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The Utility of Thyroid Transcription Factor 1 (TTF-1), Napsin A, Excision Repair Cross-Complementing 1 (ERCC1), Anaplastic Lymphoma Kinase (ALK) and the Epidermal Growth Factor Receptor (*EGFR*) Expression in Small Biopsy in Prognosis of Patients with Lung Adenocarcinoma – A Retrograde Single-Center Study from Croatia

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Background: The present study was carried out in order to evaluate our institutional experience with small biopsy in diagnosis and molecular testing of lung adenocarcinoma. Few specific and predictive markers have been evaluated and correlated with clinicopathologic characteristics and survival in patients with lung adenocarcinoma who received platinum-based chemotherapy. There have not been such reports from Croatia.

Material/Methods: A total of 142 cases of lung adenocarcinoma were retrospectively investigated in small biopsies for the immunohistochemical expression of TTF-1, napsin A, ERCC1, ALK, and the *EGFR* mutation by real-time polymerase chain reaction (rtPCR).

Results: TTF-1, napsin A, and ERCC1 expression was found in 81%, 78%, and 69% of patients, respectively, and the expressions were not significantly associated with subtype. Expression of ALK was found in 4% and *EGFR* mutation in 10% of patients. Exon 19 deletions were the most common. Longer survival was significantly associated with TTF-1 positivity (p=0.007) and napsin A positivity (p=0.026). Higher relative risk of death significantly correlated with positive expression of ERCC1 (p=0.041).

Conclusions: Positive TTF-1 and napsin A expressions in lung adenocarcinoma tissues were useful diagnostic and favorable prognostic parameters. Positive ERCC1 expression was identified as a negative prognostic marker in patients treated with platinum-based chemotherapy. The percentages of EGFR and ALK mutations corresponded to those in previously published reports for Caucasians.

MeSH Keywords: Carcinoma, Non-Small-Cell Lung • Diagnosis • Immunohistochemistry • Pathology • Prognosis • Real-Time Polymerase Chain Reaction

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Background

In Croatia, lung cancer is the most frequently diagnosed cancer and the leading cause of cancer death in men. In women, it is the third most frequently diagnosed cancer and the second cause of cancer death, with poor overall survival [1]. Adenocarcinoma is the most common type of lung carcinoma, usually presenting in the advanced stage as an inoperable disease [2]. Therefore, minimally invasive procedures have to be employed in order to obtain tumor tissue for histological and molecular analysis [3]. The combination of TTF-1 and napsin A immunohistochemistry shows highly improved sensitivity and specificity for lung adenocarcinoma diagnosis [4,5]. TTF-1 is a nuclear tissue-specific DNA-binding protein mainly expressed in thyroid follicular cells, type II pneumocytes, and nonciliated bronchiolar epithelial cells [6]. Napsin A is an aspartic proteinase involved in the maturation of the surfactant B [7]. Platinum-based chemotherapy is still the first-line therapy for advanced non-small cell lung cancer (NSCLC) [8], but in some patients chemotherapy does not have any clinical benefit [9]. ERCC1 is a nuclear protein involved in the nucleotide excision repair pathway essential for the repair of platinum-DNA adducts. ERCC1 is associated with resistance to platinum-based chemotherapy [10], so could be a prognostic and predictive biomarker [11,12]. *EGFR* and *ALK* molecular testing is recommended to select patients with lung adenocarcinoma for targeted therapy with thyrosine kinase inhibitors (TKIs) [13]. *EGFR* mutation has been associated with lung adenocarcinoma, female sex, non-smokers, and Asian ethnicity, ranging from 24% to 66% [14]. Studies from the USA and Europe reported lower mutation rates, ranging from 13% to 17% [15,16]. *ALK* rearrangement is another important finding in lung adenocarcinoma, ranging from 3% to 13% across different ethnicities, in young adult patients who never smoked [17]. Unfortunately, according to guidelines of Croatian health insurance, TKIs are not yet the first line of treatment for the patients with *EGFR* or *ALK* mutation, whose treatment still rests on platinum-based therapy. The present study was carried out in order to evaluate our institutional experience in daily practice and to provide an evidence-based approach for the utilization of immunohistochemical biomarkers and molecular testing in lung adenocarcinoma on small specimens, according to the recommendations for tissue management and guidelines for molecular testing [13].

