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Influence of biological markers on overall survival in surgically treated patients with non-small cell lung cancer

Uticaj bioloških markera na ukupno preživljavanje operisanih bolesnika sa nesitnoćelijskim karcinomom pluća

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Abstract

Background/Aim. Non-small cell lung cancer (NSCLC) is one of the most common malignant tumors and a leading cause of cancer-related deaths. The aim of this study was to assess the impact of biological markers on the overall survival rate in surgically treated NSCLC patients who received adjuvant chemotherapy and/or radiation therapy. **Methods.** This retrospective case series study included patients with NSCLC treated in the period between 2008 and 2017 at the Pulmonology Clinic and the Clinic for Chest Surgery, Military Medical Academy, Belgrade, Serbia. The survival analysis performed was based on immunohistological findings, histology type, and tumor, node, metastasis (TNM) stages. **Results.** The mortality rate was higher in the adenocarcinoma patient group compared to the squamous cell carcinoma group, albeit without statistical significance (58.3% vs. 31.2%, respectively; $p = 0.175$). Overall survival was shorter in the adenocarcinoma patient group compared to the squamous cell carcinoma group by approximately 750 days. Likewise, overall survival was shorter in the adenocarcinoma patient group compared to the squamous cell carcinoma group for CD31 positive ($p = 0.029$), p-63 positive ($p = 0.049$), MMP-9 positive ($p = 0.032$), and matrix metalloproteinase (MMP)-2 positive patients ($p = 0.016$). **Conclusion.** Adenocarcinoma is a more aggressive cancer type compared to squamous cell carcinoma with shorter overall survival. Our research showed a poorer overall survival in the adenocarcinoma group of patients compared to the squamous cell carcinoma group in CD31, p-63, MMP-9, and MMP-2 positive patients.

Key words:
biomarkers; carcinoma, non-small-cell lung; immunohistochemistry; survival; neoplasm staging; surgical procedures, operative.

Apstrakt

Uvod/Cilj. Nesitnoćelijski karcinom pluća (NSĆKP) je jedan od najčešćih malignih tumora i vodeći je uzrok smrti povezane sa karcinomima. Cilj ove studije bio je da se analizira uticaj bioloških markera na stopu ukupnog preživljavanja kod bolesnika sa NSĆKP koji su posle operacije dobijali adjuvantnu hemioterapiju i/ili radioterapiju. **Metode.** Sprovedena je retrospektivna studija tipa serije slučajeva na Klinici za pulmologiju i Klinici za grudnu hirurgiju Vojnomedicinske akademije u Beogradu, Srbija. Bolesnici sa NSĆKP lečeni su tokom perioda od deset godina (2008–2017). Analiza preživljavanja je bila zasnovana na imunohistohemijskim nalazima, patohistološkom tipu i tumor, nodus, metastaze (TNM) stadijumu. **Rezultati.** Stopa mortaliteta bila je viša u grupi bolesnika sa adenokarcinomom u poređenju sa grupom bolesnika sa skvamocelularnim karcinomom pluća, ali razlika nije bila statistički značajna (58,3%, odnosno 31,2%; $p = 0,175$). Ukupno preživljavanje bilo je kraće kod bolesnika sa adenokarcinomom u odnosu na bolesnike sa skvamocelularnim karcinomom za oko 750 dana. Ukupno preživljavanje je bilo kraće kod bolesnika sa adenokarcinomom u poređenju sa preživljavanjem bolesnika sa skvamocelularnim karcinomom kod CD31 pozitivnih ($p = 0,029$), p-63 pozitivnih ($p = 0,049$), metaloproteinaze matriksa (MMP)-9 pozitivnih ($p = 0,032$) i MMP-2 pozitivnih bolesnika ($p = 0,016$). **Zaključak.** Adenokarcinom pluća je značajno agresivniji karcinom u poređenju sa skvamocelularnim karcinomom pluća i oboleli imaju kraće vreme ukupnog preživljavanja.

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Ukupno preživljavanje je bilo kraće kod bolesnika sa adenokarcinomom u poređenju sa bolesnicima sa skvamocelularnim karcinomom kod CD31, p-63, MMP-9 i MMP-2 pozitivnih bolesnika.

Ključne reči:
biomarkeri; pluća, nesitnoćelijski karcinom; imunohistohemija; preživljavanje; neoplazme, određivanje stadijuma; hirurgija, operativne procedure.

Introduction

Lung cancer is one of the most common malignant tumors and a leading cause of cancer-related deaths [1–3]. About 80% of all lung cancers are non-small cell lung cancer (NSCLC), ie. squamous cell carcinoma and adenocarcinoma [4, 5].

The NSCLC significantly decrease overall survival, life quality, and working ability of patients, but increase direct and indirect medical cost [6]. Treatment options for patients with NSCLC consist of combined surgical treatment, radiation therapy, and/or one of the chemotherapy treatment protocols based on the stage of illness, histology type and tumor marker findings, and other parameters [6].

Surgery represents a treatment of choice for patients with NSCLC stage I-IIIA according to the tumor, node, metastasis (TNM) 8th edition classification [7, 8]. In addition to surgery, patients with resected NSCLC stage II-IIIA, who have a high risk of relapse, are treated with adjuvant chemotherapy and/or radiation therapy [6, 9]. Patients with stage IIIB and IV NSCLC are generally treated with chemotherapy and radiation therapy. For NSCLC stages I and II, radiation therapy alone is considered effective only when surgical resection is not possible either due to limited pulmonary reserve or the presence of comorbidities [10].

