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ABSTRACT

REALITIES AND PERSPECTIVES IN EARLY DIAGNOSIS OF LUNG CANCER IN GORJ COUNTY

Scientific coordinator:
Professor CRISTIANA SIMIONESCU, PhD

PhD Student:
VLAD ELENA

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SUMMARY

Key words: lung carcinoma, risk factors, mortality, carcinogenesis, thoracic CT, bronchoscopy, non-small cell LC, small-cell LC, TTF1, CEA, p63, CK5/6, CK7.

IMPORTANCE OF CARRIED OUT STUDY

Lung cancer (LC) is one of the most important and frequent malign tumours, representing over 90% lung tumours and in the last years had the most expressed increasing tendency of all neoplasias. The clinical signs in this disease appear extremely late in evolution, less than 1/3 of cases being in a useful therapeutic stage with a survival rate of 6-18 months and only approximately 20% live more than 1 year.

In the first part of the work are reviewed notions about the current level of knowledge, with reference to possibilities of diagnosis and symptomatology of lung cancer. The second part of the work constitutes the experimental study, carried out on 780 patients, investigated during the period 2005-2012. In the researches carried out within the Doctoral Thesis we proposed to determine in what manner risk factors have a significant impact in LC pathogenesis, to analyse the contribution of different diagnostic methods and to create a group of patients with increased risk of LC and the modalities to monitor them.

GENERAL PART – CURRENT LEVEL OF KNOWLEDGE

Yearly, lung cancer causes over 1,2 millions of deaths registered at global level. Morbidity and mortality caused by LC are increased in most countries in male persons, with an increased frequency at the age group 65-74. Although at present the bronchopulmonary localization remains more frequent in men, the increase rate is much higher in women, possibly due to the role of estrogens in increasing risk of lung adenodarcinoma **[17,32].**

Most of epidemiological studies concerning LC are centred on the proved role of tobacco in carcinogenesis. The risk of LC is at least 20 times higher in smokers than in non smokers. This risk is demonstrated through the rate of deaths because of LC, which is in non smokers of approximately 10/100 000 inhabitants, but of 140/100 000 inhabitants in cigarette smoker, on the whole, and of 251/100 000 inhabitants in heavy smokers **[4,9].** Epidemiological studies demonstrated that the risk for LC is correlated to the cumulative dose of cigarettes (the number and type of cigarettes, number of packages-year), to nicotine content, to use of filtered and unfiltered cigarettes. It is estimated that 1 of 7 persons smoking more than 2 cigarette packages/days develop LC **[48].**

The exact mechanism of carcinogenesis through tobacco is unknown; it is possible that certain chemical constituents of tobacco be carcinogenic or co-carcinogenic along with other inductive factors. Carcinogens in tobacco smoke are not only absorbed by the surface epithelium

of aerial ways and alveoli, but also are metabolized, fact which is emphasized by the presence of mutagens in the urine of smokers **[15].** Researches showed a succession of histological changes which occur following the long-term exposure to tobacco smoke: initially appear hyperplasia of surface bronchial epithelium and of basal cells and subsequently a malpighian metaplasia; then appear cell dysplasias (non malignant), possible to frequently emphasize it in smokers' sputum, *in situ* cancer and relatively late, real cancer (extensive) **[12].**

Professional and industrial factors are involved in LC etiology, thus long-term exposure to asbestos, especially in construction workers, significantly increases the risk of LC, besides the well known risk for pleural malignant mesothelioma. Exposure to radiations in mines of uranium, radium increases the incidence of LC by 10-30 times, but also other industrial pollutants – beryllium, chrome, nickel, arsenic – are involved in LC etiology. Atmospheric pollution in big cities, by aromatic hydrocarbons resulting from incomplete combustion of fuel has carcinogenic properties **[15,45].**

Field factors are also important in LC etiology, without being possible the determination of their sublayer, thus lung carcinoma is 2 times more frequent in persons with chronic bronchitis or bronchiectasis **[39].**

Pathogenesis of lung cancer still has many unknown aspects regarding molecular changes and their frequency in carcinogenesis. The malignant transformed cell loses the response possibility to control mechanisms of cell and tissue life and appears a knew cell antigenic configuration which gives to malignant cells a "*non-self*" character, triggering immune reactions of rejection or destruction **[12].** Between 2 and 10% of cases there is a combination of hystopathological types, most of them epidermoid carcinoma with adenocarcinoma, but also microcellular aspects may co-exist with those non-microcellular **[13].**

Due to prognostic, evolution and different treatments, LC classifies in two major groups, with practical clinical importance:

- *non small cell carcinoma* (NSCLC), comprising squamous carcinoma, adenocarcinoma, large cell carcinoma;
- *small cell carcinoma* (SCLC).

