ORIGINAL ARTICLE

Rate Between Examination of EGFR Mutation Blood Plasma Sample (ctDNA) With Cytological/Histopathological Sample in Adenocarcinoma Lung Cancer

Dian Utami Wulaningrum*, Ana Rima Setijadi, Suradi

[1]Department of Pulmonology and Respiratory Medicine Faculty of Medicine, Universitas Sebelas Maret, dr. Moewardi Hospital, Surakarta

ABSTRACT

Background: Most adenocarcinoma lung cancer which is found at an advanced stage with cytology / histopathological samples is hardly available. Examinations EGFR mutations as a biomarker for adenocarcinoma lung cancer using cytological/histopathological sample (tissue biopsy or resection and cytologi) and ctDNA blood plasma. Examination of EGFR mutations in ctDNA blood plasma sampling is simpler and easier, which also can be used as predictive and prognostic markers in non-small cell carcinoma lung cancer patients. The purpose of this study is to determine and analyse the degree of compatibility between examination of EGFR mutations by blood plasma (ctDNA) samples with the examination of cytology/histopathological EGFR mutations in adenocarcinoma lung cancer.

Methods: Diagnostic test research, by taking medical records of patients with adenocarcinoma lung cancer from January to September 2019 at Dr. Moewardi Surakarta, who was examined by EGFR mutations in cytology / histopathology and ctDNA samples.

Result: The Subjects of this study were 73 patients with adenocarcinoma lung cancer. The level of compatibility of ctDNA with EGFR mutations in cytology/histopathology samples was categorized as moderate and statistically significant (Kappa=0.459; P=0.0001), with a sensitivity value of 54.5% and a specificity of 90%.

Conclusion: The high/moderate concordance between the different DNA sources for egfr mutations (cytology/histopathology) and ctDNA.

Keywords: lung cancer, adenocarcinoma, EGFR, ctDNA

*Correspondence: Dian Utami Wulaningrum - dianutamiw@gmail.com

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INTRODUCTION

Non-small cell carcinoma lung cancer is the leading cause of cancer-related death. Lung cancer cases are found at an advanced stage due to non-specific symptoms (57% of lung cancer cases in the US are detected by metastasis). Pulmonary adeno-carcinoma represents about 50% of lung cancers and 60% of LCMC. EGFR examination becomes a routine test after the diagnosis of pulmonary adenocarcinoma has been established for the selection of patients receiving tyrosine kinase inhibitors (TKIs). EGFR mutation examination samples derived from tissue biopsy/resection and cytology are often not available because tumor biopsy is an invasive and high-risk procedure. Liquid biopsy can detect biomarkers associated with tumors to diagnose lung cancer earlier and more safely.[1–7]

The ASSESS study in the subset of Spanish patients, the good concordance (almost 90%) between the different DNA sources supports the use of plasma samples when tumor tissue is not available. Zhang et al's study about ctDNA assessment of EGFR mutation status in Chinese patients with advanced non-small cell lung cancer in real-world setting conclude ctDNA based EGFR mutation test is feasible and could be a surrogate when tissue biopsy is not available.[1–7]

The purpose of this study was to determine and analyze the degree of concordance of the EGFR mutations examination originating from blood plasma samples (ctDNA) with examinations derived from cytology /histopathology. This study hope to be able to provide scientific information regarding the concordance of EGFR mutations examination originating from blood plasma (ctDNA) with cytology/histopathology samples and as basic data for further research.

METHOD

This research is a diagnostic test research. The study population was all lung cancer populations with adenocarcinoma types, naïve treatment. The diagnosis was confirmed by the presence of lung mass images on CT-scans and cytology/histopathology examinations were performed for both primary tumours and metastatic lesions and EGFR mutations were examined from cytology/histopathology samples and blood plasma (ctDNA) who underwent outpatient and inpatient care at Dr. Moewardi Surakarta from January-September 2019.

