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Expression of p63 as predictive and prognostic factor in advanced non-small-cell lung cancer

Ekspresija p63 kao prediktivnog i prognostičkog faktora kod uznapredovalog nesitnoćelijskog karcinoma pluća

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Abstract

Background/Aim. Serbia belongs to the group of countries with a high lung cancer incidence and mortality rate. p63 gene plays an important role in development of lung cancer and immunohistochemical expression of p63 is considered to be a reliable marker for squamous histology. The results of some *in vitro* studies show a significant association of p63 expression and cisplatin chemoresistance. The aim of this study was to estimate the significance of p63 expression as predictive and prognostic factor in advanced non-small-cell lung cancer (NSCLC). **Methods.** Expression of p63 in 85 NSCLC (stages III, and IV) was investigated by the use of immunohistochemistry. Four weeks after the completion of 2 cycles of platinum-based doublet chemotherapy all the patients were evaluated based on the treatment response. Kaplan-Meier analysis with log-rank tests were used for overall survival (OS) and progression free survival (PFS) calcultations. **Results.** The expression of p63 was present in 49.4% of the patients out of whom 38.8% were with positive expression (p63+) and 10.6% of the patients were with weak expression (p63+-). Positive expression of p63 was seen in 93.9% of squamous cell carcinomas (SQCC), 5% of adenocarcinomas (AC), and in no patient with not otherwise specified (NOS) NSCLC. Weak expression of p63 was found in 12.5% of AC, 25% of NOS and only in 3% of SQCC. Analysis of the impact of the presence of p63 expression on the initial response to chemotherapy showed no statistical significance. The patients with weak p63 expression had a significantly shorter OS than the patients with no p63 expression ($p = 0.049$), and the tendency of shorter OS than the patients with p63 expression ($p = 0.068$). **Conclusion.** This study shows that p63 expression has no predictive significance for tumor response to initial chemotherapy regimen gemcitabine/cisplatin or paclitaxel/cisplatin observed in advanced NSCLC. Weak expression of p63 have a negative prognostic effect in stage III and IV NSCLC.

Key words:
carcinoma, non-small-cell lung; neoplasm staging; immunohistochemistry; disease progression; predictive value of tests.

Apstrakt

Uvod/Cilj. Srbija se ubraja u grupu zemalja sa visokom incidencom i stopom mortaliteta od karcinoma pluća. Značajnu ulogu u nastanku karcinoma pluća ima gen p63. Imunohistohemijska ekspresija p63 je značajan marker za dijagnostiku skvamocelularnih carcinoma (SCK) pluća. Rezultati nekih *in vitro* istraživanja ukazuju na značajnu vezu ekspresije p63 i rezistencije na cisplatin. Cilj ovog istraživanja bio je da se proceni značaj ekspresije p63 kao prediktivnog i prognostičkog faktora kod uznapredovalog nesitnoćelijskog karcinoma pluća (NSĆKP). **Metode.** Imunohistohemijski je analizirana ekspresija p63 kod 85 NSĆKP pluća u III i IV stadijumu bolesti. Četiri nedelje nakon završetka 2 ciklusa hemioterapije na bazi platinskog dubleta vršena je procena odgovora na terapiju. Preživljavanje bez progresije bolesti i dužina preživljavanja izračunavani su primenom Kaplan-Meierove analize i log rang testa. **Rezultati.** Ekspresiju p63 imalo je 49,4% bolesnika. Pozitivnu ekspresiju (p63+) imalo je 38,8%, a slabu ekspresiju (p63+-) 10,6% bolesnika. Pozitivna ekspresija je ustanovljena kod 93,9% SCK, kod 5% adenokarcinoma (AC) i nijednog neklasifikovanog (NK) NSĆKP. Slaba ekspresija je nađena kod 12,5% AC, 25% NNS i kod 3% SCK. Analizom uticaja prisustva ekspresije p63 na inicijalni odgovor na hemioterapiju nije utvrdjena statistička značajnost. Bolesnici

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sa slabom ekspresijom p63 imali su značajno kraće vreme ukupnog preživljavanja u odnosu na bolesnike bez ekspresije p63 (p = 0.049) i tendenciju kraćeg vremena ukupnog preživljevanja u odnosu na bolesnike sa ekspresijom p63 (p = 0.068). **Zaključak.** Ovim istraživanjem ustanovljeno je da ekspresija p63 nema prediktivni značaj za odgovor na inicijalnu hemioterapiju po gemcitabin/cisplatin ili paklitaksel/cisplatin protokolu kod uznapredovalog NSĆKP. Slaba ekspresija p63 ima negativan prognostički značaj u III i IV stadijumu NSĆKP.