In the last 20 years in the USA, Western Europe and Japan, the most frequent histological type of LC is adenocarcinoma (40%), followed by squamous carcinoma (25%), small cell carcinoma (20%) and large cell carcinoma (10-15%), the other types representing approximately 5% **[17].** In Romania, the first is, for the moment, epidermoid carcinoma (45%), followed by adenocarcinoma (25%), large cell carcinoma (10%) and small cell carcinoma (20%) **[22,49].** WHO/IASLC classification provide more precise criteria to classify dysplasia in mild, moderate and severe dysplasia. Besides the categories of squamous dysplasia and *in situ* carcinoma, in the

group of pre-invasive lesions have been added other two lesions which are atypical adenomatous hyperplasia (AAH) and diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) **[13,53].**

The most frequent manifestations of LC are: cough, haemoptysis, localized wheezing, dyspnoea, thoracic pain, dysphonia, and asthenia, lack of appetence and weight loss **[3]**. Metastatic and paraneoplastic manifestations are frequent in LC, as 60-70% of LC patients are detected in advanced stages of disease **[15]**.

The radiological examination is the most used method to diagnose LC, being superior to cytology in early detection. Computerized technology is extremely useful in emphasizing pleural tumours or in mediastinal structures, as well as in specifying the characteristics of lymph node lesions, unique or multiple. Nuclear magnetic resonance, as compared to CT, has the advantages of the lack of irradiation and the neutralization of iodine contrast solution **[2,16]**.

In case of lung cancer, fibrobronchoscopy is used to diagnose and to sample bioptic fragments, to stage cancer, as well as to perform palliative interventions in surgically overcome cancers, such as: mounting of stents or endobronchial prostheses, endobronchial tumour resections, cryotherapy, laser therapy or resections with electrocautery **[44].** Endoscopic ultrasound is an investigation which combines ultrasound and endoscopy in order to obtain images and anatomic information, normal or pathological, about different organs **[20]**. Autoflorescence bronchoscopy is a minimum invasive method used to diagnose lung cancer in early stages. It is an endoscopic procedure using blue light **[25]**.

The cytological examination of sputum is a method used in LC diagnostic. This method is based on the cell exfoliation capacity at the level of bronchial tumour and on the identification of malignant cells in sputum or intratracheal instillation fluid, after fixing and staining the preparation. The test is diagnostic in 70-80% of cases and the sensitivity may be increased even to 90%, under special technical and professional experience conditions, but in approximately 10% of cases the method may give negatively false results **[8,14]**.

LC staging is done based on TNM classification (tumour-node-metastasis) adopted in 1974 by the American Commission on cancer staging and subsequently revised. The last revision in 2012 comprised the definition of stages of lung carcinoma, the definition of lymph node stations, the definition of metastases and appropriate staging **[53]**.

SPECIAL PART – PERSONAL CONTRIBUTIONS

OBJECTIVES OF THE STUDY

This work has as main purpose correlation of clinical aspects with those provided by paraclinical and imaging explorations, in order to draw up a correspondence between risk factors, malignity scores, medical imaging results and histopathological evaluation in order to set up a correct and complete protocol for diagnostic.

Attaining of these objectives will be based on the following methods:

- clinical evaluation;
- imaging evaluation;
- morphological evaluation;
- immunohistochemical evaluation;
- statistical analysis of results.

MATERIAL AND METHODS USED IN THE STUDY

Our study was carried out during a period of 8 years, corresponding to the time interval 2005-2012 and included 780 patients aged between 35 and 86, having the diagnostic of lung cancer, hospitalized in "Tudor Vladimirescu" Pneumo-phthsiology Hospital, Runcu commune, Gorj county and Emergency Town Hospital of Tg-Cărbunești, Gorj county; patients in the oncological records of the Oncology Division of Tg-Jiu, Gorj county. These patients underwent bronchial aspirate, bronchial microlavage, bronchial brush and bronchial biopsy, for the purpose of diagnostic.

