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THE ROLE OF K-ras, p53 AND CLINICOPATHOLOGICAL FACTORS IN SUBSTAGING OF RESECTED PATIENTS WITH pIIIa – N2 NSCLC

ULOGA K-ras I p53 GENA, KLINIČKIH I PATOLOŠKIH PARAMETARA U SUBSTEJDŽINGU OPERISANIH PACIJENATA SA pIIIa – N2 NESITNOĆELIJSKIM KARCINOMOM PLUĆA

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non-small cell lung cancer, K-ras, p53, substaging

Ključne reči
nesitnoćelijski jkarcinom pluća, K-ras, p53, substejdžing

Abstract

The present study comprised 60 consecutive patients with a median age of 58 years (range 43 – 69) and histopathologically proven NSCLC (postoperative hystopathological diagnosis confirmed 20 adenocarcinomas, 32 squamous cell, 3 adenosquamous and 5 large cell carcinomas, respectively) who underwent a thoracotomy with a curative intent and were confirmed to have positive ipsilateral mediastinal lymph nodes. Patients were submitted to postoperative adjuvant radiotherapy according to split-course protocol. Apart clinical parameters (age, clinical stage, clinical N and T status, hystological type, grade of differentiation), we assesed K-ras and p53 genes mutations and correlated those items with overall survival of the patients. The follow-up period was 72 months. The Kaplan-Mayer method was used to estimate the probability of disease-free period and survival as a function of time. Statistically significant influence on survival was assignated for age, clinical nodal and tumor status, and also for K-ras and/or p53 genes mutations presence in tumor. Conclusion: This study has identified K-ras mutation presence, p53 mutations presence, N2, T3, advanced age (over 65 years) NSCLC prognostic subgroups and suggests different therapeutic approach according to the subgroup profile.

INTRODUCTION

Lung cancer is by far the leading cause of cancer death worldwide. According to the World Health Organization lung cancer killed 1.2 million people all over the world, and according to the American Cancer Society caused 157,000 deaths solely in the U.S. in 2010. The five-year survival rate is only 15 percent. Lung cancer is the leading cause of cancer death not only in men but also in women and is responsible for as many deaths as breast cancer and all gynecological cancers combined (1). Women even may be more susceptible to tobacco smoke and potentially at higher risk to lung cancer development, due to induction by sex hormons and higher expression of some genes – e.g. cytochrome P4501A1 gene and gastrin-releasing peptide receptor gene (2). Lung cancer deaths rates among women leveled off for the first time between 1995 and 2001, after continuously increasing for many decades. The therapy of non-small cell lung cancer (NSCLC) has reached a plateau in improving patient survival, with overall disappointing results. Clinical data are currently available on targeted agents in the treatment of NSCLC, focusing on epidermal growth factor receptor family inhibitors, angiogenesis inhibitors, signal transduction inhibitors, eicosanoid pathway inhibitors, vaccines and gene therapy.

Accurate staging of non-small-cell lung cancer (NSCLC) determines prognosis and facilitates decisions regarding treatment options. Since novel therapeutic strategies did not improve survival considerably, early diagnosis and creation of individual therapeutic protocols based on more precise subclassification profiles still remain the only promising tools for attempting to attain better outcome. It is exceedingly important to identify high-risk populations and to better understand the carcinogenic process.

Patients suffering from N2 non-small-cell lung cancer (NSCLC), with ipsilateral mediastinal lymph node involvement, are generally considered by most physicians to have a locally advanced disease resulting in a poor outcome, although these

patients can be divided into heterogeneous subpopulations, such as those with bulky extranodal metastases and those with either multiple or single N2 involvement (3). A number of controversies plague this subgroup of patients with respect to staging and treatment, which often makes it difficult to interpret the large amount of literature data concerning these patients. Two decades ago, Naruke et al., Martini et al. and Pearson et al. reported that surgery could cure a small proportion of these patients (4, 5, 6). Since then, many series (7) have reported 5-year survival rates ranging from 6% to 35% after surgery. These series suggested that preoperative detection of N2 disease, involvement of multiple lymph node levels, subcarinal involvement, and an adenocarcinoma subtype were associated with a worse prognosis (7). The small number of patients included in these studies and the differences in inclusion criteria clearly account for the heterogeneity of these results and for the confusion concerning the prognosis of N2 patients. Against this background, the indications for surgery for N2 NSCLC remain somewhat ambiguous. Several prognostic factors have recently been defined by historical studies, which have shown that complete resection, single lymph node metastasis, cN0-1 factor, low pT factor, and small tumor size are predictors of better prognosis in these patients (3, 8, 9). However, the type of patients with N2 NSCLC who can benefit most from a thoracotomy has not been clarified.

A recent meta-analysis found no benefit for resected N2 disease treated with postoperative radiotherapy (10).

K-ras and p53 genes and their role in cancerogenesis of lung cancer

Family of ras oncogenes

A distinguishing biochemical hallmark of members of the Ras superfamily is their ability to bind guanine nucleotides with high affinity and their ability to hydrolyze bound GTP to GDP and phosphate. Ras serves as a molecular switch for cellular growth and differentiation by cycling between an active, GTP-bound and an inactive, GDP-bound form. Upon stimulation of membrane receptors, growth-factor-induced activation of Ras is reflected in an observed increase in cellular Ras-GTP content.