Ključne reči:
pluća, nesitnoćelijski karcinom; neoplazme, određivanje stadijuma; imunohistohemija; bolest, progresija; testovi, prognostička vrednost.

Introduction

Lung cancer is the most frequently diagnosed cancer and the leading cause of cancer death among males worldwide. Among females, lung cancer is one of the leading cause of cancer death in more developed countries, and the second leading cause of cancer death in less developed countries. In males, the highest lung cancer incidence rates are in Europe, Eastern Asia, and Northern America [1]. Serbia befalls in the group of Central and South Eastern European countries with the high lung cancer incidence and mortality rate [2, 3]. Non-small-cell lung cancer (NSCLC) accounts for 80–85% of lung cancers, while small-cell lung cancer has been decreasing in frequency over the last two decades [4]. NSCLC is usually in an advanced stage not amenable to surgical resection when first diagnosed. About 40% of patients with newly diagnosed NSCLC first present with locally advanced disease, and most are inoperable [5]. The median survival of patients with untreated metastatic NSCLC is only 4 to 5 months, while the 1-year survival rate is only 10% [6]. In spite of the progress of targeted therapy, platinum-based doublet chemotherapy still represents the standard of initial care for advanced NSCKC [7].

Small biopsy specimens are the primary method for the diagnosis in the majority of lung cancer patients. In 2011 the new lung cancer classification was developed by the International Association for the Study of Lung Cancer, American Thoracic Society and European Respiratory Society, and it provides for the first time a proposed set of terms and criteria for all major histologic types of lung cancer in small byopsies and cytology [8]. One of the major changes in this approach to classification of lung cancer is greater use of special stains to classify difficult cases further into adenocarcinoma (AC) or squamous cell carcinoma (SQCC) and one of the important recommendations is to preserve as much tissue as possible for molecular testing in small biopsies. At present time, thyroid transcription factor (TTF-1) appears to be the single best marker for adenocarcinoma and p63 is a reliable marker for squamous histology [8]. When morphology and/or immunohistochemistry (IHC) are not clear to recognize AC or SQCC, carcinoma should be termed as NSCLC Not Otherwise Specified (NSCLC-NOS) [8].

p63 is a member of p53 genes family. Its basic role is to form squamons epithelial phenotype [9]. The p53/p63/p73 family binding sites modulate promoter activity of miRNAs of the miR-200 family which are known regulators of cancer stem cells and epithelial-mesenchymal transitions [10]. p63 is located in chromosome 3q27-29. p63 has 6 different isotypes. One of the isotypes – Tap63 activates p53 reporter genes and makes the cell turn to apoptosis. The isoform ΔNp63 suppresses transactivation of p53 and triggers cell proliferation [11]. Although ΔNp63 and TAp63 splice variants are expressed in NSCLCs, ΔNp63α is the predominant isoform, and in contrast to TAp63 is selectively expressed in SQCC [12]. Of clinical relevance is the fact that Tap63 is induced by many chemotherapeutic agents and that inhibiting Tap63 function leads to *in vitro* chemoresistance [13]. On the other hand, *in vitro* studies show ΔNp63 expression as a regulator of increased cell survival and cisplatin chemoresistance [14].

The aim of this study was to estimate the significance of p63 expression (determined by immunohistochemistry) as predictive and prognostic factor in advanced NSCLC.

Methods

Study design and patients selection

This prospective study included 85 patients. The study was approved by the Ethical Committee of the Military Medical Academy (MMA) in Belgrade. The patients were included in the study only if they met the following criteria: older than 18 years; the histological diagnosis of NSCLC, stage IIIa if inresectable or inoperable, IIIb or IV, according to World Health Organization (WHO) Tumor-Node-Metastasis (TNM) classification; adequate bone marrow reserve (white blood cell count ≥ 3.5×10^9/L ; platelets ≥ 100×10^9/L hemoglobin ≥ 100 g/L); adequate liver and renal function (bilirubin < 1.5 times than normal; aspartate aminotransferase (AST) and alamine aminotransferase (ALT) < 3 times than normal; normal serum urea and creatinine levels); no central nervous system metastasis; no prior chemotherapy or radiation therapy; Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 2 [15].

All the patients had available diagnostic tissue specimens obtained by bronchoscopy, and were diagnosed and treated at the Pulmonology Clinic of the MMA between 2011 and 2015. The diagnosis of lung cancer was made by endobronchial biopsy by fiberoptic bronchoscopy, or tru-cut biopsy.

