HISTOPATHOLOGICAL CHANGES IN LUNG CANCER, IN CORRELATED WITH PROGNOSTIC ASPECTS

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Abstract
Globally, about 1.3 million people die annually from lung carcinoma. Despite prophylactic and diagnostic advances, current therapy provides a low survival rate (15% at 5 years - American Institute for Cancer), because of the late diagnosis.
Our study was performed on a 5-year case studies on lung cancers in County Hospital of Oradea, diagnosed on histological (endobronchial biopsies, autopsies prelevation) and cytological examinations (brushing). The statistics from the National Cancer Institute were a comparative assessment guide in our study. We have examined the incidence of histological types of lung carcinoma correlated with prognostic aspects. Microscopic diagnosis was based on Papanicolaou (cytological preparations), hematoxylin-eosin (HE) staining and immunohistochemical (IHC) methods for biopsy specimens. For primary lung adenocarcinomas was performed the expression of human epidermal growth factor receptor 2 gene (HER2/neu) by IHC, in order to assess the need for Herceptin therapy. Histopathological diagnosis of lung cancer dictates predominant type of therapeutic conduct.

Key words: statistics, histopathology examination, IHC, HER – 2/neu gene, Herceptin

INTRODUCTION

The clinical stage of the disease is an essential factor in survival; cases diagnosed in stage I had a survival rate of 70 – 80% over five years, and cases diagnosed in stage IV had a survival rate of 2% over five years. For prognosis it is best to correlate clinical staging with histology of the tumor. In Non Small Cell Lung Carcinoma (NSCLC) survival rate ranges from 31% in stage IIB to 1% in stage IV (a mean survival rate of 8 months). In Small Cell Lung Carcinoma (SCLC) the survival rate drops to 6% over 5 years. The survival in women is 4% higher than in men in the same stage and histology (www /lungcancersurvivalrates.htm/2015).

Cancer treatment is dictated by the type of lung cancer and clinical stage. All major types of lung carcinomas are: squamous cells carcinoma and adenocarcinoma (with variants of papillary, mucinous and lepidic growth). Other type of carcinoma are adenosquamous carcinoma, poorly differentiated neuroendocrine carcinoma with small or large cells and others (Suster S, et al 2013).

Adenocarcinoma affects nonsmokers patients, may present as a solitary pulmonary mass and may be located peripherally. Squamous cell carcinoma is common in smokers, is centrally located, grows in the main

bronchus and can have early clinical symptoms (cough, hemoptysis, dyspnea) (Suster S, et al 2013, www.guide/lung-cancer-types/2015).

SCLC usually is related to smoking, has a rapid grow, a central location and spreads early into the mediastinum. Most cases are diagnosed in an inoperable stage. This type of tumor responds well to the new palliative treatment and has prolonged survival for some patients (Suster S, et al 2013, www.guide/lung-cancer-types/2015). For confirming the primary origin of NSCLC is required an IHC panel of at least 4 antibodies: Thyroid Transcription Factor – 1 (TTF – 1), Cytokeratin 7 (CK 7), Cytokeratin 20 (CK 20), p63. If the first 2 are positive and the third is negative, then probably, it is a pulmonary origin. The last marker (p63) is for squamous cell origin. For SCLC is needed a panel for neuronedocrine tumors (Chromogranin, Synaptofysin, Neuron Specific Enolase) (web/ histopathology_help/2015).

HER 2/neu is a member of ERBB 2 receptor of tyrosine kinase family. HER 2 protein overexpression and gene amplification are present in 6% to 35% and in 10% to 20%, respectively, of NSCLC. HER 2 mutations were identified in approximately 2% to 4% of NSCLC (Mazieres J, et al 2013). HER2 overexpression or gene amplification known to be associated with sensitivity to antiHER 2/neu drugs (trastuzumab, lapatinib) in breast cancer. In lung adenocarcinoma with HER 2/neu positivity, adding trastuzumab to gemcitabine-cisplatin or to docetaxel showed a little survival benefits in patients (a medium of 5.1 months) (Mazieres J, et al 2013).

The main purpose of this study is to analyse the histopathological changes in lung cancer, correlated with prognostic aspects.

MATERIAL AND METHOD

The study included 292 patients from County Hospital of Oradea with malignant endobronchial specimens (biopsies and brushings) and tissue prelevation from patients from who was performed autopsies in our hospital in a period of 5 years (2009 – 2013). From the total amount of patients, 130 were cases of primary lung carcinoma and 162 with secondary pulmonary malignancies. We appreciated the incidence of primary lung carcinomas according to sex, age and place of origin of the patients.