It is now believed that tyrosine kinases e.g. EGF receptor serve to phosphorylate some intermediary proteins which stimulate GDP dissociation from inactive Ras protein, and GTP binding, thus promoting Ras protein activation. When activated, Ras allows further signal transduction in a process of kinase cascade. The last kinase results in the phosphorylation of several cellular proteins including the transcription factors Myc and Jun (11). In this manner activated Ras ultimately results in altered gene expression. If ras gene is somehow altered it encodes for mutated protein which is constitutively active – it keeps on transmitting growth signal either stimulated by growth factor or not. In this way, malignant cell is constantly proliferating, which is one of crucial characteristics of malignant phenotype (12).

The predominant type of abnormalities of ras genes in human cancers is gene amplification or point mutations at codons 12, 13 or 61. In non-small lung cancer (NSCLC) it*s just the point mutations at the codons mentioned above that were detected (13). The predominant type of base substitution is G*T transversion (11), which is rarely detected in other tumor types (colorectal carcinoma or adenocarcinoma of pancreas). This suggests that different etiologic factors induce different types of base exchanges. The ras genes have been shown to be mutationally activated in a number of environmental cancers in humans and in a carcinogen-specific manner in experimental animals. There are a lot of evidences that chemical carcinogens (benzoapyrens) from tobacco smoke induce G*T base substitution in ras genes in lung carcinoma (14).

The incidence of ras gene mutations detected in resected specimens of NSCLC is relatively high, and varies among different histological types: in epidermoid carcinoma of the lung it*s 12 – 42%, in adenocarcinoma is even higher 30 – 56% (15), and in large cell carcinoma is rather low 27% (or even much lower according to other authors). Ras gene mutations in NSCLC are associated with shortened survival (16).

K-ras mutations were found in plasma DNA, so detection of tumor DNA in plasma is feasible using molecular techniques and this approach shows promise for monitoring patient response to therapy (17). A patient with clinical progressive disease retained the mutant DNA, while in a patient with a complete response , the K-ras mutation was no longer detectable.

One of the ways to improve the effects of radiotherapy should be the prediction of the response of the tumor and normal tissue in individual patients that would lead to tailored treatments (18).

Although radioresistance mediated by ras oncogenic transformation has been demonstrated in a number of different cell lines (19), little is known about mechanisms that underlies the phenomenon. Radiation kills cells almost entirely due to DNA damage, and the double strand break is the critical lesion for cell lethality. Since they are direct stimulus for apoptosis, demonstrated to be the main mechanism of radiation-induced cell death. Cellular resistance to radiation therefore results from impairment of apoptotic cell death. The role of ras genes in apoptosis is not clear yet. Undoubtedly, it is not directly involved in induction, but promotes apoptosis in an indirect way. If Ras is constitutively expressed (when ras gene is mutated) it lowers constitutive levels of Ca2+/Mg2+ endonuclease activity, the enzymes that execute apoptosis (20).

The maintenance of cellular response to radiation is dependent on the interaction of a cell membrane components with exogenous (or endogenous) thiols, and this signal has to be transmitted from the plasma membrane into the cell (20). Considering its strategic localization (at the inner surface of cell membrane) and function, we speculate that Ras may be involved in the control of this signal transmission and thus in the cell response to radiation.

The Ras-like small GTPases RalA and RalB are downstream signaling molecules that exert Ras function and are critical for both tumor growth and survival. In animal studies, the Ral effector RalBP1/RLIP76 mediates survival of mice after whole-body irradiation, but the role of the Ral GTPases themselves in response to ionizing irradiation is still unclear (22).

Oncogenic Ras can be a potent trigger of p53, but lately it was observed that p53-mediated tumour suppression is triggered only when oncogenic Ras signal pathway exceeds a critical threshold indicating that low-level oncogenic Kras could not engage p53 sufficiently to restrain early tumour evolution (23).

p53 tumor suppressor gene

The p53 gene encodes a transcription factor that binds specifically to DNA as a tetramer. The p53 protein has a short half life and is normally expressed at low levels in most cells and tissues. Cellular stress such as DNA damage, hypoxia, and certain oncogenes, causes accumulation of the p53 protein by protein stabilisation due to the prolonged half-life of the protein, thus triggering a p53-dependent biological response. Protein p53 initiates these responses by transcriptional transactivation of a set of target genes containing specific p53 binding motifs.

Wt p53 gene seems to act as a recessive tumor suppressor gene whereas mutant forms can behave as dominant oncogenes. Gene mutations seem to be the predominant mechanism of p53 oncogenic transformation. Nonfunctional or mutated p53 protein is found in a myriad of solid tumors in humans. Depending on the tissue source and the method of detection, findings indicative of abnormal p53 function are present in 50% of non-small-cell lung cancer patients up to 70% of small cell lung cancer specimens studied. The normal protein (wild type – wtp53) has been shown to play a role in cell cycle control, arrest in G1 checkpoint, DNA repair, apoptosis and genomic fidelity – thus called “guardian” of the genome (24).

Although the effect of loss of p53 function on outcome in NSCLC has yet to be fully determined, there are some indications as to why it may be deleterious in this neoplasm. Exposure to either radiation or other DNA damaging agents is known to induce both a G1 and G2 arrest. Recent studies have shown that the role of wt p53 is in part to function as part of feedback control pathway leading to the G1 arrest observed after exposure to DNA damaging agents (25). Tumor cells that either lack p53 expression or that express dominant negative mutants of the protein do not exibit a radiation-induced G1 arrest, although they continue to arrest in G2 in response to radiation. These findings suggest that the normal p53 gene product may function to allow cells to repair damaged DNA before entering S phase; entry into S phase once DNA has been damaged has been suggested to lead to both increased aneuploidy and increased cell killing by DNA-damaging agents.

