

JURNAL RESPIROLOGI INDONESIA

Majalah Resmi Perhimpunan Dokter Paru Indonesia
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An Evaluation of Short-Acting β 2-Agonist Prescriptions and Associated Clinical Outcomes in Asthma Management in Indonesia – The SABINA Indonesia Study

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Association Between CEA Serum Level on NSCLC Patients with EGFR Mutation from Tissue and Plasma Sample

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Abstract

Background: Lung cancer is one of the leading causes of cancer deaths in the world. The most common type of lung cancer is non-small cell lung cancer (NSCLC). Patients with NSCLC can have epidermal growth factor receptor (EGFR) mutation and increased level of CEA. Test for EGFR mutation on NSCLC has a very important role for EGFR tyrosine kinase inhibitor (TKI) therapy. CEA also can be used as a predictor for treatment efficiency of EGFR-TKI therapy. Tissue biopsy is the main diagnostic method for lung cancer but it's invasive and has some limitations. Circulating tumor DNA (ctDNA) is a new and less invasive for detecting EGFR mutation using plasma sample. In this study, we investigated the correlation between serum CEA and EGFR mutations in tissue and plasma in patients with NSCLC.

Methods: This cross-sectional observational study was conducted in Dr. Saiful Anwar Hospital, Malang from August 2018 until July 2019. 76 NSCLC patients underwent tests for EGFR mutation from tissue, ctDNA, and serum CEA level respectively. Extracted DNA from plasma and tissue samples from cytology or biopsy was checked for the EGFR mutation. The serum CEA levels were analyzed using electrochemical luminescence.

Results: From 76 participants, positive EGFR mutation from tissue samples was detected on 34 subjects and ctDNA in 19 subjects. Serum level of CEA >5 ng/ml was significantly associated with EGFR mutation from tissue sample ($P=0.037$) with an odds ratio (OR) of 2.778 (95% CI=1.050-7.348), the area under curve (AUC) for CEA was 68,8% and the cut-off was 9.14 ng/ml. Serum level of CEA >5 ng/ml was also significantly associated with ctDNA ($P=0.015$) with an OR of 4.8 (95% CI=1.259-18.299), the AUC for CEA was 78,1% and cut-off=14.8 ng/ml.

Conclusion: Serum CEA level has poor association with EGFR mutation from tissue and moderate association with EGFR mutation from ctDNA in NSCLC patients. Patients with increased level of CEA>5 ng/ml are 2.778 times more at risk to have positive EGFR mutation and 4.8 times more at risk to have positive ctDNA mutation. (*J Respirol Indones* 2022; 42(2): 97–106)

Keywords: lung cancer, NSCLC, EGFR, ctDNA, CEA

Hubungan Kadar CEA dalam Serum pada Pasien KPKBSK dengan Mutasi EGFR yang didapatkan dari Sampel Jaringan dan Darah

Abstrak

Latar belakang: Kanker paru merupakan penyebab kematian tertinggi akibat kanker di dunia. Jenis yang paling sering dijumpai adalah kanker paru karsinoma bukan sel kecil (KPKBSK). Pasien dengan KPKBSK dapat mengalami mutasi epidermal growth factor receptor (EGFR) dan peningkatan kadar CEA. Pemeriksaan mutasi EGFR pada pasien KPKBSK memiliki peranan penting untuk terapi target dengan tyrosine kinase inhibitor (TKI). CEA juga bisa digunakan untuk memprediksi efisiensi terapi EGFR-TKI. Biopsi jaringan merupakan pemeriksaan utama untuk mendiagnosis kanker tetapi metode ini invasif dan memiliki beberapa kendala. Circulating tumor DNA (ctDNA) merupakan pemeriksaan yang baru dan lebih aman untuk mendeteksi mutasi EGFR dengan sampel darah. Dalam penelitian ini, kami meneliti hubungan kadar CEA serum pada pasien KPKBSK dengan mutasi EGFR yang didapatkan dari sampel jaringan dan darah.

Metode: Jenis penelitian yang digunakan adalah observasional potong lintang. Penelitian dilakukan di Rumah Sakit Dr. Saiful Anwar Malang dari bulan Agustus 2018 sampai Juli 2019. Sebanyak 76 pasien dengan KPKBSK dilakukan pemeriksaan mutasi EGFR dari jaringan tumor, ctDNA dan CEA. Pemeriksaan mutasi EGFR dilakukan pada DNA yang diekstraksi dari darah dan sampel jaringan tumor dari sitologi atau biopsy. Kadar CEA dianalisis menggunakan electrochemical luminescence.

