficiency. This is not much different from the results of research conducted by Jemmy on “Vitamin D levels were associated with clinical response to neoadjuvant chemotherapy in postmenopausal women with locally advanced breast cancer”, it was found levels of vitamin D before chemotherapy as much as 43,3% patients had vitamin D insufficiency levels and 56,7% patients with vitamin D deficiency levels.[13] Similar studies conducted by Rachmanto et al also showed the same percentage.[14] There have been no studies explaining the effect of TKIs on vitamin D metabolism, but a study by Ying Gao et al stated that cisplatin chemotherapy in endometrial cancer patients affected calcitriol levels but did not on calcidiol. This is due to the suppression of 1α-OHase activity not due to global protein synthesis by anti-cancer agents but due to renal damage that occurs.[15]

Although at this time there is still no data regarding the baseline value of vitamin D in lung cancer patients, existing research shows that in general, vitamin D levels in patients with cancer are at the level of insufficiency or deficiency. This is consistent with the concept of dysregulation of vitamin D activity in cancer or the mechanism of vitamin D resistance by cancer cells. According to literature, cancer cells develop resistance to vitamin D by modulating the expression of VDR, CYP27B1, CYP24A1 in the vitamin D metabolic pathway. VDR is widely expressed in several tissue cells, one of which is in the lung, but this expression decreases during tumor differentiation and development in cancer cells. Cancer cells bind or ligate on E-boxes (E-boxes are one of the DNA response elements present on the binding-site protein which functions to regulate gene expression in tissue) which are present in the proximal promoter region of the VDR gene to recruit co-repressors so that there is inhibition of transcription of the VDR gene.[16] Cancer cells can also modulate VDR by suppressing VDR transcription through the expression of the K-RAS gene mutation in cancer cells. Cancer cells inhibit CYP27B1 expression so that the active formation of $1,25(OH)_2D_3$ is reduced to prevent the anti-proliferative effect caused by $1,25(OH)_2D_3$. Mawer et al found that only 1 cell out of 16 small cell lung cancer cells was capable of synthesizing $1,25(OH)_2D_3$. This shows that lung cancer cells inhibit CYP27B1 expression, so that the active formation of $1,25(OH)_2D_3$ is reduced and this is necessary for cancer cells to prevent anti-proliferative effects. Even so, increased CYP27B1 expression can occur in cancer which is useful for suppressing and avoiding the immune system by increasing the secretion of active vitamin D and also as a feedback from the body's response to activate $1,25(OH)_2D_3$.[16,17] Cancer cells increase the expression of CYP24A1, Chen et al. showed that CYP24A1 expression was 8-50x higher than normal lung tissue and the worse the degree of differentiation of a tumor, the higher the expression of the CYP24A1 enzyme. There are several mechanisms of CYP24A1 induction by cancer cells. A study by Anderson et al showed an increase in CYP24A1 micro-RNA (mRNA) in lung tumors compared to normal lung tissue.[17] Another study found that the miR-17 to miR-92 clusters regulate CYP24A1 expression in lung cancer cells.[16]

