ORIGINAL ARTICLE

Correlation of demographic, histological and molecular characteristics and clinical course of the disease in patients with lung adenocarcinoma

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ABSTRACT

Aim Retrospective analysis of disease clinical course with a purpose of defining potential prognostic parameters which are essential for optimal target therapy.

Methods The study involved 29 patients with histologically confirmed lung adenocarcinoma and existing epidermal growth factor receptor (EGFR) mutations, which are treated by tyrosine kinase inhibitors (TKI).

Results Allegations of a larger prevalence in women and non-smokers were found. The study confirmed dominance of mutations on exon 19 and exon 21. Usefulness of the treatment with erlotinib in terms of increasing survival time was evident. Median survival of patients in the survey sample was 14.5 months. Median survival in relation to gender or smoking status did not show statistical significance.

Conclusion Considering obtained results, we have confirmed that patients with advanced lung adenocarcinoma and present mutations of epidermal growth factor receptor, who had been treated with first generation TKI, had median survival time of over a year.

Key words: epidermal growth factor, mutation, reaction time, receptor

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INTRODUCTION

Detection of molecular mutations is still a novelty in understanding the treatment of malignant tumours since they represent potential prognostic parameters and points of action for new therapeutics (1). Individual treatments are becoming care standard for patients with non-small cell lung cancer (NSCLC), with the goal to improve the survival rate, slow down disease progression and improve patients' life quality (2,3).

Adenocarcinoma is a predominant histological NSCLC subgroup, which can be linked with the smoking habit, although it is more common in non-smokers and women in diseased population (4,5). The existence of distant metastases is very characteristic and very common in adenocarcinoma (6). Molecular pathology of adenocarcinoma is better defined if compared with other types of tumours because of its prevalence, surgical accessibility and due to the success in identification of clinically important mutations (7).

Epidermal growth factor receptor (EGFR) mutations are an important predictive marker for target therapy. The transmembrane glycoprotein from the EGFR group is presented in 10%-15% of cases of advanced. About 90% of all NSCLC do not have mutations in the EGFR gene, so called wild-type EGFR (EGFR wt) (8). Irrespective of the ethnic affiliation, EGFR mutations are more common in tumours of female, non-smokers (defined as less than 100 cigarettes in the life of each patient) with adenocarcinoma histology. A small subgroup of smokers, of which 5-10% are test positive, means that they will have benefits from tyrosine kinase inhibitors therapy. In the vast majority of cases, EGFR mutations are not matched with other mutations that are found in non-small cell lung cancer (e.g. KRAS mutations, ALK rearrangement) (9).

Approximately 90% of EGFR mutations are in the exon 19 or exon 21 (1,10,11). Mutations within the EGFR exons 20, are associated with EGFR TKI resistance (1).

Traditionally treated patients with metastatic lung carcinoma did not have overall survival longer than a year (12). Recent data show that the median survival time in patients with advanced non-small cell lung cancer and EGFR present mutations, which have been treated with the first generation EGFR tyrosine kinase inhibitors (TKI) is extended to 2 years, which is twice as long as compared to the overall survival rate in patients treated with traditional treatment methods (13,14). It is important to emphasize usefulness of the target therapy in terms of keeping good quality of patients' lives. The target therapy does not have side effects like standard therapy (anaemia, leukopenia, thrombocytopenia, nausea, vomiting, hair loss), and drug administration is easier, drugs are taken orally, it does not require premedication or any treatment of prolonged nausea (12).

Due to the actuality of the target therapy in patients with detected EGFR mutations, we wanted to analyse and compare data obtained in the Clinic for Lung Diseases with literature data. These data are related to the population of important patients who have not been processed yet with the goal of improving the therapy, clinical course and patients' lives.

The aim of our work is a retrospective analysis of the correlation between demographic, histological and molecular characteristics and clinical course of the disease in patients with lung adenocarcinoma.