The material was processed in the Pathology Department. For brushing specimens we used conventional smears with Babes – Papanicolaou staining. The samples from biopsies or autopsies prelevation were fixed in 10% neutral buffered formaldehyde for 12 – 24 hours, embedded in paraffin, sectioned at 4 microns with a Microm HM 325 microtome and stained with classical Hematoxylin – Eosin (HE). The slides were examined with Nikon Eclipse E200 and E600 light microscope and the photos were taken with Nikon Coolpix 4500.

We used IHC techniques for differentiating from primary and secondary lung malignancies with specific antibodies (TTF – 1, CK 7, p63, CK 20, Carcinoembrionar antigen –CEA, CDX2, Chromogranin, Synaptofysin and others). For histology typing we used the protocols of Lung Cancer from the College of American Pathologists revised in October 2013 (www /cancer_protocols/2013).

On 20 cases of primary lung adenocarcinomas we tried to establish the expression of HER2/neu gene in lung tissue using IHC antibody c–erbB – 2 Oncoprotein (clone CB 11) for a better correlation with other studies.

We obtained the approval from the patients who were included in the study and from the ethical committee of the hospital. We do not have any conflict of interest with any pharmaceutical or immunostains companies.

RESULTS AND DISCUSSION

In our study were included 130 patients with primary lung carcinoma from which 82 were males and 48 were females, a M:F ratio of approximately 2:1 (figure 1).
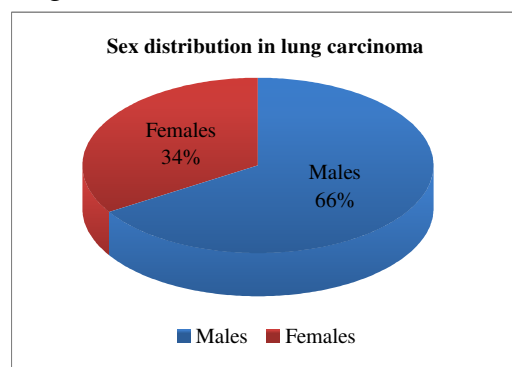

Fig. 1. Sex distribution in lung cancer patients (82 males and 48 females)

The peak age of lung cancer in our patients were between 51-70 years with a total of 95 patients (73%). The second preferred age in this type of tumor was after 71 years (23 patients – 18%). Patients who were least affected from this disease were those with age bellow 50 years (12 patients – 9%) (figure 2).

If we analyze the place of origin of our lung cancer patients, we observe that 91 of them were living in urban areas and 39 were from countryside (figure 3).
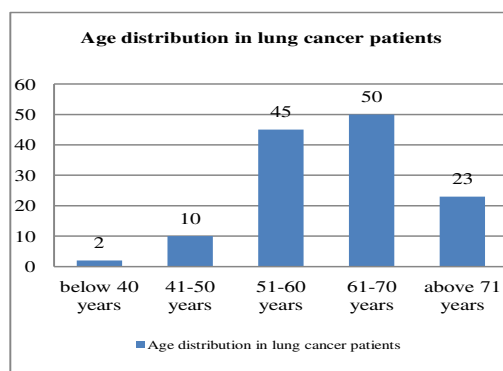
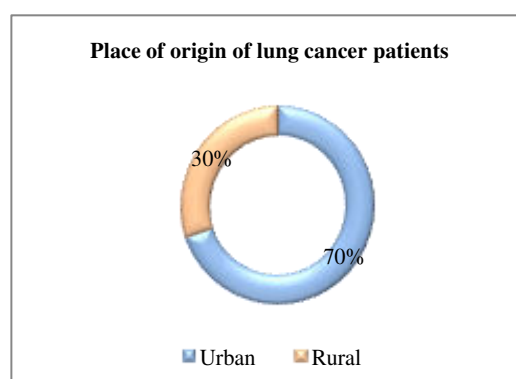Fig. 2. Age distribution in lung cancer patients

Fig. 3. Place of origin of lung cancer patients (91 patients lives in the city and 39 lives in the countryside)

