Evaluation of Clinical and Genetic Determinants of Treatment Outcome In EGFR Mutation Positive Advanced Lung Adenocarcinoma

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Abstract

Background: The aim of this research was to evaluate clinical and low-cost genetic determinants of treatment outcome in EGFR mutation positive advanced lung adenocarcinoma patients.

Material and Methods: EGFR mutation testing and EGFR 181946C>T genotyping were performed in 101 advanced lung adenocarcinoma patients using qRT-PCR and PCR-RFLP, respectively. Progression-free survival was defined as the time from the start of TKI therapy to date of progression, and overall survival as the time from diagnosis to death from any cause. Pain level was evaluated using a Numerical Rating Scale and the Verbal Descriptor Scale. Statistical significance was considered for \( P < .05 \).

Results: Patients were treated with EGFR-TKIs for a period of 1–39 months (median 9), with a median PFS of 12.0 months (10.4-13.6, CI 95%), and a median OS of 19.0 months (15.1-22.7, CI 95%). The presence of pain was significantly correlated with the existence of bone \( (P < .001) \) and adrenal glands metastases \( (P = .029) \). Genetic factors did not have a direct impact on pain management but had a significant effect on the response to TKIs leading to pain alleviation.

Conclusions: EGFR mutation subtype and the EGFR 181946 C>T SNP had a significant effect on the response to TKI inducing an indirect anti-dolorous effect.

Keywords

EGFR, lung cancer, pain, tyrosine kinase inhibitors

Introduction

Lung cancer (LC) prevention measures are not sufficiently effective even in economically developed countries. Specific clinical symptoms are absent in early stages of lung cancer and uptake of screening is inadequate even in countries which perform them, often not including individuals without smoking history.1,2 Thus, around 75% of LC cases are diagnosed in advanced stages when the curative success rate is very low due to high tumor burden and the presence of cancer-related pain is higher.3 As a temporary stop/slowing down of LC screening programs and diagnostic procedures worldwide

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during the COVID-19 pandemic has been reported,\(^{4,5}\) concerns have been raised that lung cancer-related pain can originate from direct effects of the tumor, its regional or distant spread, as well as from anti-cancer treatment.\(^{6}\) Pharmacotherapy is the standard approach for the treatment of cancer pain, but a combination of pharmacological and non-pharmacological measures should be applied when needed.\(^ {7,9}\) Bone metastases represent one of the most common sites of metastatic spread of LC and they are usually associated with shorter survival.\(^ {10}\) Radiation therapy (RT) remains the golden standard of palliative management of painful bone metastases and it is performed to relieve pain, prevent pathologic fractures, spinal cord compression as well as to maintain or improve patients’ quality of life.\(^ {11}\) Optimal medical analgesic therapy is essential alongside RT.\(^ {12}\) An individual approach to each patient is necessary, as well as constant monitoring by a multidisciplinary team which includes a pain management specialist.

Serbia was among the highest ranked countries in the world in 2018 when age-standardized rate of lung cancer occurrence was taken into account,\(^ {13}\) mostly due to the combined effect of very low tobacco cost and the lack of a structured national lung cancer screening program. As a higher percentage of Serbian lung cancer patients are diagnosed in advanced stages, around 20–30% exhibit chest pain at diagnosis, and around 6–13% bone pain.\(^ {14}\) Recently, we and others have made investigator-initiated efforts to explore genetic risk factors for LC in Serbia, in an effort to decipher the mechanisms driving its carcinogenesis, reduce the national mortality rate, and lower the number of patients who are diagnosed with advance stage lung cancer.\(^ {15-18}\) Advancement in diagnosis and treatment have also been introduced, but the accessibility of new molecular techniques and drugs still needs improvement.\(^ {19,20}\) Although many discrepancies are present between various countries in availability of innovative drugs, EGFR mutation testing is regularly applied as companion diagnostics for tyrosine kinase inhibitors (TKIs) in advanced stages of lung cancer.\(^ {21}\) In Serbia, EGFR mutation testing of lung cancer patients was initiated in 2011 from tumor tissue and in 2016 from liquid biopsy.\(^ {19,22}\) Patients with confirmed EGFR mutations are treated with first- and second-generation EGFR-TKIs until progression, at which point they are tested again for the presence of a resistant EGFR T790 M mutation which renders them candidates for third generation EGFR-TKIs.\(^ {23}\) All symptoms of the disease, including the pain level, as well as adverse effects and patient performance status are evaluated at diagnosis and closely monitored and managed during this entire TKI treatment course, contributing to the overall patients’ quality of life.

