ORIGINAL ARTICLE

Prevalence of epidermal growth factor receptor (EGFR) mutations and correlation with histological patterns in lung adenocarcinoma in patients from Bosnia and Herzegovina

Dalma Udovičić-Gagula[1], Nejra Džananović[2], Mirsad Dorić[3]

[1]Department of Pathology and Cytology, [2]Department of Clinic Biochemistry and Immunology; Clinical Centre University of Sarajevo, [3]Department of Pathology, School of Medicine, University of Sarajevo; Sarajevo, Bosnia and Herzegovina.

ABSTRACT

Aim Lung adenocarcinoma (ADC) is a leading subtype of lung cancer, histologically defined with five different architectural growth patterns: lepidic, acinar, papillary, solid and micropapillary. The aim of this study was to explore the prevalence of epidermal growth factor receptor (EGFR) mutation and a relationship between the specific histological patterns of lung ADC in the population of Bosnia and Herzegovina.

Methods The study included tumour tissue from 102 patients with completely resected lung ADC from 2015 to 2020. Molecular testing for the presence of EGFR mutations was performed by real-time PCR method. The relationship between EGFR mutation status and clinicopathological parameters was analysed.

Results The EGFR mutation was detected in 12 (11.8%) cases of ADC, more often in non-smokers (p=0.007). A higher percentage of solid growth pattern presented in ADC may be an indicator of EGFR negativity (p=0.039), while a higher percentage of micropapillary growth pattern more common in the presence of EGFR mutation (p=0.047).

Conclusion The prevalence of EGFR mutation is in accordance with the expected prevalence considering our studied population, Caucasians from South Europe. Better understanding of the relationship between histological patterns and molecular characteristics of lung ADC will enable earlier diagnosis and optimal treatment for patients.

Key words: architectural growth pattern, oncogenic mutation, lung cancer

Corresponding author:
Dalma Udovičić-Gagula,
Department of Pathology and Cytology,
Clinical Centre University in Sarajevo
Bolnička 25, Sarajevo,
Bosnia and Herzegovina
Phone: +387 33 298 694;
Fax: +387 33 257 826;
E-mail: dalma.udovicic@gmail.com
ORCID ID: https://orcid.org/0000-0003-3047-0962

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INTRODUCTION

Lung cancer has the highest incidence and mortality worldwide, with 2.2 million newly diagnosed cases in 2020 (1). According to data from Globocan, the highest incidence of lung cancer was found in Hungary with 50.1 cases per 100 000, while Bosnia and Herzegovina (B&H) it is on the 8th place with 37.8 patients per 100,000 inhabitants (2).

Adenocarcinoma (ADC) is a leading subtype of lung cancer and accounts for about 40% of all lung carcinomas (3). Histologically lung ADC is defined with five different architectural growth patterns: lepidic, acinar, papillary, solid and micropapillary (3). Several different patterns are usually presented in single ADCs and each pattern is estimated and recorded in 5–10% increments totalling 100% (3).

Extensive research of lung cancer led to the identification of genomic alterations known as "driver mutations" that initiate the evolution of a non-cancerous cell to malignancy through processes critical to cell growth and survival, and patients harbouring some of these mutations may benefit from targeted therapies (4). Epidermal growth factor receptor (EGFR) mutation is the most important targetable mutation in lung ADC, and its presence in tumours is closely related to the efficacy of EGFR tyrosine kinase inhibitor (TKI) (5).

Oncogenic EGFR mutations occur almost exclusively in lung ADC, with a frequency that varies greatly depending on the population. They have the highest frequency in non-smokers, females, younger age, and in the Asian population where the frequency is around 50% (6,7).

The presence of EGFR mutations in lung ADCs often correlates with tumour aggressiveness, a higher incidence of metastases and a worse prognosis, even with malignant pleural effusion (8). Some studies suggest that the presence of EGFR gene had a higher mutation rate in a specific subtype of pattern, in ADC with predominantly lepidic, papillary, micropapillary and acinar pattern (9-13), but results are still not consistent.

The aim of this study was to investigate the prevalence of EGFR mutation and a relationship between the specific histological patterns in lung ADC, and to determine its ability to serve as a biomarker for the prediction of presence of oncogenic EGFR mutations.