4.3. Therapeutic Response in Advanced-Stage Pulmonary Adenocarcinoma Patients

The tumor response for each individual to targeted therapy varies. In this study, an objective assessment of therapeutic response after 3 months of targeted therapy using RECIST version 1.1 was read by the same radiologist and the target therapy given was the type of Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitors (EGFR-TKIs). Tyrosine Kinase Inhibitors (TKIs) are the main line therapy in NSCLC and can give a better response when given to lung cancer patients with EGFR mutations exon 18, exon 19 and exon 21. In this study, EGFR-TKIs were used in accordance with the hospital policy program access, namely TKIs from the first and second generations. A total of 19 patients used Gefitinib and 11 patients used Afatinib, whereas Erlotinib was only in 1 patient. The results of the evaluation of the RECIST-based therapeutic response in the form of Partial Response (PR) were 18 patients (58,1%). In this study, there were 9 patients with Partial Response who used Gefitinib, while for Afatinib, 9 patients with Partial Response were obtained. For the response to Stable Disease (SD) therapy, there were 10 patients (32,3%) using Gefitinib in 7 patients, Afatinib in 2 patients. LUX-Lung 7 study comparing "head-to-head" between Afatinib and Gefitinib, Afatinib showed a slight extension of Progression Free Survival / PFS (time from initial diagnosis of disease to progression or disease severity), 11 months versus 10,9 months compared to Gefitinib. Median time-to-treatment failure (time to treatment failure) was also longer for Afatinib (13,7 months versus 11,5 months, $p = 0.0073$) with a better objective response rate (70% versus 56%, $p = 0.0083$).[18] The superior effect of Afatinib is known because the EGFR mutations that occur mostly on exon 19.[19] In addition, Afatinib irreversibly and equipotently inhibits the intrinsic activity of all receptors in the ErbB group (EGFR, HER2 and ErbB4) and indirectly inhibits ErbB3 by prevent binding (ligand-dependent) phosphorylation of ErbB3.[20] In this study, the

response to Progressive Disease (PD) therapy was only found in patients using Gefitinib, namely 3 patients or about 9,7% and PD was not found in patients using Afatinib.

4.4. Relationship between Serum Vitamin D Levels and Treatment Response in Advanced Stage Pulmonary Adenocarcinoma Patients

In this study, is not found a meaningful relationship between vitamin D levels in the serum with the response to therapy of patients adenocarcinoma of the lung advanced stage ($p>0.05$). It is indicated in patients who experienced a partial response with insufficiency as many as 14 patients (77,8%) and 4 patients (22,2%) sufficiency, while patients who experience progressive disease with insufficiency as much as 1 patients (33,3%) and 2 patients (66,7%) sufficiency. It is also indicated on the average value (the mean) levels of vitamin D in patients with progressive disease is the most high, which is 28,500 ± 9,297 ng/mL.

This study, besides showing that Afatinib can provide a better therapeutic response than Gefitinib, also shows better vitamin D levels, the percentage of sufficiency is higher than Gefitinib (27,3 vs 15,8) and the percentage of insufficiency is lower than Gefitinib (72,7 vs 84,2). Although there is not enough evidence regarding the involvement of TKIs in influencing vitamin D levels, the use of Afatinib provides a therapeutic response in the form of a partial response more than Gefitinib when it is associated with vitamin D sufficiency (27,3 vs 5,3) and in patients with Afatinib there is no progressive disease, either sufficiency or insufficiency. Progressive disease with vitamin D sufficiency in 2 patients. In this group, 1 patient had the same target lesion size in the lung organs on the chest contrast evaluation CT scan compared to the baseline contrast chest CT scan of 3.1 cm, but there was a 55% enlargement of the metastatic mass in the liver as the target lesion. Meanwhile, 1 other patient had a target lesion size in the lung organs that increased from 8.0 cm to 10.2 cm after 3 months of TKIs use. It is not certain whether this is related to mechanisms of tumor resistance against TKIs or comorbid factors which were not investigated further by the investigators. The literature states that adenocarcinoma patients with EGFR mutations will initially respond to EGFR-TKIs therapy, but subsequently will experience resistance to TKIs within a period of 9-14 months.[21,22]

There were 14 partial response patients with insufficiency, namely 6 patients (54.5%) using Afatinib and 8 patients (42.1%) using Gefitinib. In this group, 8 patients had vitamin D values ≥ 20 ng/mL and 6 patients had vitamin D values < 20 ng/mL. According to the research literature of Kewei Ma et al, there was a slight difference in the overall survival (OS) of NSCLC patients who were given platinum-based chemotherapy in the group with serum vitamin D values < 20 ng/mL compared to the group with vitamin D values ≥ 20 ng/mL (19,5 vs 22.6 months) and for progression free survival (PFS) in the group, the serum vitamin D value < 20 ng/mL compared to the group vitamin D values ≥ 20 ng/mL were almost the same, namely with a median value of 9,4 vs 9,8 months.[23] This is consistent with the results of the study, in patients with vitamin D insufficiency with serum vitamin D values < 20 ng/mL compared to those in the group with vitamin D values ≥ 20 ng /mL can give the same therapeutic response.