PATIENTS AND METHODS

Patients and study design

This retrospective study included 29 patients who underwent diagnostic and therapeutic treatment of NSCLC in the period from May 2012 to December 2016 in the Clinic for Lung Diseases, University Clinical Centre of the Republic of Srpska (RS), Bosnia and Herzegovina (B&H). The study included patients with histologically confirmed lung adenocarcinoma with detected EGFR mutations. The data relating to age, gender, smoking status, detected mutation, stage of the disease and survival time were taken from the medical history of each individual patient. A stage of the disease was determined according to 7-th TNM classification (15). Based on a decision of pulmonary council, oncological treatment was carried out by tyrosine kinase inhibitors as the first line therapy, adjuvant therapy treatment or maintenance. All patients with activating EGFR mutations (exon 18, exon 19 and exon 21), regardless of the stage of disease are treated with TKI. It is important to emphasize that patients with detected mutation at exon 20 were not treated with TKI because that is the mutation with resistance to first generation TKI. These patients were treated conventionally

with cisplatin doublet. Some patients could not be surgically treated due to associated diseases and are included in TKI treatment.

Methods

Pathohistological verification of lung cancer was done after bronchoscopy, transthoracic biopsy or surgeries. Histological type was determined on hematoxylin and eosin stained tiles (H&E staining). In cases where diagnosis was not possible with H&E stained samples, recommended antibody panel (TTF1, CK7, CK5/6 and/or p63, napsin) was used instead for immunophenotyping of non-small cell lung cancer.

From May 2012 the molecular testing of existing material had been considered as a standard procedure in the University Clinical Centre of the RS. Testing was performed on samples obtained by surgical removal of the primary tumour or metastasis, small biopsies and cytological samples. Molecular testing was done with Roche Cobas reagents (DNA samples Preparation Kit, Cobas EGFR mutational test) with PCR in real time, according to manufacturer's recommendations. Analysis of the results was carried out automatically on the Cobas 4800 software (Cobas z 480, Roche Diagnostics, Rotkreuz/Swiss).

Determining a stage of lung adenocarcinoma was done based on clinical and radiological findings (bronchoscopy, RTG or lung CT scan, CT of abdomen, head CT, scintigraphy).

Survival time is defined as a period from the date of the diagnosis to the death or the last control.

Statistical analysis

Arithmetic mean and median, i.e. the average value in proper deployed series (from smallest to largest data or by any other logical order) were used. The categorical variables were displayed as an arithmetic mean (MEAN) ± standard deviation (ST.DEV.) or median (MEDIAN) and were compared to the Student's t-test, ANOVA (variance analysis) and Mann Whitney. From the method of multivariate statistics, the Kaplan-Meier method for the evaluation of survival functions was used, log rank test to determine significant differences in survival function depending on the selected factor or risk factors for the Cox redistribution of multilateral analysis. Values of $p<0.05$ were considered statistically significant.

RESULTS

The average age of patients was 61 years (age range 45 to 77); the average age of female patients was 61.5 years and 60 years for males. Females dominated, 21 (72.5%), over males, 8 (27.5%). Non-smokers were dominant with 21 (72.5%), and smokers were represented with eight (27.5%) (Table 1).

Table 1. Baseline characteristics of 29 patients with confirmed lung adenocarcinoma and detected epidermal growth factor receptor (EGFR) mutations

Variable	Patients with adenocarcinoma
Median age (range) (years)	61 (45–77)
Gender (No, %)	
Females	21 (72.5)
Males	8 (27.5)
Smoking status, n (%)	
Non-smoker	21 (72.5)
Males	5 (17.2)
Females	16 (55.2)
Active smoker	8 (27.5)
Males	3 (10.3)
Females	5 (17.2)
Stage of disease	
Unknown	1 (3.4)
IA	1 (3.4)
IB	4 (13.8)
IIA	1 (3.4)
IIB	1 (3.4)
IIIA	4 (13.8)
IV	17 (58.6)

Female dominance was noticed in both categories, non-smokers, 16 (76.2%), and smokers, five (62.5%) (Table 1).

According to the stage of the disease, four (13.8%) patients were in stage IIIA and 17 (58.6%) patients in stage IV disease (Table 1).

The most frequently detected mutation was in exon 19, in 14 (48.3%) patients, followed by mutation in exon 21, in 11 (37.9%) patients. Next mutations were at exon 20, in 3 patients (10.3%), and mutation at the exon 18, in one (3.5%) (Table 2).

Mutations were detected in 21 (72.4%) female and in 8 (27.6%) male patients. Dominant mutation in females was on exon 21, in 9 (43%), and in males on exon 19, in 6 (75%) patients (Table 2).