The aim of this research was to detect the possible clinical and low-cost genetic determinants of treatment outcome in EGFR mutation positive advanced lung adenocarcinoma patients treated at our Institute, in an effort to provide data from the Balkan area, which is usually underrepresented in larger meta-analyses.

**Methods**

**Patient Samples**

This retrospective study included 101 patients, with inclusion criteria of a minimum age of 18 years and clinically and histologically confirmed primary advanced lung adenocarcinoma according to the current WHO classification (stage III B/IV, ECOG performance status 0, 1, or 2).\(^ {24}\) According to data that LC risk slowly diminishes and is reduced to 50% after 30 years of cessation of active smoking\(^ {4}\) the subjects were divided into 2 groups, non-smoker, that is, never smoker or ex-smoker ≥30 years, and smoker, that is, current smoker or ex-smoker <30 years, and further into ex-smoker and smoker <15 and ≥15 years. The procedures used in this study were approved by the Ethics Committee of the Institute for Oncology and Radiology of Serbia (No. 5665-01) and were in accordance with the Helsinki Declaration of 1975, as revised in 2000. All patients had a minimum age of 18 years and signed an informed consent.

**Pain Level Assessment and Management**

Pain level was evaluated using 2 approaches—a Numerical Rating Scale (NRS) and the Verbal Descriptor Scale (VDS), at baseline and continuously during TKI treatment. For the purpose of this study, a second time-point (after baseline) of official recording of pain level was the time of the first computed tomography (CT) evaluation of the efficacy of TKI treatment, which was after 3 months of treatment initiation.\(^ {25}\) Numeric rating scale involved patients’ self-reported pain rating from 0 to 10, with 0 representing no pain and 10 representing the worst pain. Mild category of pain had NRS values from 1 to 3, moderate pain had NRS values from 4 to 6, and severe pain had NRS values from 7 to 10. In patients who had difficulty responding to NRS, the VDS was used that included several descriptive phrases that referred to different levels of pain severity or intensity (e.g., “no pain,” “mild pain,” “moderate pain,” “severe pain,” and “the worst pain imaginable”). Pain management was achieved using acetaminophens, non-steroidal anti-inflammatory, and weak and strong opioids\(^ {7}\) (Supplementary Table 1). Single 8-Gy dose radiation therapy and 2 long-course regimens including 20 Gy in 5 fractions and 30 Gy in 10 fractions were used in patients with painful bone metastases as previously described.\(^ {26,27}\)

**EGFR Mutation Testing and TKI Treatment**

Formalin-fixed and paraffin-embedded (FFPE) tumor samples were obtained by biopsy/resection and used for routine **EGFR** (LRG_304, NM_005228.5) mutation testing using the Cobas® EGFR Mutation Test v2 on Cobas® 4800 (Roche Diagnostics). Patients with sensitizing EGFR mutations were treated with EGFR TKIs in the first line until progression or unacceptable toxicity. Progression-free survival (PFS) was
defined as the time from the start of TKI therapy to date of progression, and overall survival (OS) as the time from diagnosis to death from any cause. Primary resistance to TKIs was defined as the absence of any response, that is, progression of disease within the first 3 months of therapy.

**EGFR 181946C>T Genotyping**

EGFR 181946C>T (rs2293347, NM_005228.5(EGFR): c.2982 C>T) polymorphism was analyzed from FFPE derived DNA using a standard PCR-RFLP approach. A previously sequenced heterozygote sample was used as a control to assure adequate genotyping.

**Statistical Analysis**

Descriptive methods of statistical analysis (frequencies, percentage, mean, median, and standard deviation/SD/and range) were used to summarize the sample data. The associations between the patients’ and tumor characteristics were analyzed using Pearson Chi-Square, Fisher’s exact, t-test, and Mann-Whitney tests and the analyses were performed IBM SPSS Statistics 22 (SPSS Inc, Chicago, IL, USA). Survival analysis was performed using a standard Kaplan–Meier product limit method for graphical presentation; median with corresponding 95% confidence interval (95%CI) was used for description and the Log-rank test for the analysis of difference. Descriptive analyses included genotype and allelic frequencies, and their distribution between groups was tested by Fisher’s exact test; logistic regression was used to calculate the odds ratio (OR) and the 95% confidence interval (CI) to estimate the strength of associations. Two-sided P values <.05 were considered to indicate statistical significance.