In our study, squamous cell carcinoma (75 cases) and adenocarcinoma (41 cases) predominate in the specimens collected. Less frequent were the findings of small cell carcinoma (6 cases), adenosquamous carcinoma (6 patients) and giant cell carcinoma (2 cases) (figure 4).
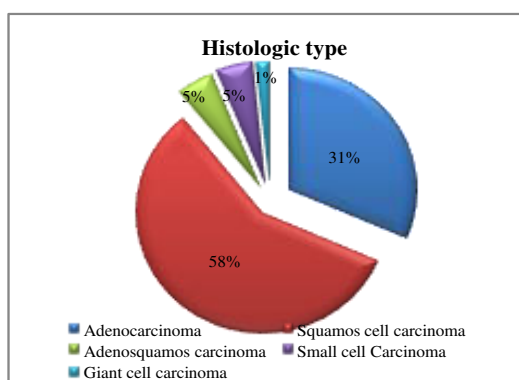

Fig. 4. Histologic types of lung carcinoma

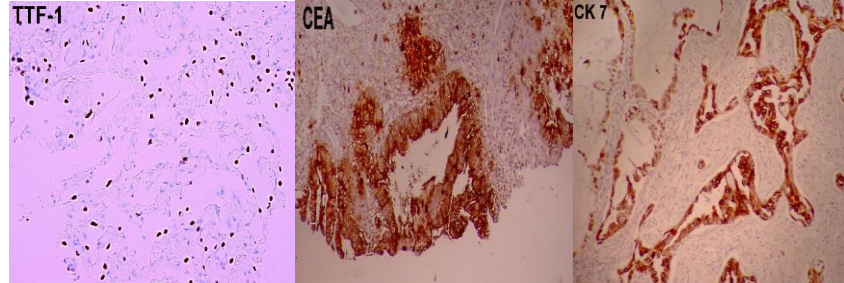Below there are some immunostains types of NSCLC (figure 5-6).

Fig. 5. Immunohistochemical panel with positive TTF – 1, CEA and CK 7 supports pulmonary origin of lung carcinoma.
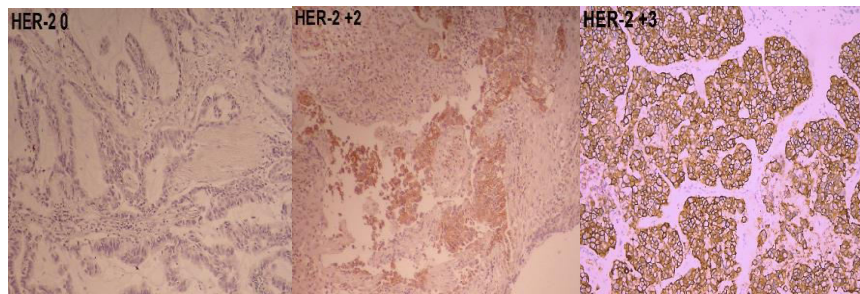

Fig. 6. HER 2/ neu expression in different cases of lung adenocarcinoma from (no expession to full expression)

We observed 20 cases of adenocarcinoma in which we tested HER 2/neu overexpression using IHC technique. The outcome of the study revealed us that 4 cases (20%) were negative, 15 were weakly positive (75%) and only 1 (5%) was strongly positive (figure 7).

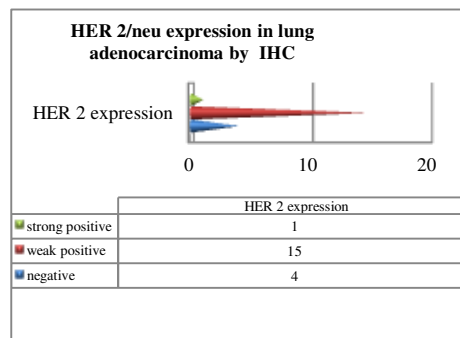|	HER 2 expression
strong positive	1
weak positive	15
negative	4

Fig. 7. HER 2/neu expression in lung adenocarcinoma

Morphological differences between SCLC, NSCLC and secondary malignancies sometimes can be difficult, being necessary to call on IHC.

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Our study includes 6 cases of SCLC (5%), compared to 10-15% of the data in the literature. SCLC are aggressive cancers in 99% are related to smoking, centrally located, metastasize quickly and usually are diagnosed in metastatic stage. For positive diagnosis are required IHC determinations, the confirmation of two neuroendocrine markers is mandatory. For grading we used a proliferation index, which we determined by IHC with Ki67 (using MiB1 antibody).