That made it plausible to assume that the efficiacy of clinical radiotherapy is, at least in part, caused by tumor cells without functioning p53 being more susceptible to the lethal effects of DNA-damaging agents as they would replicate in spite of unrepaired DNA damage. p53 is believed to confer radioresistance through inhibition of radiation-induced apoptosis (25).

Considering cancer prograssion and metastatic process, it was shown that p53 mutations usually occur before the establishment of lymph node metastasis, and subsequently persist in the metastatic nodes (26)

The identification of novel reliable prognostic factors in pIII – N2 NSCLC is important for improval of prognosis and many studies dealing with its detection are being conducted. In addition to a variety of clinical characteristics, it has recently been demonstrated that biological features of the tumor may affect the postoperative survival and sensitivity to different therapeutic modalities apart surgery. Molecular findings could even have greater impact on patients survival than clinical and pathological characteristics of the tumor.

We prospectively studied patients with N2 NSCLC treated at Clinic for Cardiothoracic Surgery at Military Medical Academy by analyzing prognostic factors to identify subpopulations with good operative indications and those who need adjuvant therapeutic approaches.

Objective: In the present study we attempted to clarify the role of different clinical (age, cTNM, cN, pT) and pathological (histological type of tumor and grade of differentiation) factors as well as K-ras and p53 genes mutations which separate patients into high and low - risk groups based on the probability of survival.

PATIENTS AND METHODS

Patients

The records of 60 patients with histopathologically verified NSCLC who underwent a thoracotomy and were confirmed to have positive ipsilateral mediastinal lymph nodes between January 1997. and September 2000. at Clinic for Cardiothoracic Surgery at Military Medical Academy, were reviewed. Forty eight (80%) were men and 12 (20%) were women. Median age for all patients was 58 years (range 43 – 69 years).

Clinical staging was decided by bronchoscopy, chest CT scan, brain CT or magnetic resonance imaging results, as well as those from upper abdominal CT and bone scintigraphy examinations, that were used to detect distant metastases. A preoperative histological and cytological diagnosis (bronchoscopical tissue sample and sputum cytology) were performed and indicated histological subtype of NSCLC. Postoperative hystopathological diagnosis confirmed 20 adenocarcinomas, 32 squamous cell, 3 adenosquamous and 5 large cell carcinomas, respectively.

According to preoperative (clinical) stage of the disease, 16 had cIIIa (26.7%), 12 had cIIb (20%), 12 were cIIa (20%) and 20 were cIb stage (33.3%) of the NSCLC respectively. Among the cohort of patients 20 of them (33.3%) had cN0, 31 patient (51.7%) had cN1 and 9 patients (15%) had cN2 disease respectively. Postoperative determination of T status confirmed 16 patients (26.7%) having pT1, 33 (55%) having pT2 and 11 (18.3%) having pT3 status, respectively.

We considered patients to be completely resected since we performed complete tumor extirpation (without a macroscopic residual lesions and with microscopic free margins around the tumor site) and radical lymph node dissection. As for lymph node dissection, in patients with a tumor in right upper-middle lobe, the superior mediastinal, paratracheal, pretracheal, tracheobronchial, and subcarinal nodes were removed. In those with a tumor in the left upper lobe, the tracheobronchial, subaortic, para-aortic, and subcarinal nodes were removed. In addition to these nodes, paraesophageal and pulmonary ligament nodes were dissected in patients with tumors in both lower lobes. The pretracheal node in patients with a left-side tumor and the anterior and posterior mediastinal nodes were optional. Patients with pleural dissemination or malignant pleural effusion were excluded from the study as they were considered as having incomplete resection, even if there was no macroscopic residual lesions after the pulmonary resection. The mediastinal lymph node was pathologically confirmed to be metastatic in all patients who underwent pulmonary resection. Preoperative diagnosis was performed by using chest radiography and computed tomographic (CT) imaging, as well as fiberoptic bronchoscopy for pulmonary nodules. Mediastinal nodes larger than 1 cm in the short axes were defined as clinical N2 disease. A mediastinoscopy was carried out in selected patients with suspected cN2 disease in imaging study. Postoperative staging was performed according to the 1997 TNM classification (27). Adjuvant radiation therapy was performed in all cases, according to split-course protocol.

Tumor specimens were obtained at thoracotomy with curative intent. The resected material was snap frozen in liquid nitrogen and stored at -70ºC until analysis. Genomic DNA was extracted according to standard procedure with fenol/chloroform/isoamylalcohol. Concentration and purity of isolated DNA was measured by spectrophotometer. First exon of K-ras gene and exons 5, 6, 7 and 8 of p53 gene were amplified utilizing designed specific primers. Mutations were detected by SSCP.

The follow-up period was 72 months. We chose date of tumor acquisition as the time point for calculation of disease free period and survival. Control exams were performed in three months intervals to evaluate remission period and time of relapse of malignant disease.

Statistical Analysis

Disease-free survival was defined as the period from the date of initial surgical treatment to the development of disease recurrence or distant metastasis. Overall survival denotes the period from the date of initial treatment. The probability of survival was calculated by using the Kaplan-Meier method (28). The prognostic influence of variables on survival was analyzed by using a log-rank test and Willcoxon test.

RESULTS

The median follow-up period was 36 months (range 24 – 72months) for 55 patients who were eligible.