**Results**

In the period from 2011 to 2020, 5650 EGFR mutation analyses were performed, and 10% of EGFR mutated samples were detected, which is in good accordance with literature data for the Caucasian population.

Patients were treated with first-line EGFR-TKIs for a period of 1–39 months (median 9 months). Median PFS in the whole group was 12.0 months (10.4-13.6, CI 95%), with 33% patients still alive at the time of data analysis. Patients who presented with pain at diagnosis did not have a significantly lower PFS compared to those without pain (11.0 (6.8-15.2 95%CI) vs 11.0 (6.3-15.7 95%CI), P = .153) (Figure 1A). Median OS in the whole group was 19.0 months (15.1-22.7, CI 95%). Median OS for patients who presented with pain at diagnosis was 19.0 months (16.7-21.3, 95%CI) and 17.0 months (9.2-24.8, 95%CI) for patients without pain, and the difference was not statistically significant (P = .906) (Figure 1B). Primary resistance to TKIs was detected in 15% of patients, and it was not correlated with the presence of pain at diagnosis (P = .904). Smoking status had no significant effect on the occurrence of primary resistance to TKIs (<30 vs ≥30 years, P = .752; <15 vs ≥15 years, P = .173). AEs were detected in 72% of TKI-treated patients with 97% of them presenting toxicity of a maximum grade 2. The presence of pain at diagnosis was not correlated with higher probability of AEs (P = .557). Smoking status had no significant effect on the occurrence of AEs (<30 vs ≥30 years, P = .918; <15 vs ≥15 years, P = .240).

At diagnosis, pain was detected in 41 (41%) patients, and it was significantly correlated with the presence of metastases (P < .001) (Table 1). Upon analysis of the presence of various metastatic sites, it was found that pain was significantly more common in patients with bone and adrenal gland metastases (Table 2). EGFR mutation type was not associated with the

![Figure 1.](image-url) (a) Progression free survival (PFS) and (b) overall survival (OS) curves of patients with and without pain at diagnosis.
presence of pain at baseline when most common EGFR mutations (exon 19 deletion, L858 R point mutation in exon 21) and rare mutations (L861Q, G719X, exon 20 insertion, S768I, double mutants) were compared ($P = .351$). The frequency of the EGFR 181946T allele was .25 in the whole group, that is, .23 in the group of patients that exhibited pain at diagnosis and .26 in the group without pain (Figure 2). The EGFR 181946T allele was not associated with the presence of pain at baseline in both the dominant (CC vs CT/TT) [$P = .657$; OR (95%CI) = 1.31 (.58-2.98)], and the recessive models (CC/CT vs TT) [$P = .846$; OR (95%CI) = .87 (.21-3.47)].

Patients with pain at baseline were treated with analgesics (and radiation in 21 patients with bone metastases) (Supplemental Table 1 and 2) and good pain management was achieved. At the second time-point of official recording of pain level (3 months after the initiation of TKI treatment and the first CT evaluation of its efficacy), 20% of patients manifested pain which was statistically lower compared to baseline ($P = .011$), and there was a statistically significant improvement of the pain level ($P < .001$) (Table 3).

Of the 41 patients that presented with pain at diagnosis, 20 patients had a worsening of pain after having achieved good