There 6 patients who experienced partial response therapy with vitamin D insufficiency when using Afatinib, with 5 patients mutating in exon deletion 19 and 1 patient on exon 21. While Gefitinib was 8 patients, with 7 patients mutated in exon deletion 19 and 1 patient mutation in exon 21. This shows that most patients with vitamin D insufficiency can respond to therapy in the form of a partial response because most patients have exon deletion 19 mutations and this is consistent with a study by Titin et al. compared to exon 21 (9.05 vs 6.76 months).[24] Zhang et al study showed that patients with exon 19 mutation EGFR gave a significantly better response after giving TKIs than exon 21.[25] The literature states that there are 3 hypotheses that underlie the better effectiveness of exon 19, namely first because exon 19 has a greater affinity for the EGF receptor than exon 21 so that the bonds formed between TKIs and exon 19 are stronger and signal barriers to cancer cell growth will be lasts longer. The second hypothesis is that the secondary mutation

T790M occurs more frequently with exon 21, especially L858R, which is a point mutation with the largest proportion in exon 21. The third hypothesis is that mutations in exon 18 (G719S) occur more frequently with exon 21 (L858R).[25,26]

5. CONCLUSION

There was no significant relationship between vitamin D levels in serum and response to therapy in patients with advanced lung adenocarcinoma with targeted therapy for TKIs for 3 months. The value of vitamin D levels in patients with advanced pulmonary adenocarcinoma is mostly insufficient. Mean level of vitamin D in patients with progressive disease was the highest, which was 28,500 ± 9,297 ng / mL. Response therapy in patients with advanced lung adenocarcinoma was obtained mostly in the form of a partial response of 58.1%, 32.3% of other therapeutic responses to stable disease and 9.7% of progressive disease.