Histology and immunohistochemistry (IHC) analyses, as well as specific gene expression assessment may become a predictive factor for response to chemotherapy in future clinical research and patient treatment [11]. Expression of biomarkers [for example, human epidermal growth factor receptor (HER)-2, B-cell lymphoma (BCL)-2, cluster of differentiation 31(CD31), p-63, v-raf murine sarcoma viral oncogene homolog B1 (BRAF), Kirsten rat sarcoma 2 viral oncogene homolog (KRAS), etc.] can be tested at a protein level using IHC, while messenger ribonucleic acids (mRNA) levels can be determined through reverse transcriptase-polymerase chain reaction (RT-PCR)-based assays. Therefore, these biomarkers are currently not in use in daily practice. Many biomarkers in lung cancer were point mutations and rearrangements in specific genes including epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase, HER-2, BCL-2, CD-31, p-63, matrix metalloproteinases (MMPs), BRAF, NUT, MET, ROS1, DDR2, fibroblast growth factor receptor 1 (FGFR1), KRAS, and phosphatase and tensin homolog (PTEN) [11]. These biomarkers might potentially provide additional information for clinical decision-making.

The overall five-year survival rate for all lung cancer in stage with localized disease is about 52.2%, in stage with the regional metastatic disease 25%, and in stage with distant metastatic disease 4% [12].

The aim of this study was to assess the impact of biological markers on the overall survival rate in surgically treated NSCLC patients, both after the adjuvant chemotherapy and/or radiation therapy.

Methods

Patients' data

This retrospective case series study of patients with NSCLC was designed as survival analysis based on immunohistological findings, histology type, and TNM stages. Forty NSCLC patients (17 females and 23 males; average age 59.22 ± 8.31 years) were treated at the Pulmonology Clinic and Chest Surgery Clinic of the Military Medical Academy in Belgrade, Serbia and were followed up over the period between 2008 and 2017.

Clinical files from all patients with clinically confirmed lung cancer admitted between 2010 and 2015 to the institutional healthcare network of the Military Medical Academy were accessed in both hard and electronic copies from the hospital registries. The following data were analyzed: demographic characteristics (age, gender), overall survival rate, immunohistological findings, histology type, and TNM stages of NSCLC.

TNM stage and patients treatment

The first step done in our hospital was to classify patients with NSCLC according to the TNM stages. T1N0M0 was classified as stage IA; T2N0M0 as stage IB; T1N1M0 as stage IIA; T2N1M0 and T3N0M0 as stage IIB; and T3N1M0, T1N2M0, T2N2M0, T3N2M0 as stage IIIA. Stage IIIB was classified as T4 any N M0 and any T N3M0, whereas stage IV was classified as any T any N M1 [13].

The second step consisted of treating patients with NSCLC according to their TNM stages. TNM stage I patients were only treated surgically. TNM stage IIA to IIIA patients were treated surgically and with adjuvant chemotherapy (etoposide and cisplatin – EP/PE protocol) and/or radiation therapy.

The above chemotherapy protocol was applied as follows: cisplatin at 60 mg/m^2 intravenous (*iv*) administered on day 1, plus etoposide at 120 mg/m^2 *iv* administered on days 1, 2, and 3, every 21 days for 4 cycles. Alternatively, cisplatin at 80 mg/m^2 *iv* was administered on day 1, plus etoposide at 100 mg/m^2 *iv* on days 1, 2, and 3, every 28 days for 4 cycles.

Radiotherapy was applied in patients with positive resection surface for malignancy and with N2 TNM stage [6].

Histology and IHC

Following tumor excision, collected tissue was formalin-fixed and paraffin-embedded (FFPE) as described below. Tissue slides were morphologically diagnosed at the Institute for Pathology and Forensic Medicine of the Military Medical Academy in Belgrade, Serbia and subsequently tested for a series of biomarkers using IHC standard protocol developed at the Laboratory for Immunohistochemistry and Electron Microscopy of the Institute for Medical Research of the Military Medical Academy in Belgrade, Serbia.

Excised tissue was fixed in 5% neutral-buffered formalin and processed in VIP Sakura apparatus for automatic fixation, dehydration, and paraffin embedding. Tissue blocks were cut at 5–7 μm and sections mounted on separate adherent chips (Super-Frost) and then dried at 56 °C for one h prior to staining.

Antibodies for immunostaining were applied according to manufacturers' recommendations. The following primary antibodies were used for IHC: HER-2, BCL-2, CD31, p-63, MMP-2, MMP-9, and MMP-14.

Statistical analyses

Continuous variables were presented as median with interquartile range (IQR). Categorical variables were reported as frequencies. Differences between categorical variables were tested by the χ^2 test, while the significance of the difference between continuous variables was tested by the non-parametric Mann-Whitney *U* test. Overall survival estimates were calculated using the Kaplan-Meier method [mean with 95% confidence interval (CI)] and log-rank (Mantel-Cox) test to assess differences between NSCLC groups. A *p*-value of less than 0.05 was considered statistically significant.

Ethics Committee approval

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was reviewed and approved on September 6, 2015, by the Ethics Committee of the Military Medical Academy.

Results

We analyzed 40 patients with NSCLC (16 patients with squamous cell carcinoma and 24 with adenocarcinoma). Females were frequently in the adenocarcinoma group (13 or 54.2% out of 24 patients), while males were frequently in the squamous cell carcinoma group (12 or 75.0% out of 16 patients) (χ^2 test; $p = 0.133$). Differences according to age were not shown between groups [median age 61.04 (IQR 52.90-65.64) in patients with squamous cell carcinoma vs. median age 58.57 (IQR 54.16-66.09) in patients with adenocarcinoma; Mann-Whitney test: $p = 0.924$].

Overall survival for all patients followed according to biomarker type was not statistically different (log-rank (Mantel-Cox) test: $p > 0.05$) (Table 1).

The mortality rate was higher in the group of patients with adenocarcinoma compared to the squamous cell carcinoma patient group (58.3% or 14 out of 24 patients, and 31.2% or 5 out of 16 patients, respectively). However, this difference was not significant (χ^2 test: $p = 0.175$).