The Kaplan-Mayer method was used to estimate the probability of survival as a function of time. Median value of survival for all patients (pts.) was 19 months (confidence interval 16 to 22 months), probability of survival p=0.1164, and median value of recurrence-free period for 55 patients with pIIIa – N2 NSCLC was 14 months with confidence interval 11 to 17 months, p=0.115 (figures 1 and 2). During 72 months of follow up 48 pts. (77.27%) got a relapse of malignant disease (36 pts. got distant metastasis, and

12 pts. suffered from loco-regional recurrence). Seven pts. (12.73%) were free of malignant disease at the end of the period. At the end of the follow up period 6 pts. were alive (11%), and 49 pts. died (89%).

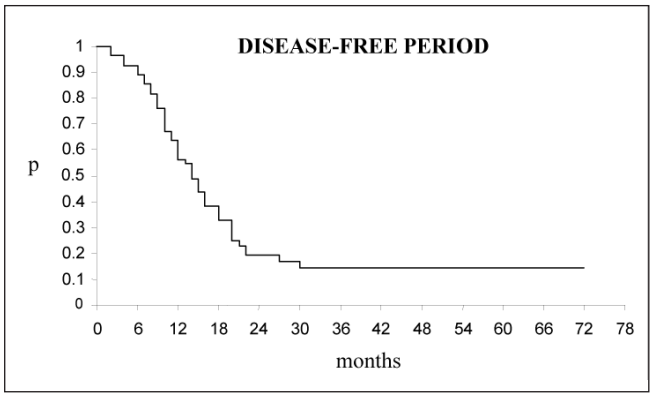

Figure 1 – Recurrence-free period – probability of disease-free period for patients with pIIIa – N2 NSCLC who underwent complete resection

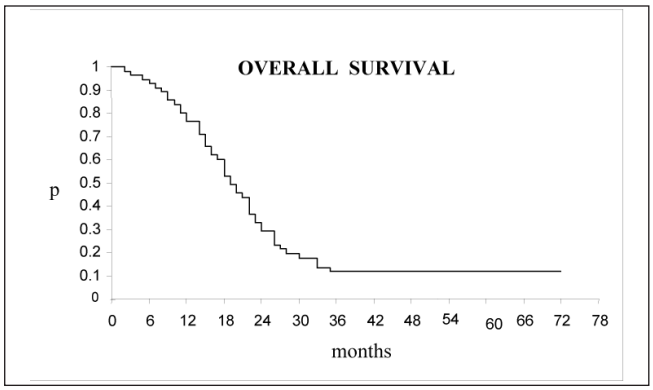

Figure 2 – Overall survival for patients with pIIIa – N2 NSCLC who underwent complete resection

The group of patients (pts.) who were eligible for follow up consisted of 1 patient with adeno-squamous carcinoma (1.8%), 18 pts. with adenocarcinoma (32.7%), 31 pts.with squamous cell carcinoma (56.4%) and 5 macrocellular carcinoma (9.1%). Probability of survival did not show significant differences according to histological type of tumor (figure 3).

Probability of survival for patients with pIIIa – N2 NSCLC who underwent complete resection also did not show statistically significant difference with relation to grade of differentiation (fig. 4). Characteristics of pathological findings showed no statistically significant prognostic factors.

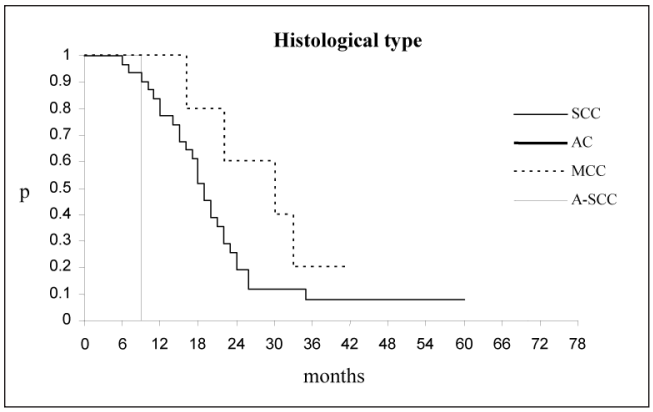

Figure 3 – Probability of survival (p) for patients with pathological stage IIIa – N2 NSCLC who underwent complete resection according to histological type of tumor

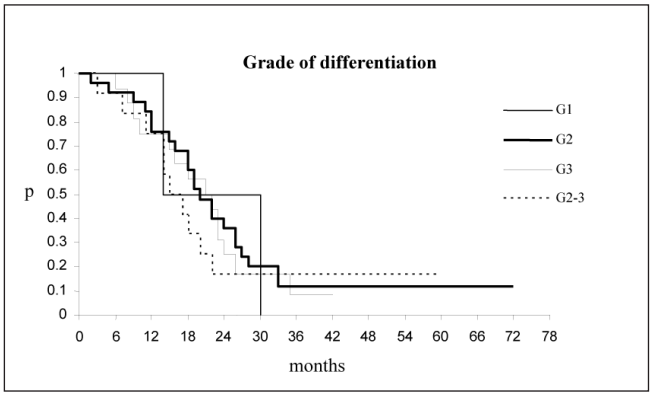

Figure 4 – Probability of survival (p) for patients with pathological stage IIIa – N2 NSCLC who underwent complete resection according to grade of differentiation

With respect to age of patients we found significant difference in probability of survival for patients older than 65 years who had significantly worse prognosis compared to those under 65 years (p=0.008) which is presented at figure 5. There was no significant difference in survival between pts. with different clinical stages (fig. 6). Regarding other clinical characteristics of patients, we found statistically significant difference in prognosis of patients with different cN status (p=0.0185), presented at figure 7. We confirmed that patients with cN0 had median survival 18 months with 95% confidence interval 13 to 23 months, whilst cN2 patients had median value of survival probability 15 months (12 to 16 months). Other significant difference was found between patients with different T status: T3 patients had unfavourable prognosis compared to pts. with T1 status (p=0.046) according to Willcoxon test (fig. 8).