Biopsy specimen was fixed with 5% buffered formalin solution, dehydrated, paraffin-embedded in Leica ASP 300. Paraffin-embedded tissue sections were cut to 4 micron thick tissue sections using a microtome and applyed to Superfrost+ glass slides. Immunohistochemical staining was preformed following the Dako immunohistochemistry protocol

(Glostrup, Denmark). To unmask epitop for p63 Target Retrieval Solution pH 9.0 (Dako catalog number S2367) was used, followed by heating in a microwave. Anti-human p63 protein (Dako catalogue, number M 7247, Clone 4A4, 1:300 dilution) was used as a primary antibody. For visualization we used EnVisionDetection Systems Peroxidase (Dako catalogue number K5007) and chromogen DAB Liquid (Dako catalog number K3466), and than observed by light microscopy. p63 expression was graded as negative (-) if there was no reactivity; weak (+-) if there was up to 10% positive staining tumor cells; and positive (+) if there was more than 10% positive tumor cells.

The patients were randomised to receive either gemcitabine/cisplatin (GC) or paclitaxel/cisplatin (PC). Gemcitabine 1000–1250 mg/m² was given on the days 1 and 8, and cisplatin 75 mg/m² was given on the day 1 of a 21-day cycle. Paclitaxel 135 mg/m² was given on the day 1, and cisplatin 75 mg/m² was given on the day 1 of a 21-day cycle.

Four weeks after the completion of 2 cycles of chemotherapy, all the patients were evaluated according to treatment response. The responses were categorized as: complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) according to the Response Evaluation Criteria in Solid Tumors (RECIST) [16]. Further treatments of all the patients were in acordance with the National Comperhensive Cancer Network guidelines [7].

All data analyses were processed using the Statistical Package for Social Sciences, version 18 (SPSS, Chicago,IL). Data are presented as mean ± standard deviation (SD), and median with 95% confidence interval. The normality of the data was assessed using Kolmogorov-Smirnov test. The difference between the groups was tested by Student's *t*-test (alternatively Mann-Whitney test) or by one-way analysis of variance (ANOVA) or alternatively Kruskal-Wallis test. χ^2 test were used to detect singificant differences among the frequences of some categories. Kaplan-Meier analysis with Log-rank tests were used for Overall Survival (OS) and Progression Free Survival (PFS). A *p*-value of 0.05 or less was considered indicative of a statistically significant difference.

Results

A total of 85 patients were included in the study. Their basic demographic characteristics are shown in Table 1. The mean age of the patients of both sexes was 62.9 years (median 63 years), women 64.6 years (median 66 years), and for men 62.5 (median 63 years). Using *t*-test for independent characteristics it was found that there was no statistically significant difference in age between the sexes. Most patients (66 or 77.6%) at the time of the diagnosis had ECOG PS 1. PS 0 had 14 (16.5%) and PS 2 5 (5.9%) of the patients. AC was diagnosed in 40 (47.1%) of the patients, NOS NSCLC was diagnosed in 12 (14.4%), and SQCC in 33(38.8%) of the patients (Table 2). When it comes to age at the diagnosis there was no statistically significant difference among the three histological types of cancer (ANOVA).

At the time of the diagnosis most of the patients (45.9%) had T2 disease; 28.2% had T3, 16.5% T4 and 9.4% had T1 disease. N2 disease had 58.8% of the patients; the same percentage (16.5%) had N1 and N3 and 8.2% of the patients had N0 disease. No distant metastasis was found in 50.6% of the patients, M1a disease was found in 17.6% of the patients, whereas M1b disease was detected in 31.8% of the patients. Most of them, 42 (49.4%), were in clinical stage IV of the disease, followed by 31 (36.5%) in stage IIIa, while the smallest number of the patients, 12 (14.1%) were in clinical stage IIIb.

Table 1

Basic demographic data of patients

Gender	Number (%) of patients	Age, (years) r ± SD	Median	Minimum	Maximum
Male	69 (81.2)	62.5 ± 9.1	63.0	43	79
Female	16 (18.8)	64.6 ± 6.8	66.0	53	75
Total	85 (100)	62.9 ± 8.7	64.0	43	79
t-test		$p = 0.400$			

r ± SD – mean ± standard deviation;

Table 2

Histological type of carcinoma and patients ages

Histological type of carcinoma	Number (%) of patients	Age, (years) r ± SD	Median	Minimum	Maximum
AC	40 (47.1)	62.9 ± 9.0	65.5	43	77
NOS	12 (14.1)	61.2 ± 9.2	62.0	50	79
SQCC	33 (38.8)	63.4 ± 8.3	64.0	43	77
Total	85 (100)	62.9 ± 8.7	64.0	43	79
ANOVA		$p = 0.756$			

r ± SD – mean ± standard deviation; AC – adenocarcinoma; NOS – not otherwise specified; SQCC – squamous cell carcinoma; ANOVA – Analysis of variance.

