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Expression and significance of Ki-67 in lung cancer

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Abstract

Ki-67 parameter is a proliferation marker in malignant tumors. The increased proliferation activity and the decreased prognosis in lung cancer determined us to investigate different parameters connected to the tumor's aggression, such as cellularity, Ki-67 positivity rate, and proliferating cell nuclear antigen (PCNA). We evaluated the proliferative activity in 62 primary lung tumors by determining the cell's percentage of Ki-67 and immunoreactive PCNA (using MIB-1 and PCNA monoclonal antibodies), classifying Ki-67 and PCNA immunoreactivity into three score groups. The results obtained emphasized a linkage between Ki-67 score with the histological tumor subtype, tumor cellularity and degree of differentiation and with other proliferation immunohistochemistry (IHC) markers, such as p53 cellular tumor antigen. The tumor's cellularity, the Ki-67 positivity rate and PCNA, together with the clinical stage and the histological differentiation bring extra pieces of useful information in order to anticipate the evolution and the prognosis of lung cancer.

Keywords: Ki-67 antigen, lung cancer, MIB-1 primary antibody, IHC markers, p53 cellular tumor antigen.

⬚ Introduction

Lung cancer was a rare disease in the early of the 20th century, but due to new causative agents and an increasing lifespan it became pandemic between the 20th and 21st centuries. Lung cancer is the biggest cancer killer in Europe, accounting for approximately 20% of total cancer deaths.

The incidence and mortality of the histological subtypes of lung cancer in Europe is permanently changing; for this reason, we aim to study and investigate relevant aspects in diagnostic and survival rates [1].

The used current methods in order to estimate how malignant a tumor is and the prognosis of patients with lung cancer also include the proliferation of the tumor cells. The tumor proliferation activity can be investigated through different methods, the immunohistochemistry (IHC) being proved to be the simplest and most reliable for the evaluation of cell proliferation.

Ki-67 antigen is a marker of cellular cycle and proliferation, usually used to estimate the cell's population proliferation, also indicating the cell growth ratio [1].

In malignant tumors, the percentage of Ki-67 positive cells can be linked to the tumor aggressiveness or to the tumor's development parameters; thus, this protein plays a practical role in the tumoral histopathology, even though its biochemical nature and its functions are very little known.

Ki-67 is a nuclear antigen expressed in all cell cycle stages, with the exception of stage G0; it is detected in stages G1, S, G2 and M of the cell cycle.

The risk factors have to be known and evaluated and the therapeutic approach must be ethical with the lowest number of encountered adverse events.

The attitude must take into account, with great attention, all the present comorbidities, associated pathologies, the medical family history and the heredo-collateral antecedents.

Our aim was to investigate the expression and significance of Ki-67 in lung cancer and to implement a flexible, personalized approach, targeted in function of the etiopathogenic aspects, in order to offer an integrative, complex research.

Our present study is very useful, original and of great impact, because it brings an interesting approach in this field, concerning the expression and significance of Ki-67 in lung cancer. Further studies are needed, in order to offer a complex integrative proper image [1].

To our knowledge, this type of studies approaching the expression and significance of Ki-67 are not so frequent in Romania.

⬚ Materials and Methods

The study has been performed on 62 cases of bronchial biopsies and tissue samples prelevated from surgical resection, from patients clinically diagnosed with dysplastic lesions or lung cancer.

The study period was done between 2009–2014 and the histopathological identification of lesions was done according to the 2011 *International Association for the Study of Lung Cancer/American Thoracic Society/ European Respiratory Society* (IASLC/ATS/ERS) lung cancer classification.

On the total number of biological samples, we tested a variety of antibodies with specificity for p53 protein (clone DO-7, 1/500 dilution, DAKO, Glostrup, Denmark) and for the proliferating cell nuclear antigen (PCNA) (clone PC10, 1/800 dilution, DAKO, Glostrup, Denmark).

In 15 cases of non-small cell lung cancer (NSCLC), we examined the expression of the epidermal growth factor receptor (EGFR).

The study material was prepared through fixating in 10% formaldehyde, paraffin inclusion and the general morphological evaluation was done on Hematoxylin– Eosin (HE) and Gömöri trichrome stained sections.

