JBUON 2019; 24(4): 1626-1637
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

# Immunohistochemical characteristics of brain metastases and corresponding primary lung cancer

Dora Marinova Marinova[1,2], Evgeni Vitkov Mekov[1,2], Denitsa Gosheva Dimitrova[1,2], Plamen Todorov Titorenkov[3], Miroslav Martinov Mihailov[1,2], Sevdalin Slavov Nachev[4], Vania Milosheva Youroukova[1,2], Dimitar Temelkov Kostadinov[1,5], Yanina Georgieva Slavova[1,6]

[1]Clinical Center for Lung Diseases, Medical University, Sofia, Bulgaria; [2]Department of Pneumonology, University Hospital for Pulmonary Diseases "St. Sofia", Sofia, Bulgaria; [3]Clinic for Pulmonary Diseases, Military Medical Academy, Sofia University, Faculty of Medicine, Sofia, Bulgaria; [4]Department of Pathology, University Hospital St Ivan Rilski, Sofia, Bulgaria; [5]Department of Bronchology, University Hospital for Pulmonary Diseases "St. Sofia", Sofia, Bulgaria; [6]Department of Pathology, University Hospital for Pulmonary Diseases "St. Sofia", Sofia, Bulgaria

## Summary

***Purpose:*** *To study brain metastases (BM) and their corresponding primary lung cancers (LCs).*

***Methods:*** *Surgically resected BMs and their corresponding primary LCs from 30 patients (25 men, 83%, age 55±9 years) were studied: 21 adenocarcinomas (ACs), 5 squamous cell carcinomas (SCCs), 4 small cell lung carcinomas (SCLCs). The histological subtype, immunohistochemical expression of TTF1, p63, Ki67 (proliferative activity), CD31, number of intratumoral microvessels, (NIM) and survival were evaluated.*

***Results:*** *There was a different histological structure in 47% of the cases of ACs of the lung in comparison with the corresponding metastasis, but none in SCC and SCLC. TTF-1 was expressed in a greater number of ACs (n=20; 95%), with lower mean expression levels, while the corresponding BM expressed the marker less frequently (n=16;76%) with higher mean expression values (p=0.011). P63 was expressed in all SCCs (p=0.68). Cytokeratin 7 was expressed equally in all ACs. Ki-67 proliferative index (PI) was higher in SCLC than in AC (p=0.008), in SCLC BM than in AC BM (p<0.001), and in SCLC BM than in SCC BM (p=0.008). The Ki-67 PI in BM was higher than in AC (p=0.003), SCC (p=0.048), but without difference in SCLC (p=0.141). CD31 NIM was higher in AC than in SCLC (p=0.003), in SCC than in SCLC (p=0.009), while no difference between AC and SCC was found (p=0.467). There were no differences between LC/BM in the NIM.*
*Survival after surgery for LC was significantly longer in AC than in SCLC (p=0.017). SCLC histology and Ki67>18% were established as negative prognostic factors after surgery for LC. Such factors were not found after surgery for BM.*

***Conclusion:*** *There are differences between primary LC and corresponding BM – in histology, immunohistochemical expression and proliferative activity, but there are no significant differences in vascularization. SCLC histology and Ki67>8% may represent negative prognostic factors after surgery for LC with BM.*

***Key words:*** *brain metastases, immunohistochemistry, lung cancer*

## Introduction

Lung cancer (LC) is the most common cause of cancer death worldwide, which places it in the group of the so-called 'socially significant diseases'. The survival rate varies widely depending on its histological type and stage. One of the main problems is the lack of sufficiently effective methods for early detection, and often treatment is ineffective at advanced stages [1].

LC often metastasizes in the brain [2]. Primary LC is found in approximately 28-68% of patients

*Corresponding author:* Dora Marinova Marinova, MD, PhD. Department of Pneumonology, "St. Sofia" University Hospital for Pulmonary Diseases, 19 Acad. Ivan Geshov blvd, Clinical Center for Lung Diseases, Medical University of Sofia, Sofia, Bulgaria. Tel: +359 888027907, Email: dmusic@abv.bg;
Received: 30/03/2018; Accepted: 17/05/2018

with solitary metastasis in the brain [2-4]. It is estimated that about 40% of LC patients develop brain metastases (BM) during their illness [5]. Brain metastasis is the primary manifestation of LC in 10% of the cases [6]. About 30-35% of BM are of pulmonary origin [7]. Most commonly, brain metastases are due to LC, followed by breast cancer, melanoma, renal cell carcinoma and colorectal cancer [8].

The prognosis of patients with BM from LC is poor. Progressive neurological deterioration with an average survival of 1-2 months is observed without treatment [9]. The median survival of patients undergoing surgical treatment for BM from LC is 4-7.7 months [3]. Therefore, the study of BM from LC is important for a more complete understanding of the LC biology [10].

Immunohistochemical (IHC) methods are extremely effective imaging methods commonly used in the diagnosis of lung and pleural tumors. They are used in cases difficult to diagnose. Through these methods, the exact histological type of the carcinoma and the grade of its differentiation is established [11]. In addition, IHC methods are suitable for the study of LC biology, including cell proliferation.

There is no systematic IHC study on the biology and morphological features of the metastatic tumors in the brain from primary LC. Ki-67 proliferative index and the number of intratumor microvessels had not been determined. The importance of the problem gives us reason to do a parallel retrospective study of the primary LC and its metastasis in the brain as this would help understand the biology of these tumors.

The purpose of our work was to study the IHC expression of TTF-1, p63, Cytokeratin 7, Ki-67 and CD31 in BM and their respective primary NSCLC to determine and compare both their proliferative activity and the number of intratumor microvessels and to define cell clones with high metastatic potential.

## Methods

*Compliance with ethical standards*

The authors declare that the experiments comply with the current laws of Bulgaria in which they were performed. The study obtained ethical approval from the Ethics Committee for Scientific Research of the Medical University, Sofia.

*Selection of biological material for the study*

Thirty patients operated for BM and primary lung cancer were selected - 13 ACs and 17 SCCs. The main characteristics of the patients included in the study are presented in Table 1. After performing IHC studies, the diagnosis changed in 14 cases (47%) and was confirmed in 16 cases (53%). The SCC diagnosis was confirmed in 5 out of 17 cases (30%); in 8 of them, it was found to be AC and in 4 small cell lung cancer (SCLC). In morphologically diagnosed AC there was no change in diagnosis after performing IHC. For the diagnosis and differentiation of tumors, a panel of the following 4 antibodies was used: Ki-67, TTF-1, p63, and Cytokeratin 7. Usually, AC is Ki-67 <50%/TTF-1+/p63-/Cytokeratin 7+; SCC is Ki-67 <50%/TTF-1-/p63 +/Cytokeratin 7-; SCLC is Ki-67 >50%/TTF-1-/p63-/Cytokeratin 7-.

