ALK Mutation Status in EGFR-negative Non-small-cell Lung Cancer Patients in Bulgaria

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Background: Patients with non-small-cell lung cancer (NSCLC) with anaplastic lymphoma kinase (ALK) rearrangement mutation are found to be 3–13%.

Aim: To evaluate the prevalence of ALK mutations in EGFR-negative NSCLC patients in Bulgaria.

Materials and methods: One hundred and thirty-two patients with EGFR-negative NSCLC were examined for ALK mutation analysis between January and June 2016. Data were obtained from patients’ register of four major oncological hospitals in Bulgaria.

Results: Data were available for 124 (93.9%) patients, tumor mass was insufficient for analysis in 8 (6.1%) patients. Most of the patients were with adenocarcinoma (82 patients, 62.1%); 11 patients (8.3%) were with squamous histology and 2 patients (1.5%) were with other type of NSCLC. Histology data were missing in 37 patients (28.0%). ALK mutation was confirmed in 5 patients (3.8%), 119 (90.2%) patients had ALK wild type. ALK positive patients were with adenocarcinoma (n=3), squamous cell carcinoma (n=1) and other type (n=1) NSCLC. All ALK mutations were observed in never smokers (n=3) and former smokers (n=2).

Conclusion: The present study is the first of this kind in Bulgaria – it investigates the prevalence of ALK mutation rate in EGFR-negative NSCLC patients, which was found to be 3.8%. The presence of EGFR, ALK or other driver mutations is a prerequisite for targeted therapy and thus needs to be accurately assessed in NSCLC.
in NSCLC patients is found to be 3%–13%.\textsuperscript{7-15} The ALK gene is found in the short arm of chromosome 2 and encodes a tyrosine kinase. ALK mutations are caused by fusion between ALK gene and echinoderm microtubule-associated protein-like 4 (EML4) gene. This abnormal gene fusion leads to the production of a protein (EML4-ALK) that appears, in many cases, to promote and maintain the malignant behavior of the cancer cells.\textsuperscript{14}

There is no data on ALK mutation rate in Bulgaria. The presence of EGFR, ALK or any driver mutation is a prerequisite for targeted therapy and thus needs to be accurately assessed in NSCLC.\textsuperscript{16-18} Because EGFR and ALK mutations are mutually exclusive, patients with ALK mutation are not thought to benefit from EGFR-targeting tyrosine kinase inhibitor’s therapy. Instead, a treatment with an ALK inhibitor (crizotinib, ceritinib) is indicated.\textsuperscript{19-21} The aim of the current study is to estimate the prevalence of ALK mutations in EGFR-negative patients with NSCLC in Bulgaria.

**MATERIALS AND METHODS**

**PATIENTS**

One hundred and thirty-two patients with EGFR-negative NSCLC were examined for ALK mutation analysis between January and June 2016. Data were obtained from patients’ register of four major oncological hospitals in Bulgaria (Serdika Hospital, Sofia; Central Oncological Hospital, Plovdiv; Nadezhda Hospital, Sofia; Tsaritsa Yoanna University Hospital, ISUL, Sofia).

Data on patients’ characteristics were collected including age, gender, smoking status, stage and histology, if available. All patients were staged according to seventh revision of TNM classification for NSCLC.

All patients with NSCLC were tested for EGFR mutation (Cobas). EGFR-negative NSCLC samples were subsequently tested for presence of ALK mutations (EML4-ALK Fusion Gene Detection Assay; AmyoDx).

**STATISTICAL ANALYSIS**

Data are described as numbers (percentages) for categorical and median (interquartile range) for not normally distributed numerical variables. The distribution of the variables among the different groups were compared with Mann-Whitney U test for quantitative and Pearson’s chi square (Fisher’s Exact test, if applicable) for qualitative variables. Statistical analyses were performed with the SPSS package for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA).