The proliferation potential was evaluated by identifying the Ki-67 nuclear antigen, using the IHC staining method with Avidin–Biotin complex (ABC). After the sections pre-treatment through boiling for 60 minutes, at 80–90^{0}C, in citrate buffer, with pH 6, the sections were incubated over night, at 4^{0}C, with the primary MIB-1 antibody (MIB-1, Dianova, Hamburg, Germany), with a dilution of 1/800 [2].

The visualization with 3-amino-9-ethylcarbazole (AEC), followed by light counterstaining with Hematoxylin of the nuclei, allowed Ki-67 to be distinguished as a final reaction product of red color, with nuclear localization.

Immunohistochemistry

IHC marking with MIB-1 monoclonal antibody of sections identified a limited staining pattern for nuclei. We identified Ki-67 immunoreactivity, evaluating the tumoral cells from the uniformly stained areas, ruling out bleeding and necrosis cores, as well as the inflammation areas. We considered as a positive response any detectable nuclear staining (dotted or diffuse).

In order to quantify Ki-67 immunoreaction, we determined the reactive cells' percentage. Taking into consideration the heterogeneity of distribution and staining intensity, we used a semi-quantitative score (corresponding to the staining intensity and the reactive nuclei percentage), which helped classifying Ki-67 immunoreactivity in three scoring groups:
▪ Low score (<25% of tumor cells were reactive);
▪ Medium score (26–75% of tumor cells became colored);
▪ High score (76–100% of tumor cells were reactive).

P53 was determined as another IHC proliferation marker, being the cellular tumor antigen. It was translated as positive p53 reaction the distinct, homogenous, and granular nuclear staining.

Statistical analysis

Based on these materials, data was collected and organized in a Microsoft Excel 2007 database and analysis was performed using Statistical Package for the Social Sciences (SPSS) version 17.0 and EpiInfo 7. The cases were compared using the χ^2 (chi-square) test, where the value of *p* is considered as follows: $p > 0.05$ – not significant (NS) differences; $p < 0.05$ – significant differences; $p < 0.01$ – very significant (VS) differences; $p < 0.001$ – extremely significant (ES) differences.

⬚ Results

In the majority of examined cases, we noticed the immunoreaction heterogeneity (concerning the intensity of staining and the nuclear marking) and a variety of marking types: nucleolar isolation (delicate, point-like), nucleolar and nucleo-cytoplasmatic marking, intense and diffuse marking of the whole nucleus together with intense marking of the nucleus and cytoplasm (Figure 1).

We also noticed the absence of Ki-67 immunoreaction in normal lung tissue.

On the surface of the normal lung epithelium and in the hyperplasic basal cells, we noticed a rare presence of dispersed Ki-67 positive nuclei.

MIB-1 immunostaining marked isolated reactive nuclei in the atypical squamous metaplastic lung epithelium, in the atypical squamous metaplasia or the dysplastic one, and in the associated squamous cell carcinoma (SCC).

An increased number of nuclei, positive for Ki-67 was noted, with a random distribution in the epithelial thickness (Figure 2).

We also noticed the Ki-67 positive reaction (with rare reactive nuclei) in the bronchial glands and in stromal lymphocytes nuclei, and in the same time in a low number of cases, we observed a clear and consistent staining of the elastic fibers of both alveolar septum and vascular walls.

In 53 of 62 (85.4%) analyzed cases of primary lung carcinoma, we identified the Ki-67 expression, its positive reaction being defined by nuclear immunostaining with variable intensity, from case to case.

The percentage of reactive Ki-67 nuclei varied within large limits, being different, directly proportional to the histological type.

In typical carcinoids and bronchioloalveolar carcinoma (BAC) we analyzed, they presented low values of proliferative activity, with rare Ki-67 reactive nuclei (Ki-67 mitotic index – MI <5%), while small cell lung cancer (SCLC) had the highest growth rate, most of the cases (83.3%) representing the intermediate immunoreactivity, by >25% Ki-67 reactive nuclei (Table 1) and an intense marking of the whole nucleus (Figure 3).