| Characteristic                  | No Pain N (%) | With Pain N (%) | P value |
|--------------------------------|---------------|-----------------|---------|
| Age (mean)                     |               |                 |         |
| Gender                         |               |                 |         |
| Male                           | 18 (30)       | 17 (41.5)       | .234    |
| Female                         | 42 (70)       | 24 (58.5)       |         |
| Performance status (PS)        |               |                 |         |
| ECOG 0                         | 5 (8.3)       | 0 (0)           | .080    |
| ECOG 1                         | 48 (80)       | 33 (80.5)       |         |
| ECOG 2                         | 4 (6.7)       | 5 (12.2)        |         |
| ECOG 3                         | 2 (3.3)       | 3 (7.3)         |         |
| ECOG 4                         | 1 (1.7)       | 0 (0)           |         |
| Smoking status                 |               |                 |         |
| Non-smoker                     | 30 (53.6)     | 15 (39.5)       | .179    |
| Smoker                         | 26 (46.4)     | 23 (60.5)       |         |
| TNM staging                    |               |                 |         |
| III B                          | 8 (13.3)      | 2 (4.9)         | .195    |
| IV                             | 52 (86.7)     | 39 (95.1)       |         |
| Presence of metastases         |               |                 |         |
| Without mets                   | 8 (13.3)      | 2 (4.9)         | <.001   |
| M1a                            | 29 (48.3)     | 9 (22.0)        |         |
| M1b                            | 14 (23.3)     | 14 (34.1)       |         |
| M1c                            | 9 (15)        | 16 (39)         |         |

M1a - tumor in contralateral lung or pleural/pericardial nodule/malignant effusion. M1b – single extrathoracic metastasis including single non-regional lymph node. M1c – multiple extrathoracic metastases in one or more organs. Statistically significant values are presented in bold.

| Metastatic Site                  | No Pain N (%) | With Pain N (%) | P value |
|----------------------------------|---------------|-----------------|---------|
| Liver                            |               |                 |         |
| No liver mets                    | 46 (76.7)     | 35 (85.4)       | .281    |
| Liver mets                       | 14 (23.3)     | 6 (14.6)        |         |
| Bone                             |               |                 | <.001   |
| No bone mets                     | 55 (91.7)     | 19 (46.3)       |         |
| Bone mets                        | 5 (8.3)       | 22 (53.7)       |         |
| Non-regional lymph nodes (Ln)    |               |                 |         |
| No ln mets                       | 58 (96.7)     | 38 (92.7)       | .393    |
| Ln mets                          | 2 (3.3)       | 3 (7.3)         |         |
| Pericard                         |               |                 |         |
| No pericard mets                 | 53 (88.3)     | 36 (87.8)       | 1.000   |
| Pericard mets                    | 7 (11.7)      | 5 (12.2)        |         |
| Brain                            |               |                 |         |
| No brain mets                    | 53 (88.3)     | 38 (92.7)       | .736    |
| Brain mets                       | 7 (11.7)      | 3 (7.3)         |         |
| Lung                             |               |                 |         |
| No lung mets                     | 34 (56.7)     | 24 (58.5)       | .852    |
| Lung mets                        | 26 (43.3)     | 17 (41.5)       |         |
| Adrenal gland                    |               |                 |         |
| No adr.gl.mets                   | 58 (96.7)     | 34 (82.9)       | .029    |
| adr.gl. Mets                     | 2 (3.3)       | 7 (17.1)        |         |
| Malignant pleural effusion       |               |                 |         |
| No pleural eff                   | 33 (55)       | 21 (51.2)       | .708    |
| Pleural eff                      | 27 (45)       | 20 (48.8)       |         |

Statistically significant values are presented in bold.

Table 1. The Presence of Pain in EGFR Mutated Patients at Diagnosis.

Table 2. Localisation of metastases associated with the presence of pain at baseline.
pain control initially, probably due to disease progression (14 cases of progression of the primary lesion in the first three months). Additional 6 patients who did not present with pain at diagnosis developed pain, again mostly due to disease progression despite TKI treatment. The remaining 21 patients achieved optimal pain control throughout the treatment course, although 17 of them eventually progressed (6 cases of a new lesion and 11 cases of progression of the primary lesion). There was no significant difference in mPFS of patients who had a worsening of pain after having achieved good pain control initially and those who achieved optimal pain control throughout the treatment course ($P = .779$).

Underlying genetic variations in the $EGFR$ gene (mutations and small nucletide polymorphisms) are known to alter protein function and affect the biology of the tumor, inducing a different symptom presentation in patients and altering the therapeutic efficacy of EGFR-TKIs.30-32 We analyzed the effect of the most common $EGFR$ mutations in exons 18-21 and the polymorphism rs2293347 (181946G>A) on pain occurrence and combined analgesic/TKI treatment efficacy. $EGFR$ mutation subtype and the type of the $EGFR$181946 CT polymorphic variant did not have a direct impact on pain occurrence and management, but had a significant effect on response to TKI indirectly affecting patients’ symptoms. Thus, the presence of specific $EGFR$ genetic changes induces a different response to TKIs which ultimately led to a decrease in the disease burden and general pain alleviation during treatment.