**RESULTS**

Data were available for 124 patients; tumor mass was insufficient for analysis in 8 (6.1%) patients. ALK mutation was confirmed in 5 patients (3.8%), 119 (90.2%) patients were diagnosed with ALK wild type (Fig. 1). ALK mutation prevalence only in adenocarcinoma is 3.9% (3/76).

Baseline characteristics of the population are summarized in Table 1. Patients were predominantly male (84 patients, 63.6%), 34 patients (25.8%) were female, gender information was missing in 14 patients (10.6%). Median age was 62.5 years (range 55-68 years). Almost half of the patients (55 patients, 41.7%) were in NSCLC stage IV; 17 patients (12.9%) - in stage III; 2 patients (1.5%) - in NSCLC stage II and 2 patients were with confirmed relapse. For 56 patients (42.4%), information for the NSCLC stage was missing. Most of the patients had adenocarcinoma (82 patients, 62.1%), 11 patients (8.3%) had squamous cell carcinoma, and 2 patients (1.5%) had another type of NSCLC.

ALK positive patients had adenocarcinoma (n=3), squamous cell carcinoma (n=1) and another type (n=1) of NSCLC. All ALK mutations were observed in never smokers (n=3) and former smokers (n=2). ALK mutations do not show difference between the genders (60 vs. 66.4%, p=0.609).

**DISCUSSION**

ALK rearrangement occurs in 3% to 13% of NSCLC patients and it is more prevalent in never/former smokers patients with adenocarcinomas. This study showed ALK mutation prevalence of 3.8% which tends to be low compared to other stud-
ALK mutation prevalence in adenocarcinoma is similar - 3.9%. However, ALK mutations occur predominantly, but not only in adenocarcinoma. Therefore, further analysis is required for identifying subgroups of patients which may benefit from this investigation, i.e. in the unusual case of relatively young non- or ex-smoker with squamous cell carcinoma, as this study suggests.

ALK mutation status is not related to gender, which could be partially explained with lack of significant difference in smoking status and age between males and females (all \( p > 0.05 \)). The ALK fusion oncogene in patients with NSCLC is strongly associated with a history of never or light smoking.\(^{13,22}\) The present study also confirms these observations. The patients with ALK rearrangements tended to be younger and they have predominantly adenocarcinoma which is also reported in other studies.\(^{19}\)

An ALK inhibitor is preferred as a first-line therapy for patients with ALK positive NSCLC. It is associated with prolongation in progression-free survival (PFS) and improved response rate and quality of life.\(^{23}\) The present study did not report increased OS, but other studies reported 1-year survival of 74.8%\(^{24}\) and median OS of 49.4 months with PFS 8.2 months.\(^{25}\)

This study determines the ALK mutation prevalence in a large number of patients, similar to this of other studies. This is the first study in Bulgaria

### Table 1. Patients’ characteristics and ALK status

| Characteristic                | ALK mutation status (n=5) | ALK wild type (n=119) | \( p \) | ALK status unknown (n=8) |
|------------------------------|--------------------------|----------------------|--------|-------------------------|
| **Gender**                   |                          |                      |        |                         |
| Male (%; n)                  | 60%; 3                   | 66.4%; 79            | 0.609**| 25%; 2                  |
| Female (%; n)                | 40%; 2                   | 23.5%; 28            |        | 50%; 4                  |
| Missing data (%; n)          | 0                        | 10.1%; 12            |        | 25%; 2                  |
| Age (median; range)          | 55; 45-64.5              | 63; 56-68            | 0.200* | 62.5; 57.5-71.75        |
| **Smoking status**           |                          |                      |        |                         |
| Current smokers (%; n)       | 0                        | 37%; 44              |        | 37.5%; 3                |
| Former smokers (%; n)        | 40%; 2                   | 16%; 19              | 0.017***| 0                       |
| Never smokers (%; n)         | 60%; 3                   | 10.1%; 12            |        | 37.5%; 3                |
| **TNM stage**                |                          |                      |        |                         |
| Stage II (%; n)              | 0                        | 1.7%; 2              |        | 0                       |
| Stage III (%; n)             | 40%; 2                   | 11.8%; 14            | 0.597***| 12.5%; 1                |
| Stage IV (%; n)              | 40%; 2                   | 40.3%; 48            |        | 62.5%; 5                |
| Relapse (%; n)               | 0                        | 1.7%; 2              |        | 0                       |
| Missing data (%; n)          | 20%; 1                   | 44.5%; 53            |        | 25%; 2                  |
| **Histology type**           |                          |                      |        |                         |
| Adenocarcinoma (%; n)        | 60%; 3                   | 61.3%; 73            |        | 75%; 6                  |
| Squamous cell carcinoma (%; n)| 20%; 1                  | 8.4%; 10             | 0.018***| 0                       |
| Other (%; n)                 | 20%; 1                   | 0.8%; 1              |        | 0                       |
| Missing data (%; n)          | 0                        | 29.5%; 35            |        | 25%; 2                  |