The seven differentiated carcinomas with large cells, Ki-67 positive, had an obvious heterogeneity of Ki-67 expression, with variations of the staining intensity, but especially of marking types (Figure 4).

We noticed an intense proliferative activity and a similar heterogeneity of marking in adenocarcinoma (ADC) (Figure 5) and in SCC (Figure 6).

Ki-67 was expressed in the primary lung tumors, but also in the corresponding metastases, such as hepatic metastasis and lymph node metastasis.

Table 1 – *Ki-67 positivity rate correlated with the cellularity and the histological degree*

Variable (*n*)	Ki-67 negative *n* (%)	Ki-67 positive *n* (%)	Ki-67 group scores *Reduced (<25%), n (%)*
Histological type			
SCLC (12)	0	12 (100%)	2 (16.6%)
NSCLC (47)	8 (17%)	39 (83%)	9 (48.7%)
Carcinoid (3)	1 (33%)	2 (66%)	1 (50%)
Cellularity (C)			
C1 (15)	4 (26.6%)	11 (73.3%)	9 (81.8%)
C2 (25)	3 (12%)	22 (88%)	7 (31.8%)
C3 (22)	2 (9%)	20 (90.9%)	6 (30%)
Histological degree (G)			
G2 (17)	7 (41.1%)	10 (58.8%)	5 (50%)
G3 (45)	2 (4.1%)	43 (95.5%)	17 (39.5%)
Total *n* (%)	9 (14.5%)	53 (85.4%)	22 (41.5%)

n: No. of cases; SCLC: Small cell lung cancer; NSCLC: Non-small cell lung cancer.

The lung carcinomas with Ki-67 positive immuno-reaction were grouped according to the histological type and the degree of tumoral differentiation.

Based on tumoral cellularity, the studied tumors were divided into three groups; the tumor cellularity was appreciated on a scale from 1 to 3, degree 3 having the highest level of cellularity.

Analyzing the link between the morphological para-meters mentioned and the tumoral proliferative activity, it was noticed that there was a significant relation between cellularity (C), tumoral histological degree (G), and the rate of growth assessed through Ki-67 score (Table 1).

The percentage of Ki-67 positive cells was correlated with the lowest degree of differentiation (G3) ($p < 0.001$; ES), third degree of cellularity (C3), being higher in SCLC and the differentiated areas of NSCLC.

Out of the tumors with G2 histological degree, seven (41.1%) cases were Ki-67 negative and 10 (58.8%) cases were Ki-67 positive ($p = 0.672875$; NS); two (4.1%) G3 tumors were Ki-67 negative and 43 (95.5%) cases were positive for this antigen ($p < 0.001$; ES).
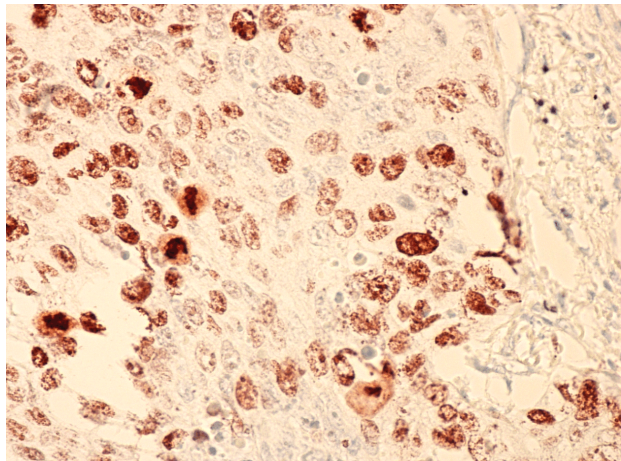