Overall survival of patients according to histology type of NSCLC (adenocarcinoma vs. squamous cell carcinoma) was not statistically different (log-rank (Mantel-Cox) test: $p = 0.057$) (Figure 1). However, cumulative survival was lower by approximately 750 days in the patient group with adenocarcinoma in comparison to the group with squamous cell carcinoma (estimated mean of 817.0 (561.2–1,072.7) vs. 1,566.4 (1,149.4–1,983.4) days respectively with 95% CI).

Overall survival of patients with adenocarcinoma and squamous cell carcinoma according to the interval from surgery to recurrence was presented in Table 2. A statistically significant difference was not observed between the groups. Cumulative survival was lower in the patient recurrence group with adenocarcinoma in comparison to the group of patients with squamous cell carcinoma by approximately 810 days (Figure 2).

Table 1

Overall survival in all patients with lung cancer according to biological markers tested (censored – alive at the end of the follow-up period)

Marker tested	Total number	Number of death events	Censored n (%)	Survival (days) – estimated mean (95% CI)	*p*-value*
HER-2 negative	32	14	18 (55.2)	1262.8 (929.7–1596.0)	0.316
HER-2 positive	8	5	3 (37.5)	655.0 (354.2–955.8)	
BCL-2 negative	1	1	-	540.0 (540.0–540.0)	0.447
BCL-2 positive	39	18	21 (53.8)	1230.9 (928.8–1533.1)	
CD31 negative	5	2	3 (60.0)	766.8 (506.1–1027.5)	0.728
CD31 positive	35	17	18 (51.4)	1194.4 (881.4–1507.4)	
p-63 negative	2	1	1 (50.0)	756.5 (456.4–1056.5)	0.875
p-63 positive	38	18	20 (52.6)	1206.6 (901.6–1511.5)	
MMP-9 negative	7	4	3 (42.9)	720.6 (533.6–907.5)	0.829
MMP-9 positive	33	15	8 (54.5)	1240.9 (911.8–1570.0)	
MMP-2 negative	19	7	12 (63.2)	1113.6 (817.0–1410.2)	0.303
MMP-2 positive	21	12	9 (42.9)	1017.7 (625.4–1410.0)	
MMP-14 negative	33	15	18 (54.5)	1243.7 (919.4–1568.0)	0.594
MMP-14 positive	7	4	3 (42.9)	663.3 (329.6–923.3)	

HER – human epidermal growth factor receptor; BCL – B-cell lymphoma; CD – cluster of differentiation; MMP – matrix metalloproteinase; CI – confidence interval.
***log-rank (Mantel-Cox) test.**

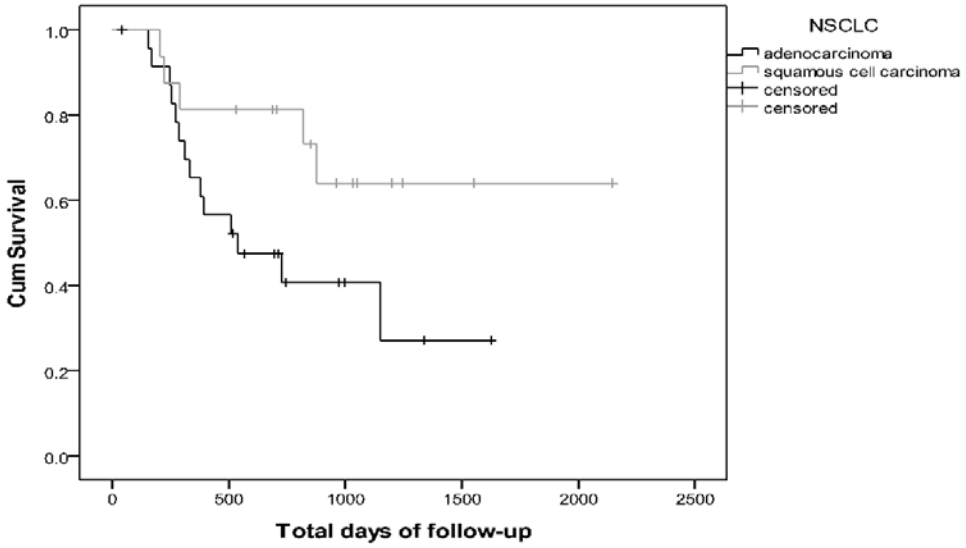

Fig. 1 – Kaplan-Meier analysis – survival curves of patients according to histology type of non-small cell lung cancer (NSCLC) (censored – alive at the end of the follow-up period).

Table 2

Distribution of overall survival of patients with non-small cell lung cancer (NSCLC) according to the interval from surgery/surgical resection to recurrence

Type of NSCLC	Recurrence	Total number	Number (%) of death events	Censored n (%)	Survival (days) – estimated mean (95% CI)	*p*-value*
Adenocarcinoma	Yes	17	11 (64.7)	6 (35.3)	803.4 (521.7–1,085.1)	0.940
	No	7	3 (42.9)	4 (57.1)	616.7 (328.4–904.9)	
Squamous cell carcinoma	Yes	6	2 (32.3)	4 (66.7)	1,615.8 (995.1–2,236.3)	0.814
	No	10	3 (30.0)	7 (70.0)	949.0 (707.0–1,191.0)	

CI – confidence interval.