As study material we have used the **medical chart** from which we have obtained data relating to the studied pathology, namely the age, the profession, the residence, the hereditary-collateral antecedents, the pathological personal antecedents, the habits (smoking, toxic environment, radiations), the anamnesis and the physical examination, the blood tests (VSH, fibrinogen, TGO,TGP,LDH), the tumour markers (alpha 1-fetoprotein, carcinomembrionic antigen, neuron-specific enolase, CYFRA 21-1), the cytology of sputum examination, the pulmonary radiography (front and profile), the fibrobronchoscopy with sampling of bronchial material, the computerised tomography, abdominal, cardiac ultrasound, lymph node biopsy; **the registers with cytopathological, anatomopathological and immunohistochemical results.**

Tissue fragments obtained through bronchial biopsy from patients diagnosed on histopathological basis with lung carcinoma, initially processed, to this purpose, through the usual technique of inclusion to paraffin up to the stage of paraffin block within the Pathological Anatomy Laboratory of Tg-Cărbuneşti Emergency Town Hospital, have subsequently been compared to tissue fragments obtained from pneunomectomy pieces, interventions carried out within the Clinic of Thoracic Surgery of and the Clinical Division of Thoracic Surgery within Marius Nasta Institute of Bucharest.

Out of the samples to be studied, 62 of them have been immunohistochemically studied within the Morphopathology Laboratory of Craiova University of Medicine and Pharmacy.

RESULTS

The studied group comprised 780 patients during the period 2005-2012, aged between 19 and 82. LC was most frequent for the age group 60-69 (195 cases – 25,10%). Most of patients came from urbanzones (565 cases -72,43%), and most of them were men (624 cases - 80%). Comparing the distribution by sex in the years at the beginning and the end of the study, it has been found out the change of percentage between men and women for the year 2005 as compared to 2012. Thus, if men/women percentage at the beginning of the study was of 69/17, at the end of the study was of 67/55.

We have classified patients depending on the stage of the disease at the beginning of screening. Thus, the proportion between operable stages/inoperable stages was of 17/69 for the year 2005, as compared to 42/80 for the year 2012.

Analysing the role of smoking in LC etiology, we have tracked the age of beginning of smoking, the duration of smoking, the number and type of smoked cigarettes, the way of inhalation, the existence and duration of the status of ex-smoker.

Thus, 89,90% of the men in the study were smokers and 67,30% were women. Most of the smokers began to smoke at the age between 10-19 (327 cases-49,09%), more than half of them smoked for more than 40 years (56,60%) and most of them smoked over 25 cigarettes/day (53,01%). The percentage of those who smoked 40-49 packages/year was more important in the studied group (486 cases -72,97%), and depending on the way of inhalation, those who profoundly inhaled were more numerous (506 cases -75,97%). Most of them smoked filtered cigarettes (599 cases-89,93%), the status of ex-smoker was present in 200 patients (30,03%), and passive exposure was significant in 23 patients (2,94%).

Professional factors were present in 179 patients (22,94%), of these 125 (69,83%) associating exposure to professional factors with smoking.

Pre-existent diseases of COPD type were met in 398 patients (51,02%), namely tuberculosis in 65 patients (8,33%), and nontuberculous pulmonary fibrosis was present in 94 patients (12,05%). In 20 patients (2,56%) in anamnesis was met another primary neoplasia other than the pulmonary one, and in 203 patients (26,04%) has been identified no disease in pathological personal antecedents. Hereditary-collateral antecedents of neoplasia, regardless the localization, have been positive for 90 patients (11,53%).

The predominant symptom of the disease was cough and change of cough character (663 patients -85%), then haemoptysis (262 patients -33,59%), thoracic pain (195 patients – 25%) and dyspnoea (180 patients -23%).

Standard radiological examination carried out the screening role in detecting LC in 768 patients (98,47%), the rest of the cases being screened after thoracic CT examination. A significant number of patients (560 -71,79%) presented non-compliance between lesion extension seen on standard radiography and thoracic CT examination.

Most of radiological changes were central (518 – 66,42%), most of them were unique radiological changes (692 -88,71%) and, similarly, most of them were nodular changes (356 - 45,64%).