*Immunohistochemical analysis*

In the present study, a DAKO EnVision TM FLEX visualization system (code no. K8024) was used. The IHC study was performed at the Department of Pathology, "St. Sofia" University Hospital for Pulmonary Diseases, according to the manufacturer's protocol with optimized conditions. Deparaffinization, rehydration and antigen recovery were performed with PT Link (Dako, Code No. PT10126) according to the manufacturer's protocol. The following monoclonal murine antibodies from DAKO were used: Ki-67 (MIB-1), TTF-1 (8G7G3/1), p63 (DAK-p63), Cytokeratin 7 (OV-TL 12/30) and CD31 (JC70A).

The reporting of IHC reactions was performed by 2 independent pathologists. A Carl Zeiss microscope was used. Quantitative measurements were made at x20 magnification for lens and x10 for an eyepiece. The pro-

**Table 1.** Characteristics of patients included in the study

| *Characteristics* | *Total N=30, n (%)* | *ADC N=21, n (%)* | *SCC N=5, n (%)* | *SCLC N=4, n (%)* |
|---|---|---|---|---|
| Changed diagnosis | 14 (43) | 10 (48) | 0 | 4 (100) |
| Males, gender | 25 (83) | 17 (81) | 4 (80) | 4 (100) |
| Age, years, mean (±SD) | 55 (±9) | 52 (±8) | 61 (±4) | 65 (±10) |
| Lung cancer surgery - first | 24 (80) | 16 (76) | 5 (100) | 3 (75) |
| Dead | 27 (90) | 18 (86) | 5 (100) | 4 (100) |
| Differentiation, low | 13 (46) | 8 (42) | 1 (20) | 4 (100) |
| N0 status | 12 (40) | 8 (38) | 3 (60) | 1 (25) |
| N1 status | 4 (13) | 2 (9.5) | 0 | 2 (50) |
| N2 status | 12 (40) | 9 (43) | 2 (40) | 1 (25) |
| N3 status | 2 (7) | 2 (9.5) | 0 | 0 |
| Surgery (pneumonectomy) | 16 (53) | 11 (52) | 3 (60) | 2 (50) |

ADC: Adenocarcinoma, SCC: Squamous-Cell carcinoma, SCLC: Small Cell Lung carcinoma

tocol of the study was approved by the ethics committee for Scientific Research of the Medical University Sofia and is part of the Grant 360/2015, Contract no. 76/2015.

*Statistics*

The data were analyzed using SPSS software, version 13.0 for Windows. To determine the distribution in the groups we used the Shapiro-Wilk test. When the distribution was normal we used the Student's t-test for the comparison of two means. The Mann-Whitney U test was used for non-normal distribution. Comparisons between different histological groups and between primary and metastatic tumors were calculated using Wilcoxon test. Means and standard deviations were calculated. For survival analysis we used Kaplan-Meier survival method and log-rank test to compare differences between groups. 1-year and 5-year survival rate were also calculated. Cox regression analysis was performed to search for prognostic factors. In all tests, two-sided p values less than 0.05 were considered statistically significant.

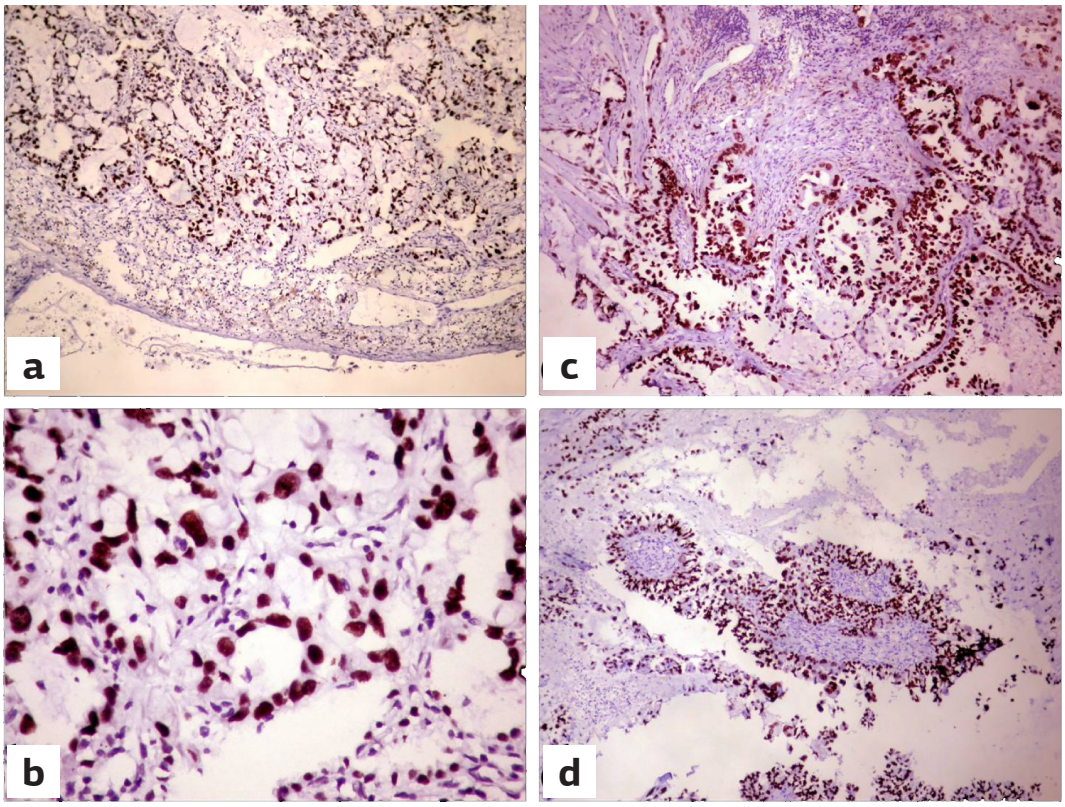
## Results

*Histological subtypes - Analysis of differences between primary lung tumor and metastasis*

Regarding AC as the primary tumor, tumors with predominant acinar cell component were the most common - 10 out of 19 (53%), in 7 out of 19 (37%) tumors the solid carcinoma component predominated, one of the tumors was mainly papillary and one had a predominantly crystalline construction. Tumors consisting of 2 components (n=16;84%) predominated, but there were also 3 components (n=3;16%). Tumors composed of 2 components were mostly acinar-papillary (5 out of 16;31%) or solid-papillary (4 out of 16;25%).