Table 2. Epidermal growth factor receptor (EGFR) mutation frequency according to gender and smoking status

	No (%) of patients				
	Males		Females		
EGFR mutation	Non-smokers	Active smokers	Non-smokers	Active smokers	Total
exon 18	0	0	0	1 (100)	1 (3.5)
exon 19	4 (28.6)	2 (14.3)	7 (50)	1 (7.1)	14 (48.3)
exon 20	0	0	2 (66.7)	1 (33.3)	3 (10.3)
exon 21	1 (9.1)	1 (9.1)	7 (63.6)	2 (18.2)	11 (37.9)
Total	5 (17.2)	3 (10.3)	16 (55.2)	5 (17.2)	29 (100)

The mutation at exon 19 and exon 21 occurred in three (21.4% and 27.3%) patients of both genders, respectively, who were referred as smokers. Mutation at exon 18 and exon 20 was observed in 1 (12.5%) patient in the ‘’smokers’’ group. In the population of patients who were referred as non-smokers, the dominant mutation was in exon 19 detected in 11 (52.4%) patients.

The mutation at exon 21 was detected in eight (38%) patients, and mutation at the exon 20 in 2 (10%) patients (Table 2).

The median survival of patients was 14.5 months. Survival time >1 year was found in 13 (48%) patients, and 7 (26%) patients had survival time >2 years. In one (7%) patient the length of survival time was not defined (the patient’s treatment continued in another State). The median survival rate for females was 12.6 months, and in males 21 months (p=0.158). The median survival in the category of non-smokers was 16.2 months, and in the category of smokers 10.4 months (p=0.465).

Median survival time of the patients in relation to the mutational status was longer in patients with detected mutation at exon 19 and exon 21. Patients with detected mutation at exon 19 who were treated by tyrosine kinase inhibitors had the median survival of 16.6 months. Patients with detected mutation at exon 21 who had been treated by tyrosine kinase inhibitors had the median survival of 18.2 months (Figure 1).

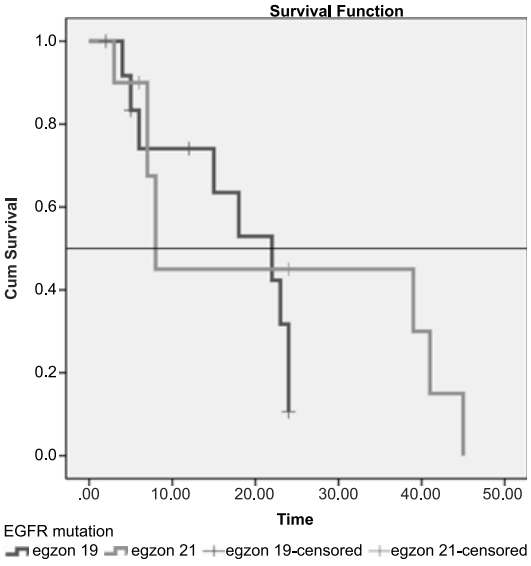

Figure 1. Kaplan-Meier survival curve in relation to epidermal growth factor receptor (EGFR) mutation

Median survival time of patients with the mutation on exon 19-was 16.6 in males and 16.5 months in females. The median survival time in the patients with detected mutation at exon 21 was 18.5 months in females (one male subject in which TKI therapy had not started yet).

The median survival time of smokers was 12.6 months in the patients with mutation on exon 19 and 8 months in the patients with mutation on exon 21 (Figure 2).

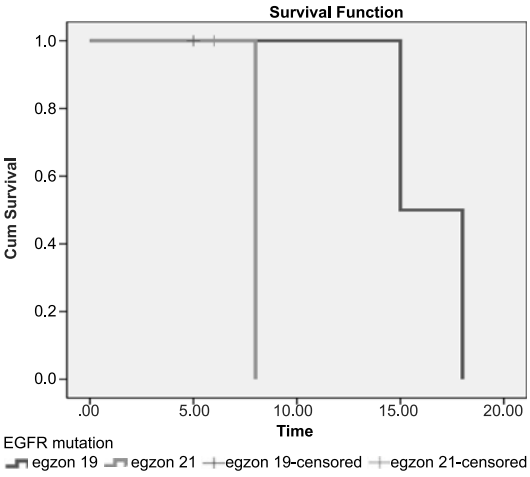

Figure 2. Kaplan-Meier survival curve for smokers in relation to epidermal growth factor receptor (EGFR) mutation

The median survival time of non-smokers was 18.3 months in the patients with mutation on exon 19 and 21.8 months in the patients with mutation on exon 21 (Figure 3).