The inclusion criteria is patients with adenocarcinoma lung cancer, the diagnosis is confirmed by the presence

of lung mass images on CT-scan and cytology/histopathological examination of both primary tumors and metastatic lesions with adenocarcinoma results, which are subjected to ctDNA examination and examination of EGFR mutation cytology/histopathology. The exclusion criteria are the patient's medical records are missing or incomplete, examination of EGFR mutations is performed in only one of the two tests for EGFR mutations (examination of EGFR mutations from cytology/histopathology only, or blood plasma ctDNA only). The sample of this study were obtained from the medical record data of adenocarcinoma lung cancer patients at Dr. Moewardi Hospital.

Examination of EGFR mutations with cytological samples (pleural effusion, bronchus rinse, bronchial swab, FNAB) or histopathology fixed with a neutral buffer of 10% formalin on cell blocks or slides stained / not stained with tumor cell counts greater than 200 cells and containing more From 50% tumor cells, both primary and metastatic lesions using the DNA extraction method with the Qiagen QIAamp® DNA Micro kit, mutation analysis using PCR HRM, fragment analysis, direct sequencing and amoyDx. 100% specificity. ctDNA examination using Therascreen EGFR Plasma RGQ PCR Kit.

Sample criteria were plasma EDTA from adenocarcinoma positive patients. Minimum plasma volume of 4.5 ml. One job can be done up to a maximum of 16 samples or 1 kit can be divided by 3x runs with samples per one run. Plasma sample separation and storage procedures prior to EGFR examination was fresh blood samples should be centrifuged immediately to separate the plasma within 2 hours if stored at room temperature or 8 hours if stored at 4^{o}C. Fresh blood centrifuge at 2000x g for 10 minutes. Separate the plasma into a new tube. The separated plasma can be stored at -20^{o}C for 4 weeks or -80^{o}C for longer storage. Avoid re-freezing. Only thaw if an examination is to be performed. Thaw the plasma at room temperature on the day of the EGFR examination. Centrifuge a second time at a speed of 16,000 x g for 10 minutes at 4^{o}C (at fixed angle rotor). Separate the clear portion of the plasma into a 14 ml sample tube (14 ml Falcon® polystyrene 17 x 100 mm round bottom tube (BD, paint no. 352051)) to continue the extraction process. Ethical clearance approved by The Health Research Ethics Committee Dr. Moewardi General Hospital.

Data analysis was performed using SPSS 21 for Windows. In this

study, the analysis is presented with a frequency distribution and percentage. The degree of concordance between the results of the EGFR mutation examination with blood plasma samples (ctDNA) and cytology/histopathology samples was calculated by means of a test of agreement (Kappa Cohen) with kappa values:> 0.8 (very good suitability), kappa: 0.6 - 0.8 (good suitability), kappa: 0.4 - 0.6 (moderate suitability) and kappa: <0.4 (less suitability). The degree of concordance between ctDNA examination results and EGFR mutations (cytology/ histopathological samples) is presented in the 2x2 table. We calculate sensitivity, specificity, positive and negative predictive value.

RESULT

The research was conducted in the medical record room of Dr. Moewardi Surakarta from October 2019 to November 2019. The results of recording the medical records of patients with adenocarcinoma lung cancer that were carried out by EGFR examination were obtained from cytology/histopathology and blood plasma (ctDNA) samples from both primary tumors and metastatic lesions obtained by 73 people. The scheme sample collection is on Figure 1.