Among the performed bronchoscopic manoeuvres, the bronchial aspirate was positive for 558 cases-71,53%, the bronchial microlavage in 692 cases-88,71%, the bronchial brush in 516 cases- 66,155, and the bronchial biopsy in 578 cases-74,10%. In 222 cases (28,46%) was performed pulmonary resection.

The endoscopic direct signs were present in 248 cases (31,79%), the endoscopic indirect signs were met in 212 cases (27,17%), and the entanglement of the two was met in 320 cases (41,04%).

Benign cytologic smears were in amount of 154 (19,76%), atypical smears in amount of 146 (18,71%), and malignant smears were in amount of 480 (61,53%). Malignant smears, based on morphological features and taking into consideration the current therapeutic implications were comprised in the following categories: non-small cell carcinomas – suggestive for squamous carcinoma (201 cases-25,76%), non-small cell carcinomas – suggestive for adenocarcinoma (120 cases-15,38%), non-small cell carcinomas - „nos” (69 cases -8,84%) and small cell carcinomas (90 cases -11,59%).

The most frequent histopathological form was NSCLC with 670 cases, out of which 454 cases NSCLC- suggestive for squamous carcinoma, representing 58,20%, 142 cases were NSCLC- suggestive for adenocarcinoma, representing 18,20% and 74 cases (9,50%) has as histopathological diagnostic NSCLC – without other specifications (NSCLC-„nos”- no otherwise

specified). SCLC carcinoma was diagnosed as histopathological form in 110 cases, representing 14,10%.

Out of 780 patients comprised in the study, the cytological examination was positive in 626 patients (146 atypical smears and 480 malignant smears) (80,25%), and out of 90 pleural effusion sampled in the study, the cytology was positive in 73 effusions (81,11%).

The immunohistochemical study comprised 62 cases, which included both squamous carcinomas and adenocarcinomas, both poorly differentiated. All selected cases were investigated for TTF1, CEA, p63, 34βE12, CK5/6 and CK7.

The analysis of TTF1 immunoexpression indicated for adenocarcinomas positivity in 95% of cases, while squamous carcinomas were negative in all investigated cases. If for squamous carcinomas was found negativity in this marker, adenocarcinomas were in most of cases intense and diffusely positive.

The analysis of CEA immunoexpression indicated positivity in both categories of carcinomas, but with different pattern. For squamous carcinomas was found positivity in 64,2% (27 cases) with staining pattern predominantly cytoplasmatic or cytoplasmatic with peripheral intensification, while adenocarcinomas were positive in 85% of cases (17 cases) with membrane staining pattern.

Immunoexpression of p63 was positive in 92,8% of squamous carcinomas, while adenocarcinomas were positive in 25% cases. In 59,5% cases, tumours were positive in more than three quarters of cells, in 23,8% of cases staining was present in 50-75% of cells and in 4 cases was found the presence of marker in less than half of tumour cells.

Immunoexpression of 34βE12 indicated positivity both for adenocarcinomas and squamous carcinomas. In squamous carcinomas the marker was positive in 41 of 42 tumours investigated (97.6%), but also in 6 (30%) of adenocarcinomas.

The analysis of CK5/6 expression also indicated positivity for both types of carcinomas. Thus, we found positivity in 95,3% of cases for squamous carcinomas and in 35% of cases of adenocarcinomas.

The analysis of immunoexpression for CK7 indicated positivity in all adenocarcinomas (100%), but only in 4,7% of squamous carcinomas.

Analysing the predominant histological type of lung cancer and the presence of smoking, we have noticed that the NSCLC-squamous carcinoma type was the most frequent in smokers included in the study. The statistical analysis of the association of the two factors showed a value of $p<0.01$ much smaller than 0.001 of p, indicating a highly statistical significant difference of distribution.

If we take into consideration the histological type of NSCLC- squamous carcinoma associated to the nodular radiological aspect and the endoscopic aspect of mixed changes, we have found that there is a strong influence between the 2 factors ($p<0.01$).

Analysing the histopathological type of NSCLC-adenocarcinoma by taking into consideration the peripheral nodular radiological aspect and the endoscopic aspect of mixed changes, it is noticed that there is a strong influence between the 2 factors ($p<0.01$).