Material and Methods

Patients and tissue procurement

From January 2013 till December 2014, 196 cases of new primary lung adenocarcinomas, 133 (68%) in males and 63 (32%) in females, were diagnosed at the Institute of Pathology, Forensic Medicine and Cytology, Clinical Hospital Center Split, Croatia. Among them, 142 (72%) were diagnosed on small biopsy, 36 (18%) on the surgical resection, 10 (5%) on transthoracic needle biopsies, and 8 (4%) on cytology samples. A total of 142 patients diagnosed only on small biopsy who did not undergo previous therapy were investigated in this study. None of the patients received neoadjuvant therapy. Original slides and paraffin blocks were collected from the archive, clinical data were collected from the hospital records of the Department of Pulmonology, and the time of death from the Mortality Registry. TNM staging of the disease was performed by imaging radiologic techniques. Overall survival was evaluated from the date of diagnosis until the end of March 2015 or the time of death, and patients treated with symptomatic therapy were excluded. Diagnosis of lung adenocarcinoma was performed based on morphology and immunohistochemical markers if necessary: cytokeratin (CK) 7, TTF-1, and napsin A. CK7 was obligatorily positive. Adenocarcinomas were subclassified into histological subtypes according to the latest World Health Organization (WHO) classification of lung carcinoma from 2015 [18]. Approval for the study was obtained from the Hospital Ethics Committee (code 500-03/15/01/42).

Immunohistochemistry

The slides of tumor tissue were stained with monoclonal mouse antibodies napsin A (1:200), TTF-1 (1:100), and ERCC1 (4F9, ready to use) (all reagents DAKO, Glostrup, Denmark), and rabbit monoclonal antibody ALK (D5F3, Ventana, Tucson, Arizona). TTF-1, napsin A, and ERCC1 were visualized with the iVIEW DAB Detection Kit (Ventana, Tucson, Arizona) and ALK with the OptiView DAB ICH Detection Kit and the OptiView Amplification Kit (Ventana, Tucson, Arizona) on the automatic stainer BenchMark GX (Ventana, Tucson, Arizona). A total of 89 patients had a sufficient sample for immunohistochemical analysis for napsin A, 118 for TTF-1, 102 for ALK, and 73 for ERCC1. The results were analyzed by two pathologists on a light microscope Olympus 51 BX (Olympus, Tokyo, Japan). A positive expression of napsin A and ALK was shown as brown granular cytoplasmic staining and positive expression of TTF-1 as homogenous nuclear staining in any cell. ERCC1 staining was estimated on 100 tumor cells under light microscope at magnification of 400×. Percentages of positive tumor nuclei were determined, and staining intensity was graded on the scale 0 to 3. Respiratory epithelium with staining intensity of 2 was used as an internal positive control. By multiplying the staining intensity by the percentage of positive nuclei, the ERCC1 H score was determined, according to Besse et al. [12]. Median value of all H scores was used in order to classify tumors as ERCC1 positive or negative. Another way of discrimination was to use 0 to indicate ERCC1-negative tumors (no immunohistochemical reaction in any cell) and ERCC1 >0 to indicate ERCC1-positive tumors (positive immunohistochemical reaction in any cell).

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EGFR mutation detection by rtPCR

A total of 113 patients had a sufficient sample for reflex molecular analysis of the *EGFR* mutation. Molecular analysis of the *EGFR* mutation was done at the time of diagnosis with a cobas EGFR Mutation Test (Roche, Basel, Switzerland) real-time polymerase chain reaction (rtPCR). A cobas Sample Preparation Kit (Roche, Basel, Switzerland) was used for sample preparation and DNA extraction. Automatic amplification and detection were done on a cobas z 480 Analyzer (Roche, Basel, Switzerland).

Statistical analysis

Statistical significance was set at $p<0.05$, and all confidence intervals (CIs) were at the 95% level. Statistical significance of the difference in categorical characteristics was calculated by using the χ^2 test. Analysis of statistical significance of differences in numerical groups was performed with the Kruskal-Wallis test and of differences between two groups with the Mann-Whitney test. The Cox proportional model and logistic regression model were used to identify factors that might significantly influence survival. Survival curves were calculated according to the Kaplan-Meier method, and differences between curves were evaluated with the log-rank test. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software (version 19 for Windows; SPSS Inc., Chicago, Illinois, USA).

Results

Clinicopathologic characteristics of lung adenocarcinoma patients and distribution of subtypes