PATIENTS AND METODS

Patients and study design

The retrospective study included 102 resection specimens of patients with lung ADCs treated surgically from 2015 to 2020 in the Clinical Centre University of Sarajevo, B&H, confirmed by pathohistological examination. The inclusion criteria were patients with surgically completely resected ADCs of the lung, including resected regional lymph nodes. The exclusion criteria were patients who had a previous treatment with neoadjuvant chemotherapy or radiotherapy (Figure 1).

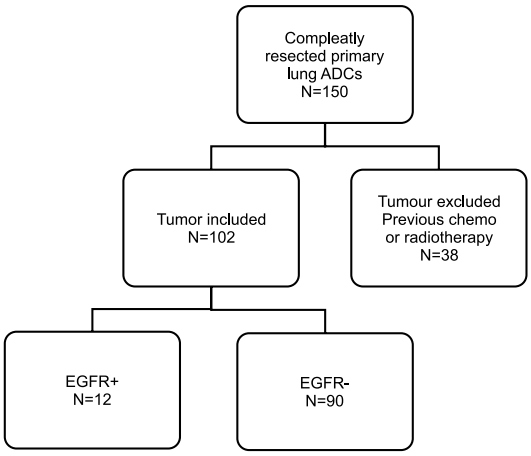

Figure 1. Distribution of different tumour groups of 102 patients with lung adenocarcinoma (ADC);
EGFR+, EGFR mutation positive; EGFR-, EGFR mutation negative

Tumour staging was performed according to the 8th edition of the American Joint Committee on Cancer's AJCC Cancer Staging Manual (14).

This study was conducted in compliance with the ethical principles originating in or derived from the Declaration of Helsinki and was approved by the Ethics Committee of the Clinical Centre, University of Sarajevo (No: 0302-53944).

Methods

Histologic evaluation. All resected specimens were fixed in 10% formalin, (a minimum of three tumour blocks not thicker than 5 mm and relation of the tumour with surrounding structures were taken, and in cases if the tumour had a diameter ≤3 cm they were completely taken over), embedded in paraffin blocks, and stained with haematoxylin and eosin, in the standard manner (15). The histological slides were evaluated by two

pathologists. All tumours were histologically classified according to the 4th WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart (3). Each tumour sample was reviewed using comprehensive histological subtyping, recording the percentage of 5% increments for each component of the histological pattern.

Pathohistological factors (significant prognostic factors for lung adenocarcinomas) were also investigated: lymphovascular invasion, pleural invasion, spread through alveolar spaces (STAS) (14).

EGFR mutation analysis. An analysis of EGFR mutations was performed with Cobas EGFR Mutation Test v2 (Cobas 4800 System, Roche Diagnostics, USA); the analysis of raw data and reporting of results were fully automated. Real-time PCR test for qualitative detection of defined mutations of the EGFR gene identified 42 mutations in exons 18, 19, 20 and 21, including the T790M resistance mutation. Genomic DNA was isolated and purified from formalin-fixed, paraffin-embedded tumour tissue using a Cobas DNA Sample Preparation Kit (Roche Diagnostics, USA) according to the manufacturer's instructions.

Statistical analysis

Before the analysis, the data were entered into a database created in Microsoft Excel program. The impact of the following factors on the presence and type of EGFR mutation was evaluated: gender, age, smoking status, tumour size, pathological stage, lymphovascular invasion, and pleural invasion. Fisher's exact tests and $\chi 2$ tests were used with categorical data. For continuous variables, first, we analysed the symmetry of distribution using the Kolmogorov-Smirnov test, and since it showed an asymmetric distribution, non-parametric tests were used. Correlations and linear regression were used for the connection and direction of the connection between the variables. Binary logistical regression was used to determine whether evaluated clinic-pathological examination had a significant effect on EGFR status, and to determine the influence of the percentage of histological patterns presented in ADC on the presence of an EGFR mutation. For comparison between independent variables to determine which one has the most influence on the dependent variable, logistic regression coefficient (B) was used. To determine whether a variable can be a good marker, the ROC curve was used. Statistical significance was set to $p \leq 0.05$.