***Log-rank (Mantel-Cox) test; yes adenocarcinoma/ yes squamous cell carcinoma log-rank (Mantel-Cox) test: $p = 0.147$; no adenocarcinoma/ no squamous cell carcinoma log-rank (Mantel-Cox) test: $p = 0.316$.**

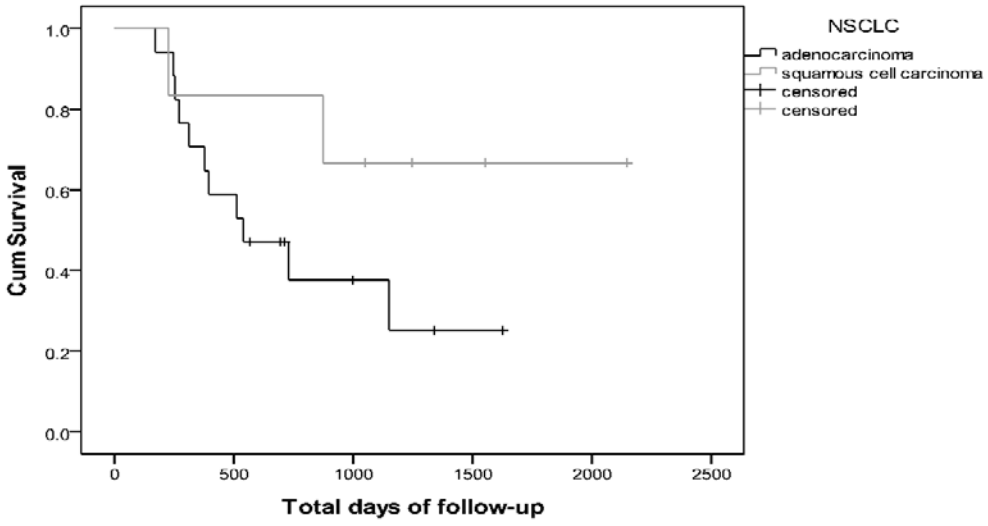

Fig. 2 – Kaplan-Meier analysis – survival curves in patients with recurrence according to histology type of non-small cell lung cancer (NSCLC) (censored – alive at the end of the follow-up period).

Table 3

Distribution of overall survival in patients with non-small cell lung cancer (NSCLC) according to clinical initial tumor, node, metastasis (TNM) stage

Type of NSCLC	TNM stage	Total number	Number (%) of death events	Censored n (%)	Survival (days) – estimated mean (95% CI)	*p*-value*
Adenocarcinoma	IA,IB	6	3 (50.0)	3 (50.0)	1101.5 (708.8–1494.2)	
	IIA,IIB	12	6 (50.0)	6 (50.0)	810.4 (508.9–1112.0)	0.060
	IIIA	6	5 (83.3)	1 (16.7)	374.4 (186.6–562.2)	
Squamous cell carcinoma	IA,IB	-	-	-	-	
	IIA,IIB	9	3 (33.3)	6 (66.7)	971.0 (702.1–1239.9)	0.970
	IIIA	7	2 (28.6)	5 (71.4)	1586.4 (325.6–947.9)	

CI – confidence interval.
***Log-Rank (Mantel-Cox) test; IIA, IIB adenocarcinoma/ IIA, IIB squamous cell carcinoma log-rank (Mantel-Cox) test: $p = 0.380$; IIIA adenocarcinoma/ IIIA squamous cell carcinoma log-rank (Mantel-Cox) test: $p = 0.007$;**

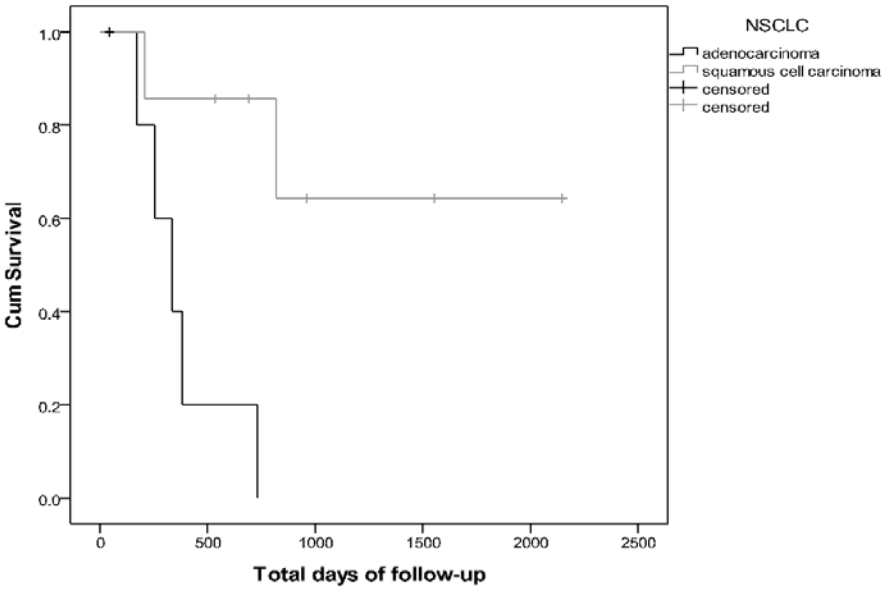

Fig. 3 – Kaplan-Meier analysis – survival curves in patients with non-small cell lung cancer (NSCLC) in IIIA tumor, node, metastasis (TNM) stage according to histology type (censored – alive at the end of the follow-up period).

Overall survival was estimated and compared among patients according to preoperative TNM stage in both patient groups (Table 3). There was no statistically significant difference in survival between patients in stages I, II, and IIIA within the adenocarcinoma ($p = 0.060$) and the squamous cell carcinoma group ($p = 0.970$). However, overall survival between patients with adenocarcinoma and those with squamous cell carcinoma according to the initial TNM stage showed that patients with adenocarcinoma had a statistically significantly lower survival rate compared to the patients with squamous cell carcinoma in TNM stage IIIA [log-rank (Mantel-Cox) test: $p = 0.007$; mean 374.4 days vs. 1,586.4 days, respectively] (Figure 3).