In 24 months, among 196 newly diagnosed patients with lung adenocarcinoma (ages 44–89 years), 133 (68%) were males and 63 (32%) were females. The cumulative incidence per 10,000 inhabitants was 9.5 (95% CI: 8.1–10.8); in females it was 5.6 (95% CI: 4.2–7) and in males it was 13.9 (95% CI: 11.5–16.2), which was 2.9 times more in males than in females. In our study, 142 patients with lung adenocarcinoma diagnosed only on small biopsy were analyzed: 95 (67%) males and 47 (33%) females. The median age was 64 years (minimum-maximum: 44–89 years). Within 27 months of follow-up, 76 (53.5%) patients died. Median survival was 8 months (SE: 0.87; 95% CI: 6.3–9.7). Solid subtype was found in 80 patients, acinar in 42, papillary in 15, and lepidic subtype in 5 patients. Patients were analyzed according to histologic subtype in relation to clinicopathologic characteristics (Table 1). No statistically significant difference was observed between solid and other subtypes in sex ($\chi^2=0.061$; $p=0.805$) and age ($\chi^2=0.030$; $p=0.843$). No statistically significant difference was observed between solid and other subtypes in TNM stage ($\chi^2=5$; $p=0.171$), tumor size ($\chi^2=5.96$; $p=0.133$), and lymph nodes ($\chi^2=0.761$; $p=0.383$). Nevertheless, when the stage I disease was excluded, the solid subtype was 2.4 times more common in the stage III disease with a level of significance of 92% ($\chi^2=4.99$; $p=0.082$). Metastases were observed 1.8 times more often in the acinar subtype compared with the solid subtype, with a level of significance of 94% ($\chi^2=3.46$; $p=0.063$). Napsin A expression correlated with TTF-1 positivity ($\chi^2=28.25$; $p<0.001$). Among 18 napsin A-negative cases, 10 (55%) were TTF-1 negative, which was 13.7 times more than in napsin A-positive cases. Among 85 napsin A-positive cases, 64 (95%) were TTF-1 positive, which was 2 times more than in napsin A-negative cases. Among 68 TTF-1-positive cases, 41 (60%) had distant metastases, and among 16 TTF-1-negative cases, 14 had distant metastases (87%), which was 1.5 times more, with a level of significance of 92% ($\chi^2=3.1$; p=0.077). ERCC1 expression was negative in 23 (31%) patients. No statistically significant difference was found in ERCC1 expression between solid and other subtypes (z=0.148; $p=0.882$), as well as EGFR status (z=1.54; $p=0.122$). ALK expression was found in 4% of patients (Figure 1A, 1B).

EGFR mutation status

EGFR mutation was confirmed in 11 patients: 6 males and 5 females. Nine patients had exon 19 deletion (19 del), one had exon 21 L858R point mutation (L858R mutation), and one had a mutation on exon 18 G719X. They all had positive expression of TTF-1 and napsin A in tumor cells. The positive ALK expression and *EGFR* mutation were exclusive.

Survival analysis

Overall survival (Table 2) was twice longer in TTF-1 and napsin A positive cases ($p=0.007$ and $p=0.026$, respectively). Overall survival was significantly associated with a lower TNM stage ($p=0.001$), negative lymph nodes ($p=0.04$), and negative distant metastasis ($p<0.001$). The patients with stage II disease lived 1.6 times longer than the patients with stage III disease, and 2.8 times longer than the patients with stage IV disease. The patients treated with chemotherapy after surgical resection had an average survival 1.9 times longer than patients treated with chemotherapy alone, and 3.7 times longer than patients treated with the combination of chemotherapy and radiotherapy ($p=0.001$). No statistically significant difference in overall survival according to age (LR 0.13; $p=0.722$), sex (LR 1.5; $p=0.218$), smoking status (LR 0.59; $p=0.439$), tumor subtype (LR 0.483; $p=0.487$), tumor size (LR 3.8; $p=0.285$), and ERCC1 expression (LR 1.6; $p=0.210$) was found. Average survival of patients with *EGFR* mutation was 11 (9–13) months, and average survival of patients with wild type was 14 (7–12) months (LR 0.56; $p=0.456$). All variables were correlated with death (Table 3). The patients without expression of TTF-1

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Table 1. N (%) of patients with lung adenocarcinoma according to histologic subtype in relation to clinicopathologic characteristics.

Clinicopathologic characteristics		Patients N (%)	Histologic subtype			
			Lepidic	Acinar	Papillary	Solid
Age (years)		64 (44–89)	62 (50–75)	63 (44–82)	72 (59–89)	63 (47–88)
Sex	Male	95 (100)	2	24 (25)	10	59 (62)
	Female	47 (100)	3	18 (38)	5	21 (45)
TNM stage	1	2 (100)	0	1	0	1
	2	11 (100)	2	2 (18)	2	5 (45)
	3	24 (100)	1	5 (22)	0	18 (75)
	4	70 (100)	1	26 (37)	8	35 (50)
Tumor*	T1	11 (100)	1	3 (27)	3	4 (36)
	T2	31 (100)	1	10 (32)	3	17 (55)
	T3	26 (100)	1	14 (54)	1	10 (38)
	T4	38 (100)	1	68 (22)	4	25 (66)
Lymph nodes	Positive	92 (100)	3	29 (32)	6	54 (59)
Metastasis	Yes	64 (100)	1	25 (39)	6	32 (50)
Therapy	CH	71 (100)	1	26 (37)	4	40 (56)
	CH + SR	11 (100)	2	1 (9)	2	6 (56)
	CH + RT	15 (100)	1	3 (20)	3	8 (53)
	ST	5 (100)	0	1 (20)	0	4 (80)
TTF-1	Negative	22 (100)	0	8 (36)	1	13 (59)
	Positive	96 (100)	3	27 (28))	9	57 (59)
Napsin A	Negative	20 (100)	0	4 (20)	1	15 (75)
	Positive	69 (100)	2	20 (29)	7	40 (58)
ERCC1	Median (min–max)	73		0.1 (0–3)	1 (1–3)	0.75 (0–3)
EGFR	Wild Type	102 (100)	4	32 (31)	10	56 (55)
	Mutated	11 (100)	1	3 (27)	2	5 (45)
ALK	Negative	98 (100)	3	26 (26)	11	58 (59)
	Positive	4 (100)	0	2	1	1