Hasil: Dari 76 pasien, ditemukan mutasi EGFR positif dari sampel jaringan sebanyak 34 pasien dan ctDNA positif sebanyak 19 pasien. Kadar CEA >5 ng/ml dalam serum memiliki hubungan bermakna dengan mutasi EGFR di jaringan ($P=0.037$) dengan odds ratio (OR)=2.778 (95% CI=1.050-7.348), area under the curve (AUC) untuk CEA 68,8% dan cut-off CEA 9.14 ng/ml. Kadar CEA >5 ng/ml juga berhubungan dengan ctDNA ($P=0.015$) dengan OR=4.8 (95% CI=1.259-18.299), AUC untuk CEA=78,1% dan cut-off=14.8 ng/ml.

Kesimpulan: Kadar serum CEA memiliki hubungan lemah dengan mutasi EGFR dari jaringan dan hubungan sedang dengan mutasi EGFR dari ctDNA pada pasien KPKBSK. Pasien dengan peningkatan CEA>5 ng/ml memiliki risiko 2.778 kali mengalami mutasi EGFR dan 4.8 kali berisiko mengalami mutasi positif ctDNA. (*J Respirol Indones* 2022; 42(2): 97-106)

Kata kunci: kanker paru, KPKBSK, EGFR, ctDNA, CEA

INTRODUCTION

Lung cancer is one of the leading causes of cancer death globally. The main risk factor for lung cancer is smoking. Other factors that are also known to influence the development of lung cancer include exposure to radon gas, asbestosis, air pollution and genetic factors.^{1,2}

Lung cancer is divided into two main types, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The latter constitutes 80% of all lung cancers, with adenocarcinoma as the most common histological type.^{1,2}

Recent studies have found that genetic aberrations play a role in controlling cell survival in NSCLC. This aberration increases cell division and induces tumors. One of the pathways that can deviate is the epidermal growth factor receptor (EGFR), a transmembrane receptor tyrosine kinase found in normal epithelial, mesenchymal and neurogenic tissues. The overexpression of EGFR is thought to play a role in the development of lung cancer.³

Mutations of EGFR are usually detected from tumour tissue samples obtained by biopsy or surgery. These mutations are primarily found in Asian ethnicity and lung cancer patients with adenocarcinoma type (around 51.4%), while there's no significant association with gender and smoking status. The best samples for examination of EGFR mutations were obtained surgically. However, 70–80% of NSCLC patients cannot undergo surgery at diagnosis while biopsy carries a high risk of bleeding in advanced cancer. Therefore, a more accessible and safer way is needed in estimating the occurring mutations.^{4,5}

A new method to measure circulating free tumor-derived DNA (ctDNA) has been developed. In cancer patients, dead tumor cells release DNA into the bloodstream, and these DNA fragments bring about changes in tumor-specific sequences. Several studies have demonstrated the easiness and predictive value of using ctDNA to monitor tumor dynamics in various solid tumors. The degree of correlation between EGFR mutations and ctDNA is highly dependent on the detection method used, varying between 66–100%.^{5–7}

Carcinoembryonic antigen (CEA) is an adhesion protein whose expression can be activated and regulated through the P13-K/Akt and STAT 3/5 signalling pathways on EGFR. Activation of the P13-K/Akt signalling pathway is involved in regulating cell differentiation, proliferation and apoptosis. CEA acts as an adhesion molecule and is involved in cell aggregation. CEA has a role in antiapoptotic and prometastatic in colon cancer. In lung cancer, CEA levels were also elevated, especially in adenocarcinoma. Overexpression of CEA levels can protect tumor cells against apoptosis induced by loss of cell contact with the extracellular matrix and inhibit cell death.^{6,8,9}

EGFR mutations can cause aberrations in the P13-K/Akt and STAT 3/5 signalling pathways, thereby reducing signal transduction and causing antiapoptotic activity in tumor cells. It is suspected that the antiapoptotic signalling pathway that occurs due to aberrations in the activation of Akt and STAT 3/5 molecules can affect CEA expression. In addition, EGFR mutations are autophosphorylated in the absence of interleukin-3 without EGFR stimulation. It is suspected that continuous signalling from EGFR mutations can stimulate antiapoptotic activity; as a result, the expression of CEA protein as an antiapoptotic agent may appear to be increased in patients with EGFR gene mutations.^{8–10}

One of the proteins that can be affected by activation of the EGFR pathway, an elevated serum CEA level, may be a sign of a mutated EGFR. However, this assumption requires further research. Although EGFR and CEA belong to different protein groups, studies in recent years have found a relationship between the expression of CEA levels and EGFR mutations.¹¹