In the SCLC histological type, taking into consideration the radiological aspect of mixed peripheral changes and the mixed endoscopic aspect, it is also noticed that there is a strong influence between the two factors ($p<0.01$).

Between mixed peripheral radiological changes and those endoscopic of extrinsic compression with the histological type NSCLC-„nos", there is a strong influence, fact which is statistically emphasized by the value of $p<0.01$.

The analysis of the immunoexpression of analyzed markers for adenocarcinomas and squamous carcinomas poorly differentiated indicated significant differences of markers as compared to the lesion type. Thus, in case of adenocarcinomas, the distribution of positive TTF1 markers was significantly superior as compared to analyzed squamous carcinomas ($p=<0.001$, Fischer exact test), aspect found also in case of markers CK7 ($p=<0.001$, Fischer exact test)

In the case of CEA, also the percentage of positive adenocarcinomas was superior to squamous carcinomas, the statistical differences were not significant ($p>0,05$, Fischer exact test). As for the analyzed squamous carcinomas, the distribution of positive p63 markers was significant as compared to analyzed adenocarcinomas ($p=<0.001$, Fischer exact test), aspects also found in case of markers 34βE12 and CK5/6 ($p=<0.001$, Fischer exact test).

The analysis of percentage values of markers p63, 34βE12 şi CK5/6 indicated a positive linear distribution in case of p63/CK5/6 [$r(40)= 0.856$, $p=0.000$], respectively 34βE12 /CK5/6, [$r(40)= 0.304$, $p=0.049$] and non significant in case of p63/ 34βE12 (test Pearson).

The analysis of markers used in this study indicates an immunophenotype TTF1+CEA+CK7+ specific to adenocarcinomas and p63+, 34βE12+ and CK5/6+specific to pulmonary squamous carcinoamas.

DISCUSSIONS

Following the distribution of patients depending on calendar years, it has been noticed a progressive increase of patients yearly investigated, most of them being registered in the year 2012, with a difference of 36 patients as compared to the first year, 2005. This is explained both by the increase of LC frequency due to cumulation of factors, by increasing addressability of

patients to doctor, and possibly due to anti-tobacco campaign in the last years, and also by improvement of diagnostic methods.

The increase of number of cases diagnosed with lung carcinoma was more acute for age groups older than 50, most of epidemiological studies considering the fact that most of lung cancer cases are met at these ages, due to the cumulation of risk factors with long evolution of dysplastic and pre-cancerous metaplastic lesions in cancerous lesions **[4,49]**.

By analysing the distribution of patients depending on the origin area, it has been noticed that origin from urban zones was more frequent. This is explained by the increased addressability of persons living in urban areas as compared to the rural ones, both due to a higher educational and intellectual level and to means of diagnostic, of prevention**.**

On the other hand, the bigger number of patient from urban area would be due to the easy access of the population in this area to means of investigation, and to a higher exposure to air pollutants, the risk factors having an increased incidence in urban zones due to the existent industrialization **[49]**.

By analysing the distribution by sex in different years and especially the limit years in the followed interval 2005 and 2012, we have found out the change of the male/female proportion corresponding to year 2005, regarding the increase of the number of women diagnosed with LC.

The prevalence of lung cancer in men is explained by the cumulation of risk factors for this disease in men, who are subject to air and industrial pollutants more due to the profession, subject to stress more due to social position and smoking habit, prevalent in this sex **[4]**. The increase of number of women diagnosed with LC in the last years may be the result of the increasing number of smokers and the increase of the woman's role in social life, almost identical to that of man **[49]**.

The increased number of LC in women in the last years was due to the increase of number of adenocarcinomas diagnosed in this sex, fact which is due to the role of estrogens in the increase of risk in lung adenocarcinoma **[28]**.

Relating to the staging on screening of cases in the study, most of them were detected late, stages of disease considered inoperable - IIIB and IV-, as compared to operable stages - I, II and IIIA. The proportion between inoperable and operable cases changed in the year 2012 by the increase of number of operable cases. This was due to improvement of means of diagnosis due to technical progress, increase of addressability, the echo of the anti-smoking campaign in the last years, as well as the careful follow-up of patients with increased risk of lung cancer **[6]**.

