Clinical Characteristics, Tumor, Node, Metastasis Status, and Mutation Rate in Domain of Epidermal Growth Factor Receptor Gene in Serbian Patients with Lung Adenocarcinoma

Bojan Zaric, Vladimir Stojsic,* Tomi Kovacevic,* Tatjana Sarcev,* Aleksandar Tepavac,* Radmila Jankovic,† Jelena Spasic,† Davorin Radosavljevic,† Paul Zarogoulidis,‡ Nevena Vukobradovic-Djoric,§ and Branislav Perin**

Objective: Mutation rate in domain of *EGFR* gene varies between populations of lung cancer patients. Primary aim of this study was to analyze clinical and pathological characteristics, and tumor, node, metastasis status and stage of diseases, in relation to mutation status.
Methods: After histological confirmation of lung adenocarcinoma tissue obtained during bronchoscopy was consecutively sent for EGFR testing. Genomic DNA extraction was performed with the QIAamp DNA FFPE Tissue kit. Clinical data for multivariate analysis were extracted from hospital based-lung cancer registry.
Results: Among 360 tested patients, there was 67.8% males and 32.2% females, aged 61 ± 9.8 years. Majority of patients were smokers (57.0%) with Eastern Cooperative Oncology Group 1 performance status (92.2%). Mutation in EGFR gene was detected in 42 (11.7%) patients. Deletion in exon 19 was detected in 24 (6.7%) patients, mutation in exon 21 in 17 (4.7%), and mutation in exon 18 in one patient (0.3%). Patients were mostly diagnosed in stage IV adenocarcinoma (74.4%). Statistically significant differences were determined in relation to smoking ($p < 0.001$), T descriptor (size; $p = 0.019$) and gender ($p = 0.002$).
Conclusions: Mutation rate in domain of EGFR gene in investigated lung cancer population is in range with reported data in Caucasian race. Smoking, T descriptor and gender were found to be related to the EGFR status.

Key Words: Adenocarcinoma of the lung, Epidermal growth factor receptor, lung cancer, Mutation, non–small-cell lung cancer, targeted therapy

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*Institute for Pulmonary Diseases of Vojvodina, Clinic for Pulmonary Oncology, Faculty of Medicine, University of Novi Sad, Sremska Kamenica; †Institute for Oncology and Radiology of Serbia, Belgrade, Serbia; ‡Pulmonary Department-Oncology Unit, "G. Papanikolaou" General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece; and §ROCHE, Beograd, Serbia.

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Address for correspondence: Bojan Zaric, MD, PhD, Department for Invasive Diagnostics, Faculty of Medicine, Institute for Pulmonary Diseases of Vojvodina, Clinic for Thoracic Oncology, Clinical Trials Unit, University of Novi Sad, Put doktora Goldmana 4, 21204 Sremska Kamenica, Serbia. E-mail: bojanzaric@neobee.net

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Testing of tumor tissue for presence of activating mutations in domain of EGFR gene became routine in the diagnostic algorithm for evaluation of lung adenocarcinoma.[1–5] General recommendations of expert societies state that clinical characteristics (such as gender, age, ethnicity, and smoking history) are not sufficiently sensitive to select patients for molecular testing. However, it is well known that mutation rate in domain of the EGFR gene in adenocarcinoma of the lung is higher in Asian population, women, and non-smokers.[6–12] In a limited resource setting, especially in low-income countries, molecular tests are not readily available to test all patients. In that case, some kind of clinical or clinicopathological selection must be imposed. Major aims of this study were determination of mutation rate in domain of EGFR gene in patients with adenocarcinoma of the lung among Caucasian Serbian population, and evaluation of relationship between clinical characteristics and tumor, node, metastasis (TNM) status on one side and mutation rate on the other.

In a situation when EGFR mutation testing rate drops significantly, mainly because of discontinuation of support from pharmaceutical industry, it is essential to know how many patients absolutely need to be tested to receive the most appropriate treatment. Knowing the EGFR mutation rate in lung adenocarcinoma population and its relation with clinical characteristics and TNM staging might facilitate creation of appropriate national strategy to implement molecular testing in routine medical oncology diagnostics.