A study conducted by Abdurahman et al suggested a significant association between EGFR mutations and serum CEA levels. However, this study had a relatively small sample size.¹² Another study by Normawati et al regarding the association between tissue EGFR mutations and CEA levels in patients with lung adenocarcinoma at Dr. Saiful Anwar Hospital, Malang stated that lung adenocarcinoma patients with EGFR mutations had a 3.4 times

increased risk of CEA compared to patients without EGFR mutations.¹³

So far, no study has been conducted on the association between serum CEA levels and EGFR mutations in tissue and blood (ctDNA) in patients with NSCLC. Therefore, we meant to find the association between serum CEA levels with ctDNA. This study would be conducted in NSCLC patients at Dr. Saiful Anwar Hospital, Malang. It is hoped that the result of this study can be used as a consideration for patients who do not consent or are unable to undergo ctDNA EGFR mutation tests to make it easier for patients to receive appropriate therapy.

METHOD

The research was conducted in the pulmonary clinic and inpatient ward of Dr. Saiful Anwar Hospital, Malang from August 2018 to September 2019. This study was conducted using a cross-sectional observation method to determine the association between EGFR mutations in tissue and blood with serum CEA levels in newly diagnosed NSCLC patients treated at dr. Saiful Anwar Hospital, Malang who had never received chemotherapy. The inclusion criteria for this study were patients aged >18 years who were diagnosed with NSCLC through cytology and histopathology examination and had never received any kind of anti-cancer treatment. Patients whose tumor tissues did not meet the requirements for EGFR testing due to low cell counts, patients who had received anti-cancer treatment before CEA levels were examined and patients with secondary lung cancer were not included in this study.

The sampling of this study was carried out at the pulmonary clinic and the inpatient ward of Dr Saiful Anwar Hospital, Malang. Each subject signed

the informed consent. Eighty patients who met the inclusion criteria and were willing to participate in the study were subjected to EGFR examination both with tissue and blood samples (ctDNA). An independent sample T-test was done to determine the association between EGFR mutations and CEA levels. Processing of data analysis using IBM SPSS software version 24.0 with a 95% confidence level, alpha = 0.05.

The area under the curve (AUC) value is obtained by analyzing the receiver operating characteristics (ROC) curve and then determining the cut-off point; after finding the cut-off point, the sensitivity, specificity, accuracy, positive predictive value and negative predictive value are calculated.

RESULTS

During the study, 80 subjects who were willing to participate in the study and signed the informed consent were obtained. In the course of the study, the examination of EGFR mutations from tissue samples could not be carried out in four patients because the number of cells was insufficient, so they were excluded from the study. At the end of the study, there were a total of 76 subjects who were included in the statistical analysis. Among them, 34 positive EGFR mutations were found in tissues and 19 in ctDNA.

The gender distribution showed that 36 (47.4%) of the subjects were female and 40 (52.6%) were male, as shown in Table 1. The association between gender and EGFR mutations was determined by chi-square test which showed $P=0.072$. It can be concluded that gender is not associated with EGFR mutations in the tissue.

Table 1. Characteristics of Gender, Smoking Status and Occupation on EGFR and ctDNA Mutations

Characteristics		EGFR Mutations						P	ctDNA				P
		Positive		Negative		Total			Positive		Negative		
		n	%	n	%	N	%		N	%	N	%	
Gender	Male	14	18.4	26	34.2	40	52.6	0.072 ^b	7	9.2	33	43.4	0.111 ^b
	Female	20	26.3	16	21.1	36	47.4		12	15.8	24	31.6	
Smoking Status	Smokers	11	14.5	24	31.6	35	46.1	0.031 ^b	7	9.2	28	36.8	0.352 ^b
	Non-Smokers	23	30.3	18	23.6	41	53.9		12	15.8	29	39.2	
Occupation	High Risk	12	15.8	16	21.1	28	36.8	0.816 ^b	6	7.9	22	28.9	0.583 ^b
	Low Risk	22	28.9	26	34.2	48	63.2		13	17.1	35	46.1	