Relating to the distribution of patients depending on the presence of active smoking as risk factor, it has been found out that many of the patients included in the study were smokers, both women and men. All retrospective studies found that the risk of lung cancer is higher if the

smoker began to smoke at a young age, if the duration of smoking was longer, if the number of smoked cigarettes/day was big, if the number of cigarette package/year was big and if the inhaling way was profound **[9,19]**. Smoker's behaviour was changed lately, possibly as a consequence of the anti-tobacco campaign in the last years, most of smokers using filtered cigarettes **[47]**.

Although many previous studies related the occurrence of lung cancer to the status of ex-smoker, the occurrence prevalence being in the first 5 years as of quitting smoking, it is not clearly related to this status of the occurrence of LC **[9]**. Previous studies consider passive smoking as having the same risk of occurrence of LC as active smoking **[47]**, but the difference between the number of active smokers having LC and the number of passive smokers having LC is significant ($p<0,05$).

It has been demonstrated the fact that the concomitant exposure to smoking and professional pollutants, especially asbestos, would increase LC risk by 90 times. Aromatic hydrocarbons resulted from incomplete combustions (benzopyrene and dibenzanthracene) are binding with cellular DNA, initiating carcinogenesis **[15]**.

Previous studies showed the association between COLD and LC, also due to the implication of smoking in the etiology of both diseases **[3]**. Post-tuberculous scars, chronic interstitial lung diseases (FID, scleroderma, rheumatoid polyartritis, sarcoidosis, interstitial pneumonia) are associated to LC, especially to NSCLC- adenocarcinomas. The term of ,,cicatricial carcinoma" is used for adenocarcinomas occurring in association to pulmonary scars due to chronic diseases and probably the neoplasia develops as a result of lesions determined by the basic disease **[6]**. The treatments followed by the patients included in the study for the first diagnosed neoplasia, especially those of radiotherapy, seem to be involved in the occurrence of lung cancer as a second neoplasic localization **[15]**.

Genetic factors are also important in the etiology of lung cancer. TERT and CRR 9 genes situated on the fifth pair of chromosomes process telomerase, the enzyme considered a determinant factor in the occurrence of LC. Genetic variations of these genes increase the risk of occurrence of lung cancer up to 60%, regardless the persons are smokers or not **[12]**.

Our study showed that the symptomatology given by the syndrome of malignant impregnation was prevalent, as 2/3 of the patients with LC present symptomatology due to intrathoracic local extension or extrathoracic metastases **[3,15]**.

The small percentage of undetected radiological lesions through standard radiology, both due to the size of lesion (under the limit of visual perception of 1,5 mm) and to the position of lesion behind the cardiac shadow, under the right diaphragmatic cupola or lesion pertaining to

mediastin, difficult to interpret on standard radiography, is within the limits described for this examination of previous studies **[42]**.

The category of nodular radiological lesions was the most important from the numerical standpoint, being followed by the category of nodular lesions entangled with those interstitial. The prevalence of entangled lesions is given by the prevalent late screening of studied cases, most of them metastases and with local extension **[2]**.

The percentages of positivity obtained of 74,10% for bronchial biopsy and the highest 88,71% for the cytological examination of bronchial microlavage are comparable to data in the specialty literature **[8]**. Although cytology on different sampled products does not confer the safety of histopathological diagnostic, as it raises only one suspicion of malignity, it remains an indispensable examination within lung cancer management **[14]**.

Direct endoscopic signs were both endoluminal vegetations and endobronchial stenosis in our study, which correspond to the data in the specialty literature **[44]**. Indirect signs of lung neoplasm were as follows: extrinsic compression, bronchial rigidity and alterations of statics of tracheobronchial axis. Direct and indirect signs of tumour process were the most frequently entangled.

The existence of entanglement of endoscopic direct and indirect signs is correlated to the high percentage of cases of lung cancer detected in late stages of the disease, given the fact that such disease stage associates both endoscopic manifestation determined by the tumour itself and endoscopic manifestation determined by neoplastic adenopathies and diffuse infiltrations of mucous **[50]**.

The frequency of malignant smears of 61,53% obtained in our study in the cytology performed from biological products is situated under the lower limit of positivity for malignity in previous studies, comprised between 70-90% **[14]**. If to this frequency we add the frequency of atypical smears obtained of 18,71%, we will reach a value of 80,24% for the suspicion of malignity given by the cytological examination, value which is situated between the values previously reported **[8]**.