References

1. Liu J, Dong Y, Lu C, Wang Y, Peng L, Jiang M, et al. Meta-analysis of the correlation between vitamin D and lung cancer risk and outcomes. Oncotarget. 2017;8(46):81040-81051.
2. Chen GC, Zhang ZL, Wan Z, Wang L, Weber P, Eggersdorfer M, et al. Circulating 25-hydroxyvitamin D and risk of lung cancer: a dose–response meta-analysis. Cancer Causes Control, Springer. 2015:1-10.
3. Norton R, O'Connell MA. Vitamin D: Potential in the Prevention and Treatment of Lung Cancer. Anticancer Research. 2012;32:211-222.
4. Zhang L, Wang S, Che X, Li X. Vitamin D and Lung Cancer Risk: A Comprehensive Review and Meta-Analysis. Cell Physiol Biochem. 2015;36:299-305.
5. Gandini S, Boniol M, Haukka J, Byrnes G, Cox B, Sneyd MJ, et al. Meta-analysis of observational studies of serum 25-hydroxyvitamin D levels and colorectal, breast and prostate cancer and colorectal adenoma. Int. J. Cancer. 2011;128:1414–1424.
6. Fong KM, Sekido Y, Gazdar AF, Minna JD. Lung cancer 9: Molecular biology of lung cancer: clinical implications. Thorax. 2003;58:892–900.
7. Soeroso NN, Soeroso L, Syafiuddin T. Level of Serum Carcinoembryogenic Antigen (CEA) in Non Small Cell Lung Cancer (NSCLC) at Adam Malik Hospital. J Respir Indo. 2014;34(1):17-25.
8. NCCN Clinical Practice Guidelines in Oncology. Non-Small Cell Lung Cancer Version 3. NCCN Guidelines. 2018.
9. Skeel RT, Khleif SN. Handbook of Cancer Chemotherapy 8th edition. LWW-Wolters Kluwer. 2011.
10. Riihimäki M, Hemminki A, Fallah M, Thomsen H, Sundquist K, Sundquist J, et al. Metastatic Sites and Survival in Lung Cancer. Elsevier, Lung Cancer. 2014;86:78-84.
11. Rahmayani TP. Proporsi Hasil Pemeriksaan Epidermal Growth Factor Receptor (EGFR) Pada Pasien Kanker Paru Jenis Adenokarsinoma di RSUP Haji Adam Malik Tahun 2017. Skripsi, Prodi Pendidikan Dokter FK USU Medan. 2018.
12. Kobayashi Y, Mitsudomi T. Not all epidermal growth factor receptor mutations in lung cancer are created equal: Perspectives for individualized treatment strategy. Japanese Cancer Association, Cancer Sci. 2016:1-8.
13. Sutantio JA. Hubungan antara kadar vitamin D darah dan respons klinis kemoterapi neoadjuvant pada wanita pasca menopause dengan locally advanced breast cancer di RSUD Dr. Soetomo Surabaya. Tesis - Program studi Ilmu Kedokteran Klinik jenjang Magister-FK Unair. 2019
14. Rachmanto AN, Ishardyanto H, Ali I, Setiawati R. Relationship of Blood Vitamin-D Levels on Neoadjuvant Chemotherapy Response of Caf (Tumor Size Based on Ultrasonographic Examination) in Post Menopause Women with Locally Advance Breast Cancer in Dr. Soetomo General Hospital Surabaya. Indian Journal of Public Health Research & Development. 2020;11(10):203-209.
15. Gao Y, Shimizu M, Yamada S, Ozaki Y, Aso T. The Effects of Chemotherapy Including Cisplatin on Vitamin D Metabolism. Endocrine Journal. 1993;40(6):737-742.
16. Jeon SM, Shin EA. Exploring Vitamin D Metabolism and Function in Cancer. Experimental and Molecular Medicine. 2018;50(20):1-14.
17. Parise RA, Egorin MJ, Kanterewicz B, Taimi M, Petkovich M, Lew AM, et al. CYP24, the enzyme that catabolizes the antiproliferative agent vitamin D, is increased in lung cancer. Int. J. Cancer. 2006;119:1819–1828.
18. Park K, Tan EH, O'Byrne K, Zhang L, Boyer M, Mok T, et al. Afatinib versus gefi tinib as fi rst-line treatment of patients with EGFR mutation-positive non-small-cell lung cancer (LUX-Lung 7): a phase 2B, open-label, randomized controlled trial. Lancet Oncol. 2016;17:577-89.
19. Badawy AA, Khedr G, Omar A, Bae S, Arafat W, Grant S. Site of Metastases as Prognostic Factors in Unselected Population of Stage IV Non-Small Cell Lung Cancer. Asian Pac J Cancer Prev. 2018;19(7):1907-1910.

20. Hirsh V. Afatinib (BIBW 2992) development in non-small-cell lung cancer. Future Oncol. 2011;7(7):817–825.
21. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatinpaclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009;361(10):947-57.
22. Sequist LV, Waltman BA, Santagata DD, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and Histological Evolution of Lung Cancers Acquiring Resistance to EGFR Inhibitors. Sci Transl Med. 2011;3(75):1-27.
23. Ma K, Xu W, Wang C, Li B, Su K, Li W. Vitamin D deficiency is associated with a poor prognosis in advanced non-small cell lung cancer patients treated with platinum-based first-line chemotherapy. Cancer Biomarkers. 2016;1:1-7.
24. Agustina TS, Wulandari L. Perbandingan respons terapi gefitinib pada pasien KPKBSK EGFR mutasi exon 19 dan exon 21. J Respir Indo. 2017;37(3):232-240.
25. Zhang Y, Sheng J, Kang S, Fang W, Yan Y, Hu Z, et al. Patients with Exon 19 Deletion Were Associated with Longer Progression-Free Survival Compared to Those with L858R Mutation after First-Line EGFR-TKIs for Advanced Non-Small Cell Lung Cancer: A Meta-Analysis. PLoS ONE. 2014;9(9):e10716.
26. Harari PM. Epidermal Growth Factor Receptor Inhibition Strategies in Oncology. Endocrine-Related Cancer. 2004;11:689–708.