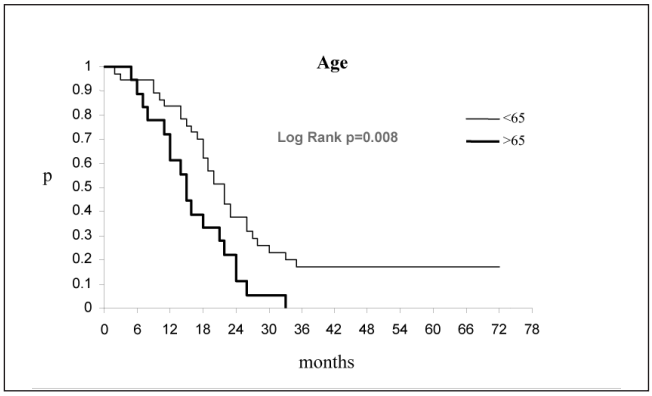

Figure 5 – Probability of survival (p) for patients with pathological stage IIIa – N2 NSCLC who underwent complete resection according to age of the patient

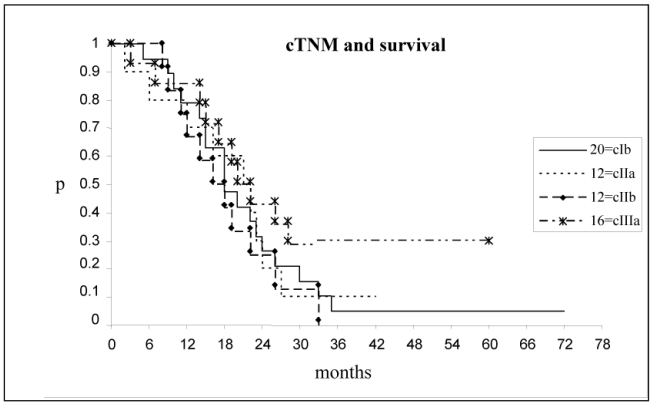

Figure 6 – Probability of survival (p) for patients with pathological stage IIIa – N2 NSCLC who underwent complete resection according to clinical stage

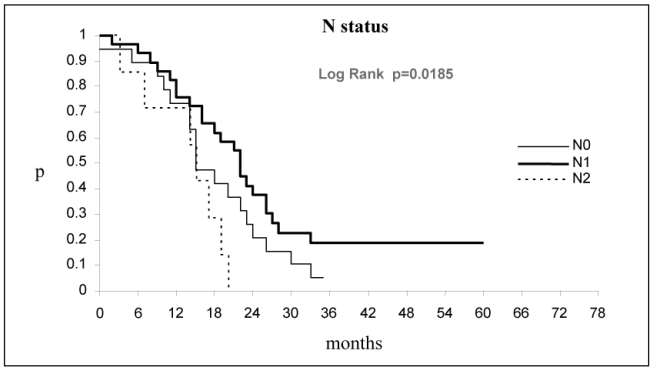

Figure 7 – Probability of survival (p) for patients with pathological stage IIIa – N2 NSCLC who underwent complete resection according to clinical nodal status

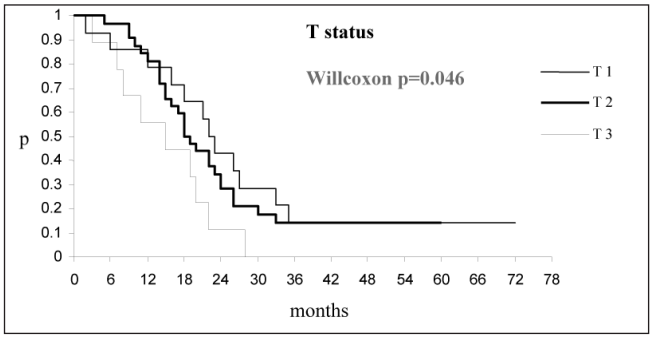

Figure 8 – Probability of survival (p) for patients with pathological stage IIIa – N2 NSCLC who underwent complete resection according to postoperative T status (Willcoxon p= 0.046)

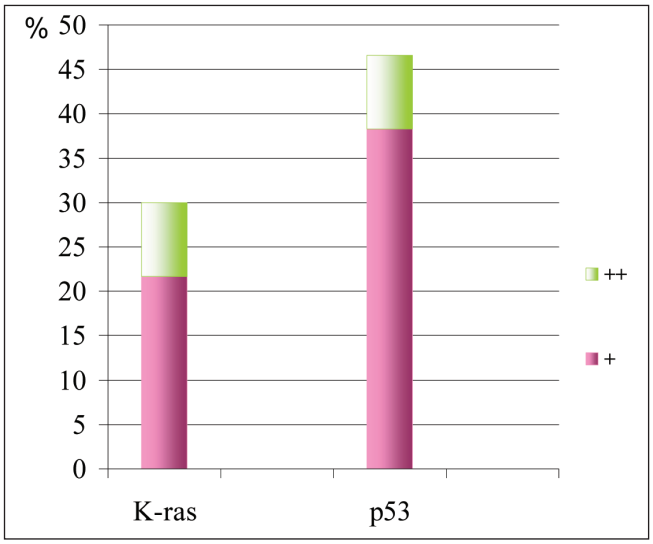

Figure 9 – Detection of mutation in K-ras and p53 genes (+ mutation in one gene, ++ mutations in both genes)

We examined 60 tumor specimens for K-ras and p53 mutations and detected K-ras exon I point mutations in 18 tumors (30%) and p53 mutations in exons 5, 6 or 7 in 28 tumors (46.7%). Mutations in both oncogenes were present in 5 tumor tissues (8.3%).