Figure 1 – *Ki-67 immunoreaction with intense staining and granular nuclear pattern (Anti-Ki-67 antibody immunomarking, ABC method, AEC visualization, ×400). ABC: Avidin–Biotin complex; AEC: 3-Amino-9-ethyl-carbazole.*
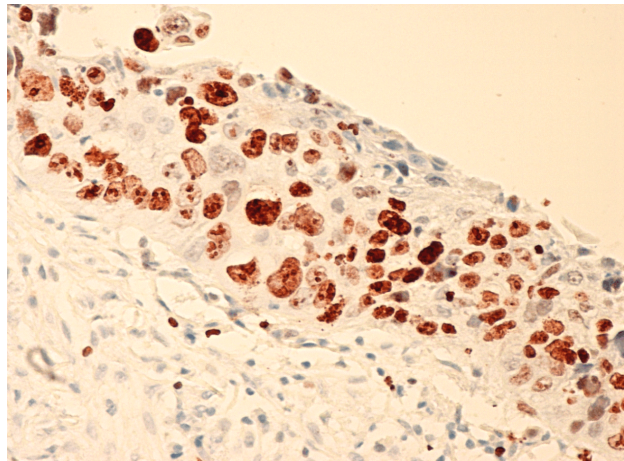

Figure 2 – *Intraepithelial carcinoma with Ki-67 nuclei dispersed in the entire thickness of the epithelium (Anti-Ki-67 antibody immunomarking, ABC method, AEC visualization, ×400). ABC: Avidin–Biotin complex; AEC: 3-Amino-9-ethylcarbazole.*

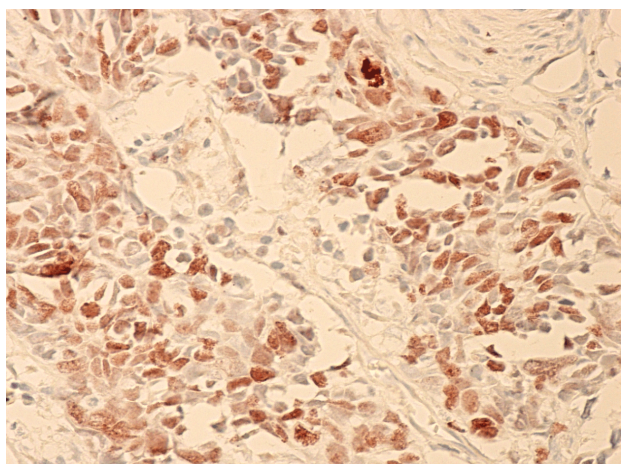**Figure 3 – *SCLC with Ki-67 high score (>75% reactive tumoral cells) (Anti-Ki-67 antibody immunomarking, ABC method, AEC visualization, ×400). SCLC: Small cell lung cancer; ABC: Avidin–Biotin complex; AEC: 3-Amino-9-ethylcarbazole.***
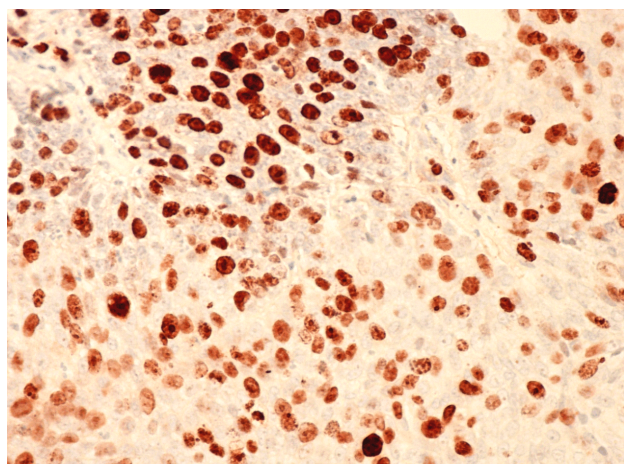