Cancer-related pain is very common in lung cancer patients as majority of cases are diagnosed in advanced stages especially in countries like Serbia without appropriate lung screening programs and smoking cessation measures. As early palliative care and symptom management have proven effects on the improvement of the quality of life and longer survival they are an essential part of lung cancer patients’ care.33 Although other countries have performed multicenter prospective studies,34 no detailed studies that evaluate the factors associated with pain in $EGFR$ mutated advanced lung adenocarcinoma patients treated with EGFR-TKIs have been performed in our region so far.

Most commonly described causes of pain in patients with advanced LC are related to skeletal and adrenal metastases and pancoast tumors.35 In our study, the presence of pain at diagnosis was significantly correlated with the presence of metastases, especially in the bones and adrenal glands, which
is in accordance with previously published data.\textsuperscript{10,36} The World Health Organization (WHO) recommends use of Three-Step Analgesic Ladder model for adequate pain management in cancer patients.\textsuperscript{37} Paracetamol is the mainstay of the first two steps of the WHO Analgesic Ladder in many countries as well as in our study.\textsuperscript{38} Taking into account the well-known side effects of long-term use of NSAIDs they were not used regularly in our study except as a single intermittent therapy or in combination with paracetamol for mild pain relief. Since some authors suggested replacing weak opioids with low doses of oral morphine thereby eliminating step 2 of the Ladder, the cornerstone of analgesic therapy in our study was oral morphine.\textsuperscript{39} Nevertheless, 10\% of our patients had adequate pain relief control with tramadol as a mild opioid of step 2 in maximum used dose 200 mg per day. Oxycodone or hydromorphone, in both immediate-release and slow-release formulations for oral administration are effective alternatives to oral morphine.\textsuperscript{40,41} In this study, oral methadone was used only in a small set of patients (2\%) with severe pain resistant to previous pharmacotherapy. Transdermal fentanyl was used as an efficient option for patients with stable opioid requirements, as well as for patients that had problems with oral administration of drugs or patients. Adjuvant analgesics that were used in this study were pregabalin, gabapentin, and dexamethasone. Overall, good pain management was achieved, with a significant decrease of patients who still had pain during treatment, and with a statistically lower pain level. In patients who developed a new lesion or had progression of the primary tumor during TKI treatment, we also observed worsening of pain despite having previously achieved good pain control.

Radiotherapy has an important role in bone pain management, treatment of metastatic spinal cord compression and palliative treatment of pathological bone fractures that cannot be surgically stabilized.\textsuperscript{11,42} Various palliative radiotherapy regimens are recommended for this indication, but an 8 Gy single dose should be considered as a regimen of choice for patients with painful bone metastases according to newest ESMO Clinical Practice Guideline for management of pain in adult cancer patients.\textsuperscript{38} In this study, the most commonly used regimens for palliative antidolorous radiation therapy were short-course 8 Gy single fraction as well as two long-course regimens including 20 Gy in 5 fractions and 30 Gy in 10 fractions.\textsuperscript{27} Although the presence of painful bone metastases is usually associated with shorter survival,\textsuperscript{43} in our study no difference in PFS or OS was observed in patients who presented with bone metastases-related pain at diagnosis.

\textbf{Conclusions:} \textit{EGFR} mutation subtype and the \textit{EGFR} 181946 C>T SNP had a significant effect on the response to TKI inducing an indirect anti-dolorous effect and might be useful for accessible, low-cost prediction of treatment.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{The effect of (a) \textit{EGFR} mutation types and (b) \textit{EGFR} 181946 CT polymorphic variants on pain management and (c) response to TKI treatment.}
\end{figure}
outcome in advanced NSCLC, especially in low-income countries.

**Declaration of Conflicting Interests**
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**Ethics Approval**
The procedures used in this study were approved by the Ethics Committee of the Institute for Oncology and Radiology of Serbia and were in accordance with the Helsinki Declaration of 1964 and its later amendments or comparable ethical standards. All patients signed an informed consent.

**Data Availability**
Data The data that support the findings of this study are not publicly available due to ethics restrictions, and can be obtained from the corresponding author upon reasonable request.

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**Supplemental Material**
Supplemental material for this article is available online.

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