CH – chemotherapy; SR – surgical resection; RT – radiotherapy; ST – symptomatic therapy. * WHO 2015 [18].

and napsin A had higher relative risk of death than those with a positive expression (p=0.011 and p=0.036, respectively) (Figure 2A, 2B). The best discrimination between ERCC1-positive and ERCC1-negative tumors was obtained when any positive cell was used as the definition of an ERCC1-positive tumor (ERCC1>0) and when no immunohistochemical reaction in any cell was used as the definition of an ERCC1-negative tumor (ERCC1=0). Using this definition, patients with ERCC1-positive tumors had 1.9 times higher relative risk for death (p=0.041) than patients with ERCC1-negative tumors (Figure 3A, 3B). Relative risk of death rose by 1.2 times whenever ERCC1 expression rose (p=0.12), with a level of significance of 90%. Relative risk of death was strongly associated with distant metastasis (p=0.001) and lymph nodes (p=0.059), with a level of significance of 94%. All patients were treated with the chemotherapy based on platinum, alone or in combination with surgical resection or radiotherapy. The patients who received only chemotherapy were compared with others. The patients who

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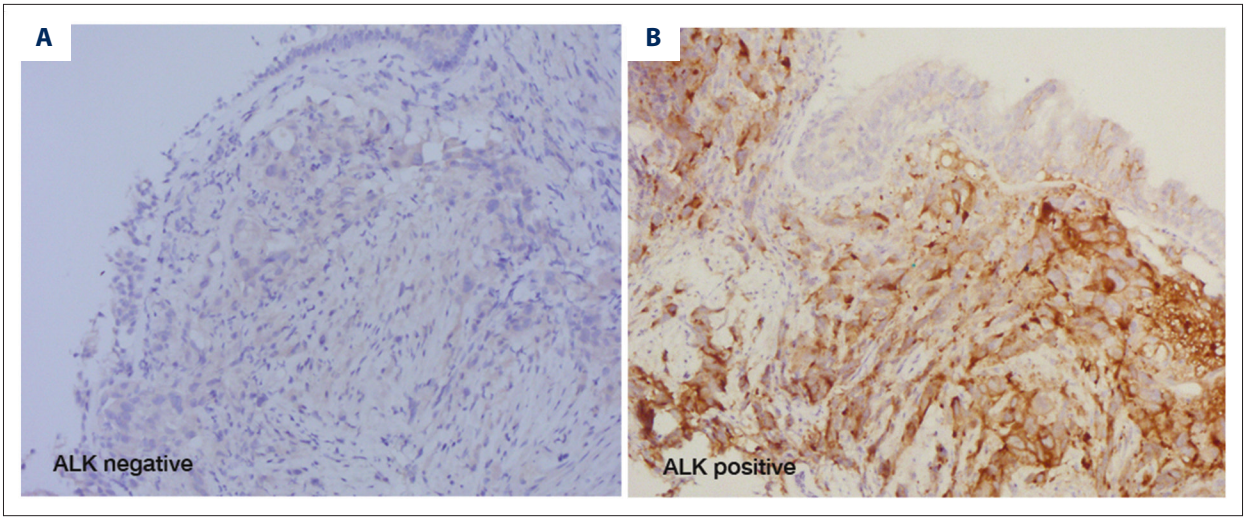

Figure 1. ALK immunohistochemistry in lung adenocarcinoma: (**A**) negative expression and (**B**) strong and diffuse positive cytoplasmic expression in *ALK* rearranged lung adenocarcinoma (ALK/HRP 200×).

Table 2. Mean and median of overall survival for patients with lung adenocarcinoma according to statistically significant variables.

Variable		Mean(SE); 95%CI	Median(SE); 95%CI	LR	*p*
TTF-1	Positive	13.3(1.2); 11–16	10(1); 8–12	7.3	0.007
	Negative	6.4(1,2); 4–8.9	5(2.3); 0.5–9.5		
Napsin A	Positive	13.4(1.4); 10.8–16	9(1.2); 6.6–11	4.9	0.026
	Negative	6(0.96); 4–7.9	8(5); 0–18		
N	No	19(2.5); 14–24	8(0.75); 6.5–9.5	4.1	0.042
	Yes	10(1.1); 8–12			
M	No	17.3(2); 13–21	19	15.6	<0.001
	Yes	8(1); 6–10	5(1); 3–7		
TNM	2	22.4(2,4); 17–27		16	<0.001
	3	13.7(2,4); 8.7–19	10(1.2); 7.7–12		
	4	8.1(1); 6–10	5(0.9); 3–7		
Therapy	CH	11.3(1.2); 9–14	8(0.8); 6–9.6	13.5	0.001
	CH + SR	22.4(2.4); 17–27			
	CH + RT	6(2.2); 1.7–10	2(0.5); 1.1–2.9		

CH – chemotherapy; SR – surgical resection,; RT – radiotherapy.

were treated with chemotherapy and radiotherapy had a 2.4 times higher relative risk of death than the patients treated only by chemotherapy (p=0.009). The patients who received only chemotherapy had a 5.7 times higher risk of death than patients who underwent surgical resection and chemotherapy (p=0.087), with a level of significance of 91%.