Note: (* Significant when $P<0.05$; ^b Chi-square Test

Table 2. Clinical Characteristics Associated with EGFR and ctDNA Mutations

Characteristics		EGFR Mutations				Total		P	ctDNA					
		Positive		Negative					Positive		Negative		P	
		N	%	N	%	N	%		N	%	N	%		
Stadium	III	2	2.6	5	6.6	7	9.2	0.450 ^c	0	0.0	7	9.2	0.182 ^c	
	IV	32	30.9	37	48.7	69	90.2	0.182 ^c	19	25	50	65.8		
Histological type	Adenocarcinoma	29	28.2	35	46.1	64	84.2	Reff	16	21.1	48	63.2	Reff	
	Squamous Cell Carcinoma	2	2.6	2	2.6	4	5.3	Reff	1	1.3	3	3.9	1.00 ^c	
	Adenosquamous	3	3.9	5	6.6	8	10.5	1.00 ^c	2	2.6	6	7.9	1.00 ^c	
Sampling technique	FNAB	14	18.4	31	40.8	45	59.2	1.00 ^c	-	-	-	-	-	
	Bronchial washing/brushing	3	3.9	0	0	3	3.9	0.114 ^c	-	-	-	-	-	
	Pleural fluid cytology	9	11.8	8	10.5	17	22.4	0.075 ^c	-	-	-	-	-	
	Biopsy FOB	7	9.2	3	3.9	10	13.2	0.033 ^c	-	-	-	-	-	
	TBNA	1	1.3	0	0	1	1.3	0.326 ^c	-	-	-	-	-	

Note=* Significant when $P < 0.05$; ^c Fischer's exact Test

Another chi-square test was conducted to determine the association between gender and ctDNA results which shows $P=0.111$. It can be concluded that there is no association between gender and ctDNA results.

Smoking history was divided into smoker and non-smoker. In this study, 36 subjects were smokers (46.1%) and 41 subjects never smoked (53.9%) (Table 1). A significant association was found between non-smoker and positive EGFR mutation ($P=0.031$) but none between smoking history and ctDNA ($P=0.352$).

Among all research subjects, homemakers make up the biggest part of them with amounted to 21 people (27.6%), with 13 people (17.1%) tested positive for EGFR mutations and 8 (10.5%) were negative. Aside of them, 19 subjects work as farmers (25%), 3 as household assistants (3.9%), 7 as office employees (9.2%), 5 as carpenters (6.6%), 11 are self-employed (6.6%) and 10 have jobs other than those mentioned (13.2%). For statistical reasons, the occupations were divided into those with a high risk of lung cancer (farmers and artisans) and those with lower risk (other professions). Chi-square test shows $P=0.816$, so it can be concluded that occupation was not associated with EGFR mutations, nor was there a significant association with ctDNA results ($P=0.583$).

In this study, the majority of patients were in stage IVa, which amounted to 63 people (82.9%), while at stage IIIb there were 0 people (0%) (Table 2). EGFR mutations in tissue were also found mostly in stage IVa, with 31 people (40.8%) yielded positive

results. Subjects were divided into two major groups in regards to their cancer stage for statistical analysis purposes: stage III (IIIa, IIIb and IIIc) and stage IV (IVa and IVb). Fischer's exact test showed no association between cancer stage and EGFR mutation ($P=0.450$). The association between histological cancer type and EGFR mutation was also studied, with 64 people were found to have adenocarcinoma (84.2%), 4 have squamous cell carcinoma (SCC) (5.3%) and 8 adenosquamous cell carcinoma (10.5%). Chi-square test with adenocarcinoma as a reference showed that $P=1.000$ for SCC and $P=0.725$ for adenosquamous. It was concluded that cancer type was not associated with EGFR mutations (Table 2).

Most of the materials for EGFR examination were taken by FNAB technique, which consisted of 45 samples (59.2%), while the least one came from TBNA (1 sample). A chi-square test with FNAB as a comparison showed that tissue sampling via biopsy was more likely to get positive EGFR mutation results compared to FNAB ($P=0.033$). Meanwhile, there are no positive or negative impacts on other examination materials such as bronchial washing or brushing, pleural fluid cytology and TBNA.

Positive ctDNA results were mostly found at stage IVa (Table 2), which amounted to 18 people (23.7%), while at stage III there was no positive result at all. Fischer's exact test showed no association between cancer stage and ctDNA results ($p=0.182$). In regards to the association between cancer type and ctDNA, $P=1.00$ both in adenosquamous and SCC type as determined by Fischer's exact test with

adenocarcinoma as reference. It was concluded that cancer type was not related to the ctDNA results (Table 2).

As mentioned before, positive EGFR mutations from tissue samples were found in 34 patients (44.74%), which consisted of mutations in exon 18 in 1 patient (2.94%), exon 19 in 26 patients (76.47%), exon 20 in 2 patients (5.88%) and exon 21 in 5 patients (14.71%). On the other hand, EGFR mutations via ctDNA were found in 19 patients (25%), consisting of mutations in exon 18 in 1 patient (5.26%), exon 19 in 14 patients (73.68%), exon 21 in 3 patients (15, 79%) and double mutations in exons 19 and 21 in 1 patient (5.26%).