No significant differences were observed between adenocarcinoma and squamous cell carcinoma by the distribution of patients according to biological markers (Table 4). The patients were most frequently positive for BCL-2, CD31, p-63, MMP-9, and MMP-2, but rarely for HER-2 and MMP-14.

Overall survival was estimated and compared among NSCLC patients according to the status of biological markers in two patient groups studied (Table 4). There was a statistically significant difference in survival between patients with positive and negative biological markers regardless of NSCLC type. Cumulative survival was lower in the adenocarcinoma patient group compared to the squamous cell carcinoma group for CD31 positive (log-rank (Mantel-Cox) test: $p = 0.029$) (Figure 4), p-63 positive ($p = 0.049$) (Figure 5), MMP-9 positive ($p = 0.032$) (Figure 6) and MMP-2 positive patients ($p = 0.016$) (Figure 7).

Table 4

Overall survival in the patients with non-small cell lung cancer (NSCLC) according to biological markers (censored – alive at the end of the follow-up period)

Type of NSCLC	Marker[1] tested	Total, n (%)	*p*-value#	Number (%) of death events	Censored n (%)	Survival (days) – estimated mean (CI 95%)	*p*-value*
Adenocarcinoma	HER-2 negative	18 (75.0)	0.572	9 (50.0)	9 (50.0)	922.8 (621.3–1224.2)	0.103
	HER-2 positive	6 (25.0)		5 (83.3)	1 (16.7)	398.0 (241.9–554.1)	
Squamous cell carcinoma	HER-2 negative	14 (87.5)		5 (35.7)	9 (64.3)	All censored patients in HER-2 positive group	0.307
	HER-2 positive	2 (12.5)		-	2 (100.0)		
Adenocarcinoma	BCL-2 negative	1 (4.2)		1 (100.0)	-	540.0 (540.0–540.0)	0.735
	BCL-2 positive	23 (95.8)	1.000	13 (56.5)	10 (43.5)	833.0 (566.8–1099.2)	
Squamous cell carcinoma	BCL-2 negative	-		-	-	-	-
	BCL-2 positive	16 (100.0)		5 (31.2)	11 (68.8)	1566.4 (1149.4–1983.4)	
Adenocarcinoma	CD31 negative	3 (12.5)		1 (33.3)	2 (66.7)	847.3 (601.4–1093.2)	0.339
	CD31 positive	21 (87.5)	1.000	13 (61.9)	8 (38.1)	772.1 (504.6–1039.5)	
Squamous cell carcinoma	CD31 negative	2 (12.5)		1 (50.0)	1 (50.0)	572.5 (182.4–962.6)	0.451
	CD31 positive	14 (87.5)		4 (28.6)	10 (71.4)	1624.5 (1197.6–2051.5)	
Adenocarcinoma	p-63 negative	2 (8.3)		1 (50.0)	1 (50.0)	756.5 (456.4–1056.5)	0.618
	p-63 positive	22 (91.7)	0.657	13 (59.1)	9 (40.9)	799.4 (531.8–1067.0)	
Squamous cell carcinoma	p-63 negative	-		-	-	-	-
	p-63 positive	16 (100.0)		5 (31.2)	11 (68.8)	1566.4 (1149.4–1983.4)	
Adenocarcinoma	MMP-9 negative	4 (16.7)		2 (50.0)	2 (50.0)	749.7 (530.8–968.7)	0.531
	MMP-9 positive	20 (83.3)	1.000#	12 (60.0)	8 (40.0)	785.7 (505.5–1065.9)	
Squamous cell carcinoma	MMP-9 negative	3 (18.8)		2 (66.7)	1 (33.3)	681.7 (239.6–1123.7)	0.215
	MMP-9 positive	13 (81.2)		3 (23.1)	10 (76.9)	1710.4 (1278.8–2141.9)	
Adenocarcinoma	MMP-2 negative	9 (37.5)		3 (33.3)	6 (66.7)	1152.2 (725.0–1580.0)	0.141
	MMP-2 positive	15 (62.5)	0.219#	11 (73.3)	4 (26.7)	609.7 (384.7–834.7)	
Squamous cell carcinoma	MMP-2 negative	10 (62.5)		4 (40.0)	6 (60.0)	1041.7 (659.5–1423.9)	0.276
	MMP-2 positive	6 (37.5)		1 (16.7)	5 (83.3)	1882.4 (1416.8–2348.0)	
Adenocarcinoma	MMP-14 negative	18 (75.0)		10 (55.6)	8 (44.4)	852.6 (554.6–1150.6)	0.721
	MMP-14 positive	6 (25.0)	0.269#	4 (66.7)	2 (33.3)	572.2 (329.6–814.7)	
Squamous cell carcinoma	MMP-14 negative	15 (93.8)		5 (33.3)	10 (66.7)	All censored patients in MMP-14 positive group	0.494
	MMP-14 positive	1 (6.2)		-	1 (100.0)		

[1]For explanation see under Table 1.