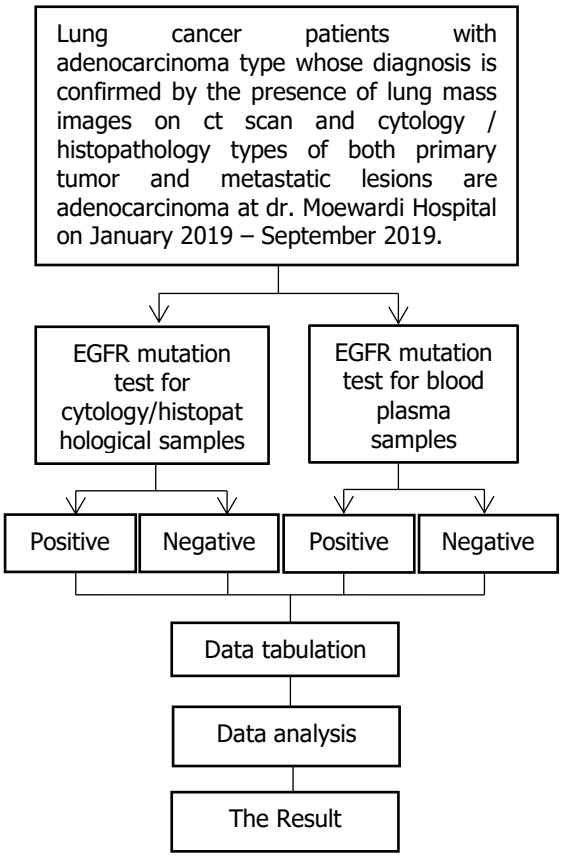

Figure 1. The scheme of sample collection

Lung cancer patients with adenocarcinoma types based on the table above were 35 men (47.9%) and 38 women (52.1%). The majority of patients with adenocarcinoma lung cancer in the study were ≥40 years by 67 people (91.8%), while the rest <40 years were 6 people (8.2%).

Table. 1 Basic characteristics of research subjects

Characteristics	Frequency	Percentage
Gender		
Male	35	47.9
Women	38	52.1
Age		
<40 years	6	8.2
≥40 years	67	91.8
EGFR Mutation Results		
No mutations	39	53.4
Del Exon 19	18	24.7
Exon 21 L861Q	1	1.4
Exon 21 L858R	10	13.7
Exon 20 T790M + Exon 21 L858R	1	1.4
Del Exon 19 + Exon 21 L858R	2	2.7
Exon 21 L858R + Exon 21 L861Q	2	2.7
ctDNA results		
No mutations	48	65.8
Del Exon 19	16	21.9
Exon 21 L858R	7	9.6
Exon 20 T790M + Exon 21 L858R	2	2.7
Smoking History		
Yes	30	41.1
Not	43	58.9
Stadium		
IIIC	4	5.5
IV	69	94.5
Metastasis		
There is no	4	5.5
Pleural effusion	25	34.2
Bone metastasis	6	8.2
Metastatic liver	3	4,1
Brain metastasis	2	2.7
Pneumonic type	3	4,1
Pleural effusion, bone, liver metastases	5	6.8
Pleural effusion, bone metastasis	8	11
Pleural effusion, bone, brain metastasis	1	1.4
Pleural effusion, colli lymphadenopathy	2	2.7
Bone, brain metastasis, pneumonic type	2	2.7
Bone metastases, colli lymphadenopathy	1	1.4
Bone metastasis, pneumonic type	2	2.7
Pleural effusion, liver metastases	5	6.8

Characteristics	Frequency	Percentage
Bone, liver metastases	3	4,1
Bone, brain metastasis	1	1.4
Cytology / Histopathology Samples		
TTNA	40	54.8
Bronchial brushing	7	9.6
Bronchial washing/BAL	4	5.5
Endobronchial biopsy	6	8.2
FNAB	7	9.6
Thoracocentesis	8	11.0
Core Needle Biopsy	1	1.4

The results of ctDNA examination were obtained as follows, there were no mutations in 48 people (65.8%), 16 people with exon 19 deletions (21.9%), 7 people with exon 21 L858R (9.6%), 20 T790M exon. + Exon 21 L858R there are 2 people (2.7%). Smoking history was also found on the patients there were 30 people (41.1%) smoked while 43 people (58.9%) did not smoke. The stage in the subjects of this study was an advanced stage, there were 4 people (5.5%) with stage IIIC, while in stage IV there were 69 people (94.5%).

The table above also shows that 4 patients (5.5%) had no metastases, while the other 69 patients had metastases (94.5%). Pleural effusion occurred in 25 people (34.2%), this is the most common metastasis in the subjects of this study. Table 1 shows that the metastases that occurred in the subjects of this study did not only occur in one place but there were also several places such as pleural effusion, bone metastasis and brain metastasis simultaneously.