SCLC ranks third in frequency between types of lung carcinoma. They should be distinguished from other types of extrapulmonary small cell carcinomas such as Merkel cell carcinomas with the coexpression of cytokeratin and neuroendocrine markers. The positivity of CK20 in Merkel cell carcinoma is very helpful in this differential (Chan JK, et al 1997).

Our 124 cases of NSCLC (95%) were compared with the data from literature of 85 – 90%, including three types: adenocarcinoma 31 % (40% in other studies), squamous cell carcinoma 58%, adenosquamous carcinoma 5% and giant cell carcinoma 1%.

Lung adenocarcinoma has no incriminated smoking in etiology, is more common in women, having peripheral location, tends to disseminate by lymph vessels. Lepidic growth adenocarcinoma frequently develops multicentricity, has maximum incidence in women, nonsmokers and higher incidence in Asia. The incidence of lung adenocarcinoma in literature data is of 28% in men and 42% women (Parkin M, et al 2002, Parkin M., 2004).

Data from the literature show significant differences between the incidence of squamous cell carcinoma and adenocarcinoma by gender (44% squamous cell carcinoma in men and 25% women) (Parkin M, et al 2002).

Giant cell carcinoma, undifferentiated variant is an aggressive type of lung carcinoma, with dissemination in the lymph nodes and distant sites. In a study made in 1995 by Travis WD showed that giant cell lung carcinoma represent approximately 9% of lung cancers (Travis WD, et al 1995). They are more common after 60 years, and most occur in men (Brambilla E, et al 1992, Iyoda A, et al 2001).

Microscopic diagnosis of lung carcinoma is based on excisional or incisional biopsy with HE stain. The next step is to determine the origin of the primary tumor requiring IHC (CK7positive, CK20 negative TTF1 +, positive CEA). TTF1 has high degree of positivity in adenocarcinoma (72%), were weakly positive (3%) in squamous cell carcinoma and strong positive (94%) in SCLC. HER – 2/neu positivity in lung adenocarcinoma gives chance to antiHer – 2 therapy in combination with chemotherapy agents, making an extrasurvival of 5.1 months (Mazieres J, et al 2013, Gatzemeier U., et al 2004). The studies in this respect are insufficient. There are studies done on large lots lung adenocarcinoma that had proven that HER – 2/neu positivity differs according to the working technique (IHC,

fluorescence in situ hybridization, serum determination by enzyme – linked immunosorbent assay). Concordance between all three techniques was made only in 3 cases (Charles Bankhead et. al., 2013).

In medical practice very often biopsy material is very small that you can not make these determinations, being limited to the usual HE stain, CK7 and TTF1. TTF1 is one of the most useful diagnostic marker IHC pathology. It regulates morphogenesis in lung upper airway branching and terminal differentiation pathways (type II pneumocytes, Clara cells and expression of surfactant factor).

Another aspect of the study was to determine HER – 2/neu expression in lung adenocarcinomas in order to correlate with the new trends in oncology therapy. The treatment with Herceptin in HER – 2/neu overexpression in adenocarcinoma, which ensures the survival for a few months in this type of carcinoma in combination with chemotherapy and radiation therapy.

The studies from the literature showed that overexpression of HER 2 in NSCLC was present only in adenocarcinoma and not in squamous cell carcinoma or adenosquamous carcinoma (www.tlcr.org/2015).

CONCLUSIONS

The diagnosis of lung cancer is the sum of clinical signs, imaging studies and microscopic appearance and IHC facts.

Microscopic examination is essential and aims mainly to establish and differentiate the two major types of lung carcinomas: SCLC and NSCLC.

Course of treatment is dictated by clinical stage and histologic type. Tumors found in stage I benefit of surgical therapy. SCLC responds well to chemotherapy and radiation therapy with 20% overall survival for 5 years.

Using IHC technique allows differentiation between different types and primarily determining the origin of the lung. The algorithm CK7 positive, CK20 negative and TTF – 1 positive supports pulmonary origin.

Because of the high prevalence of lung cancer worldwide and the availability of standard and investigational therapies targeting HER – 2/neu, routine molecular typing of lung adenocarcinoma should include HER – 2/neu.

HER 2 status in NSCLC broadens the therapeutic possibilities of increasing life expectancy by combination therapy with Herceptin.

Lung carcinoma is a form of cancer that can benefit from a highly effective primary prevention by eliminating smoking, if we consider its role in the etiology of lung carcinomas.

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