 # – χ^2 test; * – log-rank (Mantel-Cox) test; HER-2 positive adenocarcinoma/ HER-2 positive squamous cell carcinoma log-rank (Mantel-Cox) test: $p = 0.092$; HER-2 negative adenocarcinoma/ HER-2 negative squamous cell carcinoma log-rank (Mantel-Cox) test: $p = 0.330$; BCL-2 positive adenocarcinoma/ BCL-2 positive squamous cell carcinoma log-rank (Mantel-Cox) test: $p = 0.072$; CD31 positive adenocarcinoma/ CD31 positive squamous cell carcinoma log-rank (Mantel-Cox) test: $p = 0.029$; p-63 positive adenocarcinoma/ p-63 positive squamous cell carcinoma log-rank (Mantel-Cox) test $p = 0.049$; MMP-9 positive adenocarcinoma/ MMP-9 positive squamous cell carcinoma log-rank (Mantel-Cox) test $p = 0.032$; MMP-9 negative adenocarcinoma/ MMP-9 negative squamous cell carcinoma log-rank (Mantel-Cox) test: $p = 0.730$; MMP-2 positive adenocarcinoma/ MMP-2 positive squamous cell carcinoma log-rank (Mantel-Cox) test: $p = 0.016$; MMP-2 negative adenocarcinoma/ MMP-2 negative squamous cell carcinoma log-rank (Mantel-Cox) test: $p = 0.837$; MMP-14 positive adenocarcinoma/ MMP-14 positive squamous cell carcinoma log-rank (Mantel-Cox) test: $p = 0.330$; MMP-14 negative adenocarcinoma/ MMP-14 negative squamous cell carcinoma log-rank (Mantel-Cox) test: $p = 0.130$.

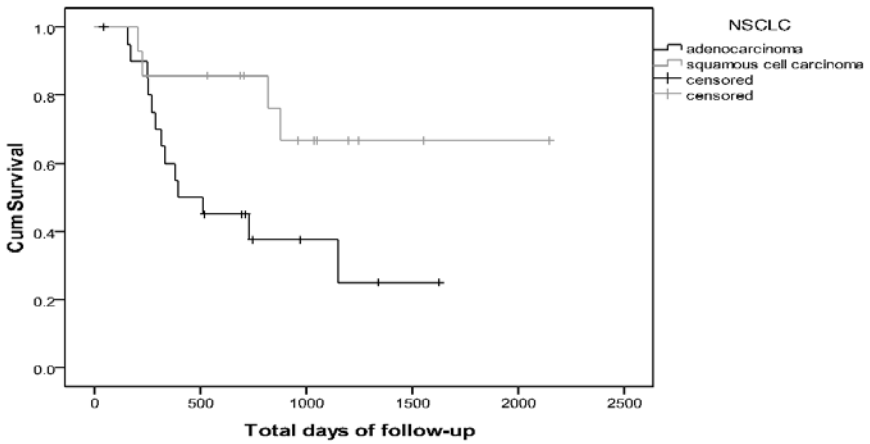

Fig. 4 – Kaplan-Meier analysis – survival curves in positive CD31 patients with non-small cell lung cancer (NSCLC) according to histology type (censored – alive at the end of the follow-up period).
CD – cluster of differentiation.

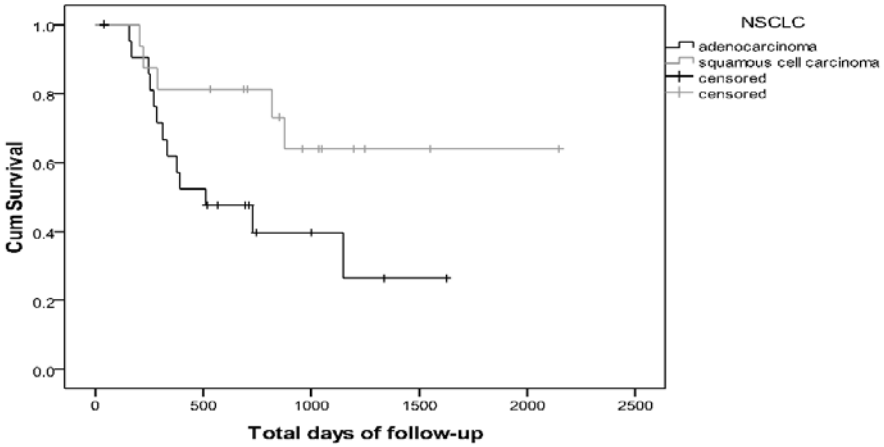

Fig. 5 – Kaplan-Meier analysis – survival curves in positive p-63 patients with non-small cell lung cancer (NSCLC) according to histology type (censored – alive at the end of the follow-up period).

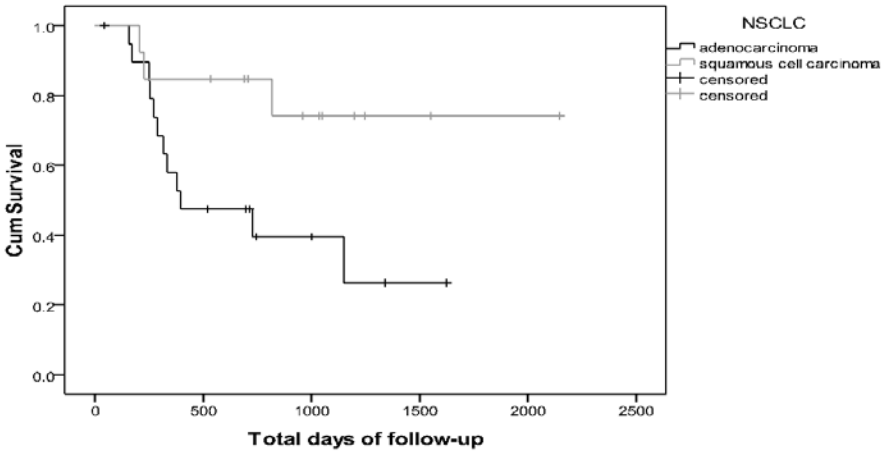

Fig. 6 – Kaplan-Meier analysis – survival curves in positive MMP-9 patients with non-small cell lung cancer NSCLC) according to histology type (censored – alive at the end of the follow-up period).
MMP – matrix metalloproteinase.