*\( p \)Mann-Whitney test; **Fisher’s exact test; ***\( \chi^2 \) analysis
on this subject. Moreover, the sample from multiple centers makes it representative and gives higher confidence of the results’ generalizability. The demographic characteristics provide useful information for identifying subgroups of patients with highest probability of ALK mutations (for example non- and ex-smokers) as well as those with lower probability (i.e. current smokers).

The limitations of this study are related to the missing information. Since the data were obtained from registers, some of the information was not available and this resulted in lower statistical power of some of the analyses.

CONCLUSION

Our study showed 3.8% prevalence of ALK mutation in EGFR-negative NSCLC patients and 3.9% in patients with adenocarcinoma which tends to be low. However, most of the patients with ALK mutation were with adenocarcinoma. ALK rearrangements tend to happen in younger patients and did not correlate with gender. The presence of EGFR, ALK or other driver mutation is a prerequisite for targeted therapy and thus needs to be accurately assessed in NSCLC. The identification of ALK mutation in patients with advanced NSCLC is associated with better prognosis if target therapy is started.

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Введение: Количество больных с немелкоклеточным раком лёгкого (НМКРЛ) с мутацией с перегруппировкой генов анапластической лимфомы киназы (ALK) составляет от 3 до 13%.

Цель: Установить распространённость ALK-мутаций среди EGFR-отрицательных больных с НМКРЛ из Болгарии.

Материалы и методы: Сто тридцать три больных с EGFR-отрицательным НМКРЛ были исследованы на предмет мутации ALK в период с января по июнь 2016 года. Данные были извлечены из регистров больных пяти основных онкологических больниц в Болгарии.

Результаты: Были установлены данные о 124 (93.9%) больных, масса опухолей была недостаточной для анализа у 8 (6.1%) больных. Большинство больных были с аденокарциномой (82 пациента, 62.1%); у 11 больных (8.3%) была плоскоклеточная карцинома, а у 2 больных (1.5%) был другой тип НМКРЛ. Отсутствовали гистологические данные у 37 больных (28.0%). ALK-мутация была подтверждена у 5 больных (3.8%), у 119 (90.2%) больных были «дикие типы» ALK-мутаций. У больных с положительной ALK-мутацией были аденокарцинома (n = 3), плоскоклеточная карцинома (n = 1) и другой тип НМКРЛ (n = 1). Все мутации ALK наблюдались у людей, которые никогда не курили (n = 3) и у бывших курильщиков (n = 2).

Выводы: Настоящее исследование является первым в своём роде в Болгaria – оно исследует частоту распространённости ALK-мутаций у пациентов с EGFR-отрицательным НМКРЛ, которое составляет 3.8%. Наличие EGFR, ALK или других мутаций является предпосылкой для целенаправленной терапии и в силу этого необходима их пунктуальная идентификация при НМКРЛ.