As for brain metastases, tumors with predominantly papillary cellular component were the most prevalent - 9 out of 19 tumors (47%), in 5 out of 19 (26%) the solid carcinoma component was predominant, in 4 out of 19 (21%) the acinar component was predominant, and one of the tumors had a predominantly cribriform pattern. Tumors consisting of 2 components (n=10;52%) predominated, but there were also tumors with 1 component (n=5;26%) and 3 components (n=4;21%). Tumors composed of 2 components were mostly solid papillary (5/10;50%) or papillary-acinar (3/10;30%). Those made up of one component were 4 papillary and 1 cribriform pattern.

Nine out of the 19 ACs examined had a changed structure in metastatic form (47%). When comparing the histological patterns of the primary site with its corresponding metastasis it was found that out of the 10 primary predominantly acinar ACs, 7




**Picture 1.** A case of an acinar-papillary adenocarcinoma. Pair of lung and corresponding brain metastasis. Immunohistochemical staining with TTF-1 antibody. **a)** the primary (lung) tumor; x10; **b)** the primary (lung) tumor; x1000; **c)** brain metastasis; x10; **d)** brain metastasis, x10.

(70%) were predominantly papillary in metastatic form, indicating that the papillary component metastasized more frequently; of the 7 primary predominantly solid ACs, 2 (28%) had a modified structure in metastatic form - 1 papillary and 1 acinar-papillary. There were no structural differences between primary and metastatic carcinoma as far as predominantly papillary and cribriform ACs were concerned.

In 3 out of 5 SCC there was a keratinization in single tumor cells, both in the primary site and metastasis.

*Expression of immunohistochemical markers*

*TTF-1 (Picture 1)*

We used the TTF-1 marker to accurately diagnose lung AC.

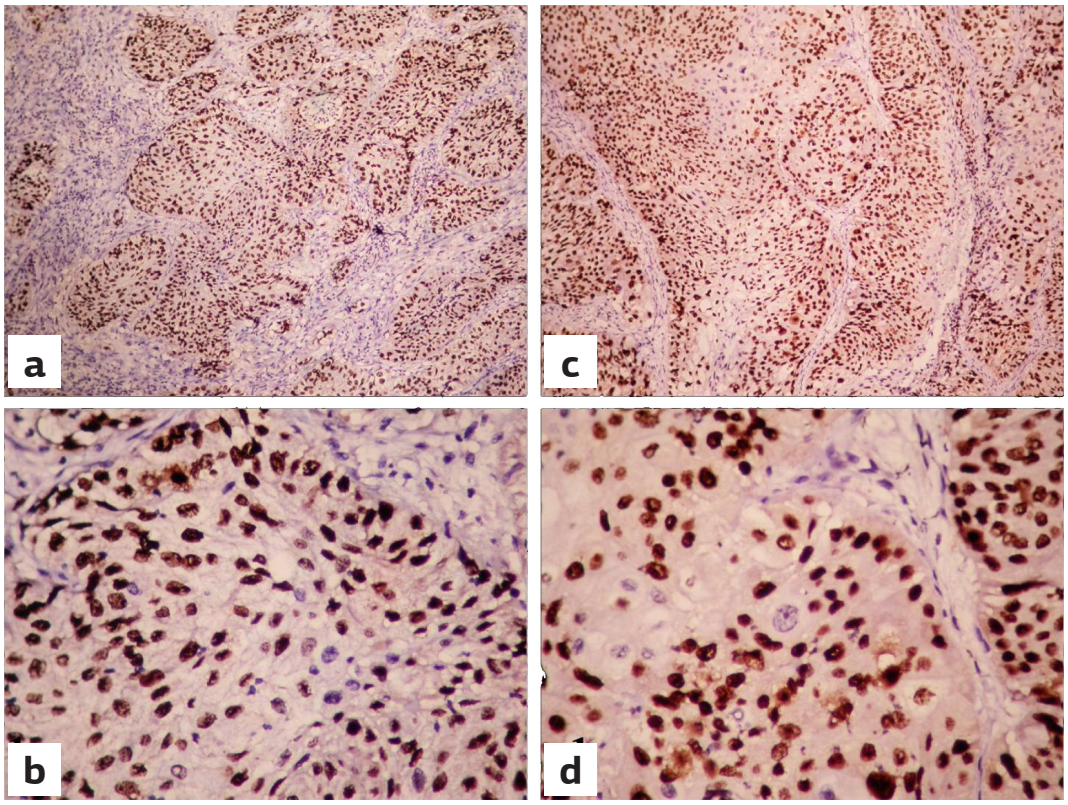
In LC, AC TTF-1 was expressed in 20 (95.2%) cases, and only 1 (4.8%) did not express the marker - solid with high mucus production (the diagnosis in this case was established with positive expression of Cytokeratin 7 and Haematoxylin/Eosin staining). Regarding the expression of TTF-1 from BM, 5 of them lacked expression of the marker, one case lacked the expression in LC and 4 other cases the expression in the primary carcinoma - 23.8% in total. Unlike AC, none of the SCC expressed TTF-1. In SCLC, TTF-1 was expressed in only one case (out of a total of 4;25%) with a low intensity in 85% in primary tumor and metastatic cells.

The mean levels of TTF-1 expression in the lung (45.44±28.98) and brain (73.88±24.85) in AC (n=16), were significantly different (Wilcoxon, p=0.011).

TTF-1 was expressed in a greater number of cases of lung AC, but with lower mean expression levels, whereas the corresponding BM marker was not often expressed, but when present it had higher mean values.

*P63 (Picture 2)*

In 2 (9.5%) primary AC, the expression of p63 was observed in single cells but not in metastases, and in both cases the expression of TTF-1 and Cytokeratin 7 was also observed. On the other hand, in 3 brain metastases p63 was expressed in single cells while no expression was present in the primary tumor. In contrast to AC, p63 was expressed in all cases of SCC (100%). SCLC did not express p63 in the primary tumor, but in 2 (50%) metastases the expression occurred in 10% of the tumor cells with low intensity in one case and in 50% of the tumor cells with high intensity in the other. When comparing the mean expression levels of p63 in the lung (80.60±25.97) and the brain (81.60±30.75) in




**Picture 2.** A case of a squamous-cell carcinoma. Pair of lung and corresponding brain metastasis. Immunohistochemical staining with p63 antibody. **a)** the primary (lung) tumor; x10; **b)** the primary (lung) tumor; x1000; **c)** brain metastasis; x10; **d)** brain metastasis, x1000.

SCC (n=5), it was found that the difference was not significant (Wilcoxon, p=0.686).

P63 was expressed at a high percentage of tumor cells in all SCCs, with no difference in the expression level between the primary tumor and metastasis.

*Cytokeratin 7*

This marker was equally expressed in 100% of AC tumor cells (n=21), both in the primary tumor and in the corresponding metastasis. Only in 1 case of low-differentiated AC the expression was moderate in 75% of the tumor cells.