Figure 4 – *Undifferentiated carcinoma with big cells. Heterogeneous Ki-67 expression with variations of the marking type (Anti-Ki-67 antibody immunomarking, ABC method, AEC visualization, ×400). ABC: Avidin–Biotin complex; AEC: 3-Amino-9-ethylcarbazole.*
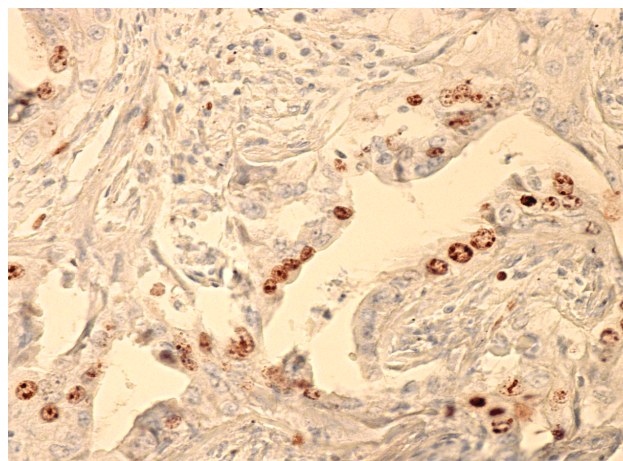
Figure 5 – *Lung carcinoma with acinar cells. Positive Ki-67 immunoreaction with isolated Ki-67 reactive nuclei (Anti-Ki-67 antibody immunomarking, ABC method, AEC visualization, ×400). ABC: Avidin–Biotin complex; AEC: 3-Amino-9-ethylcarbazole.*
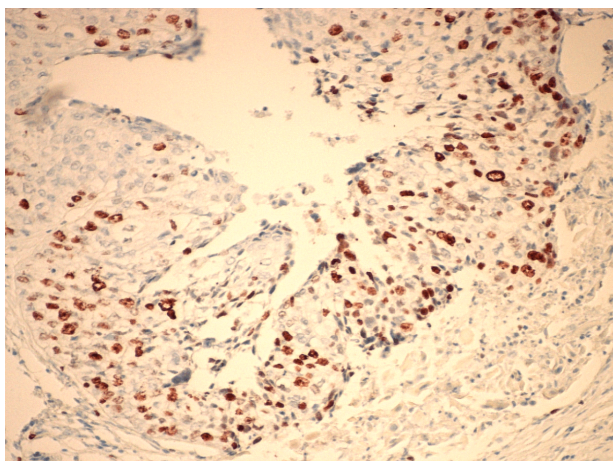

Figure 6 – *Carcinoma with squamous cells. Positive Ki-67 immunoreaction in the tumoral cell nuclei (Anti-Ki-67 antibody immunomarking, ABC method, AEC visualization, ×200). ABC: Avidin–Biotin complex; AEC: 3-Amino-9-ethylcarbazole.*

We analyzed the possible prognosis significance of Ki-67 expression on subgroups of patients classified according to the lymph nodes status and the metastasis potential (Table 2).

In 53 cases of carcinomas that emphasized Ki-67 nuclear antigen, the involvement of lymph nodes was noted in 21 (39.6%) cases, pleural invasion ± extension at the thoracic wall, as well as novelty in 11 (20.7%) cases, local relapse in six (11.3%) cases and remote metastasis in 13 (24.5%) cases; all these tumors were characterized by an unfavorable evolution, with the death of 14 patients in the first 21 months after diagnosis.

The tumoral proliferative rate was analyzed (evaluated through the Ki-67 score) in relation with the p53 immuno-reactivity. Twenty-nine of the 32 (90.6%) p53 positive carcinoma cases showed Ki-67 nuclear antigen ($p<0.001$; ES), 21 (72.4%) cases had an intermediate and high Ki-67 score, with over 25% reactive nuclei ($p=0.000465$; ES) (Table 3).

Table 2 – *Relation between Ki-67 expression and parameters of tumors evolving*

Ki-67 immunoreaction (*n*)	Positive lymph nodes	Pleural invasion ± of thoracic wall	Local recurrence	Remote metastasis
Ki-67 negative (9)	4 (44.4%)	1 (11.1%)	1 (11.1%)	1 (11.1%)
Ki-67 positive (53)	21 (39.6%)	11 (20.7%)	6 (11.3%)	13 (24.5%)
p	0.924448 (NS)	0.825267 (NS)	0.581481 (NS)	0.64627 (NS)

n: No. of cases; NS: Not significant.

Table 3 – *Ki-67 immunoreaction correlated with the presence of p53 cellular tumor antigen*

Ki-67 score *(n)*	p53 immunoreaction			
	p53 negative (n=30)	*p*	*p53 positive (n=32)*	*p*
Ki-67 negative (9)	7 (23.35%)	0.003431 (VS)	3 (9.3%)	<0.001 (ES)
Ki-67 positive (53)	23 (76.7%)		29 (90.6%)	
Low score (<25% reactive nuclei)	14 (60.8%)	0.121395 (NS)	8 (27.5%)	0.000465 (ES)
Intermediate and high score (>25% reactive nuclei)	9 (39.2%)		21 (72.4%)	

n: No. of cases; VS: Very significant; ES: Extremely significant; NS: Not significant.