Mutations in K-ras oncogene were detected in 40.6% of squamous cell carcinomas, 20% adenocarcinomas and 20% large cell carcinomas.

Out of the 60 tumor specimens mutations were detected in codons 5, 6 or 7 of p53 tumor suppresor gene: 13 mutations in exon 5, 5 mutations in exon 6 and 11 mutations in exon 7 (one tumor had double mutation in both exons 5 and 7). No exon 8 mutations were detected in our tumor collection.

Distribution of mutation according to histological type of the tumor is as follows: 59.4% in squamocellular carcinoma, 40% in large cell, 33.3% in adenosquamous and 30% in adenocarcinoma respectively.

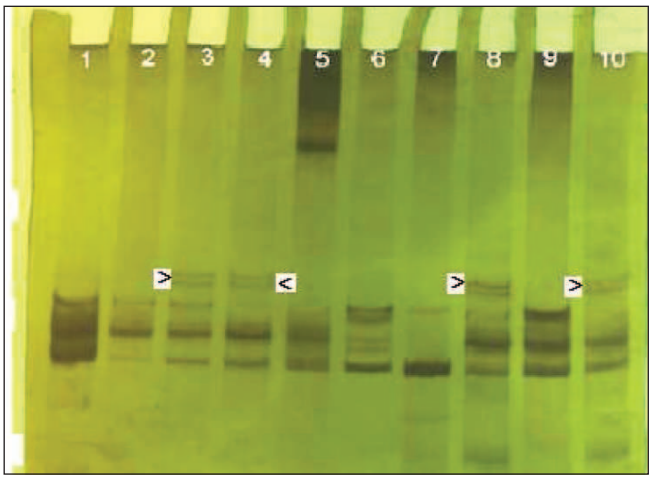

Figure 10 – SSCP of K-ras exon I mutationsLines 1 i 2 are control DNA (from lymphocytes of healthy persons) Lines 3 – 10 are genomic DNA from tumor tissues (mutations in lines 3, 4 , 8 and 10)

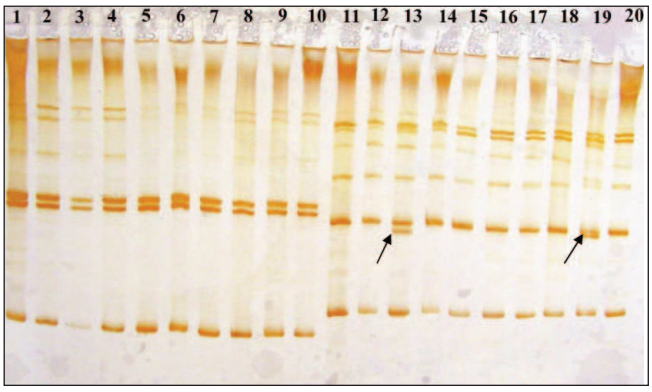

Figure 11 – SSCP analysis of exon 8 mutations (lanes 1 – 10) and exon 5 mutations of p53 gene (lanes 11 – 20). Controls in lanes 10 and 20. Mutations are detected in exon 5, lanes 13 and 19.

The Kaplan-Mayer survival curve demonstrates that K-ras positive pts. had probability of disease-free period (DFP) 11 months (confidence interval 8-14 months) whileas K-ras negative pts. had significantly longer DFP of 16 months (11-21 months) with p=0.0071 (figure 12). At the end of follow up period 100% of K-ras posiive patients died, whilst in a group of K-ras negative pts. 24.32% of pts survived.

Median value of probability of overall survival for K-ras positive pts. was 15 months (confidence interval 13 – 17 months) , and K-ras negative pts. had median value of probability of overall survival 22 months (18 – 26 months). Statistical significance is p=0.008 (figure 13).

Analysis od DFP for p53 positive and p53 negative pts. confirmed that p53 positive pts. lived considerably shorter (12 months with confidence interval 9 to 15 months) compared to p53 negative pts. (18 months with confidence interval 14 to 22 months), p=0.0177 (figure 14).

Median value of probability of overall survival for p53 positive pts. was 18 months (confidence interval 15 – 21 months) , and p53 negative pts. had median value of probability of overall survival 22 months (17 – 27 months), statistical significance is p=0.021 (figure 15). All p53 positive pts. died during the follow up period, whilst in a group of pts. without p53 mutations 21.43% of pts. were alive at the end of the period.

DISCUSSION

In the performed study overall survival for patients with pathologically proven IIIa – N2 NSCLC was 11% at the end of follow up period (6 years). In other studies which comprised 5-year prognosis, survival is a bit higher, probably due to the shorter follow up

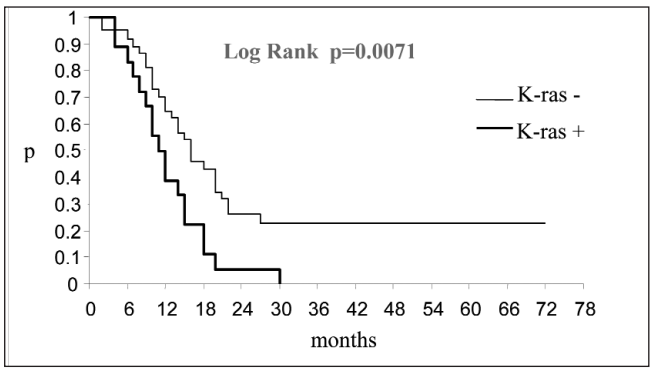

Figure 12 – Probability of disease free period (p) for patients with pathological stage IIIa – N2 NSCLC who underwent complete resection according to the presence of K-ras mutations