In one (20%) SCC it was 100% expressed with moderate intensity, both in primary and metastatic cancer. This case expressed p63 but not TTF-1.

In SCLC it was expressed in 2 (50%) cases in some tumor cells, both in the primary tumor and metastasis.

Comparison of the mean expression levels of Cytokeratin 7 in the lung (98.57±6.55) and brain (98.81±5.46) in AC (n=21) revealed that the difference was not significant (Wilcoxon, p=0.317).

Cytokeratin 7 was expressed in all ACs with the same strength regardless of the location - primary or metastatic. It was rarely expressed in SCC and slightly more often in SCLC.

*Ki-67 proliferative index (Tables 2 and 3, Figure 1, Picture 3)*

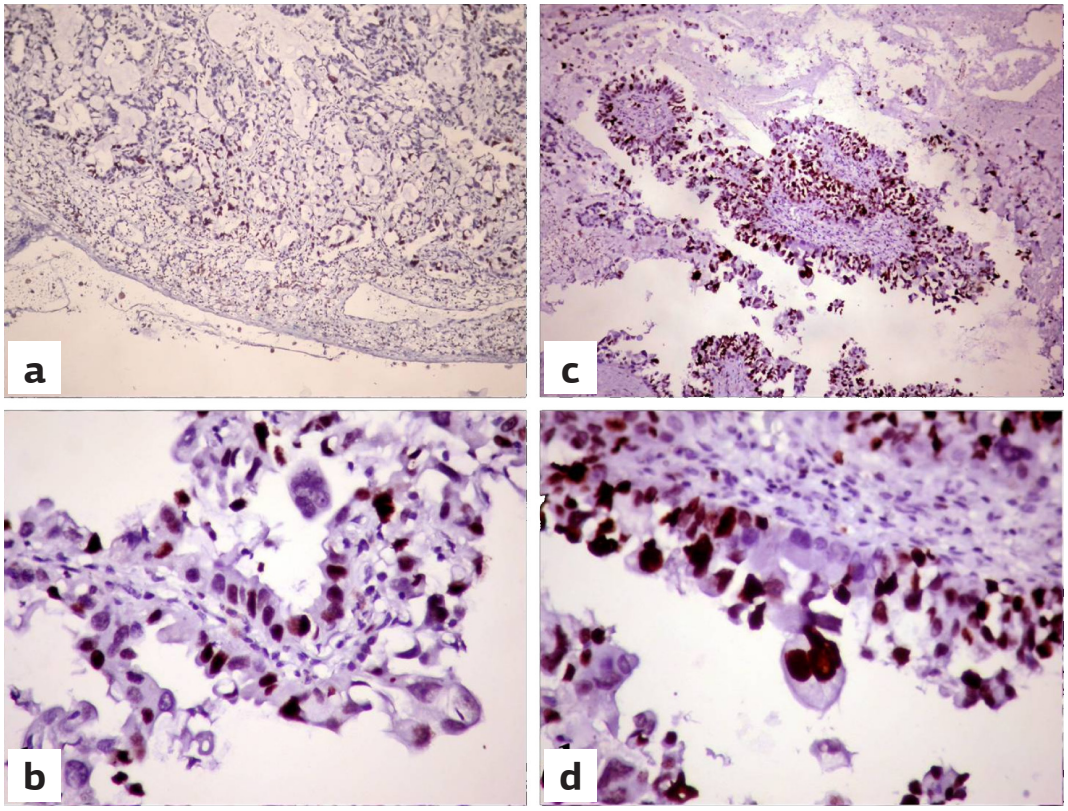
*Comparison of Ki-67 expression*

To determine the distribution of the different histological groups in primary tumors we used the Shapiro-Wil test. The Mann-Whitney U test was used for non-normal distribution. The distribution was normal only in the SCC and SCLC groups, so the comparison between these two groups was done with the Student's t-test for the comparison of two means.

The levels of proliferative activity in SCLC were significantly higher than those in AC (p=0.008). There was no significant difference in Ki-67 expression between AC and SCC, as well as between SCC and SCLC (probably because of the small number of patients in the study) (Table 3).

- Between the different histological groups in metastases: In the studied groups the distribution was normal, therefore a Student's t-test was used to compare two independent samples.

The levels of proliferative activity in SCLC were significantly higher than those in AC (p<0.001) and SCC (p=0.008) groups. No significant difference was found in Ki-67 expression between AC and SCC groups.




**Picture 3.** A case of an acinar-papillary adenocarcinoma. Pair of lung and corresponding brain metastasis. Immunohistochemical staining with Ki-67 antibody showing cell proliferative activity. **a)** the primary (lung) tumor; x10; **b)** the primary (lung) tumor; x1000; **c)** brain metastasis; x10; **d)** brain metastasis, x1000.