Discussion

Small biopsies are the most common tissue samples for the diagnosis of lung cancer, because 70% of patients present in advanced stages [13]. These data are largely in accordance with our observations: 72% adenocarcinomas were diagnosed on a small biopsy and 87.9% of patients presented in advanced stages. Solis et al. [19] found that solid subtype is a marker of unfavorable prognosis associated with advanced stages of disease compared with the acinar and lepidic subtypes. In our

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Table 3. Cox regression analysis for Oscensor in 76 patients who died of lung adencarcinoma.

Variable		RR	95% CI	*p*
Sex	Male	0.743	0.455–1.22	0.237
Age (years)	≤64	0.923	0.584–1.5	0.732
Subtype	Solid	1.12	0.876–1.42	0.376
TTF-1	Negative	2.0	1.2–3.5	0.011
Napsin A	Negative	1.9	1.0–3.6	0.036
EGFR	Mutated	0.717	0.287–1.8	0.475
T		1.24	0.938–1.64	0.131
N	Yes	2.66	0.96–7.4	0.059
M	Yes	3.5	1.75–6.9	<0.001
TNM	2			0.003
	3	3.9	0.495–32	0.194
	4	10.6	1.5–77	0.019
Therapy	CH			0.005
	CH + SR	0.176	0.024–1.3	0.087
	CH + RT	2.4	1.2–4.6	0.009
ERCC1	Positive	1.9	1.0–3.5	0.041
	Negative			

CH – chemotherapy; SR – surgical resection; RT – radiotherapy.

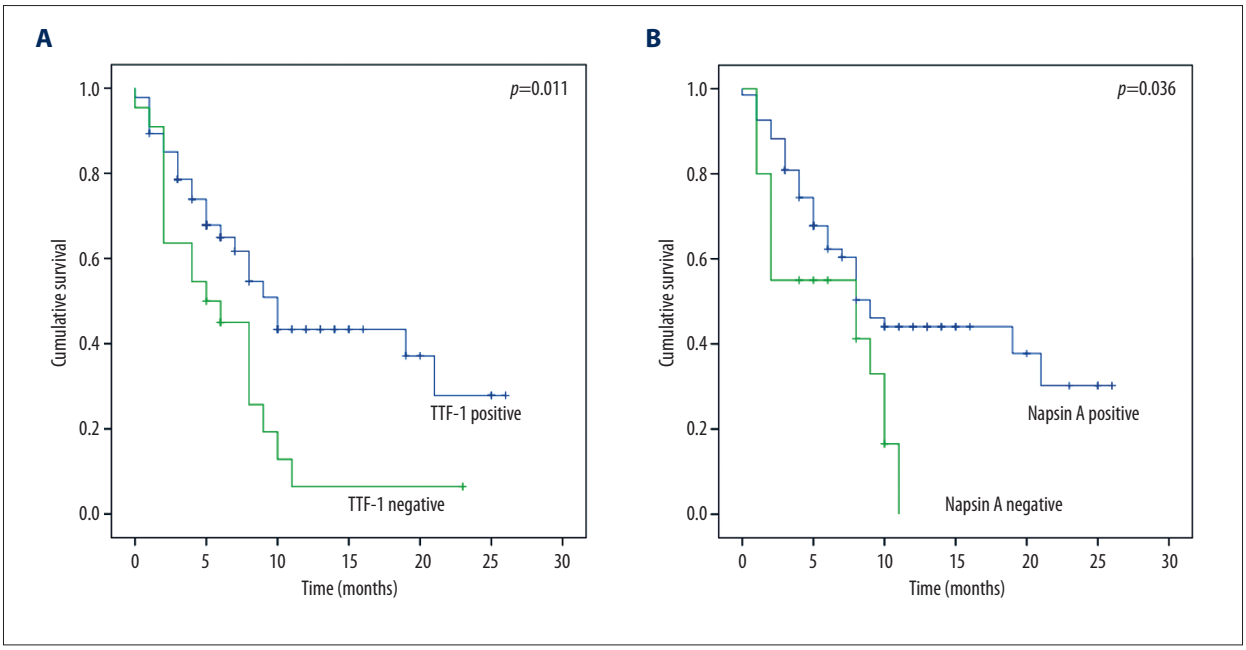

Figure 2. Analysis of lung adenocarcinoma patients survival according to TTF-1 and napsin A expression using the Kaplan-Meier method. Positive expression of TTF-1 and napsin A expression were associated with longer overall survival in patients with lung adenocarcinoma. (**A**) TTF-1 and (**B**) napsin A.