METHODS

The study was a prospective, non-randomized trial, conducted at the Institute for Pulmonary Diseases of Vojvodina and the Institute for Oncology and Radiology of Serbia in the period from January 2012 to November 2013. It was approved by the institutional review and ethics board. All of the patients screened for the enrollment were previously scheduled for routine bronchoscopy, because of high suspicion of having lung cancer established according to imaging studies. After bronchoscopy and confirmation of lung adenocarcinoma histology, all tumor tissue samples with adequate histology were consecutively sent to pathology department and genetic

TABLE 1. Demographic Characteristics

Characteristic	*N* (%)
Gender	
Male	244(67.8)
Female	116 (32.2)
Smoking history	
Active smoker	204 (57.0)
Former smoker	77 (21.5)
Non-smoker	77 (21.5)
EGFR status	
Wild type	318 (88.3)
Mutated	42 (11.7)
Type of mutation in 21 mutated patients	
Exon 19 deletion	24 (6.7)
Exon 21 mutation	17 (4.7)
Exon 18 mutation	1 (0.3)

laboratory for EGFR mutation status testing. Inclusion criteria were: age over 18 years; histological or cytological confirmation of lung adenocarcinoma; chemotherapy, targeted therapy, and radiotherapy naïve; stage IIIB or IV; and sufficient amount of tissue for genetic testing. Exclusion criteria were non-adenocarcinoma of the lung histology/cytology, previous chemotherapy, radiotherapy or targeted therapy, and insufficient amount of tissue.

Genomic DNA used for EGFR mutation analysis was isolated from formalin-fixed and paraffin-embedded tumor samples from 360 patients with non–small-cell lung cancer. Genomic DNA extraction was performed with the QIAamp DNA FFPE Tissue kit (QIAGEN, United Kindgom). For determining the 28 different mutations in exons 18–21 of the *EGFR* gene, *therascree*n EGFR PCR Kit (QIAGEN Manchester Ltd., United Kingdom) was used. All statistical analyses were performed with SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL).

RESULTS

There was 244 (67.8%) male and 116 (32.2%) female patients, average age 61 ± 9.8, range from 38 to 78, enrolled in this trial. Majority of patients, 332 (92.2%) were Eastern Cooperative Oncology Group performance status 1. Most of patients were active and former smokers 204 (57.0%) and 77 (21.5%), respectively. Demographic characteristics are given in Table 1. Among 360 evaluated lung adenocarcinoma patients, 42 (11.7%) are harboring EGFR mutation. Twenty four patients or 6.7% have deletion in exon 19, and 17 (4.7%) have mutation in exon 21, whereas one patient (0.3%) has mutation in exon 18. Two-hundred sixty-eight patients or 74.4% were diagnosed in stage IV, whereas 92 (25.6%) patients were in stage IIIB. Characteristics related to clinical staging are given in Table 2. Considering the fact that 74.2% of patients were diagnosed with metastatic (M1) disease, evaluation of subgroup with metastases was performed. Subgroup analysis was performed in relation to the site of the metastasis. Most often metastatic spread involved contralateral lung, pleural effusion, adrenal glands, brain, bones, and liver. Cough was the most common symptom in the investigated group, present in 242 (67.2%) of patients. Multivariate analysis evaluated all demographic data, clinical characteristics, symptoms and signs, and site of metastases.

TABLE 2. Characteristics Related to Clinical Staging in Patients Tested for EGFR Mutation

Characteristic	*N* (%)
T factor (TNM)	
T1a	12(3.3)
T1b	9 (2.5)
T2a	51 (14.2)
T2b	29 (8.1)
T3	101 (28.1)
T4	158 (43.9)
N factor (TNM)	
N0	66 (18.3)
N1	10 (2.8)
N2	127 (35.3)
N3	157 (43.6)
M factor (TNM)	
M0	93 (25.8)
M1a	137 (38.1)
M1b	130 (36.1)
Stage	
IIIB	92 (25.6)
IV	268 (74.4)

TNM, tumor, node, metastasis classification.

Statistically significant relation was found between positive EGFR mutation status and gender ($p = 0.002$), T status in TNM classification ($p = 0.019$), and smoking ($p < 0.001$). Results of multivariate analysis along with p values are given in Table 3.

DISCUSSION

One of the most important results of this trial is insight into mutation status of the *EGFR* gene in lung adenocarcinoma of Eastern European Caucasian population. Mutation rate in domain of *EGFR* gene is highly dependent on race, being highest in Eastern Asia population and lowest in Nordic European countries. Serbian population is relatively homogeneous because migrations bypassed Eastern Europe because of poor income of the countries. Finally, these results fill in the gap in reports from various geographical regions.