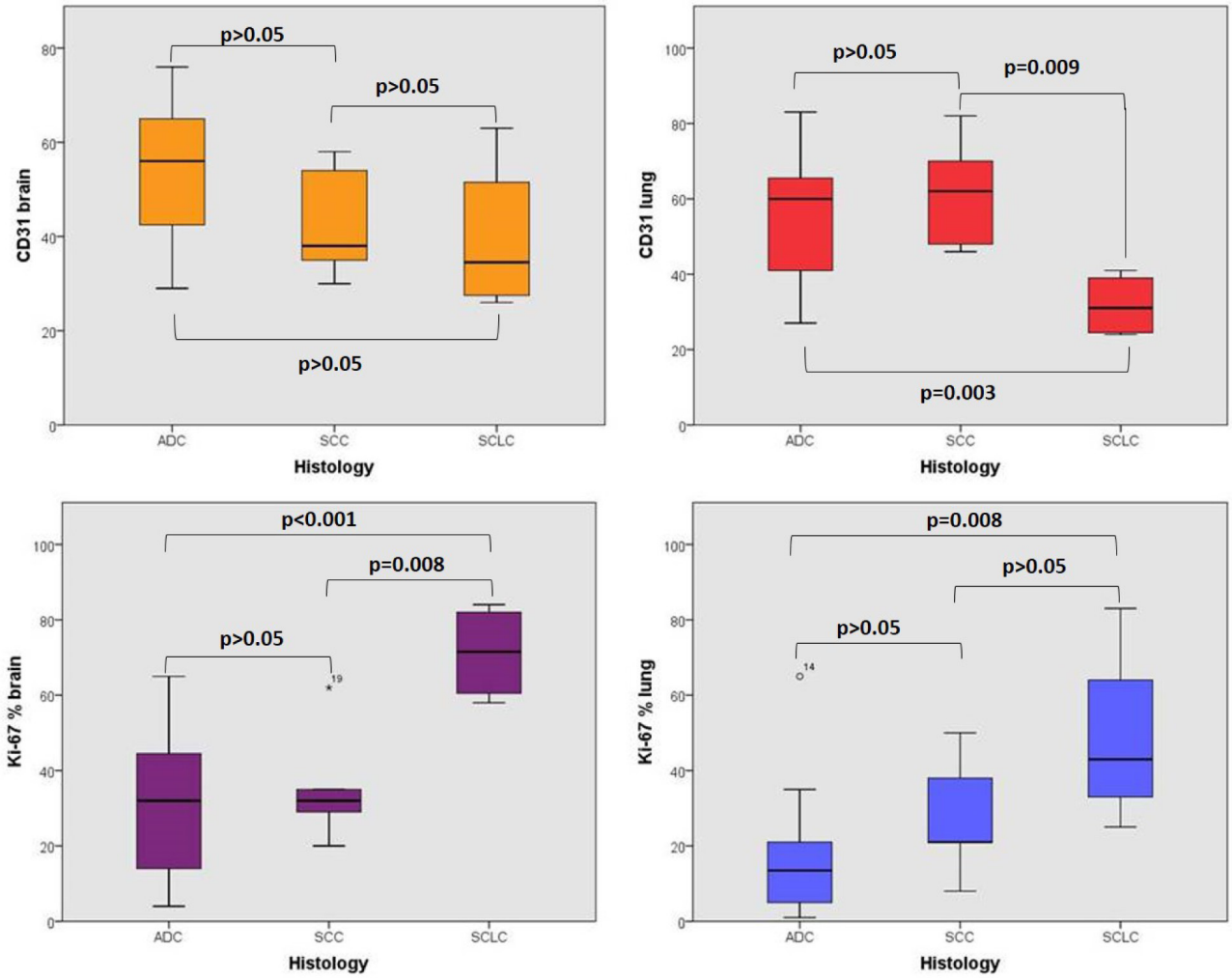



**Figure 1.** NITMV and Ki-67 expression in different histological groups in lung cancer and metastases.

**Table 2.** Ki-67 expression in the different histological groups of primary tumors and metastases

| *Groups* | *Ki-67 lung* | *p value* | *Ki-67 brain mean±SD* | *p value* |
|---|---|---|---|---|
| ADC, mean rank, n=20 | 11.95* | M-W U=28; | 30.14±17.72 | 0.535 |
| SCC, mean rank, n=4 | 17.20* | 0.153 | 35.60±15.79 | |
| ADC, mean rank, n=20 | 10.80* | M-W U=6; | 30.14±17.72 | 0.000 |
| SCLC, mean rank, n=4 | 21.00* | 0.008 | 71.25±12.69 | |
| SCC, n=4** | 27.60±16.44 | 0.169 | 35.60±15.79 | 0.008 |
| SCLC, n=4** | 48.50±24.57 | | 71.25±12.69 | |

* Mann-Whitney (M-W) U test with a mean rank. ** mean±SD

**Table 3.** Ki-67 expression between pairs of primary tumor-metastasis in different histological groups

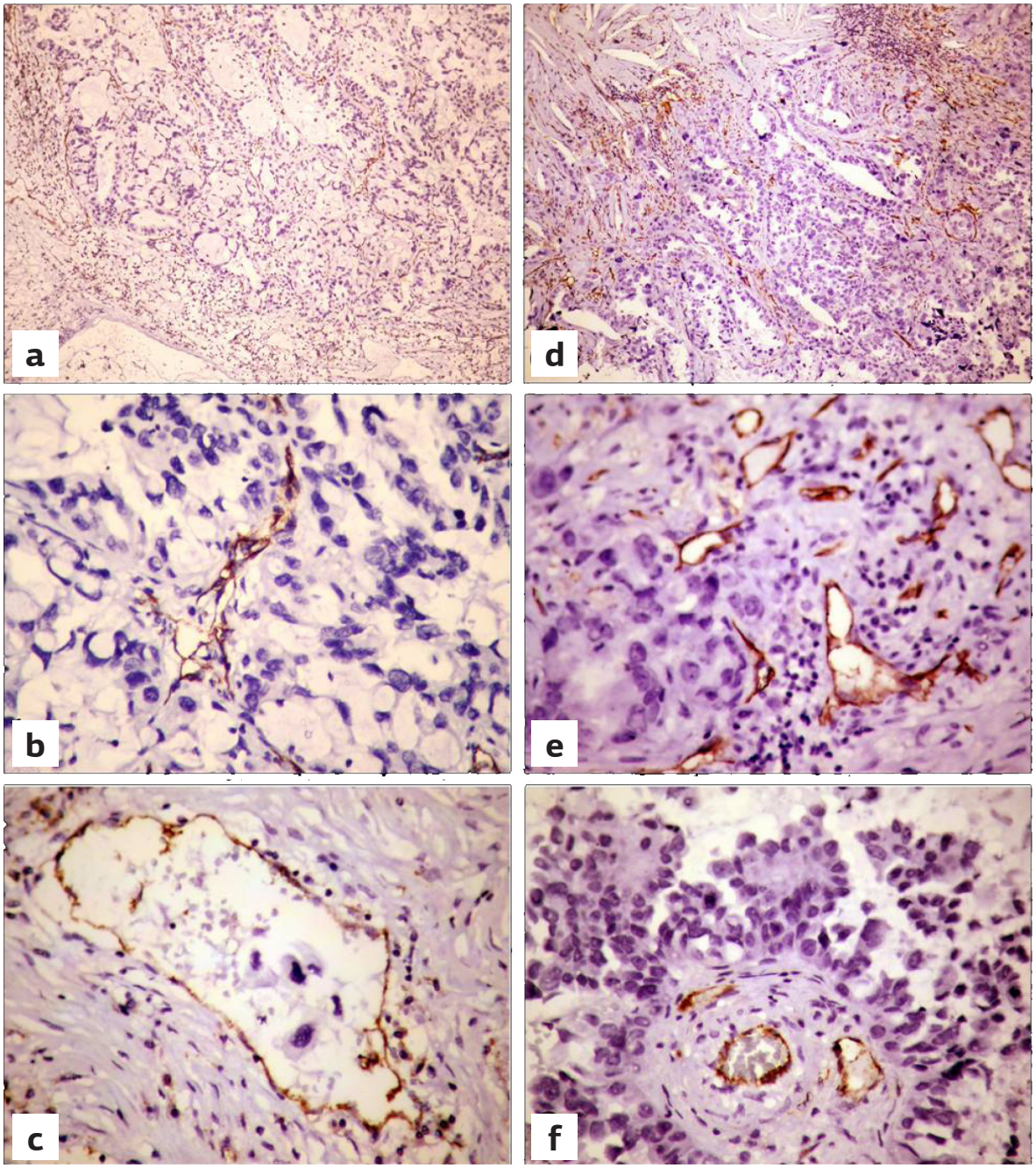
| | *Groups* | *mean±SD* | *p value* |
|---|---|---|---|
| ADC | Ki-67 lung | 17.25±15.04 | 0.003* |
| n=21 | Ki-67 brain | 30.14±17.72 | |
| SCC | Ki-67 lung | 27.60±16.44 | 0.048 |
| n=5 | Ki-67 brain | 35.60±15.79 | |
| SCLC | Ki-67 lung | 48.50±24.57 | 0.141 |
| n=4 | Ki-67 brain | 71.25±12.69 | |