RESULTS

The total number of patients was 102, with slightly more males, 58 (56.9%) than females, 44 (43.1%), and the median age was 63 years (age range 44-79 years). Of the patients with a known smoking status, 12 (11.8%) had never smoked, 51 (50%) were current or former smokers.

The mean tumour size was 42.6±19.4 mm (range 14–103 mm). Lymphovascular and pleural invasions were observed in 86 (84.3%) and 84 (82.3%) cases, respectively. Metastases in regional lymph nodes were presented in 58 (56.9%) patients.

The most common pathologic stage was pT2 in 39 (38.2%) and pT3 in 39 (38.2%) patients.

The most predominant pattern was acinar, in 69 (67.6%) patients. The percentage representation of individual patterns varied greatly in tumours, so the highest average representation had acinar pattern of 55.7%, followed by solid one, 31.4% (Table 1).

Table 1. Patients' clinicopathological characteristics

Variable	No (%) of patients		p
	Total	EGFR+	
Total	102 (100)	12 (11.8)	
Gender			
Male	58 (56.9)	5 (8.6)	0,091
Female	44 (43.1)	7 (15.9)	
Age			
< 63 y	46 (45.1)	5 (10.86)	0.403
≥ 63 y	56 (54.9)	7 (12.5)	
Smoking status			
Never	12 (11.8)	5 (41.66)	
Smoker	51 (50)	4 (7.84)	0.007
Unknown	39 (38.2)	2 (5.12)	
LN metastasis			
No	44 (43.1)	4 (9.09)	0.118
Yes	58 (56.9)	8 (13.79)	
Pleural invasion			
No	18 (17.6)	1 (5.55)	0.226
Yes	84 (82.4)	11 (13.09)	
LVI			
No	16 (15.7)	1 (6.25)	0.466
Yes	86 (84.3)	11 (12.79)	
STAS			
No	32 (31.4)	2 (6.25)	0.256
Yes	70 (68.6)	10 (14.28)	
Tumour size			
≤ 3 cm	35 (34.3)	6 (17.14)	0.446
>3 cm	67 (65.7)	6 (8.95)	
Pathological stage			
pT1	8 (7.84)	0	
pT2	39 (38.23)	3 (7.69)	0.048
pT3	39 (38.23)	5 (12.82)	
pT4	16 (15.7)	4 (25)	

EGFR+, epidermal growth factor receptor (EGFR) mutation positive; LN lymph node; LVI lymphovascular invasion; STAS Spread through alveolar spaces

Of the 102 patients, the presence of EGFR mutations was detected in 12 (11.8%). The most common mutation was classic mutation: exon 19 deletion (50%) and L858R mutation (16.7%) (Table 2).

Table 2. Detected types of epidermal growth factor receptor (EGFR) mutations

EGFR mutation	No of patients
exon 19 deletion	6
exon 21 mutation L858R	2
exon 21 (L861Q mutation)	1
exon 20 insertion	1
exon 18 G719X	1
S768I+G719X	1
Total	**12**

The patients with EGFR mutations positive ADC were significantly associated with patients who had never smoked (p=0.007), and patients with a higher pathological stage of tumour (p=0.048) (Table 2). The EGFR mutation status was not correlated with age, gender, lymph node metastasis, pleural invasion, STAS, nor tumour size.

The association of pT tumour stage to the presence of EGFR mutation was statistically significant, ADCs of a higher pT stage had more frequently EGFR mutations (p=0.048).

The EGFR mutation was most frequently found in ADC with acinar predominant pattern, 10 (83%).

The analysis of the rates of individual patterns on the presence of EGFR mutation showed that the presence of micro papillary and solid pattern in lung ADC had opposite statistical significance (p=0.047 and p=0.039, respectively). For every 10% more micropapillary pattern in lung ADC, the chance that EGFR mutation was present increased by 10.5 times, and contrary, for every 10% more solid pattern in lung ADC, the chance that EGFR mutation was present decreased by 0.4% (Table 3).