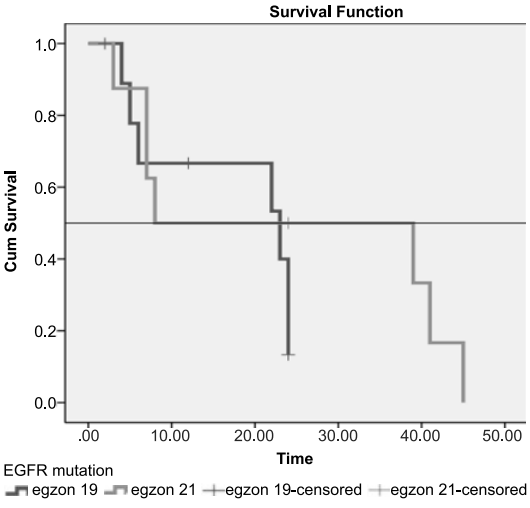

Figure 3. Kaplan-Meier survival curve for non-smokers in relation to epidermal growth factor receptor (EGFR) mutation

DISCUSSION

The results of the study are similar to the reports which suggest that bronchial cancer is a disease with prevalence amongst older patients (17). The average age of patients was 61 years; lung cancer occurred in 2.3% patients younger than 40 years of age and in 5% in patients older than 80.

Literature data on the disease in affected population show that the histological type of lung adenocarcinoma is more common in women and non-smokers (18,19). Our results showed lung adenocarcinoma in 28% of males and 42% of females resulting in the prevalence in males according to the literature data, but it was much higher (72.5%) in females. Our results confirmed the literature allegations and show the dominance of lung adenocarcinoma in non-smokers.

The disease stage of patients with non-small cell lung cancer is in correlation with the five-year survival. Patients in stage IV disease achieve five-year survival in 2% of cases (10). In the analysed population, most patients were in stage IV of disease (58.6%).

The presence of activating EGFR mutations is a key factor for integrating tyrosine kinase inhibitors in the treatment of patients with lung cancer. The most frequent mutation is a deletion at exon 19 and exon 21 mutation (L858R). These two mutations together make 90% in relation to all other mutations (11). The overall frequency of exon 19 and exon 21 mutation in our study was 86.3%, which is slightly lower in comparison with the literature data (11,20).

In the majority of published studies, female/male ratio of patients with diagnosed adenocarcinoma who underwent testing for the presence of EGFR mutation was 1.6:1 in favour of women (10-12). In our study, this ratio is 2.6:1 in favour of women.

The application of tyrosine kinase inhibitors is indicated in cases with present EGFR mutations. In our study, in 10.3% of subjects, mutation which indicates resistance to the first generation of tyrosine kinase inhibitors was detected and in 89.7% of patients mutations which indicate application of tyrosine kinase inhibitors. A previous study shows that the median of survival is longer than a year (12). In our study, median survival time of patients with EGFR mutations present at exon 19 and exon 21, who have been treated by tyrosine kinase inhibitors, is longer than 1 year, which is slightly shorter than the data shown in literature; the median survival in patients with advanced non-small cell lung cancer and present EGFR mutations, who were treated with the first generation EGFR tyrosine kinase inhibitors, (TKI) is extended to 2 years (13,14).

Observing median survival time in patients with present EGFR mutations in exon 19 and 21 did not show difference between genders. The median survival time is still over 1 year.

As far as the smoking status is concerned median survival time in patients with EGFR mutations present at exon 19 and 21 is longer in non-smoking patients. The difference in survival time, which can range from 4.6 to 21.8 months, confirms literature data of more effective therapy in non-smokers.

A shortage of this study mostly refers to lack of patients which disabled our efforts in proving statistical significance in the compared groups. The implementation of the treatment with tyrosine kinase inhibitors is difficult in our clinical practice (prolonged procedure and waiting list).

In conclusion, a part of the population with the highest risk of lung cancer are people older than 50 years of age with a history of smoking. The study confirms literature allegations on dominancy of histological type of adenocarcinoma and also on prevalence amongst female and non-smoking population. It determined the impact of the treatment on median survival in patients with EGFR mutations (over a year).

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TRANSPARENCY DECLARATON

Conflicts of interest: Nothing to declare.

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