* Wilcoxon test

**Table 4.** Comparison in NITMV between different histological groups in primary tumors and metastases

| *Groups* | *CD31 lung, mean±SD* | *p value* | *CD31 brain, mean±SD* | *p value* |
|---|---|---|---|---|
| ADC | 55.68±15.65 | 0.467 | 54.15 ±14.69 | 0.125 |
| SCC | 61.60±15.13 | | 43.00±12.28 | |
| ADC | 55.68±15.65 | 0.003 | 54.15 ±14.69 | 0.180 |
| SCLC | 31.75±8.54 | | 39.50±16.78 | |
| SCC | 61.60±15.13 | 0.009 | 43.00±12.28 | 0.740 |
| SCLC | 31.75±8.54 | | 39.50±16.78 | |

For abbreviations see text




**Picture 4.** A case of an acinar-papillary adenocarcinoma. Pair of lung and corresponding brain metastasis. Immunohistochemical staining with CD31 antibody showing intratumoral microvessels. **a)** the primary (lung) tumor; x10; **b)** the primary (lung) tumor; x1000; **c)** the primary (lung) tumor; x1000; **d)** brain metastasis; x10; **e)** brain metastasis, x1000; **f)** brain metastasis, x1000.

• Between pairs of primary tumor-metastasis in different histological groups - two dependent samples: The proliferative activity levels for Ki-67 were significantly higher in metastases than in the primary AC tumor (p=0.003) and SCC (p=0.048). In SCLC, the difference did not reach significance, probably because of the small number of cases.

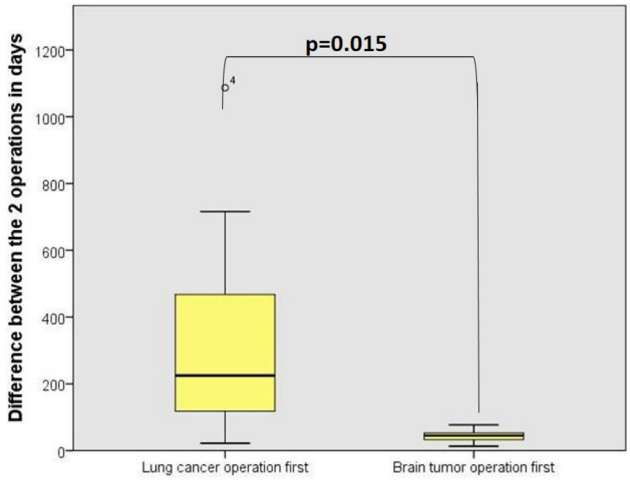



**Figure 2.** Median patient survival after lung surgery and brain metastasis surgery.

*CD31 - Comparison in the number of intratumoral microvessels (NITMV) (Table 4, Figure 1, Picture 4)*

• Between the different histological groups in primary tumors – the distribution determined by the Shapiro-Wilk test was normal in all groups, so comparison of the groups was done with Student's t-test for the comparison of two means.

The number of microvessels in SCLC was significantly lower compared to that in AC (p=0.003), and SCC (p=0.009). No significant difference was found in the number of microvessels between ACs and SCCs.

• Between different histological groups in metastases:

In the studied groups, the distribution was normal, so Student's t-test was used to compare two independent variables. No significant difference was found in the mean number of tumor microvessels between different histological groups in metastases.

• Between pairs of primary tumor-metastasis in the different histological groups - two paired samples: There was no difference between the number of microvessels in the primary site and metastasis in AC. There were more tumor microvessels in the

**Table 5.** Comparison in NITMV between pairs of primary tumor-metastasis in different histological groups

| | *Groups* | *mean±SD* | *p value* |
|---|---|---|---|
| ADC | CD31 lung | 55.68±15.65 | 0.710 |
| n=19 | CD31 brain | 54.15 ±14.69 | |
| SCC | CD31 lung | 61.60±15.13 | 0.092 |
| n=4 | CD31 brain | 43.00±12.28 | |
| SCLC | CD31 lung | 31.75±8.54 | 0.439 |
| n=4 | CD31 brain | 39.50±16.78 | |

**Table 6a.** Survival after lung surgery according to histological type

| *Histology* | *Median* | | *95% confidence interval* | |
|---|---|---|---|---|
| | *Estimate* | *Standard error* | *Lower bound* | *Upper bound* |
| ADC | 712 | 169.441 | 379.896 | 1044.104 |
| SCC | 417 | 132.549 | 157.204 | 676.796 |
| SCLC | 150 | 43.274 | 65.182 | 234.818 |
| Total | 554 | 133.227 | 292.876 | 815.124 |

**Table 6b.** Survival after lung surgery according to histological type

| *Pairwise comparisons* | *Histology* | *ADC* | | *SCC* | | *SCLC* | |
|---|---|---|---|---|---|---|---|
| | | *Chi-square* | *Sig.* | *Chi-square* | *Sig.* | *Chi-square* | *Sig.* |
| Log rank | ADC | | | 1.308 | 0.253 | 5.684 | 0.017 |
| (Mantel-Cox) | SCC | 1.308 | 0.253 | | | 0.566 | 0.452 |
| | SCLC | 5.684 | 0.017 | 0.566 | 0.452 | | |

primary site, but the difference did not reach statistical significance, probably because of the small number of cases. There were more microvessels in the SCLC metastasis, but the difference did not reach statistical significance.

*Survival analysis*

With regard to patient survival, information was received for 28 of them from the National Cancer Registry. Only 3 (10.7%) patients were still alive. In 23 patients (82.1%), a primary LC surgery was firstly performed and after an average of 308 ± 271 days a BM operation was performed. In 5 (17.9%) patients BM surgery preceded LC surgery with a mean of 44±23 days. The observed time difference between the two operations, depending on whether the primary LC or metastasis was first operated, was significant (Mann-Whitney U test, p=0.015; Figure 2).

Survival analysis did not include 3 patients who died within 1 month after surgery - so-called 'early postoperative mortality', so overall survival was calculated on 25 subjects.