On the results of the CEA examination, it was found that 46 subjects (60.5%) had increased CEA level (Table 3). Chi-square test yielded the result of $p=0.037$, which showed that elevated level of CEA has a significant association with the result of EGFR mutations. The value of the diagnostic Odds Ratio (OR) is 2.778 with 95% CI=1.050-7.348, meaning that with the increase in CEA level, positive EGFR mutation will be 2.778 times more likely.

From the data obtained, it can be calculated the value of the EGFR mutation trend in CEA:

$$\text{Sensitivity} = \frac{25}{(25+9)} = 73.5\%$$

$$\text{Specificity} = \frac{21}{(21+21)} = 50\%$$

$$\text{Positive predictive value (PPV)} = \frac{25}{(25+21)} = 54.3\%$$

$$\text{Negative predictive value (NPV)} = \frac{21}{(9+21)} = 70\%$$

$$\text{Positive Likelihood Ratio (LR+)} = \frac{0.735}{(1-0.500)} = 1.47$$

$$\text{Negative Likelihood Ratio (LR-)} = \frac{1-0.735}{0.5} = 0.53$$

$$\text{Accuracy} = \frac{13+39}{76} = 60.5\%$$

The above results show that the sensitivity of CEA levels is 73.5%, and specificity is 50%. From the

sensitivity results, it can be concluded that CEA can detect 73.5% of EGFR mutations. The specificity of CEA 50% means that the probability of CEA detecting lung cancer patients without EGFR mutation is 50%. A PPV of 54.3% means that only about 54.3% of EGFR underwent mutations. An NPV of 70% means that approximately 70% of the EGFR in patients with adenocarcinoma lung cancer are entirely unmutated. The LR+ is 1.47 and the LR- is 0.53. The accuracy of the CEA level in detecting EGFR mutations in tissue is 60.5%.

Table 4 shows that the average CEA level in people with positive EGFR mutations is 108.04; this value is higher than the group without EGFR mutations, with a mean CEA of 60.00. The Mann-Whitney test shows that $P=0.005$, so it can be concluded that there is a significant difference between the CEA values in the EGFR mutation group and the group without EGFR mutations in the tissue.

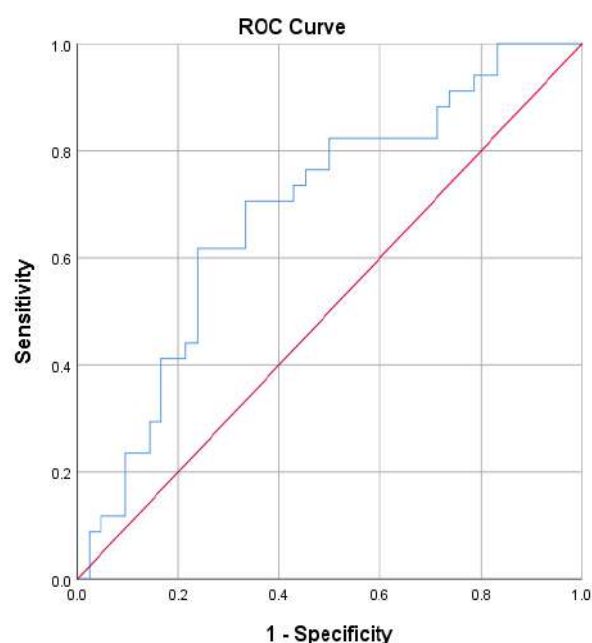


Figure 1. ROC curve of CEA value against EGFR mutations in the network

Table 3. Association between Tissue EGFR Mutations and ctDNA with CEA

Characteristics		EGFR Mutations				P	OR (95% CI)	ctDNA				P	OR (95% CI)
		Positive		Negative				Positive		Negative			
		N	%	n	%			N	%	N	%		
CEA	Elevated	25	32.9	21	27.6	0.037 ^{ab}	2.778 (1.050–7.348)	16	21.1	30	39.5	0.015 ^{ab}	4.8 (1.259–18.299)
	Normal	9	11.8	21	27.6			3	3.9	27	35.5		