The expression of p63 was present in 42 (49.4%) of the patients out of whom weak expression (p63+-) in 9 (10.6%) of the patients, positive expression (p63+) in 33 (38.8%) of the patients. Positive expression p63 was seen in 31 of the patients with SQCC (93.9% of p63 +; and 93.9% of SQCC), 2 patients with AC (6.1% of p63+; and 5% of AC), and in no patient with NOS NSCLC (Figures 1 and 2). So, there was a statistically significant difference between SQCC and AC, as well as between SQCC and NOS NSCLC ($p < 0.001$). Weak expression of p63 was found in 5 of the patients with AC (55.6% of p63+-; 12.5% of AC), 3 patients with NOS (33.3% of p63+-; 25% of NOS) and only 1 with SQCC (11.1% of p63+-; 3% of SQCC) (Table 3).

There was a favorable response to chemotherapy in 63 (74.1%) of the patients, of which a partial response in 39 patients (45.9% compared to the total number of patients), stable disease in 24 (28.25%) of the patients while 22 (25.9%) of the patients had disease progression. Gender had no significant effect on the response to chemotherapy. A total of 75% of women had a favorable response to chemotherapy, and 73.9% of the male population.

The studied patients had ECOG PS estimated in the range of 0 to 2. There was a significant influence of PS on the response to chemotherapy. A correlation of PS 2 score with adverse responses was statistically significant ($p = 0.015$). In the group of patients with an adverse response to chemotherapy, 80% showed PS score 2, accounting for 18.25% of the total number of patients.

There was no statistically significant effect of the histological type of the tumor, nor T disease on the response to chemotherapy. There was a statistically significant correlation of negative responses to chemotherapy with N2

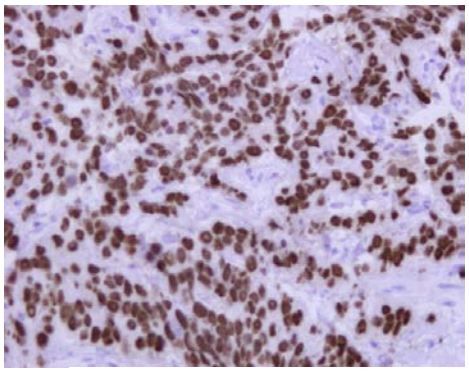

Fig. 1 – Squamous cell carcinoma/ p63 positive (p63 immunohistochemistry H&E, ×200).

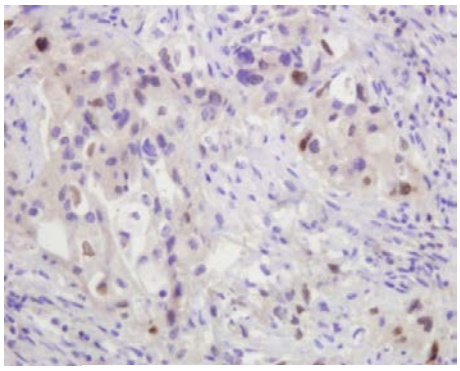

Fig. 2 – Adenocarcinoma/p63 weak positive (p63 immunohistochemistry H&E, ×200).

Table 3

P63 expression in relation to the histological type of carcinoma

p63 expression	Histological type of carcinoma			Total
	AC	NOS	SQCC	
p63-				
number of patients	33	9	1	43
(%) in p63-	76.7	20.9	2.3	100
(%) in histological type	82.5	75.0	3.0	50.6
p63+				
number of patients	2	0	31	33
(%) in p63+	6.1	0.0	93.3	100
(%) in histological type	5.0	0.0	93.3	38.8
p63+-				
number of patients	5	3	1	9
(%) in p63+-	55.6	33.3	11.1	100
(%) in histological type	12.5	25.0	3.0	10.6
Total				
number of patients	40	12	33	85
(%) in p63	47.1	14.1	38.8	100
(%) in histological type	100	100	100	100
χ^2 test			p <0.001*	

AC – adenocarcinoma; NOS – not otherwise specified; SQCC – squamous cell carcinoma.

There were no statistically significant differences in the distribution of chemotherapy protocols in relation to the histopathological type of cancer ($p = 0.116$), as well as in relation to the stage of the disease (p=0.203). The expression of p63 with respect to the stage of the disease showed no statistically significant differences ($p = 0.256$).

disease (p = 0.050). Compared to the group of patients with PD, 81.8% had N2 disease. There was no statistically significant difference among M0, M1a and M1b diseases as well as among IIIa, IIIb and IV stages of the disease, regarding the initial response to chemotherapy.