- Survival after LC operation: 1-year survival was 68% and 5-year survival 12% (median 554 ± 801 days).
- Survival after BM operation: 1-year survival was 36% regardless of histological type, and 5-year survival was 12% - it did not differ significantly from survival after LC operation with a median survival of 269 ± 809 days.
- 1-year survival after LC operation was higher and median survival was longer compared to survivals after BM operation.

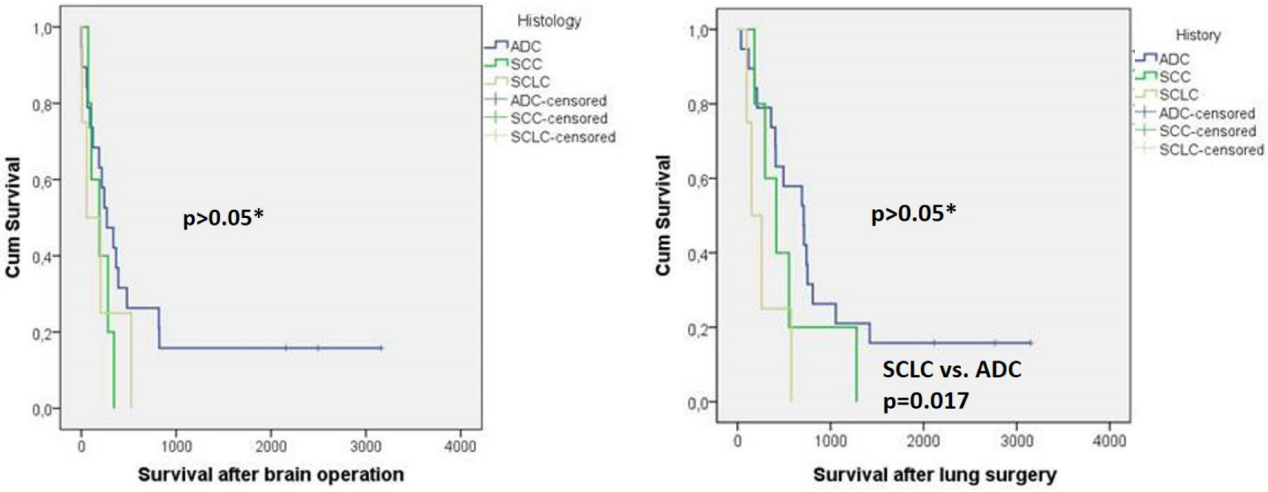



**Figure 3.** Kaplan-Meier cumulative patient survival according to histology. Cum survival: cumulative survival; ADC: adenocarcinoma; SCC: squamous cell carcinoma; SCLC: small cell lung carcinoma.

**Table 7a.** Survival after brain surgery according to histological type

| *Histology* | *Median* | | *95% confidence interval* | |
|---|---|---|---|---|
| | *Estimate* | *Standard error* | *Lower bound* | *Upper bound* |
| ADC | 339 | 85.749 | 170.931 | 507.069 |
| SCC | 192 | 93.113 | 9.499 | 374.501 |
| SCLC | 203 | 118.392 | .000 | 435.048 |
| Overall | 269 | 54.123 | 162.918 | 375.082 |

**Table 7b.** Survival after brain surgery according to histological type

| *Pairwise comparisons* | *Histology* | *ADC* | | *SCC* | | *SCLC* | |
|---|---|---|---|---|---|---|---|
| | | *Chi-square* | *Sig.* | *Chi-square* | *Sig.* | *Chi-square* | *Sig.* |
| Log rank | ADC | | | 2.856 | 0.091 | 0.849 | 0.357 |
| (Mantel-Cox) | SCC | 2.856 | 0.091 | | | 0.326 | .568 |
| | SCLC | 0.849 | 0.357 | 0.326 | 0.568 | | |

• Survival related to histological type: depending on the histological type of the tumor, the survival after LC operation was significantly longer in AC compared to SCLC group (p=0.017) (Table 6a and 6b, Figure 3). After BM operation, there was a tendency for reduced survival in SCC compared to AC group (p=0.09) (Table 7a and 7b, Figure 3).

• Survival related to Ki-67, TTF-1, CD31 and histology: Cox regression analysis found that only the histological type of the tumor and the Ki-67 proliferative index were significant survival determinants after LC operation. Negative prognostic factors were SCLC histological type (p=0.048, HR 2.28, 95% CI 1.01-5.18) and Ki-67 >18% (p=0.020; HR 1.96, 95% CI 1.11-3.47). Such factors were not established with respect to survival after BM operation.

## Discussion

According to our data, in almost half of the cases the diagnosis was changed based on IHC markers, which indicates the importance of the IHC study. To diagnose and distinguish tumors, a panel of the following 4 antibodies was sufficient: Ki-67, TTF-1, p63 and Cytokeratin 7.

When comparing the histological structure of the primary tumor and metastasis, we found that in half of the ACs there was a difference, as the pappilary component metastasizes most often. Papillary AC, and especially its micropapillary variant, are the most aggressive subtypes of lung AC. They are poorly differentiated (G3) and often at the time of diagnosis, the disease is already at an advanced stage with a poor prognosis for the patient [12]. Studies by other authors have shown that micropapillary and solid variants of lung AC occur more often in BM biopsies, even if they are poorly represented in the primary site [13,14]. Clay et al. found a significant percentage difference in histological structure between BM from lung AC and the primary site [15]. The prevalence of the more aggressive papillary variant of lung AC is consistent with other outcomes of this study - such as a higher Ki-67 proliferative index in BM biopsies.

With regard to the examined markers, the following conclusions were reached:

• TTF-1 was expressed in a greater number of cases of lung AC, but with lower mean expression levels, whereas the corresponding BM marker was not often expressed, but when it was present it had significantly higher mean values.

• P63 was expressed in a high percentage of tumor cells in all SCCs, with no difference in the level of expression between the primary tumor and the metastasis.

• Cytokeratin 7 was expressed in all ACs with the same strength regardless of the site - primary or metastatic. It was rarely expressed in SCC and slightly more often than in SCLC.

It was found that the Ki-67 proliferative activity of carcinoma cells in the metastatic site was higher than that in the primary site. Gomez-Roca et al. also found differences in Ki-67 expression between the primary site and BM - in 31% of patients with NSCLC, and in some of them the expression of the marker in the primary site was almost zero [16]. The higher proliferative activity of carcinoma cells in the metastatic site as opposed to the primary site is probably due to epigenetic factors leading to overexpression of the marker. Based on evidence that increased Ki-67 expression in NSCLC is a risk factor for BM development, we could hypothesize that it promotes a rapid growth of metastasis in size.

CD31 was induced for establishing vascular invasion in primary lung cancer. No significant differences were found between the primary tumor and the metastasis.