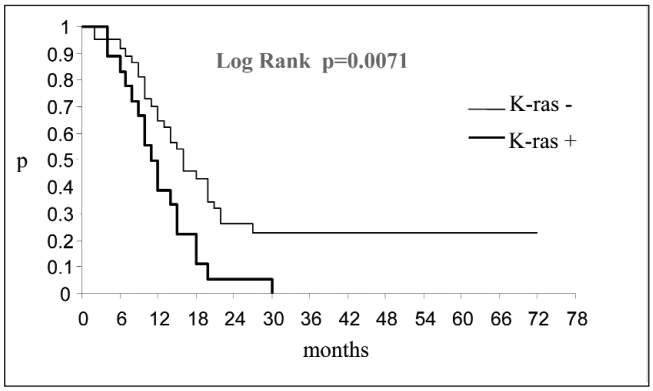

Figure 13 – Probability of survival (p) for patients with pathological stage IIIa – N2 NSCLC who underwent complete resection according to the presence of K-ras mutations

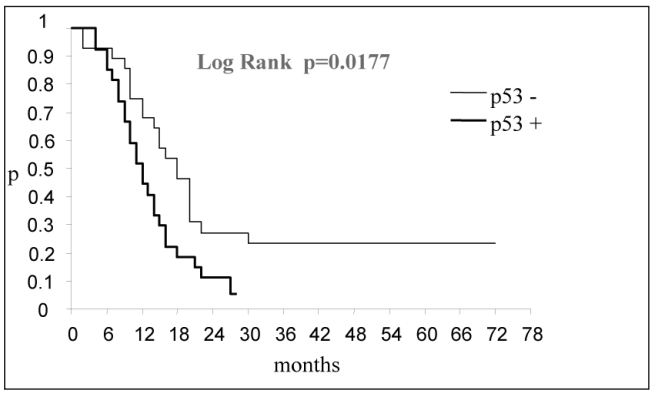

Figure 14 – Probability of disease free period (p) for patients with pathological stage IIIa – N2 NSCLC who underwent complete resection according to the presence of p53 mutations

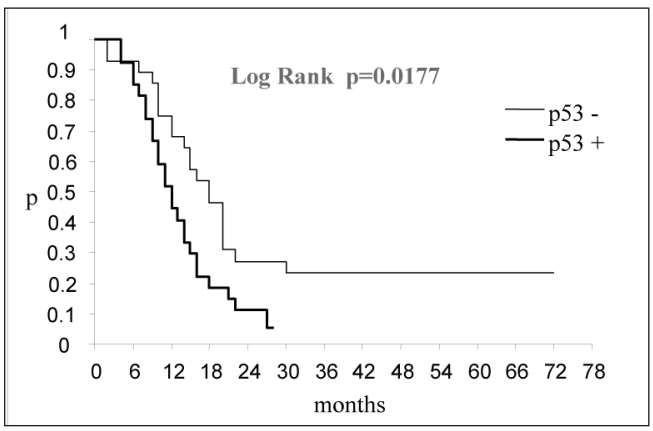

Figure 15 – Probability of survival (p) for patients with pathological stage IIIa – N2 NSCLC who underwent complete resection according to the presence of p53 mutations

period (29, 30, 31, 32) Distant metastasis comprised 65% of recurrence in our study, and this is lower compared to other reported series (33).

We assessed survival by histological type and grade of differentiation. There were no statistical difference between histological types of NSCLC. Inoue et al. found that pts. with squamous cell carcinoma had better prognosis than patients with large cell carcinoma (p=0.020). Furthermore, patients with squamous cell carcinoma also tended to live longer than adenocarcinoma patients although the difference was not significant (34).

We performed analysis of clinical N factor in correlation with survival, and found it to be a significant prognostic factor. Other clinical trials dealing with prognostic factors for pIIIa – N2 disease also confirmed that preoperative cN factor determined with a chest CT scan, was able to predict prognosis because surgically discovered patients with N2 disease had a better outcome than those given a preoperative diagnosis of cN2 disease (9, 34, 35). A lot of clinical trials exerted analyses of number of N2 stations to survival of patients with resected IIIa – N2 NSCLC and evaluated that number of involved N2 stations is crucial prognostic factor for these patients (3, 9, 36, 37).

Our analyses confirmed that patient's age is also an important prognostic factor, as patients older than 65 years had significantly worse prognosis (p=0.008) which was shown in other studies with univariate and multivariate analysis (36).

This study showed no significant difference in prognosis between clinical stages (cTNM) of the disease albeit some studies confirmed that as a significant predictor of survival (38). Larger tumor size was also referred to be of significant prognostic value in some studies (8, 34), which was also found in our study for T3 factor compared to smaller tumor size T1-2 factors.

Experience with clinically occult stage IIIa – N2 NSCLC suggests that multiple levels of mediastinal lymph node metastases predict treatment failure in patients following resection and that adjuvant mediastinal radiation improves disease free but not overall survival (39).