Note= ^a Significant when $P<0.05$; ^b Chi-square Test

Table 4. CEA values with EGFR mutations in tissue and ctDNA

Characteristics	EGFR Mutations in tissue			P-value	ctDNA			P-value
	Positive	Negative	Total		Positive	Negative	Total	
CEA Median	26.81	3.85	8.13	0.005 ^{td}	86.2	86.2	86.2	<0.001 ^{td}
(Min - Max)	(1.6–872)	(0.91–951.8)	(0.91–951.8)		(1.63–870.47)	(1.63–872.1)	(1.63–872.1)	
Mean	108.04	60.00	81.49 ±		189.88 ±	45.36 ±	45.36 ±	
± SD	± 188.22	± 166.02	176.7		239.32	134.56	134.56	

Note= (* Meaning when $P < 0,05$; ^{td} Mann-Whitney Test

In the ROC analysis (Figure 1), the AUC value is 0.688 or 68.8%. This means CEA level has weak accuracy in determining EGFR mutations from tissues. The interpretation value is that AUC >50.0–60.0% is very weak, >60.0–70.0% weak, >70.0–80.0% moderate, >80.0–90.0% good and >90.0–100.0% very good

Based on the plotting in the Table 3, the CEA cut-off value is 9.14. So, it can be concluded that the CEA cut-off to allow the positive EGFR mutation results in tissue to be 9.14 ng/ml.

Most subjects, amounting to 46 people (60.5%) had elevated CEA levels (Table 3), with 16 of them (21.1%) showed positive ctDNA. Chi-square test determined that there was a significant association between ctDNA results and elevated CEA levels, with $P=0.015$ and OR: 4,800 with 95% CI=1,259-18,299, which means that an increase in CEA level will yield 4.8 times the chance of positive ctDNA mutations.

From the data obtained, it can be calculated the trend value of ctDNA results in CEA:

$$\text{Sensitivity} = \frac{16}{(16+3)} = 84.2\%$$

$$\text{Specificity} = \frac{27}{(27+30)} = 47\%$$

$$\text{PPV} = \frac{16}{(16+30)} = 34.8\%$$

$$\text{NPV} = \frac{27}{(3+27)} = 90\%$$

$$\text{LR+} = \frac{0.842}{(1-0.470)} = 1.78$$

$$\text{LR-} = \frac{1-0.842}{0.47} = 0.34$$

$$\text{Accuracy} = \frac{13+39}{76} = 56.6\%$$

The above results show that the sensitivity of CEA levels to ctDNA is 84.2%, and specificity is 47%. From the sensitivity results, it can be concluded that CEA can detect EGFR mutations in plasma by 84.2%. CEA specificity of 47% means that the probability of CEA detecting lung cancer patients without EGFR mutations in plasma is 47%. A PPV of 34.8% means

that only about 34.8% of ctDNA are truly mutated. An NPV of 70% means that approximately 70% of the ctDNA of lung cancer patients are completely unmutated. The LR+ is 1.78, and the LR- is 0.34. The accuracy of the CEA level for detecting EGFR mutations in plasma is 56.6%.

Table 4 shows that the average CEA in subjects with positive ctDNA was 189.88; this value is higher than the group without the EGFR mutation, with an average CEA of 45.36. Using Mann-Whitney test, $P < 0.001$ was obtained, so it can be concluded that there is a significant difference between the CEA values in the group with positive and negative ctDNA.

In the ROC analysis (Figure 2), the area under curve (AUC) value is 0.781 or 78.1%. This means that CEA level has moderate accuracy in determining EGFR mutations in plasma.

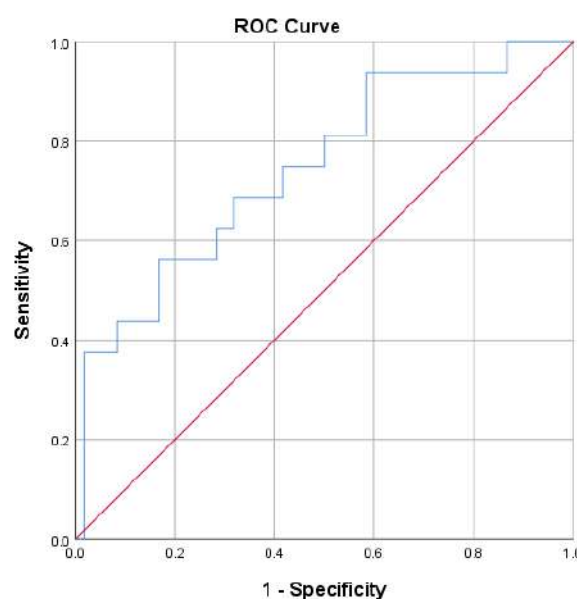


Figure 2. ROC curve of CEA value against ctDNA

Based on plotting, the cut-off value for CEA is 14.8. So, it can be concluded that the cut-off of CEA level that allows a positive result of ctDNA is 14.8 ng/ml.