The onset and development of BM is a complex multi-stage process whereby tumor cells, after reaching the brain, interact with the cells in the surrounding brain tissue. In this process they both interact with each other. This may result in differences in the expression of some biomarkers from metastatic tumor cells which could differ from those in the primary site. The differences found in our study in histology and IHC markers between the primary site and BM were also observed by other authors. Recent studies favor the hypothesis that there are differences in morphology, biomarker expression, and genotype between the primary tumor and BM in lung AC that are not well studied. Chirieac et al. found differences in mutations in ABLI, BRAAF, EGFR, FGFR3, HRAS, KIT, KRAS, MET, N-ras, PDGFRA, Pi3K and RET genes between BM and the primary site - in 39.1% of the studied patients mutations were found only in the primary site, whereas 27.9% of patients had them only in BM [17]. Gomez-Roca et al. reported differences in Ki-67 expression, vascular endothelial growth factor receptor (VEGFR), epithelial growth factor receptor (EGFR), and the protein responsible for DNA repair (ERCC1) between the primary site and BM in 30% of the studied patients with NSCLC. In their view, those differences raise the issue of the need to biopsy both the primary site and BM prior to making a decision on chemotherapy [16].

We examined the patient survival and found that 1-year survival after lung cancer surgery was higher and median survival was longer than after

brain surgery. They are consistent with data from other studies – such as Saad et al. who indicated an average time of 12.5 months [18]. In cases where BM surgery precedes LC surgery, this period is shortened due to the active search for the primary site. This lower survival observed after BM surgery is logically associated with the presented results, which shows the predominance of the more aggressive AC variant (papillary) and a higher Ki-67 expression.

According to the histological type of the tumor, survival after lung surgery was significantly longer in AC compared to SCLC. There was a tendency for reduced survival in SCC compared to AC after brain surgery.

Negative prognostic factors were found – SCLC histology and Ki-67 >18%. Such factors have not been established for survival after BM surgery. The prognostic role of Ki-67 that we have established is consistent with the results of other authors. Frank et al. showed that tumors with high Ki-67 proliferative activity are more aggressive [19]. SCLC is usually at a very advanced stage at the time of diagnosis [20]. Consistent with these data, our results showed low survival after lung surgery.

## Conclusions

The use of diagnostic markers reveals how tumor cells change their immune phenotype in metastases. This is one of the few studies presenting relevant data, thus contributing to a better understanding of the role of angiogenesis, the metastatic process and the biology of lung carcinomas.

## Acknowledgements

This study was supported by Grant 360/2015, Contract no. 76/2015 funded by Medical University, Sofia.

The results of this study were presented at a thematic poster session at the Milano 2017 ERS International Congress, European Respiratory Journal Sep 2017, 50 (Suppl 61) PA4230; DOI: 10.1183/1393003.congress-2017.PA4230 and at the ELCC, Geneva 2017, Annals of Oncology (2017) 28 (suppl_2): ii52-ii55. 10.1093/annonc/mdx094

## Conflict of interests

The authors declare no conflict of interests.

## References

1. Boyle P, Levin B. WHO/IARC World Cancer Report 2008, Lyon: IACR International Agency for Cancer Research (2008).
2. Merchut MP. Brain metastases from undiagnosed systemic neoplasms. Arch Intern Med 1989;149:1076-3.
3. Winston KR, Walsh JW, Fisher EG. Results of operative treatment of intracranial metastatic tumors. Cancer 1980;45:2639-45.
4. Veith RG, Odom GL. Intracranial metastases and their neurosurgical treatment. J Neurosurg 1965;23:375-82.
5. Rizzi A, Tondini M, Rocco G et al. Lung cancer with a single brain metastasis: therapeutic options. Tumori 1990;76:579-99.
6. Nugent JL, Bunn PA Jr, Matthews MJ et al. CNS metastases in small cell bronchogenic carcinoma. Increasing frequency and changing pattern with lengthening survival. Cancer 1979;44:1885-93.
7. Wronski M, Arbit E, Burt M, Galicich JH. Survival after surgical treatment of brain metastases from lung cancer: a follow-up study of 231 patients between 1976 and 1991. J Neurosurg 1995;83:605-16.
8. Wen PY, Loeffler JS. Management of brain metastases. Oncology 1999; 13:941-57.
9. Posner JB. Neurological complications of sytemic cancer. Med Clin North Am 1971;55:625-46.
10. Chi A, Komaki R. Treatment of brain metastasis from lung cancer. Cancers (Basel) 2010;2:2100-37.
11. Brambilla E, Lantuejoul S. Pathology and immunohistochemistry of lung cancer. Thoracic malignancies. Eur Respir Mon 2009;44:15-35.
12. Bekiesinska-Figatowska M, Kuczyinska-Zardzewiaty A, Klepacka T et al. Miliary brain metastases from papillary adenocarcinoma of the lung – unusual MRI pattern with histopathologic correlation. Pol J Radiol 2013; 78:57-60.
13. Russell PA, Barnett SA, Walkiewicz M et al. Correlation of mutation status and survival with predominant histologic subtype according to the new IASLC/ATS/ERS lung adenocarcinoma classification in stage III (N2) patients. J Thorac Oncol 2013;8:461-8.
14. Sica G, Yoshizawa A, Sima CS et al. A grading system of lung adenocarcinomas based on histologic pattern is predictive of disease recurrence in stage I tumors. Am J Surg Pathol 2010;34:1155-62.
15. Clay TD, Do H, Sundararajan V et al. The Clinical Relevance of Pathologic Subtypes in Metastatic Lung Adenocarcinoma. J Thorac Oncol 2014;9:654-63.
16. Gomez-Roca C, Raynaud CM, Penault-Llorka F et al. Differential Expression of Biomarkers in Primary Non-Small Cell Lung Cancer and Metastatic Sites. J Thorac Oncol 2009;4:1212-20.

17. Chirieac LR. Biology of Lung Cancer Metastases. In: Cagle PT, Allen TC, Beasley MB et al. (Eds): Molecular Pathology of Lung Cancer. Springer Science & Business Media, New York, 2012, pp 201-9.
18. Saad AG, Yeap BY, Thuninssen FBJM et al. Immunohistochemical Markers Associated with Brain Metastases in Patients with Non-Small Cell Lung Carcinoma. Cancer 2008;113:2129-38.
19. Frank I, Cheville J, Blute M. Prognostic value of p53 and MIB-1 in transitional cell carcinoma of the urinary bladder with regional lymph node involvement. Cancer 2004;101:1803-8.
20. Travis WD. Update on small cell carcinoma and its differentiation from squamous cell carcinoma and other non-small cell carcinomas. Mod Pathol 2012;25:S18-S30.