According to Tanaka et al. in cT1-2N2M0 or pT1-2N2M0 patients, a good prognosis can be realized by complete tumor resection with mediastinal lymph nodes dissection. Additionally, downstaging to N1 or N0 nodal status after induction chemo- or radiotherapy showed improvement in 5-year survival rate (40). In contrast, surgical treatment should not be justified in cT3N2M0 or pT3N2M0 patients (41). Our investigation confirmed poor prognosis of pT3N2M0 patients and also find surgery to be an insufficient treatment, acquiring additional treatment intents, such as chemotherapy.

The anatomical definition of N1 stations, its boundary to N2 stations, and its prognostic implication are yet to be defined in lung cancer. Metastasis in lymph nodes close to the pleural reflection has been classified differently as N1 or N2 according to the lymph node maps promulgated so far. In terms of prognosis, a pleural reflection does not seem an appropriate anatomical boundary between N1 and N2 stations in lung cancer (39). This is also to be clarified for precise staging of NSCLC and thus the decision of treatment intent for different stages and substages of the disease.

We confirmed that certain gene alterations significantly affect clinical outcome of pts. with NSCLC. Direct evaluations that K-ras and p53 genes mutations negatively influence survival of pts. with pIIIa – N2 NSCLC assessed in certain studies is also achieved in this study. Detection that p53 gene mutations are closely related to shortened survival of advanced stage pts. with NSCLC is reported in studies of Bubb et al. 2002, Yuan et al. 2002, Niklinska et al. 2001, Sanchez-Pernaute et al. 1998, Steels et al. 2001, Murakami et al. 2000, Anđelković T. et al. 2011 (43-49), but not in studies of Nishio et al. 1996 and Langendijk et al. 1996 (50, 51). Numerous studies observed the negative influence on survival of K-ras gene mutations Anđelić et al. 2001; Graziano et al. 1999; Hatzaki et al. 2001, Mitsudomi et al. 1991, both in early and late stages of NSCLC, irrespective of treatment intent (52-55). Some authors

propose K-ras gene mutations as an additional prognostic factor for lung cancer patients Rosell et al. 1996; Miyake et al. 1999 (56, 57). This study also elucidates the influence that p53 and K-ras gene mutations have on patients outcome and indicate them as additional factor for substaging of NSCLC.

Recent efforts to add new factors that could aid better staging and subclassification of NSCLC patients have been made. A group of authors from Yokohama conducted a study using cDNA microarray analysis to determine whether expression levels of genes in tumors were correlated with survival. Some survival-related genes were detected in the tumor tissue of lung cancer patients using cDNA microarray analysis. A prospective study is required to confirm whether expression levels of these genes can be used for prognosis (58). Authors from Philadelphia identified 19 novel genes that have neither been described in non-small cell lung cancer (i.e., cdc2, cullin 4A, ZAC, p57, DP-1, GADD45, PISSLRE, cdc20) nor in any other tumors (i.e., cyclin F, cullin 5, p34). These results identified several potential cell cycle genes altered in lung cancer (59).

Detection of tumor DNA in plasma is feasible using molecular techniques. A variety of tumor-genetic alterations i.e. K-ras and p53 genes, have been identified circulating free-form in the plasma and serum, detection and quantification of which shows promise for monitoring patient response to therapy. The desirable property and utility of these noninvasive, sensitive, and specific biomarkers lie in its ability to provide an early indication of disease progression (17, 60, 61).

Gene alterations in non-small cell lung cancer are a cause of intrinsic genetic differences between tumors of similar histology and clinical presentation and thus represent a promising, reliable new tool for lung cancer diagnosis, prognosis and prediction of radio- and chemosensitivity of the tumor. They may be added to clinical and pathological factors for subclassification of all stages of NSCLC.

CONCLUSION

Our study revealed subgroups of pIIIa – N2 with K-ras and/or p53 mutations respectively with particularly unfavourable prognosis that require additional postsurgical treatment other than radiotherapy. On the other hand, subgroups without K-ras or p53 mutations could benefit from postsurgical radiotherapy alone or in combination with other therapeutic modality.

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Apstrakt

Ovim istraživanjem je obuhvaćeno 60 pacijenata prosečne starosti 58 godina (od 43 do 69) i sa patohistološki potvrđenim nemikrocelularnim karcinomom pluća (postoperativnim patohistološkim nalazima je dijagnostikovano 29 adenokarcinoma, 32 planocelularna, 3 adenoskvamozna i 5 makrocelularnih karcinoma). Kod ovih pacijenata je učinjena kurativna torakotomija i potvrđeno je prisustvo sekundarnih depozita u ipsilateralnim limfaticima toraksa. Pacijenti su primili postoperativo adjuvantnu zračnu terapiju po protokolu split-course. Pored kliničkih parametara (starost, klinički stadijum, klinički N i T status, histološki tip i stadijum diferencijacije) određivali smo i prisustvo mutacija u K-ras i/ili p53 genima i korelirali kliničke i laboratorijske parametre sa preživljavanjem pacijenata. Praćenje bolesnika je trajalo 72 meseca nakon operacije. Kaplan-Majerovom metodom je ispitivena verovatnoća perioda bez bolesti i verovatnoća preživljavanja u funkciji vremena. Pokazano je da na preživljavanje imaju statistički značajan uticaj godine pacijenta, klinički nodalni i tumorski status, kao i prisustvo mutacije/a u K-ras i p53 genima.
Zaključak: U ovom istraživanju su identifikovane podgrupe pacijenata sa nesitnoćelijskim karcinomom pluća koji imaju mutaciju u K-ras genu, p53 genu, N2 bolest, T3 tumorski status ili više od 65 godina kao nezavisne prognostičke faktore, i u zavisnosti od toga se predlaže različit terapijski pristup za ove grupe pacijenata.

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