Table 3. The influence of the presence of specific histological patterns on the presence of epidermal growth factor receptor (EGFR) mutation

Histological pattern	B	p
Acinar	0.019	0.141
Solid	-0.037	0.039
Papillary	0.021	0.386
Micropapillary	0.052	0.047
Lepidic	0.007	0.814

B, estimated regression coefficient -values for the logistic regression equation for predicting the dependent variable from the independent variable

The ROC curves (Figure 2) showed that the percentage of a solid pattern in lung adenocarcinoma could be a marker for the absence of EGFR mutation (AUC=0.722; p=0.013). Further analysis showed that the "cutoff" value of 7.5% for presence of solid pattern (Youden index=0.383) had a sensitivity of 80% and specificity of 58%, in the prediction of EGFR negativity (EGFR wild type).

A regression analysis showed that non-smoking status was the most important independent positive predictor of the occurrence of EGFR mutation in lung adenocarcinoma (OR=0.119; CI:0.02-0.55; p=0.007). In smokers, the possibility of the presence of an EGFR mutation in the tumour decreased by 88% in our sample, that is, in our population of patients with lung adenocarcinoma between 45-97%.

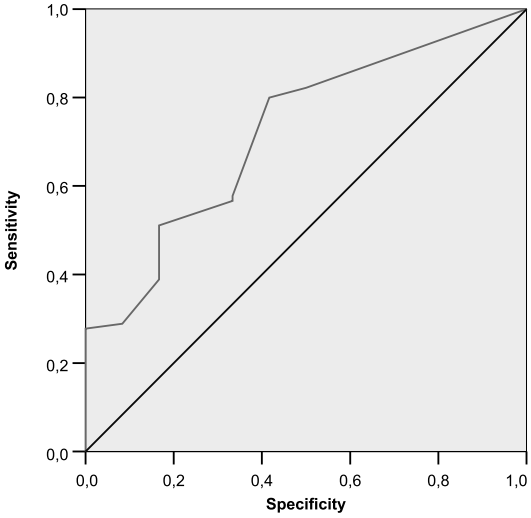

Figure 2. Receiver operating characteristic (ROC) curve shows prediction of absence of epidermal growth factor receptor (EGFR) mutation based on percentage of solid pattern in lung adenocarcinoma;
Area under the curve AUC=0.722; p=0.013

DISCUSSION

The identification of EGFR mutations has been routinely performed in B&H for years. However, the frequency and type of EGFR mutations in B&H population have not been widely investigated. The only data presented so far included just basic demographic data but no detailed analysis and a correlation with relevant clinical-pathological parameters (16). To the best of our knowledge, the current study represents the first comprehensive research of the correlation of clinicopathological characteristics and the prevalence of EGFR mutation in lung ADC patients from Bosnia and Herzegovina.

We recorded 11.8% of 102 primary lung adenocarcinoma specimens with EGFR mutation,

which was more frequently found in non-smokers. Our result is in accordance with the expected prevalence of EGFR mutations, considering the geographic location and ethnicity of our studied population, Caucasians from South Europe (7). According to previous studies, the EGFR mutation prevalence is the highest in the Pacific Asian population, more than 50%, with Taiwan having the highest rate of detection of EGFR-activating mutations, 55%, compared with the Caucasian population only 10–17% (17,18). Furthermore, the low prevalence of EGFR mutations in our population can be attributed to the high prevalence of smokers and cultural habits of our population, with most patients being active smokers or being constantly in contact with tobacco smoke in their family and work environment. Our study once again proved that EGFR mutations can be considered oncogenic driver mutations of ADC non-smokers (19,20).

In accordance with data from the literature (18,21), the most common EGFR mutations in our population were exon 19 deletion and L858R mutation, which accounted for more than 2/3 of all EGFR mutations. The presence of these "classic" EGFR mutations is clinically most significant, as they show the best clinical response and the greatest sensitivity to tyrosine kinase inhibitors (21). Exon 18 G719X mutation represented the most common "unusual" EGFR mutation (3%), which occurs in almost a third of cases as a part of complex mutation (18), while the exon 21 L861Q mutation accounts for 2% of all EGFR mutations (22). Similarly, exon 18 G719X was the most common “unusual” EGFR mutation in our study, followed by exon 21 L861Q mutation.