In the subgroup of p53 negative tumors, we noted a high number of cases (23; 76.6%) with Ki-67 positive immunoreaction ($p=0.003431$; ES), in 14 (60.8%) of these cases the used antibody (MIB-1) marking <25% of the tumor cells.

In the p53 positive group, the number of patients with high and intermediate Ki-67 score is significantly higher than the number of patients with low score ($p=0.000465$; ES).

Among the patients with p53 negative tumors, the number of Ki-67 positive cases is significantly higher than the Ki-67 negative ones ($p=0.0034$; ES).

In order to evaluate the Ki-67–EGFR co-expression, we pursued a possible relation between growth fraction determined by IHC and EGFR expression. A Ki-67 positive immunoreaction was identified especially in tumors that were discovered in III and IV stages of disease (nine cases); similarly, the EGFR expression proved to be positive more frequently in the advanced tumor stages than the ones at the beginning, thus proving a relation between these markers and invasive characteristics of tumor.

The majority of T3 and T4 tumors (seven out of nine cases; 77.7%) showed high values of proliferative activities (Ki-67 score >25%) and EGFR positive immunoreaction (with an EGFR absolute staining score average of 3), compared to the tumors that show little invasive features; the involvement of lymph nodes and remote metastasis

were noticed to be more frequently in Ki-67+ EGFR+ tumors (five cases).

For a better assessment of the prognosis significance of Ki-67–EGFR co-expression, the following two markers were analyzed according to the classification of tumors in four groups (based on immunoreaction positivity) (Table 4):

▪ Group 1 included Ki-67- EGFR- tumors;

▪ Group 2 was made up of Ki-67- EGFR+ tumors;

▪ Group 3 included tumors with Ki-67+ (reduced Ki-67 score; <25%) and EGFR+ immunoreactions.

Table 4 – *The prognosis significance of Ki-67–EGFR co-expression*

Tumor group	Ki-67- EGFR-	Ki-67- EGFR+	Ki-67 <25% EGFR+	Ki-67 >25% EGFR+
No. of patients	1	3	3	8
Survival rate [months]	40	11	9	7

EGFR: Epidermal growth factor receptor.

The relation between the Ki-67 growth ratio and the EGFR positivity rate in 15 NSCLCs revealed that all Ki-67 positive tumors expressed EGFR.

The results show the highest rate of survival (of 40 months), on the other side not encountered on the date of the last check up, in case of a patient discovered in stage II of disease, with Ki-67- EGFR- tumor.

Compared to this, the analysis of the survival rate according to the Ki-67 and EGFR expressions suggests that Ki-67 positive immunoreaction (>25%) and simultaneous EGFR overexpression anticipates a group of lung tumors with a reticent prognosis and a more reduced survival tendency.

Analyzing the relation between Ki-67 and the PCNA positivity rate (Table 5), we noticed that there was a higher percentage of tumors with intermediate and high PCNA scores (73.5%), compared to positive tumors for Ki-67 nuclear antigen from the same score groups (31 cases; 58.4%), but statistically insignificant (p=0.074989).

Table 5 – *Ki-67 and PCNA positivity rate*

Ki-67 and PCNA immunoreaction	n	Score groups		
		Reduced score (<25%)	p	Intermediate and high (25–100%)
Ki-67 positive	53	22 (41.5%)	0.215815 (NS)	31 (58.4%)
PCNA positive	53	14 (26.4%)		39 (73.5%)

n: No. of cases; PCNA: Proliferating cell nuclear antigen; NS: Not significant.

There were identified cells that express Ki-67 antigen (MIB-1 positive cells) in 53 of the 62 (85.3%) lung carcinoma studied cases. Twenty-two carcinoma cases were allocated in the low score group (Ki-67 <25%) and 31 cases in the intermediate and high score group (Ki-67 >25%).