Analysis of the impact of the presence of p63 expression on the initial response to chemotherapy showed no statistical significance.

It turned out that there was no statistically significant difference in the studied patients regarding the initial response to chemotherapy when comparing the two applied chemotherapy regimens (gemcitabine / cisplatin and paclitaxel / cisplatin). Partial response to the regimen GC was found in 44.3% of the patients and to the regimen PC in 50% of the patients, SD in the group that received GC had 26.2% of the patients and in the group which received PC 33.3% of the patients. The progression of the disease in the group with the regimen GC was found in 29.5% and with the regimen PC in 25.9% of the patients.

Analysis of the progression-free survival and overall survival was related to 68 patients (56 male and 12 female). By the end of the monitoring period, 6 (10.7%) men and 4 (33.3%) women survived. A total of 17 of the patients continued treatment in other oncology centers, so that the analysis of PFS and OS did not apply to them. The median PFS time could not be determined because the number of end-point events did not reach half the total number.

The analysis of the impact of gender on PFS showed that there were no statistically significant differences. The estimated mean PFS in men was 14.25 months and 11.42 months in women. Log-rank (Mantel-Cox) test showed a statistically significant difference in favor of females regarding the overall survival time (p = 0.037) (Table 4).

Comparing the relationship of ECOG PS and PFS, there was a statistically significant difference between the PS 2 and PS 1 (mean 3.4 months *vs* 14.8 months).

The patients with PS 2 had a significantly shorter OS than the patients with PS 0 (mean 9.40 months/median 7.00 months *vs* mean 19.52 months) (p = 0.031) and the tendency of shorter OS than the patients with PS1 (mean 16.35 months/medain 13.00 months) (p = 0.059).

The results of PFS analysis compared to histological type of carcinoma showed no statistically significance difference. When it comes to OS, however, the patients with NOS lived significantly shorter as compared to the patients with SQCC (mean 10.33 months/median 9 months *vs* 17.14 months/median 14 months), (p = 0.027). There was also a tendency of shorter OS compared to the patients with AC, but with no statistical significance (p = 0.09) (Figure 3).

There were no statistically significant differences between the III and IV stages of the disease in terms of PFS. OS was statistically more significant in the patients with IIIb than in those with clinical stage IV of the disease (mean 24.66 months/median 21 months *vs* mean 13.81 months/median 12 months) (p = 0.008).

Table 4

Overall survival (OS) in relation to the patients gender

Gender	OS (months)	
	mean (95% confidence interval)	median (95% confidence interval)
Male (n = 56)	15.25 (12.82–17.69)	13.00 (10.93–15.06)
Female (n = 12)	22.00 (15.61–28.38)	19.00 (7.11–30.88)
Total (n = 68)	16.49 (14.09–18.88)	13.00 (11.03–14.97)
Log-Rang (Mantel-Cox)	p = 0.037*	

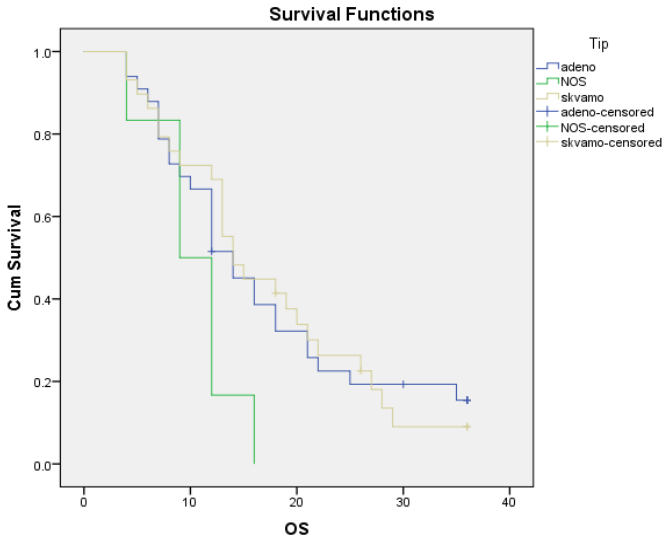

Fig. 3 – Kaplan-Meier estimates of overall survival (OS) according to the histological type of carcinoma.
NOS – not otherwise specified.
Estimation is limited to the longest survival time if it is censored.

The ratio of the expression of p63 had no statistically significant influence on PFS, but when it comes to OS there was a statistically significant difference. The patients with weak p63 expression had a significantly shorter OS than the patients with no p63 expression (p = 0.049), and the tendency of shorter OS than the patients with p63 expression (p = 0.068) (Table 5, Figure 4).

There were no statistically significant differences in PFS and OS regarding the two initially applied chemotherapy regimens.