The high degree of positive cytology of pleural effusions (81,11%) is due to the fact that under the conditions of the existence of a pleural effusion, we are speaking of metastasis of the disease, so an advanced stage of the disease which is frequently associated to positive cytology **[14]**.

The new aspects of histopathological classification followed in our study are those given by the therapeutic possibilities which a certain histopathological class gives. Thus, apart the classical separation between NSCLC and SCLC, due to special radio- and chemosensitivity of SCLC, the histopathological classification followed by us and reported in previous studies of the

American Thoracic Society and European Respiratory Society is based on the following therapeutic grounds:

1. NSCLC-adenocarcinoma and NSCLC-without specifications must be tested for EGFR mutation, because the presence of this mutation is predictable for the therapeutic responses to EGFR tirosine kinase inhibitors **[52]**.
2. NSCLC-adenocarcinoma is a histopathology predictive for the therapy with Pemetrexed combined with Cisplatin **[31]**.
3. NSCLC-squamous is a histopathology to which treatment with Bevacizumab (a blocker of VEGF-A factor, a vascular growth factor which stimulates angiogenesis in cancer) was accompanied by frequent fulminating haemoptysis **[24]**.

The data obtained in our study shows that squamous NSCLC carcinoma is the first as frequency, a frequency which exceeds the limits of WHO and in our country (58,20%), NSCLC-adenocarcinoma is the second as frequency, below WHO statistics and for Romania (18,20%). Also, the frequency of SCLC carcinoma, the third, is under the statistics of WHO and Romania (14,10%), while the frequency of NSCLC carcinoma -„nos" (9,50%) is comparable to the frequency in Romania, but smaller than WHO statistics.

In most of the cases, the differentiation of adenocarcinoma from lung squamous carcinoma is easily made based on standard morphological criteria **[43]**, respectively is helpful the presence of keratinisation and intracellular bridges as distinctive signs of squamous carcinoma and glandular architecture having the shape of acini, papilla, micropapilla or cytoplasmatic mucin, for adenocarcinoma. However, the differentiation may be difficult in case of poorly differentiated tumours, in case where glandular or squamous features are subtle or present only with focal character. These aspects are amplified on small biopsy specimens, because of insufficient cellularity, crushing of tissues through sampling artefacts, or through cell dispersion **[53]**. The rate of concordance among pathology of subtyping of these tumours on routine biopsy preparations is about 81 % **[43]**.

Immunohistochemistry represents an auxiliary instrument extremely efficient in differentiating adenocarcinoma from squamous carcinoma. Although the optimum diagnostic algorithm is not firmly fixed yet, a series of recent studies show the fact that immunohistochemistry increases the accuracy and reproducibility of diagnostic and also minimises the rate of diagnostic failure for carcinomas without small cells **[52]**. Antibody panels used included TTF1 and CEA for adenocarcinomas and CK5/6 and p63 / desmoglein 3 (DG3) for squamous carcinomas **[24]**, or TTF-1 and napsin A for adenocarcinomas and p63 together with CK5/6 for squamous carcinomas **[31]**.

The use of a minimum panel of antibodies is critical for specimens with reduced cellularity where is possible only the performance of a limited number of sections **[31]**. Moreover, the limitations of the sample with reduced size is imposed also by the necessity to preserve the tissue for additional molecular studies, which imposes the use of sensitive and specific antibody panels **[24]**.

Out of the radiological analysis of forms of lung cancer pertaining to female sex, it is noticed that, unlike men, women develop forms of cancer manifested radiologically under the shape of entangled radiological changes almost in equal number as men, as compared to the proportion 1:5 between women and men in unique radiological manifestations. Associated to the peripheral prevalence of radiological lesions in women, we may conclude that women develop severe forms of LC, detected in an advanced stage, fact which is due to the possible hormonal component which is at the basis of LC etiology in women **[42]**.

Analysing the increase rata of histopathological forms, the highest increase in our study was registered by NSCLC-adenocarcinoma, tendency showed also by reports from USA and Japan, which places this histopathological form on the first place as number of cases registered in the last 5 years **[43]**. The increase in number of all histopathological types is also the result of increase of capacity of medical services to determine the LC, due to performances of modern medical equipments purchased in the recent years, as well as the increased addressability of population already made aware of the anti-tobacco campaign in the last years **[36,37]**.