EGFR mutation rate varies between populations of patients with lung adenocarcinoma and ranges from 10% to 15% in Caucasian population to over 50% in most Asian populations. Average mutation rate detected in our study is 11.7%, what correlates with the average for Caucasian race. Only data originating from Slavic ethnicity came from a Russian trial[1] which reported high mutation rate of 20%. In the most recently published French trial,[2] EGFR mutation rate detected after testing of 1332 patients was 13.5% with slight predominance of exon 19 deletion (52.7%) over L858R mutation detected in 48.3% of patients. The same

TABLE 3. Results of Multivariate Analysis Along With *p* Values

	Multivariate Analysis
Demographic Characteristic	***p* Value**
Gender	p = 0.002*
Age	*p* = 0.243
Smoking history	p < 0.001*
Pack-years index	p = 0.019*
Tumor size	*p* = 0.247
ECOG	*p* = 0.217
Clinical characteristic	
T factor (TNM)	p = 0.019*
N factor (TNM)	*p* = 0.863
M factor (TNM)	*p* = 0.793
Stage	*p* = 0.783
Site of metastasis	
Pleural nodes	*p* = 0.756
Pericardial effusion	*p* = 0.915
Pleural effusion	*p* = 0.304
Contralateral pulmonary node(s)	*p* = 0.904
Brain	*p* = 0.363
Liver	*p* = 0.373
Adrenal gland	*p* = 0.178
Bones	*p* = 0.629
Other	*p* = 0.811
Symptoms and signs	
Cough	*p* = 0.435
Dyspnea	*p* = 0.761
Chest pain	*p* = 0.521
Hemoptysis	*p* = 0.857
Hoarseness	*p* = 0.562
Dysphagia	*p* = 0.700
Body weight loss	*p* = 0.394
Fever	*p* = 0.418
Neurological disorders	*p* = 0.404
Bone pain	*p* = 0.915

TNM, tumor, node, metastasis classification; ECOG, Eastern Cooperative Oncology Group.
*Statistically signifcant.

pattern is observed in our trial. Millela et al.[3] investigated EGFR molecular profiling in a phase II study in molecularly or clinically selected patients pretreated with chemotherapy. Comparable age was observed in our group and this Italian trial. Gender distribution in Milella's study revealed 59.6% of male and 40.4% of female patients, also comparable with the one observed in our group (67.8% male versus 32.2% female). EGFR mutation rate was detected in 9% of patients evaluated in this study, whereas we observed it in 11.7% of Serbian lung adenocarcinoma population. One of the cornerstone trials defining relations between clinicopathological characteristics and EGFR mutation status was published in 2006 by US group of authors led by Tsao et al.[4] In a general population of tested lung cancer patients with mixed ethnicity mutation rate was 8.8%, lower that observed in our cohort. One of the Norwegian studies[5] recently reported about EGFR mutation rate in unselected group of lung cancer patients of Nordic origin. The EGFR mutation rate in Helland's trial[13] was 7.5% with predominance of exon 19 deletion and exon 21 point mutation. Authors did not find significant association between the type of mutation and clinicopathological characteristics. In one of the most recent studies from India,[6] EGFR mutation rate in Indian lung adenocarcinoma patients was higher than reported in Caucasian race but lower than in Asian. EGFR mutation rate in India was detected in 25.9% of patients with adenocarcinoma of the lung. In that study exon 19 deletion was correlated with gender, never smokers, pleural effusion and distant metastases ($p < 0.05$). We also observed relation of EGFR mutation rate with smoking, but we did not find correlation between mutation rate and distant metastases ($p = 0.793$) or pleural effusion ($p = 0.304$). Frequency of exon 19 deletion in Indian trial was 51.2% in our study 66.6%, whereas point mutation in exon 21 was observed in 34.9% of Indian patients and 33.3% of Serbian patients. Sahoo et al.[7] reported slightly higher EGFR mutation rate in Indian patients than previous trial. Exon 19 deletion was detected in 52% of EGFR-mutated lung adenocarcinoma patients, whereas exon 21 mutation was present in 26% of the cases. Several Chinese studies reported similar results about EGFR mutation rate and clinicopathological characteristics in lung adenocarcinoma.[8–10] In these Chinese studies, mutation rate in the domain of EGFR gene in adenocarcinoma of the lung ranges from 37% to 66%. This is significantly higher than the rate observed in our group. The North African results were recently published by Moroccan group.[11] The overall frequency of EGFR mutation was 21%, with mainly exon 19 deletion (69%) and point mutation in exon 21 and 20 in 28%. Higher mutation rate than Western European but lower than Asian. Most comprehensive study on frequency of EGFR mutation in African American population of patients with lung adenocarcinoma was published by Reinersman.[12] Overall mutation rate in African American population was found to be 19%, with 78% of exon 19 deletions, and 22% of exon 21 point mutations. In Korean population, EGFR mutation rate among patients with adenocarcinoma of the lung was 51.3% in most recently published study.[14] This is one of the trials in which exon 19 deletion is not the most frequent type of mutation in EGFR domain. Majority of Japanese studies report higher mutation rate in the domain of the *EGFR* gene in adenocarcinoma of the lung when compared with Caucasian studies. The EGFR mutation rate in Japanese adenocarcinoma population ranges between 20% and 40%.[13,15]