Table 2. Results of EGFR mutations in cytological/histopathological samples by sex

Sex	EGFR Mutation Results		Total
	Negative	Positive	
Male	23 (31.5%)	12 (16.4%)	35 (47.9%)
Women	17 (23.3%)	21 (28.8%)	38 (52.1%)
Total	40 (54.8%)	33 (45.2%)	73 (100%)

The result of EGFR mutations based on cytology/histopathological samples based on sex was more prevalent in 21 women (28.8%) compared to men, namely 12 (16.4%) shown in Table 2.

Table 3. Results of ctDNA by sex

Sex	ctDNA results		Total
	Negative	Positive	
Male	27 (37.0%)	8 (10.9%)	35 (47.9%)
Women	21 (28.8%)	17 (23.3%)	38 (52.1%)
Total	48 (65.8%)	25 (34.2%)	73 (100%)

Table 4. Kappa test results between ctDNA and EGFR mutation examination of cytology / histopathology samples

CTDNA	EGFR mutations		Total	Kappa test	P
	Positive	Negative			
Positive	18	4	22	0.459	0.0001
Negative	15	36	51		
Total	33	40	73		

Table 5. Sensitivity and specificity tests

ctDNA	EGFR mutations		Total
	Positive	Negative	
Positive	18	4	22
Negative	15	36	51
Total	33	40	73
Sensitivity	: 0.545	Specificity	: 0.900
PPR	: 0.818	NEV	: 0.706
PLR	: 5,450	NLR	: 0.505

Note: PPR=Positive Probability Ratio, NEV: Negative Estimated Value, PLR: Positive Likelihood Ratio, NLR: Negative Likelihood Ratio

The results of ctDNA examination based on sex in Table 3 show that there were more mutations in 17 women (23.3%) while in men there were 8 people (10.9%). The ctDNA examination compared with EGFR mutations with cytology/histopathology samples is called true positive taking into account the same mutation points. The kappa test results show a value of 0.459 which means the level of concordance between the results ctDNA with EGFR examination of cytology/histopathological samples with moderate levels, P=0.0001 (P<0.05), which means that the suitability of ctDNA with cytology/histopathology was statistically significant.

The diagnosis of EGFR mutations with ctDNA samples obtained a sensitivity of 54.5%, which means that 54.5% of the diagnosis of EGFR mutations in cytology/histopathology samples with positive results could be detected by positive ctDNA examination and the specificity value of ctDNA measurements obtained in this study was 90. 0% means that 90.0% of the diagnosis of negative EGFR mutations will be excluded in ctDNA positive patients.

On examination, the NDP value was 81.8%, which means that if the ctDNA test was positive, then there was an 81.8% chance of a positive EGFR mutation diagnosis. While the NDN value is 70.6%, which means that if the ctDNA is negative, there is a 70.6% chance of diagnosing an EGFR mutation with a negative result.

The RKP value is 5.45, which means that the possibility of a patient with a positive ctDNA result will get a

diagnosis of an EGFR mutation with a positive result of 5.45 times greater than a negative ctDNA. The RKN value is 0.505, which means that the likelihood that a patient with a negative ctDNA measurement value will get a diagnosis of an EGFR mutation with a positive result is 0.505 times less than a patient with ctDNA with a positive result.

DISCUSSION

This study found a total sample of adenocarcinoma lung cancer patients who were examined for ctDNA and EGFR mutations with 73 cytology/ histopathological samples. The subjects of this study were 38 women (52.1%) who suffered from adenocarcinoma lung cancer, this is in accordance with the histological prevalence of adenocarcinoma tumors which occurred more in women over three decades, with the incidence increasing slowly.[8]

The age of the patients as the subjects in this study tend to be ≥40 years with 91.8%, where age ≥40 years was a high risk of lung cancer.[9] EGFR mutations in cytology /histopathological samples in this study occurred many deletions of exon 19, there were 18 people (24.7%) and 10 exon 21 L858R mutations (13.7%), Pal et al stated that the most common mutations were in exon 19 and exon 21 L858R2. Lyu et al's research in 2018 showed that ctDNA examination had higher accuracy against deletions of exon 19 and exon 21 L858R, in a study conducted at dr. Moewardi showed that exon 19 deletions were 16 people (21.9%) and exon 21 L858R, namely 7 people (9.6%).[10]