Although all these mutations belong to the group of activating EGFR mutations, it is important to point out that these "unusual" EGFR mutations show a variable therapeutic response to EGFR-TKI depending on which exon they occur. Several studies have pointed out that patients with "unusual" EGFR mutations show a better therapeutic response to the second-generation EGFR-TKIs (23-26).

Among EGFR-positive ADCs, we recorded one exon 20 insertion; this type of mutation accounts for 4-10% of all EGFR-mutated carcinomas (23,26). Tumours with these mutations are traditionally resistant to the treatment with currently approved TKIs, and although this mutation can structurally activate the EGFR receptor, they do it without increasing the affinity for EGFR TKIs (28,29). However, recent clinical trials of new selective EGFR/HER2 TKIs have shown strong preclinical activity in the presence of EGFR exon 20 insertions compared to traditional EGFR TKIs (30,31), and as a results, the Food and Drug Administration (FDA) (32) has recently approved two new drugs (amivantamab and mobocertinib) for the treatment of lung adenocarcinoma with EGFR exon 20 insertion (33).

The "unusual" EGFR mutations in our population accounted for 25%, which is slightly higher compared to previous studies accounting for 20% of all EGFR mutations (34,35). The reason for this could be a relatively small total number of EGFR mutated ADCs in our population, but also the higher frequency of the detection of "unusual" EGFR mutations in the elderly population as some authors have noted too (34).

We did not record the presence of T790M mutation, the most common resistant mutation that occurs frequently after the treatment with EGFR TKI, and it is rarely found in EGFR TKI-naive patients such as those in our study (36).

The results of the presented study have shown that in our population gender has no statistical significance for the presence of EGFR mutations, which is opposite to earlier studies that clearly showed that EGFR mutations occurred more often in females (7,37,38). However, considering the low frequency of EGFR mutation in our population and taking in the account absolute values, we recorded more females then males.

Comparing the presence of specific histological patterns with the presence of EGFR mutations, we recorded a significantly higher percentage of the solid pattern in ADC without EGFR mutation. This result is consistent with data from earlier studies that recorded the presence of EGFR mutations more often in ADCs with low-grade histological patterns: lepidic, acinar and papillary patterns (39-41). Additionally, the results of our study showed that the higher percentage of the micropapillary pattern significantly correlated with the presence of EGFR mutation, and that higher percentage of micropapillary pattern significantly increased incidence of EGFR mutation in lung ADC. Although most of the earlier

studies showed that EGFR positive tumours were mostly of lepidic and acinar pattern, similarly to our results, studies of Song et al. (9), Chao et al. (42) and Matsumura et al. (43) also recorded a strong correlation of the presence of micropapillary pattern with the presence of EGFR mutation. However, it is worth noting that the results of most studies were based on ADCs samples obtained mostly from small biopsies and not from completely resected samples, as is the case in our study. In addition, it well known that the micropapillary pattern is highly malignant, and such tumours are often diagnosed in non-resectable stages (42,43), which further limited its inclusion in the studies performed with completely resectable ADCs. Therefore, in our analysis we included the total percentage representation of all present histological patterns in ADC.

The results of our study showed that in completely resected lung adenocarcinoma, even a small percentage of micropapillary pattern is a predictor of the presence of EGFR mutation.

The limitations of this study include the small sample size, and inclusion of only resectable tumours influencing general conclusions. It is known that only 30% of NSCLCs are resectable at the time of clinical appearance (44). Future studies with larger sample size could help in overcoming this limitation; also, additional studies are needed to define the full molecular profile and the frequency of other targetable oncogenic mutations of lung ADCs in the population of Bosnia and Herzegovina.

In conclusion, this study of EGFR mutations in lung ADC of the population in Bosnia and Herzegovina reveals an overall prevalence of 11.8%, which is consistent with the expected prevalence considering geographic and ethnic structure.

Because the lung ADCs are tumours with unpredictable behaviour and very heterogeneous histological and molecular characteristics, better understanding of the relationship between histological patterns and molecular characteristics of ADC will enable an earlier diagnosis and optimal treatment for patients.

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TRANSPARENCY DECLARATION

Competing interest: None to declare.

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