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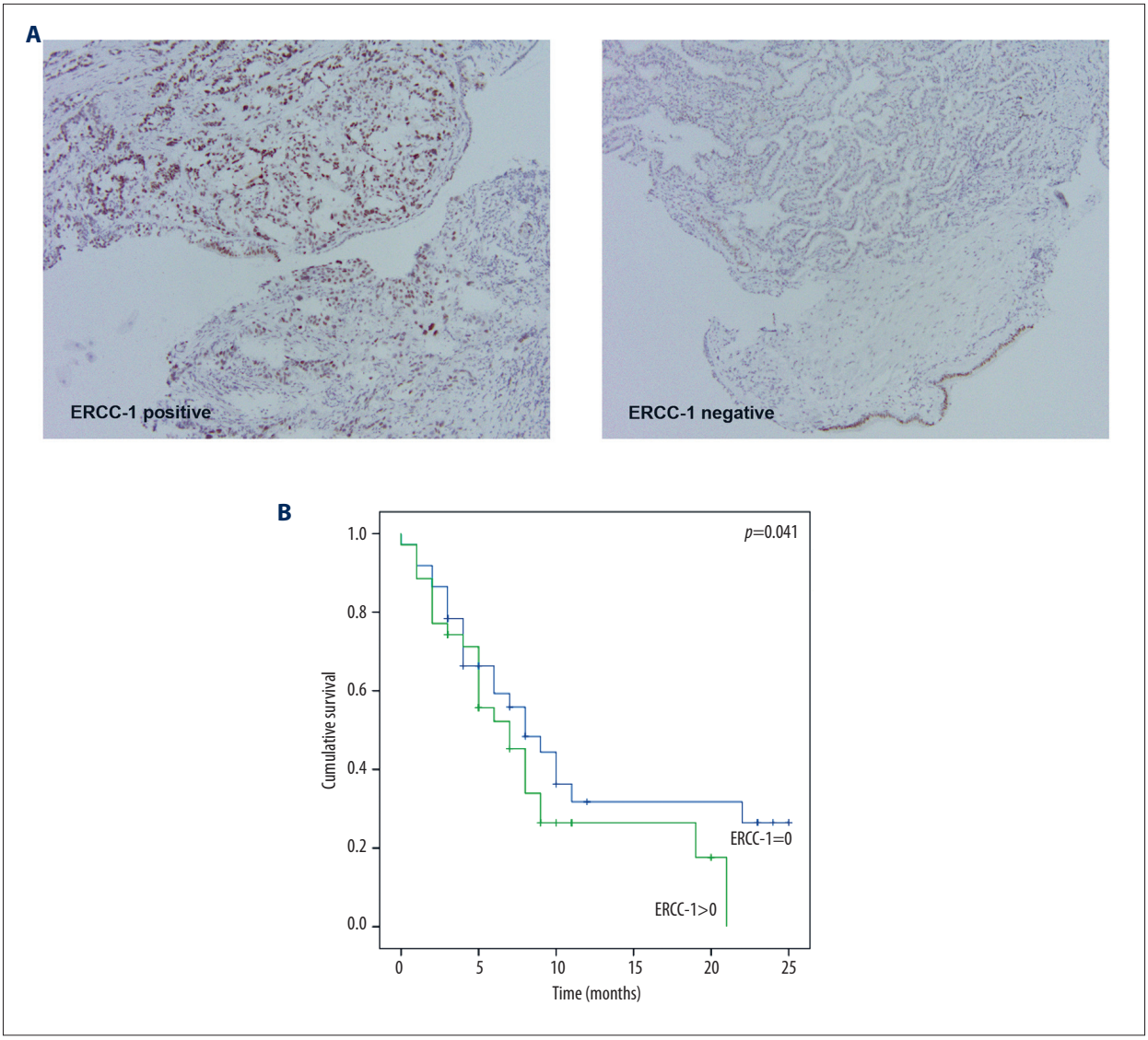

Figure 3. ERCC1 expression is associated with overall survival in patients with lung adenocarcinoma. (**A**) Positive and negative expression of ERCC1 in two cases of lung adenocarcinoma with respiratory epithelium as internal positive control (ERCC1/HRP 100×). (**B**) Among patients who were treated with platinum-based chemotherapy, the patients with negative expression of ERCC1 had longer overall survival.