NSCLC – squamous carcinoma was associated most frequently to central nodular radiological changes and mixed endoscopic changes, NSCLC-adenocarcinoma was associated most frequently to mixed peripheral radiological changes of entangled type, nodular and fluid, and endoscopically with mixed changes. SCLC histological type was predominantly presented as mixed, nodular and mediastinal central radiological changes and endoscopically as mixed signs, and the histological type NSCLC "nos" was predominantly presented as mixed peripheral radiological image and endoscopically as extrinsic compression.

If we take into consideration the majority late screening of these patients, with a surviving rate below 5 years, below 10% according to the data in special literature **[15]**, we reasoned at the necessity of a diagnostic algorithm, which could be applied by all doctors involved in detecting this diagnostic (family doctor, pneumologist, radiologist, thoracic surgeon and oncologist).

CONCLUSIONS

1. The progressive increase of cases of LC from one year to another, in the time interval of the study, was compliant with the increase of frequency of lung carcinoma in the last years in the world and in our country, due to the cumulation of risk factors and the increased incidence of smoking habit.

2. The occurrence of LC is predominantly related to age over 50, to the existence of the status of smoker, to the urban zones origin, to the existence of cancer hereditary-collateral antecedents, to the existence of pre-existent affections and to male sex.

3. Most of the cases were detected in inoperable stages, but during the studied time interval the number of these cases was reduced, due to the increase of patient addressability as well as to the increase of performances of medical equipment in the recent years, which provide surgical solutions to many cases.

4. Symptomatology in advanced stages was given by distance metastases, while in incipient stages the main symptoms were cough, dyspnoea, haemoptysis and thoracic pain.

5. Standard radiological examination performed the role of screening, despite the limits of this examination, and thoracic CT examination allowed exact diagnostic and staging of the disease.

6. In men were prevalent the radiological forms of disease localized in centre and unique, in a closely relation to the implication of smoking in LC etiology in men. In women were prevalent the peripheral and multiple radiological forms, entangled (nodular and interstitial or nodular and pleural) in closely relation to the prevalence of adenocarcinoma in this sex.

7. Bronchial endoscopy allowed the histopathological conformation of cases through sampled products, in a percentage comprised between 70 and 89%, comparable to the data in the specialty literature.

8. Distribution of histopathological results in NSCLC and SCLC, then of NSCLC in: NSCLC-squamous, NSCLC-adenocarcinoma and NSCLC-„nos" was carried out because of therapeutic reasons, these histopathological forms beneficiating from different oncological therapies. This classification simplifies the multitude of histopathological forms of lung cancer in categories important from the therapeutic standpoint.

9. The most frequent histopathological form was NSCLC-squamous, then NSCLC-adenocarcinoma, followed by SCLC and NSCLC-„nos". The percentages of these histopathological forms obtained in our study were comparable to the data in the existent specialty literature, the histopathological form NSCLC-adenocarcinoma registering an increase of incidence also in our study as in the specialty literature.

10. NSCLC-squamous carcinoma was predominantly met in male sex both due to biological differences of risk specific to sex, and to direct influence of social behaviour of man in disease triggering: smoking, professional risk, consumption of alcohol, excitant food diet. In women was prevalent the histopathological form of NSCLC-adenocarcinoma, which is closely related to the estrogenic component of female body and the implication of these hormones in triggering the disease.

11. The used markers, represented by TTF-1, CEA, p63, 34βE12, CK5/6 and CK7 may validate a diagnostic algorithm on small biopsy tissue fragments/brush, for poorly differentiated carcinomas, which are difficult to classify on usual preparations.

12. The analysis of used markers in this study indicates an immunophenotype TTF1+CEA+CK7+ specific for lung adenocarcinomas, and un immunophenotype p63+, 34βE12+ and CK5/6+ specific for lung squamous carcinomas.

13. The careful monitoring of risk groups for LC within the population, by applying the proposed algorithm, especially in the occurrence of change of cough character in a chronic smoker, could lead to the screening in operable stages of these diseases and the increase of survival rate in these patients. The corroboration between cytological results of biological results and the bronchial endoscopic aspect may raise a suspicion of malignity, which must lead to the continuation of diagnostic invasive methods to specify the histopathological form.

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