Table 5

Overall survival (OS) in relation to the p63 expression

P63 expresion	OS (months)	
	mean (95% confidence interval)	median (95% confidence interval)
P63- (n = 33)	17.36 (13.78–20.95)	14.00 (10.99–17.01)
P63+ (n = 29)	16.75 (13.16–20.35)	14.00 (11.36–16.46)
P63+ (n = 6)	10.17 (6.76–13.58)	9.00 (5.23–12.77)
Total (n=68)	16.49 (14.11–18.88)	13.00 (11.03–14.97)
Log-Rang (Mantel-Cox)	P63	
	P63+ *vs* P63-	*P = 0.049**
	P63+ *vs* P63-	P = 0.806
	P63- *vs* P63+	P = 0.068

***statistically significant**

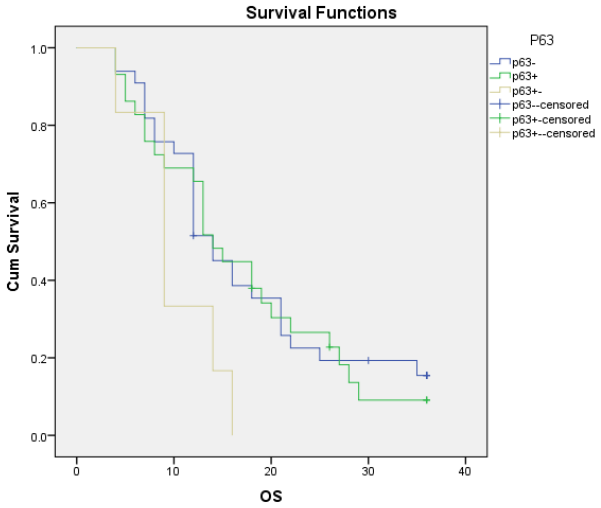

Fig. 4 – Kaplan-Meier estimates of overall survival (OS) according to the p63 expression. Estimation is limited to the longest survival time if it is censored.

Discussion

Despite the progress of the targeted molecular therapies, the most common treatment of advanced NSCLC in clinical practice is the use of chemotherapy in the first-line. Standard first-line chemotherapy regimens include combinations of third-generation agents with either cisplatin, or carboplatin. All the forms of anticancer therapy have side effects. On the other hand, the presence or development of resistance to chemotherapeutic agents is one of the major problems. That is why the search for predictors of response and for prognostic factors is still ongoing. The aim of our study was to determine whether p63, which is usually used for diagnostic purposes for NSCLC from small tissue samples, could be used as predictive or prognostic factor.

In this prospective study, analyzing a homogenous, well-defined patient population, we estimated the predicitive and prognostic significance of p63. Of the total number of 85 patients, there were 4.3 times more men than women. This ratio is in line with the epidemiological situation in the world and in our country [2, 3, 17]. Adenocarcinoma was diagnosed in 47.1% of the patients, squamous cell carcinoma in 38.8% of the patients, while unclassified non-small-cell lung cancer was diagnosed in 14.4%. The percentage of NOS in this study is similar to that published by Collins [18]. In his work, based on immunohistochemistry panel, 85% of the patients had AC or SQCC, whereas 15% of the patients were diagnosed with NOS NSCLC. On the other hand, Sigel et al. [19] are of the opinion that it is allowed to only 7% of all NSCLC from samples obtained by bronchial biopsy and cytological samples (after morphological examination, IHC staining and mucin) to remain unclassified. However, despite the use of the sophisticated methods of analysis small tissue samples NOS NSCLC diagnosis in practice occurs in about 10% to 30% [20], as in this study.

There was a highly significant expression of p63 in SQCC in comparison to AC and NOS NSCLC. Of the total number of the patients with the expression of p63, 93.6% had SQCC. On the other hand, of the total number of the patients with weak expression of p63, 55.6% had AC. Within the group of patients with SQCC, 97% had expression or weak expression of p63, and in the group with the AC expresion or weak expresion of p63 had 17.5% of the patients. p63 may show patchy and/or weak staining in 20%–30% of adenocarcinomas [8]. Bir et al. [21] in 2014 published similar results, according to which 24 out of 25 (96%) of the SQCC patients were p63 positive, and in 25%, 6 of 20 patients, AC showed weak p63 staining. According to the results of Yaman et al.[22] published in 2015, p63 staining was positive in 87.5% of SQCC and in 4.3% AC.