DISCUSSION

In this study, the gender distribution shows that 36 subjects are female (47.4%) and 40 are male (52.6%). Positive EGFR mutations were found more in females (26.3%) than males (18.4%). Similar thing can be found for ctDNA, in which the positive result can be found in 15.8% females and 9.2% males. The results of this study is similar the ones by of Reck et al and Gao et al which found that positive EGFR mutations in tissue and plasma are more commonly found in female ($P=0.072$ and $P=0.111$ respectively).^{14,15} On the other hand, a study by Feng et al revealed that the level of EGFR mutation was not related to gender.¹⁶

The study on smoking history found that higher percentage of positive EGFR mutations in the tissue was found in non-smokers (18.5%) than smokers (14.5%) with $P=0.031$. Positive mutations in ctDNA also were more common in non-smokers (15.8%) than smokers (9.2%) with $P=0.352$. Because smoking is one of the main risk factors for lung cancer, smoking is considered a reference for statistical analysis. This indicates that smoking history has a significant association with EGFR mutations in the tissue. This result is similar to other studies which said that positive EGFR mutations are more common in non-smokers.^{6,14}

Based on their occupations, the subjects were divided into two major groups: those with high risk of lung cancer (farmers and artisans) and those with lower risk (homemakers, office employees, entrepreneurs or traders, civil servants, etc). The results of this study are in accordance to the literature which shows that positive EGFR mutations are more commonly found in women, in this case, homemakers. However, there's no significant association between highrisk occupation and EGFR mutation status in tissue ($P=0.816$) and plasma ($P=0.583$).^{6,14}

In this study, positive EGFR mutations from tissue samples were mostly found in stage IVa (40.8%) and the least in stages IIIb and IIIc (0%) but there's no significant difference ($P=0.450$). In ctDNA, positive EGFR mutations were only found at stage IVa (23.7%) and IVb (1.3%), but this isn't significantly

different either ($P=0.182$). On ctDNA examination, there was no positive EGFR mutation at all for stage III. Of the 19 subjects who were tested positive for EGFR mutations from plasma, all of them were in stage IVa and IVb. Herman et al's study also found positive EGFR mutations in ctDNA in subjects with NSCLC stage IVa and IVb, with no positive ctDNA results in stage III.¹⁷ Qui et al's study said that the level of positive EGFR mutations in ctDNA tends to be higher in more advanced cancer stage.⁵ This is because ctDNA originates from tumor cells that undergo apoptosis and necrosis, thereby releasing DNA into the bloodstream. These DNA fragments then carry tumor-specific sequences (ctDNA).^{5,6,17}

Based on the histological type, positive EGFR mutations in NSCLC were most commonly found in adenocarcinoma (29 subjects), and the least in SCC (2 subjects). Likewise, on ctDNA examination, positive EGFR mutations were found most in adenocarcinoma (16 people) and least in SCC (1 person). This is in accordance to the literature where it is said that EGFR mutations are most commonly found in adenocarcinoma. Therefore, for statistical calculations, adenocarcinoma was used as a reference. With adenocarcinoma as a reference, there was no significant association between cancer type and EGFR mutation status in SCC and adenosquamous tissue samples ($P=1.000$ and $P=0.725$ respectively) or ctDNA (both $P=1.000$).^{6,14,18}

The largest amount of samples for tissue EGFR examination came from FNAB, which contributed to 45 samples (59.2%), compared to TBNA which only had 1 sample. With FNAB as a reference, it was found that the examination results from the biopsy were more likely to get positive EGFR mutation results compared to FNAB, with $P=0.033$. The study conducted by Guan et al compared EGFR mutations in tissue samples and pleural effusions in adenocarcinoma patients; the result was that 34% of tissue samples and 30% of pleural fluid tested positive for EGFR mutations.¹⁹ Though the percentage of positive mutations in tissue is higher than the ones from pleural fluid, the difference isn't statistically significant.^{19,20}

This study shows a significant association between tissue EGFR mutations and serum CEA levels ($P=0.037$) with an OR of 2.778. The average CEA in subjects with positive EGFR mutations is 108.04; this value is higher than in the group without EGFR mutations, which has an average CEA of 60.00 ($P=0.005$). It can be concluded that there is a significant difference between the CEA values in the two groups. This is similar to a previous study at Saiful Anwar Hospital, Malang by Normawati et al which found a significant association between tissue EGFR mutations in adenocarcinoma patients and serum CEA levels.¹³ Gao et al and Lv et al also revealed that elevated serum CEA levels were significantly associated with EGFR mutations.^{15,21}