study, the solid and acinar subtypes were more common in advanced stage of disease. More common distant metastases in the acinar subtype could be explained by lung adenocarcinoma histologic heterogeneity, resulting in a discrepancy with the final histologic diagnosis in resection specimen [13]. The TTF-1 and napsin A expressions were positive in 81% and 78% cases, respectively, and were not associated with the tumor subtype. Zhang et al. showed the similar results for both markers, with positivity in 85% cases [20]. TTF-1 regulates the expression of napsin A, which explain their common coexpression [4]. The lower number of napsin A-positive cases in our study could be related to technical problems in sampling [21]. To the best of our knowledge, we have not found available data on the prognostic significance of TTF-1 and napsin A expression analyzed exclusively on small biopsy specimens, but only on resected specimens [22,23]. Thus, the results reported herein extend the favorable prognosis related to a positive TTF-1 staining to the patients diagnosed only on a small biopsy specimen. The study highlights the decreased risk of distant metastases in TTF-1-positive patients. The overall survival was longer in TTF-1-positive and napsin A-positive patients. Ma et al. [23] showed that the expression levels of napsin A and TTF-1 are independent prognostic factors for survival. Zhan et al. concluded in meta-analysis that the overexpression of TTF-1 is associated with a favorable prognosis [22]. The reasons why TTF-1 is related to prognosis of patients with primary lung

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adenocarcinoma are unclear. Myong revealed that a positive TTF-1 staining has been inversely related to the proliferative activity evaluated through Ki-67 expression [24]. These observations suggest that TTF-1 and napsin A are not only useful diagnostic markers but also valuable prognostic markers in patients with lung adenocarcinoma and may play a role in molecular cancerogenesis. Overall survival was longer in patients without lymph node and distant metastasis and with a lower stage of disease, which is consistent with published data [2,14,16,17]. Patients who underwent surgical resection and chemotherapy had a lower relative risk of death. A possible explanation for this could be that surgical therapy was performed in patients with lower stages of disease. All of our patients were treated with platinum-based chemotherapy, which targets DNA and induces damage that cancer cells struggle to overcome. ERCC1 expression levels have been explored as a marker of DNA repair capacity in tumor cells [9–11]. In our study, high ERCC1 expression was associated with increased relative risk for death and decreased overall survival. This effect may be attributed to increased DNA repair of platinum-induced DNA adducts in ERCC1-positive patients. Although low ERCC1 expression is generally associated with sensitivity to platinum, the published results are not always consistent. Several studies demonstrate that NSCLC patients treated with platinum-based therapy and having ERCC1-negative tumors had an increased survival [9–12,25], but Booton et al. did not favor a prognostically better outcome [26]. Previous studies [8,27] observed that *EGFR* mutation was associated with lower level of ERCC1 expression, which was not confirmed in our study, probably because the number of patients with *EGFR* mutation was too low. It can be postulated that decreased ability to repair DNA damage may be correlated with increased genome instability and tumor mutations. We did not find a correlation between lung adenocarcinoma subtype and *EGFR* status. Chen et al. showed an association between lepidic, papillary, and acinar subtypes and higher *EGFR* mutation rate, while the solid and mucinous components were associated with lower *EGFR* mutation rate [28]. Prognostic value of *EGFR* mutation in patients who did not receive TKIs was rarely reported [29]. In our study average survival of our patients according to *EGFR* status was not significantly different. *EGFR* mutation and ALK expression were mutually exclusive, as in the published literature [30].

Conclusions

Following guidelines for good practice of small biopsy samples in patients with lung adenocarcinoma, we established a tissue management strategy to ensure the optimal treatment of patients who can benefit from new targeted therapies. Our data confirmed that TTF-1 and napsin A are not only useful diagnostic markers but also valuable prognostic markers in patients with lung adenocarcinoma. Positive ERCC1 expression was identified as a negative prognostic marker in patients treated with platinum-based therapy. The percentages of EGFR and ALK mutations corresponded to those in previously published reports for Caucasian patients.

Conflict of interest

The authors declare no conflict of interest.

References:

1. Šekerija M, Bubanović LJ, Glamočanin S et al: Cancer incidence in Croatia – 2012. Croatian institute of public health; Croatian National Cancer Registry Zagreb, 2014; 37: 3–10
2. König K, Peifer M, Fassunke J et al: Implementation of amplicon parallel sequencing leads to improvement of diagnosis and therapy of lung cancer patients. J Thorac Oncol, 2015; 10: 1049–57
3. Fassina A, Cappellesso R, Simonato F et al: Fine needle aspiration of non-small cell lung cancer: Current state and future perspective. Cytopathology, 2012; 23(4): 213–219
4. Mukhopadhyay S, Katzenstein AL: Subclassification of non-small cell lung carcinomas lacking morphologic differentiation on biopsy specimens: Utility of an immunohistochemical panel containing TTF-1, napsin A, p63 and CK5/6. Am J Surg Pathol, 2011; 35: 15–25
5. Turner BM, Cagle PT, Sainz IM et al: Napsin A, a new marker for lung adenocarcinoma is complementary and more sensitive and specific than thyroid transcription factor 1 in the differential diagnosis of primary pulmonary carcinoma: Evaluation of 1674 cases by tissue microarray. Arch Pathol Lab Med, 2012; 136: 163–71
6. Barletta JA, Perner S, Iafrate AJ et al: Clinical significance of TTF-1 protein expression and TTF-1 gene amplification in lung adenocarcinoma. J Cell Mol Med, 2009; 13: 1977–86
7. Stoll LM, Johnson MW, Gabrielson E et al: The utility of napsin-A in the identification of primary and metastatic lung adenocarcinoma among cytologically poorly differentiated carcinomas. Cancer Cytopathol, 2010; 118: 441–49
8. Ren S, Chen X, Kuang P et al: Association of EGFR mutation or ALK rearrangement with expression of DNA repair and synthesis genes in never-smoker women with pulmonary adenocarcinoma. Cancer, 2012; 118: 5588–94
9. Martin LP, Hamilton TC, Schilder RJ: Platinum resistance: The role of DNA repair pathways. Clin Cancer Res, 2008; 14: 1291–95
10. Reed E: Platinum-DNA adduct, nucleotide excision repair and platinum-based anti-cancer chemotherapy. Cancer Treat Rev, 1998; 24: 331–44
11. Ryu JS, Memon A, Lee SK: ERCC1 and personalized medicine in lung cancer. Ann Transl Med, 2014; 2: 32
12. Besse B, Olaussen KA, Soria JC: ERCC1 and RRM1: Ready for prime time? J Clin Oncol, 2013; 31: 1050–60
13. Travis WD, Brambilla E, Noguchi M 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: 668–84
14. Ma BB, Hui EP, Mok TS: Population-based differences in treatment outcome following anticancer drug therapies. Lancet Oncol, 2010; 11: 75–84
15. Gao B, Sun Y, Zhang J et al: Spectrum of LKB1, EGFR, and KRAS mutations in chinese lung adenocarcinoma. J Thorac Oncol, 2010; 5: 1130–35
16. Eberhard DA, Johnson BE, Amler LC et al: Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol, 2005; 23: 5900–9

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17. Shaw AT, Yeap BY, Mino-Kenudson M et al: Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol, 2009; 27: 4247–53
18. Travis WD, Noguchi M, Yatabe Y et al: Adenocarcinoma. In WHO classification of tumours of the lung, pleura, thymus and heart, ur. Travis WD, Brambilla E, Burke AP et al. IARC Lyon, 2015; 26–37
19. Solis LM, Behrens C, Raso MG et al: Histologic patterns and molecular characteristics of lung adenocarcinoma associated with clinical outcome. Cancer, 2012; 118: 2889–99
20. Zhang P, Han Y.P, Huang L et al: Value of napsin A and thyroid transcription factor-1 in the identification of primary lung adenocarcinoma. Oncol Lett, 2010; 1: 899–903
21. Lindeman NI, Cagle PT, Beasley MB et al: Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Thorac Oncol, 2013; 8: 823–59
22. Zhan P, Qian Q, Wan B et al: Prognostic value of TTF-1 expression in patients with non-small cell lung cancer: A meta-analysis. Trans Cancer Res, 2013; 2: 25–32
23. Ma Y, Fan M, Dai L et al: The expression of TTF-1 and napsin A in early-stage lung adenocarcinoma correlates with the results of surgical treatment. Tumour Biol, 2015; 36(19): 8085–92
24. Myong NH: Thyroid transcription factor-1 (TTF-1) expression in human lung carcinomas: its prognostic implication and relationship with expressions of p53 and Ki-67 proteins. J Korean Med Sci, 2003; 18: 494–500
25. Holm B, Mellemgaard A, Skov T, Skov BG: Different impact of excision repair cross-complementation group 1 on survival in male and female patients with inoperable non-small-cell lung cancer treated with carboplatin and gemcitabine. J Clin Oncol, 2009; 27(26): 4254–59
26. Booton R, Ward T, Ashcroft L et al: ERCC1 mRNA expression is not associated with response and survival after platinum-based chemotherapy regimens in advanced non-small cell lung cancer. J Thorc Oncol. 2007; 2: 902–6
27. Yamashita F, Azuma K, Yoshida T et al: Prognostic value of EGFR mutation and ERCC1 in patients with non-small cell lung cancer undergoing platinum-based chemotherapy. PLoS One, 2013; 8: 1–8
28. Chen Z, Liu X, Zhao J et al: Correlation of EGFR mutation and histological subtype according to the IASLC/ATS/ERS classification of lung adenocarcinoma. Int J Clin Exp Pathol, 2014; 7: 8039–45
29. Inamura K, Ninomiya H, Ishikawa Y, Matsubara O: Is the epidermal growth factor receptor status in lung cancers reflected in clinicopathologic features? Arch Pathol Lab Med, 2010; 134: 66–72
30. Doval DC, Prabhash K, Patil S et al: Clinical and epidemiological study of EGFR mutations and EML4-ALK fusion genes among Indian patients with adenocarcinoma of the lung. Onco Targets and Therapy, 2015; 8: 117–23

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]