There was a statistically significant effect of PS as the response to chemotherapy. In the group of patients with adverse response to chemotherapy (regardless of which of the two regimes applied), 80% belonged to the group of patients with PS 2. Cuyún Carter et al. [23] published a comprehensive review of non-genetic prognostic and predictive factors that had an impact on the outcome of advanced NSCLC. The results of 54 studies, published from January 2000 to November 2010, were analyzed. Two out of ten studies examined and confirmed the importance of PS as predictive factor. The results of this study are also in favor of the importance of PS as a predictive factor.

It has been confirmed that there is a statistically significant correlation of negative responses to chemotherapy with N2 status. Of the patients with PD, 81.8% had N2 disease. According to the literature, the N status is significant to the prognosis [24]. When we analyze the effect of the presence and the level of p63 expression in the patients with advanced NSCLC on the response to chemotherapy, we found no statistically significant difference.

Gender had no significant effect on PFS, but there was a statistically significant difference in favor of females when looking at OS. Of the 45 studies, 17 (38%) confirmed a significant advantage of women in relation to better outcome [23].

Of the 49 studies on evaluating the PS as prognostic factor, the results of 36 (73%) confirmed a significant correlation of ECOG PS and clinical outcomes, and that a lower ECOG PS score is associated with better outcomes [23]. In this study, as expected, the patients with PS2 had the worst prognosis.

Of the 31 studies examining the importance of histology type as prognostic factor, 4 studies (12.9%) show an advantage of adenocarcinomas compared to other histological types of NSCLC [23]. Our group of patients with NOS histological type had the shortest time for OS, that is statistically significantly lower than that in SQCC, and the tendency of lower OS compared to AC.

Of the 38 studies that compared IIIB and stage IV disease, 21 (55%) show a significant association of a lower stage with a better outcome, as in our study [23].

According to the results of this study, expression of p63 did not influence PFS. However, in relation to the OS, there was a statistically significant difference between the patients with tumors with weak expression p63 (p63 +-) and the patients with no expression of p63 (10.17 months *vs* 17.36 months). There is also a tendency for better outcome in the patients with p63 expression as compared to the patients with weak expression. Ma et al. [25] explored the significance of p63 expression in SQCC of the lung in 76 patients in the early stage of disease (I, II, and IIIA-only T4N0). Based on the postoperative follow-up the obtained results show that there was a correlation of high expression of p63 with a better prognosis. Barlisi et al. [26] show in their study that in the squamous cell carcinoma p63 amplification and staining intensity are associated with better survival independently on the stage and the degree of differentiation of the tumor. On the contrary Yaman et al. [22] found no significant effect of p63 on survival.

The key limitation of this study is the relatively small number of patients. Another important limitation are small diagnostic samples of tumor tissue or metastatic lymph node because such samples may be histologically different from the rest of the whole tumor.

Conclusion

Expression of p63 is significantly more common in SQCC than in AC and NSCLC NOS. P63 has no predictive significance for tumor response to initial chemotherapy regimen GC or TC observed in the III and IV NSCLC clinical stage. Patients in stage III and IV NSCLC with low expression of p63 have worse prognosis than patients without p63 expression. They also have the tendency to worse prognosis compared to patients with p63 expression. This data give rise to additional investigation, which may provide the foundation for generating more effective therapeutic strategies in NSCLC.

R E F E R E N C E S

1. *Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A.* Global cancer statistics, 2012. CA Cancer J Clin 2015; 65(2): 87 – 108.
2. Institute of Public Health of Serbia "Dr Milan Jovanović Batut". Department for Prevention and Control of Noncommunicable Diseases. Report No 14. Cancer incidence and mortality in Central Serbia, 2012. Belgrade: Institute of Public Health of Serbia "Dr Milan Jovanović Batut"; 2014. (Serbian)
3. *Vrdoljak E, Wojtukiewicz MZ, Pienkowski T, Bodoky G, Berzinec P, Finek J,* et al. Cancer epidemiology in Central, South and Eastern European countries. Croat Med J 2011; 52(4): 478 – 87.
4. *Peters S, Adjei AA, Gridelli C, Reck M, Kerr K, Felip E.* ESMO Guidelines Working Group. Metastatic non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012; 23 Suppl 7: vii56 – 64.