Mutations of EGFR in plasma also showed a significant association with serum CEA levels in this study ($P=0.015$) with an OR of 4.8 (cut-off=5 ng/ml). The average CEA in subjects with positive ctDNA was 189.88; this value was higher than the group without the EGFR mutation, which had an average CEA of 45.36 ($P<0.001$). A study conducted by Que et al found a significant association between increased serum CEA levels and the possibility of positive ctDNA with $P=0.034$. Another study conducted by Guo et al found the same thing, while Jin et al found that lung adenocarcinoma patients who had CEA serum levels of 20 ng/mL were the ideal population to receive targeted therapy using EGFR-TKI.^{6,22,23}

Many factors, both malignant and non-malignant might affect CEA levels. Aside from NSCLC, malignancies in the ovarium, breast and thyroid also caused an increase in CEA levels. In non-malignant cases, CEA levels were elevated in smokers, emphysematous lung, appendicitis, cholecystitis, cirrhosis of the liver, pancreatitis, inflammatory bowel disease and patients receiving orlistat therapy. So, in this study, there's a possibility that the increase in CEA levels on the subjects is due to the history of smoking in the patient, emphysematous lung on chest x-ray or an infectious process.^{24,25}

This study also examined sensitivity, specificity, accuracy, PPV, NPV, LR+, LR- and accuracy of

serum CEA levels to detect EGFR mutations in tissue and plasma. Based on the analysis, it was found that the sensitivity of CEA levels to detect EGFR mutations in tissue was 73.5% while the specificity was 50%, PPV=54.3%, NPV=70%, LR+=1.47, LR-=0.53 and accuracy 60.5%. The values obtained are almost the same as those of Pan et al and Normawati et al.^{13,26} Pan et al found that the sensitivity and specificity of CEA in estimating EGFR mutations were 76% and 45% respectively, while the PPV and NPV were 52% and 71%.²⁶ Normawati et al found the sensitivity and specificity of CEA in estimating EGFR mutations were 77% and 50%, while PPV and NPV were 53% and 76%.¹³

In regards to the examination of CEA levels to detect EGFR mutations in plasma, it's found to have 84.2% sensitivity, 47% specificity, 34.8% PPV, 90% NPV, 1.78 LR+, 0.34 LR- and 56.6% accuracy. These results are not significantly different compared to the one to detect EGFR mutations in the tissue. A study conducted by Gao et al found that the sensitivity of CEA to detect EGFR mutations was 69.6% and the specificity was 48.8%.¹⁵

The ROC curve presented in Figure 1 shows an AUC value of 68.8%, so CEA is considered weak in estimating EGFR mutations in tissues. The CEA cut-off value that allows positive EGFR mutation in tissues is 9.14 ng/ml. From the results of the new CEA cut-off value of 9.14 ng/ml, it was found that the specificity, PPV, NPV, LR+ and accuracy were better than using a CEA cut-off of 5 ng/ml in determining EGFR mutations in tissues. Figure 2 shows the AUC value of 78.1%, so CEA is considered to have a moderate level of accuracy in determining EGFR mutations in plasma. The CEA cut-off value that allows positive mutations in blood is 14.8 ng/ml. From the new CEA cut-off value, it was found that the specificity, PPV, LR+, LR- and accuracy were better than using the 5 ng/ml cut off. Research by Pan et al suggested that CEA may not be an ideal predictor for EGFR mutations with a ROC curve of 0.608; this is thought to be due to the expression of CEA levels that can be influenced by various causes and from multiple pathways.²⁶ Research conducted by Yan Ling et al found the AUC value of 59% with a CEA cut

off of 87 ng/ml, which means CEA has a weak association with EGFR mutations.²¹ Using the new CEA cut-off, better results of PPV, LR+ and accuracy were obtained, but this cannot be used globally because it is only limited to this research. Another study with larger number of samples is needed to prove this hypothesis.^{12,21}

The limitation of this study is that it only examined the association of tissue and blood EGFR mutations with a single tumor marker, namely CEA. This study also only noted the presence or absence of EGFR mutations in tissue and blood without distinguishing common mutations and uncommon mutations or which exon the mutations happen to.

CONCLUSION

This study found that EGFR mutations are most commonly found in females, non-smokers and adenocarcinoma type. Elevation in CEA level is found to be significantly associated to EGFR mutations both in tissues and serum, but CEA only has weak accuracy in determining EGFR mutation in tissues and moderate in blood. A higher cut-off value of CEA is needed to allow positive EGFR mutation.

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