This study shows that 58.9% of patients do not smoke, this may be due to the number of female subjects more than men. Table 1 shows that the subjects of this study are advanced stages patients, with stage IIIC and IV with metastases in various places, Cheng et al in 2017 stated that 57% of patients in the US were diagnosed at an advanced stage and had metastasis. Qiu et al's (2015) study cited that patients with advanced stages have high levels of circulating tumor DNA, the current hypothesis indicates that the amount of ctDNA is associated with tumor volume and metastasis.[11]

The results of this study indicate that the number of cytological samples (TTNA, bronchial brushing, bronchial rinse, FNAB, pleural fluid) was higher than the histopathological samples (core biopsy, forceps biopsy). A study conducted by Yatabe in 2015 showed that 98% of the data for examining EGFR mutations in Indonesia were

cytology samples and 2% biopsy samples.[12]

Hlinkova's research in 2013 showed that 85.9% of cytological samples were used to examine EGFR mutations. Two-thirds of patients are at an advanced stage when they were diagnosed for lung cancer, only small biopsy or cytology specimens are available for EGFR examination in the majority of patients. Any type of small biopsy or cytological specimen is suitable for examination of mutations that have been confirmed by reports from various laboratories around the world.[12]

The results of EGFR mutations in cytology/histopathology samples (table 2) and the results of EGFR mutations in blood plasma samples (ctDNA) in table 3 based on sex showed that more women were 22 subjects and 17 subjects were male. These results are in accordance with the study by Zhang et al. In 2016 which concluded that mutations were more common in women (43.7%) than in men (24%). The clinic pathological features that correlate with the EGFR mutation include East Asian ethnicity, adenocarcinoma histology, women and a history of never smoking.[13]

The kappa test results in table 4 show a value of 0.459, which means the level of concordance between the ctDNA results and the examination of the EGFR mutation in the cytology/ histopathology sample is moderate, with P=0.0001 which means statistically significant with a sensitivity of 54.5%, specificity of 90%, PPR 81, 8%, NEV 70.6% (table 5). These results differ from the 2016 ASSESS study regarding the suitability level of 90%, 50% sensitivity, 80% PPR, and 91% NEV, while the 2014 IFUM study had a 95% conformance rate, 73% sensitivity, 99% specificity, 94% PPR, and 95% NEV.[14] The IGNITE Study in 2013 – 2014, mutation status concordance between 2581 matched tissue/cytology and plasma samples: 80.5% (sensitivity 46.9%, specificity 95.6%).[15]

Zhang et al's study about ctDNA assessment of EGFR mutation status in Chinese patients with advanced non-small cell lung cancer in real-world setting, in this study 35 patients had both tissue and plasma samples and the detection concordance was 68.6% (24/35).[3] Mao et al in 2013 presents the meta-analysis of diagnostic tests for EGFR mutation in blood using EGFR mutation in tumor tissues as the gold standard, the sensitivity, specificity, and concordance rate were 0.61 (95% CI 0.50–0.71), 0.90 (95% CI 0.85–0.94), and 0.79 (95% CI 0.73–0.84), respectively.[15]

This difference in the level of concordance may be due to the fact that the specimens examined are not optimal, this can occur from blood collection, specimen treatment, delivery of specimens from the collection location to the examination site, DNA purification, and gaps from knowledge.[11,14]

The results of this study indicate that ctDNA has high specifications and is an effective biomarker for detection of EGFR mutation status. These results are consistent with research conducted by Qiu et al. 2015 with the conclusion that ctDNA is an effective method for detecting EGFR mutation status in KPKBSK, based on high diagnostic accuracy and specificity, ctDNA can be the main screening test for NSCLC and ctDNA analysis methods. Standardized and validation still needs to be developed.[11]

CONCLUSION

The level of concordance between the examination of the EGFR mutation of blood plasma samples (ctDNA) and the examination of the EGFR mutation of the cytology / histopathology sample was in the moderate category (kappa test 0.459) and statistically significant, with a sensitivity value of 54.5% and a specificity of 90%.

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