5. *Alberola V, Cortesi E, Juan O*. Weekly paclitaxel in the treatment of metastatic and/or recurrent non-small cell lung cancer. Crit Rev Oncol Hematol 2002; 44 Suppl: S31 – 41.
6. *Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J,* et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002; 346(2): 92 – 8.
7. NCCN. Non-Small Cell Lung Cancer. 2016. Guidelines. Version 4. Available from: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp
8. *Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger K, Yatabe Y,* et al. Diagnosis of lung cancer in small biopsies and cytology: Implications of the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification. Arch Pathol Lab Med 2013; 137(5): 668 – 84.
9. *Conde E, Angulo B, Redondo P, Toldos O, Garcia-Garcia E, Suarez-Gauthier A*, et al. The use of p63 immunohistochemistry for the identification of squamous cell carcinoma of the lung. PLoS One 2010; 5(8): e12209.
10. *Knout EC, Grag K, Arroyo JD, Correa Y, Sarkar D, Parkin RK*, et al. An integrative genomic approach identifies p73 and p63 as activatores of miR-200 microRNA family transcription. Nucleic Acids Res 2012; 40(2): 499 – 510.
11. *Bishop PW*. Immunohistochemistry in the diagnosis of pulmonary tumors. In*: Hasleton P, Flieder DB*, editors. Spencer`s pathology of the lung. 6th ed. New York: Cambrige University Press; 2013. p. 1015 – 42.
12. *Nobre AR, Albergaria A, Schmitt* F. p40: A p63 Isoform Useful for Lung Cancer Diagnosis: A Review of the Physiological and Pathological Role of p63. Acta Cytol 2013; 57(1): 1 – 8.
13. *Melino G*. P63 is a suppressor of tumorigenesis and metastasis interacting with mutant p53. Cell Death Differ 2011; 18(9): 1487 – 99.
14. *Sen T, Sen N, Brait M, Begum S, Chatterjee A, Hoque MO,* et al. DeltaNp63alpha confers tumor cell resistance to cisplatin through the AKT1 transcriptional regulation. Cancer Res 2011; 71(3): 1167 – 76.
15. *Soresen JB, Klee M, Palshof T, Hansen HH*. Performance status assessment in cancer patients. An inter-observer variability study. Br J Cancer 1993; 67(4): 773 – 5.
16. *Eisenhauer EA, Thetasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R*, et al. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1. 1). Eur J Cancer 2009; 45(2): 228 – 47.
17. *Schottenfeld D, Searle JG*. The etiology and epidemiology of lung cancer. In: *Pass HI, Carbone DP, Minna JD, Johnson DH, Turrisi AT* , editors. Lung Cancer: Principles and Practice. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 3 –73.
18. *Collins BT*. Endobronchial ultrasound fine-needle aspiration biopsy of pulmonary non-small cell carcinoma with subclassification by immunohistochemistry panel. Cancer Cytopathol 2013; 121(3): 146 – 54.
19. *Sigel CS, Moreira AL., Travis WD, Zakowski MF, Thornton RH, Riely GJ*, et al. Subtyping of non-small cell lung carcinoma: a comparison of small biopsy and cytology specimens. J Thorac Oncol 2011; 6(11): 1849 – 56.
20. *Celik B, Khoor A, Bulut T, Nassar A*. Rapid on-site evaluation has high diagnostic yield differentiating adenocarcinoma vs squamous cell carcinoma of non-small cell lung carcinoma, not otherwise specified subgroup. Pathol Oncol Res 2015; 21(1): 167 – 72.
21. *Bir F, Aksoy AA, Satiroglu TN, Kaya S, Baser S, Yaren A*. Potential utility of p63 expression in differential diagnosis of non-small-cell lung carcinoma and its effect on prognosis of the disease. Med Sci Monit 2014; 20: 219 – 6.
22. *Yaman B, Nart D, Ekren PK, Çok G, Veral A*. Expression of p63, TTF-1 and Maspin in Non-Small Cell Lung Carcinoma and Their Effect on the Prognosis and Differential Diagnosis. Turk Patoloji Derg 2015; 31(3): 163 – 74.
23. *Cuyún Carter G, Barrett AM, Kaye JA, Liepa AM, Winfree KB, John WJ*. A comprehensive review of nongenetic prognostic and predictive factors influencing the heterogeneity of outcomes in advanced non-small-cell lung cancer. Cancer Manag Res 2014; 6: 437 – 49.
24. *Roberts JR, Pass HI*. Lung cancer staging in non-small cell lung cancer including preoperative and intra-operative techniques. In: *Pass HI, Carbone DP, Minna JD, Johnson DH, Turrisi AT*, editors. Lung Cancer: Principles and Practice. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 372 – 86.
25. *Ma Y, Fan M, Dai L, Kang X, Liu Y, Sun Y*, et al. Expression of p63 and CK5/6 in early-stage lung squamous cell carcinoma is not only an early diagnostic indicator but also correlates with a good prognosis. Thorac Cancer 2015; 6(3): 288 – 95.
26. *Barlési F, Pinot D, Legoffic A, Doddoli C, Chetaille B, Torre JP*, et al. Positive thyroid transcription factor 1 staining strongly correlates with survival of patients with adenocarcinoma of the lung. Br J Cancer 2005; 93(4): 450 – 2.

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