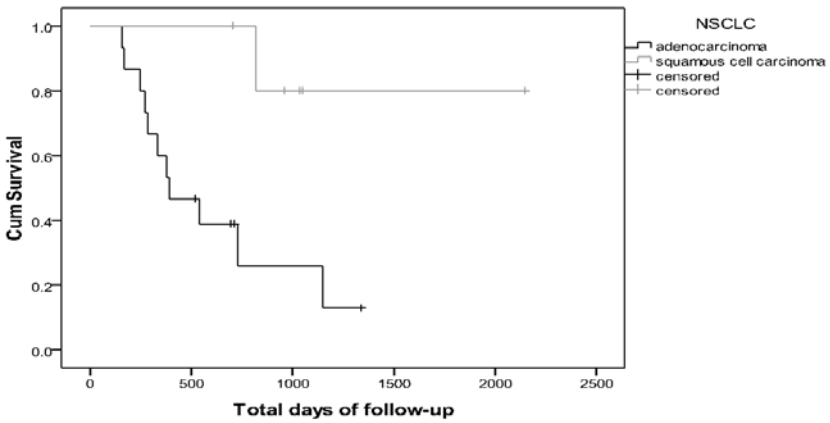

Fig. 7 – Kaplan-Meier analysis – survival curves in positive MMP-2 patients with non-small cell lung cancer (NSCLC) according to histology type (censored – alive at the end of the follow-up period). MMP – matrix metalloproteinase.

Discussion

NSCLC cancer is one of the major causes of cancer-related deaths [2]. To analyze survival in our NSCLC patients, we conducted a retrospective case-series study using the follow-up data from a period between 2008 and 2017. Our study design aimed at assessing the overall survival in NSCLC patients according to specific biomarkers expression, TNM stage, and histology type [14].

In the operable NSCLC patients, adjuvant chemotherapy has been considered a standard modality of treatment following surgical resection of the tumor [14–19]. Besides, molecularly targeted therapy has significantly improved outcomes of patients with a metastatic form of NSCLC [2, 18]. Nevertheless, for the majority of patients, platinum-based chemotherapy remains the gold standard treatment and has led to significantly improved survival outcomes with approximately 10–11 months median survival [20].

In our study, there were more male patients in the squamous cell carcinoma group, while female patients dominated the adenocarcinoma group. Men develop tracheal, bronchus, and lung cancer more often compared to women (1/18 for men; 1/45 for women) [3]. The estimated number of lung cancer cases worldwide has increased by 44% in men and 76% in women since 1985 [21]. The higher rate increase in women has been attributed to the fact that cigarette smoking in the female population peaked two decades later than in the male [21]. Our squamous cell carcinoma patients were not significantly older compared to the adenocarcinoma group (median age 61.04 vs. 58.57, respectively). Comparably, in another study, squamous cell carcinoma patients were slightly older when compared to adenocarcinoma patients (median age 69 vs. 65, respectively) [22, 23].

Patients with adenocarcinoma had a poorer prognosis compared to squamous cell carcinoma patients [22]. Similarly, in our study, the mortality rate was significantly higher in the adenocarcinoma group (58.3%) as opposed to the squamous cell carcinoma group (31.2%). According to the literature, generally, the five-year survival rate for all NSCLC patients in stage IA, IB, IIA, and IIB is about 49%, 45%, 30%, and 31%, respectively [24]. This rate for NSCLC patients in stage IIIA and IIIB is about 14% and 5%, respectively [24].

Overall survival of patients according to recurrence is of major significance [23]. Recurrence rates reported following surgical cancer resection range from 30% to 75% [25]. The majority of recurrent tumors are distant, and more than 80% of recurrences occur within the first two years after resection. Cumulative survival was lower in our patient recurrence group with adenocarcinoma compared to the squamous cell carcinoma group by about 810 days. This is in line with our findings showing adenocarcinoma as a more aggressive cancer type than squamous cell carcinoma.

The complete resection of early-stage NSCLC offers to patients high hopes for a successful therapeutic outcome. However, the recurrence rates postresection remain high [26]. For that reason, right from the beginning of therapy in NSCLC patients, a complete surgical removal needs to be ensured both macroscopically and microscopically. Often, occult micro-metastatic cancer cells, already present systemically at the time of surgery, remain undetected by standard staging methods suggesting an underestimation of the true tumor stage. Moreover, dissemination of cancer cells might occur during the handling of the tumor during surgery [26].

Overall survival according to TNM stages was observed among patients with adenocarcinoma, as well as those with squamous cell carcinoma. Statistically significant difference was not observed between our patients with adenocarcinoma and patients with squamous cell carcinoma in TNM stage groups IA, IB, IIA, and IIB, but this difference was shown between the groups in the IIIA stage. Overall survival was lower between stage IIIA adenocarcinoma patients compared to squamous cell carcinoma patients of the same stage (mean 374.4 days vs. 1,586.4 days, respectively). This evidence is also in line with adenocarcinoma being a more aggressive cancer type when compared to squamous cell carcinoma.

After curative resections, patients with lung cancer at the same TNM stage show wide variations in their recurrence onset and overall survival [26]. The current TNM staging system, based on clinical and pathological findings, may have achieved the limit of its relevance. Being able to predict the exact likelihood and timing of relapse can help guide the administration of adjuvant therapies. There are two methods for identifying factors related to recurrence and low overall survival following surgery: expression of tumor markers and molecular biological techniques. Excellent prognostic markers for predicting the postoperative recurrence of cancer are KRAS, Ki-67, p16, EGFR, and others. Since histological differentiation, vessel invasion, lymphatic permeation, and pleural invasion have been reported as poor prognostic factors for the disease-free survival [27–29], an extensive pathological investigation is also of high significance.