CONCLUSIONS

Knowing the frequency of EGFR mutation in various geographical regions might be of importance to evaluate global lung cancer burden and build national and international strategies for overcoming this disease especially through targeted therapy treatment. It is obvious that the lowest mutation rate is observed in Western European countries, that mutation rate rises over North Africa, Russia, and India and peaks in Far

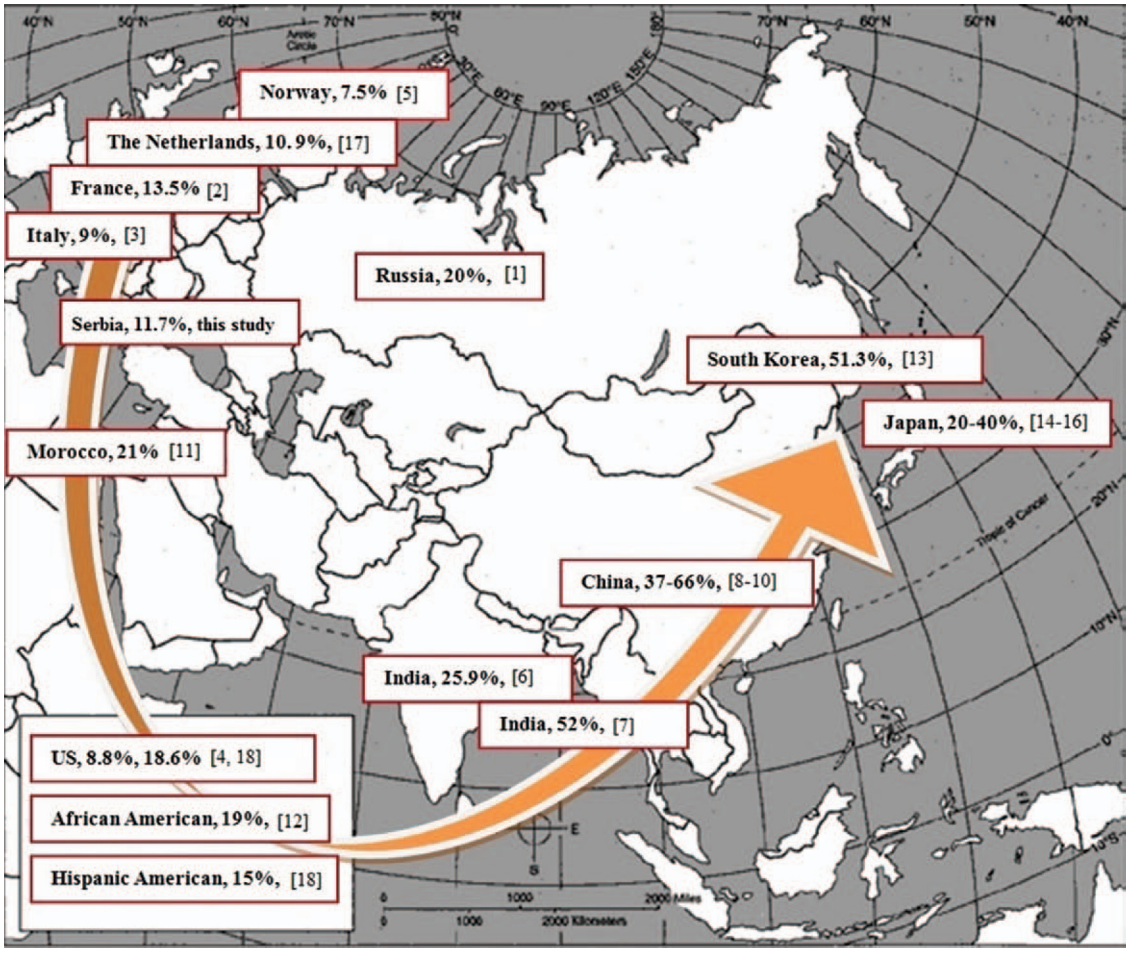

FIGURE 1. West to east increase in frequency of mutations in the domain of *EGFR* gene in Eurasian lung adenocarcinoma patients included in clinical trials analyzed in this article. Legend: Country the study originated from, frequency of EGFR mutations in percentage, reference.

East Asia (Fig. 1). There are no sufficient data on frequency of EGFR mutation in lung adenocarcinoma patients from Middle East, Eastern Europe, North and South Africa, Persian area, Central Asia, or South America. In Serbian population, we observed similar mutation rate as in Western Europe.

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