Twenty-nine of 32 (90.6%) p53 positive carcinomas expressed Ki-67 antigen, 21 (72.4%) cases had an intermediate and high Ki-67 score (with reactive nuclei >25%); the results emphasize prevalence of p53 positive immunoreaction in lung cancer weakly differentiated with an elevated proliferative rate.

⯐ Discussions

Studying the expression of Ki-67 antigen in normal bronchial epithelium and atypical squamous metaplasia, we identified dispersed reactive nuclei, the majority being found in the basal layer. We noted a high number of reactive nuclei on the entire thickness of the epithelium, in atypical squamous metaplasia and intra-epithelium carcinoma associated with invasive squamous tumors, the observations being in accordance with Pendleton *et al.* study [3] regarding Ki-67 expression in bronchial squamous metaplasia.

We noticed a positive immunoreaction and a heterogeneity of Ki-67 expression in 53 of the 62 (85.4%) MIB-1 stained primary lung tumors.

The IHC detection of Ki-67 antigen expression, correlated to other biological and clinico-morphological parameters, represent a real value in the prognosis assessment for patients with lung cancer.

A significant relation can be seen between Ki-67 staining and other prognosis factors; thus, the percentage of Ki-67 positive cells (Ki-67 score) is different among the histological lung cancer subtypes, according to the tumor stage and differentiation degree, confirming its prognostic validity.

Matching the Ki-67 score groups with the histological type of lung cancer, we noticed that the majority of SCLCs (83.3%) represents >25% of Ki-67 reactive nuclei, with intermediate and high Ki-67 score, compared to only 51.2% of NSCLCs (included in the same score group).

Typical carcinoid tumor and BAC represented a Ki-67 marking index <5% (low Ki-67 score), these values being much lower than in conventional lung SCLC and ADC, differences similar to those noted by Kitamura *et al.* [4] and Przygodzki *et al.* [5], seem to be linked to the atypia degree of the lesions, indicating the proliferation of a limited number of cells.

Other similar studies have a small number of data referring to the prognosis value of the Ki-67 marking index; also, the relation between the Ki-67 positivity rate and the prognosis is still unclear [6]. Some authors noted a lower probability of survival (five years) in patients with Ki-67 values >13% [7].

Martin *et al.* suggested the existence of an association between Ki-67 and the reduced survival in patients with lung cancer. Their analysis showed Ki-67 expression being a negative prognosis factor for the survival in patients with NSCLC [8].

The results of Nakano & Oka [9] say that the increased proliferative activity is linked to a high tumor malignity, with an increased rate of metastasis and tumor relapse.

The higher the tumoral proliferation activity and proliferative potential, the lower is the survival (from the moment the diagnosis was given) [10]. Ki-67 can be considered as a supplementary test that helps histologically classifying tumors [11].

Our results underline a relationship between Ki-67 immunostaining and the advanced stage of disease (III and IV) together with elevated cellularity (C3), histological type of SCLC, and weak tumor differentiation (according to similar observations reported by Kawai *et al.*) [12]. We also noticed that these parameters are strongly linked between them, together with the prognosis, suggesting

the possibility of using them in assessing the clinical behavior.

The implication of lymph nodes along with a high metastasis potential were tied up to tumors that show Ki-67 nuclear antigen, describing a group of neoplasia with fast evolution and exitus in the first two years after diagnosis.

Remembering the possible relation between *P53* genetic overexpression and tumor proliferative activity [24], we analyzed the relation between the growth rate and p53 immunoreactivity, and on the other hand, we pursued the relation between the proliferative activity and the EGFR positivity rate (estimated by calculating Ki-67 score and the EGFR absolute staining score).

We noticed a p53 overexpression in 29 of the 32 (90.6%) Ki-67 positive lung tumor cases and in 21 (72.4%) tumor cases with intermediate or high Ki-67 score (with reactive nuclei >25%); these results suggested that the quantity of positive p53 immunoreactions is weakly differentiated in lung tumors with a high proliferation rate.