Personalized medicine by targeting appropriate molecular targets in tumors has helped improve survival in NSCLC patients [30]. MMPs and zinc-dependent endopeptidases play roles within various areas of cancer pathology. Tumor growth, metastasis, angiogenesis, and MMP activation are increased in nearly all human cancers when compared to normal tissue [31,32]. MMPs are involved in the degradation of the extracellular matrix. In addition, MMPs are known to influence lung cancer metastatic properties and are involved in several signaling pathways [ECM, collagen regulate polarization of Th1/Th2 inflammatory response; Springolipid and Ephrin receptor-signaling pathway (ET-1); N-cadherin; N-cadherin, vascular smooth muscle cell-extracellular matrix (VSMC-ECM) attachment; IGF-2, VEGF-B and VEGF signaling pathways; p38, JNK, and NF-κB pathways] [33]. Overall, increased levels of specific MMPs have been associated with NSCLC progression [33].

MMP-14 is a critical protein in cancer invasion and metastasis [33]. Invasion through collagen networks and subsequent collagenolysis relies principally on MMP-14 and not on secreted MMPs. MMP-14 expression has been correlated with primary tumor growth and metastasis as well as angiogenesis. Detailed analysis of MMP-14-promoted tumor growth has suggested that a cytoplasmic domain is required for the MMP-14 enhanced tumor growth.

MMP-2 has a role in extracellular matrix disassembly, increased cell proliferation, invasion/migration, and angiogenesis [34]. Strong immunohistochemical staining for MMP-2 in tumor tissue predicts poor survival in lung cancer patients [32, 35]. MMP-2 has been implicated in lymphatic and vascular invasion of NSCLC, thus the prognostic value of MMP-2 expression in NSCLC is of great significance [32, 33]. MMP-2 overexpression predicts a poor prognosis in early-stage NSCLC. This study shows that MMP-2 overexpression correlates with early cancer-related death. Other MMP subclasses are also associated with a degree of lung cancer aggressiveness. It is of note that one systematic review suggests that MMP-2 expression has a poor prognostic significance of NSCLC patient's survival [36].

MMP-9 has a role in extracellular matrix remodeling, increased cell proliferation, invasion/migration, and angiogenesis [37]. Highly expressed MMP-9 correlates with shortened survival of NSCLC patients [32, 38]. MMP-9 expression is an independent prognostic marker for resected stage NSCLC. Thus, MMP-9 is a novel biomarker significantly and independently predicting a worse prognosis of resected stage NSCLC. In a different study, tumor MMP-9 expression was associated with poor outcomes in adenocarcinoma but not in squamous cell carcinoma patients [39]. MMP-9 expression was identified as an independent marker of relapse in completely resected lung adenocarcinoma.

In NSCLC patients, genetic aberrations of the human epidermal growth factor-2 (HER-2) signaling pathway are associated with different sensitivity to EGFR tyrosine kinase inhibitors (TKIs) [40]. This is a plausible mechanism and prognostic role of acquired resistance to the EGFR TKIs in EGFR-mutated tumors. Although in our study a vast majority of patients in both cancer groups were HER-2 negative, our positive adenocarcinoma patients showed a lower survival rate compared to HER-2 negative adenocarcinoma patients. Gene amplification is a well-known mechanism of proto-oncogene activation and has been described in many human malignancies, including lung tumors. However, HER-2 amplification seems far less common in NSCLC compared to other cancers. Recently, the predictive role of HER-2 overexpression has been more extensively studied with the purpose to identify anti-HER-2 agents applicable in NSCLC patients.

BCL-2 overexpression is associated with better outcome and survival of the patients with NSCLC [41, 42]. Patients with positive BCL-2 expression have a better survival rate compared to patients with negative BCL-2 expression [43]. Our study showed comparable findings.

The intensity of neoangiogenesis in a tumor can be reliably evaluated by measuring the intratumoral microvessel density of CD31 cell membrane protein. CD31 is an integral endothelial membrane protein that mediates cell-to-cell adhesion. Statistics have shown a more significant survival rate in NSCLC patients with high CD-31 expression compared to patients with lower CD-31 expression [44].

Expression of p-63, an established marker of squamous differentiation, is also present in NSCLC patients [45]. P-63 is a transcription factor that transactivates p-53 target genes and induces apoptosis when expressed in cells. The p-63 gene amplification and overexpression may have important implications in tumorigenesis [46]. In our previous study, patients with weak p-63 expression had a significantly shorter overall survival than patients with no p-63 expression and a tendency of shorter overall survival than patients with p-63 expression [47]. Patients with negative p-63 expression tend to have a worse prognosis compared to patients with p-63 expression. On the other hand, in a study by Ko et al. [48], negative expression of p-63 was associated with a short recurrence interval of the disease and shorter survival in NSCLC.

Based on the results of our study, tumor (biological) markers represent significant negative prognostic indicators in all patients with NSCLC regardless of the histological tumor subtype. All patients with positive marker expression had a short recurrence interval of the disease, as well as a short overall survival. These data should be considered when deciding on patient treatment following surgical resections. We propose that patients with positive expression of BCL-2, CD31, p-63, MMP-9, MMP-2, HER-2, and MMP-14 should receive adjuvant chemotherapy irrespective of their TNM clinical stage and tumor histological type.

Limitation of the study

Our study is limited by a low size effect and a retrospective character; the optimal management of lung cancer patients according to biomarkers needs to be determined by prospective clinical trials in large patient cohorts.

Conclusion

Adenocarcinoma is more aggressive compared to squamous cell carcinoma showing a lower overall survival in patients. Cumulative survival was shorter by approximately 750 days in the adenocarcinoma patients in comparison with squamous cell carcinoma patients. In addition, cumulative survival was shorter in adenocarcinoma patient group in comparison with the squamous cell carcinoma group in CD31, p-63, MMP-9, and MMP-2 positive patients. Therefore, these biological markers have a significant prognostic value for NSCLC patient survival. Biological marker expression may be a useful clinical prognostic tool of therapeutic outcome.

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