The observations about the relation between p53 expression and Ki-67 antigen in lung cancer are controversial [13]. Evaluating the prognostic significance of Ki-67 and p53 expressions in resected NSCLC, Scagliotti *et al.* [14] underline no significant correlation. Still, the authors notice a relatively high risk of recurrence and a low rate of survival in patients with >25% positive tumor cells. Our results confirm these observations.

In the tumor cases with Ki-67 positive immunoreaction, we noted an increased number of p53 negative cases, probably suggesting that the *P53* gene mutation is not the only mechanism involved in acquiring the proliferative potential.

The overexpression of the *P53* tumor suppressor gene and the Ki-67 cellular proliferation marker on lung biopsy are considered predictive factors for the survival of patients with lung cancer [15].

There is little data about the possible relation between the growth ratio determined by IHC and the EGFR expression in lung carcinomas. In 15 of the 62 IHC studied carcinoma cases, we analyzed the EGFR expression and its relation with the proliferative activity estimated through the Ki-67 score. We noticed that Ki-67 positivity is closely related to the EGFR positive immunoreaction; all Ki-67 positive tumors showed EGFR, while only three Ki-67 negative tumors showed EGFR positive reaction. Thus, it looks like the increased expression of Ki-67 and EGFR can be related with the tumor evolution.

The EGFR expression in highly proliferative tumors (Ki-67 score of 75–100%) diagnosed in an advanced stage, along with positive lymph nodes, suggest that the simultaneous positive expressions of Ki-67 antigen and EGFR (Ki-67+ EGFR+) reflect the invasive character of lung cancer, and are considered to be malignancy indicators. These results clearly show a strong relation between the growth ratio (Ki-67 >25%), elevated EGFR expression and the clinico-pathological characteristics of an invasive tumor behavior; thus, the Ki-67+ EGFR+ co-expression is an indicator for a reticent prognosis, of invasion and of the increased potential of metastasis in patients with lung cancer.

The other observations emphasizing the relation between PCNA expression and Ki-67 antigen in lung cancer are rather controversial [16]. Rowlands *et al.* [17] note the lack of association between Ki-67 score and PCNA.

In our study, the PCNA immunoreaction was well correlated with the ratio of Ki-67 growth; still, we found a large number of tumors with intermediate and high PCNA scores, compared to Ki-67 positive tumors in the same score groups.

The relation between PCNA positive immunoreactions, Ki-67 positive ratio and metastatic potential (lympho-nodular involvement and isolated metastasis) suggests the predictive value of these factors concerning the proliferation in lung cancer. We can state that the elevated proliferative activity of tumor cells anticipate an increased malignancy, with a higher frequency of relapses and metastasis, confirming Kawai *et al.* observations [12, 18].

Knowing these facts, also the therapeutic approach must be carefully personalized, integrative and adapted according to the associated signs, symptoms, etiopathogenic, genetic risk and environmental factors, in order to encounter the least complications and adverse events.

Conclusions

We noted a relation between Ki-67 immunostaining (tumors with intermediate and high Ki-67 score), together with an advanced stage of disease (III and IV) and an increased cellularity (C3), in the histological type of SCLC and weak tumoral differentiation, after correlating Ki-67 score group with other prognosis variables. These parameters are closely correlated between them, as well as the prognosis, suggesting the possibility of using them as factors in assessing the proliferation status and the clinical behavior. The involvement of lymph nodes and the increased potential of metastasis in patients with Ki-67 positive tumors defined a group of carcinomas with disadvantaged evolution and exitus, in the first two years after the diagnosis. EGFR overexpression in highly proliferative diagnosed advanced stage tumors with positive lymph nodes, suggests that the simultaneous expression of Ki-67+ EGFR+ are indicators of a reserved prognosis and increased metastasis potential in patients with lung cancer. PCNA immunoreaction complied rather well with the Ki-67 growth ratio; the relation between PCNA immunoreaction, Ki-67 positivity rate and metastatic potential (involvement of lymph nodes and remote metastasis) proving that the high proliferative activity anticipates an increased malignity, with a higher frequency of relapse and metastasis.

Conflict of interests
The authors declare no conflict of interests.

Author contribution
Codrina Mihaela Levai has equal contribution and thus shares first authorship.

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