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Original Paper

Presence of p53 in Tumor Cells - an Indicator of Disease Severity? Retrospective Study in Patients with NSCC (Partial Results)

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REZUMAT

Prezența p53 în celule tumorale - un indicator al severității bolii? Studiu retrospectiv la pacienți cu NSCC (rezultate parțiale)

Cancerul pulmonar este cea mai frecventă neoplazie, urmat de cancerele de sân, prostată și colon. Celulele canceroase conțin o multitudine de aberații genetice, care apar de-a lungul ciclului celular. Proteina mutantă p53 este considerată cea mai importantă dintre alterările moleculare prezente în neoplazii, model a cărui cunoaștere ar permite descifrarea mecanismelor moleculare ce determină apariția mutațiilor din cancerele umane. Am efectuat un studiu retrospectiv, în care am reținut 40 pacienți dintre subiecții adresanți în cadrul secției de pneumologie 3 a Institutului Național de Pneumologie „Marius Nasta". Aceștia au avut de parcurs un protocol, pornind de la criteriile de includere și excludere, de parcurs investigații (radiografie, bronhoscopie, tomografie computerizată, biopsie) pentru a putea stabili diagnosticul de cancer, tipul acestuia și determinări imunohistochimice. Dintre markerii utilizați în imunohistochimie, ne-am canalizat asupra p53 pentru a observa în ce măsură studiul acestui marker este util stabilirii tipului histopatologic de neoplasm pulmonar și în ce măsură este un predictor al evoluției bolii.

Cuvinte cheie: cancer pulmonar, imunohistochimie, p53

ABSTRACT

The lung cancer is the most common malignancy followed by cancers of breast, prostate and colon. The cancerous cells contain many genetic aberrations that occur throughout the cell cycle. The mutant protein and its investigation might provide insight into the molecular and histological characteristic of the disease. This retrospective study we established 40 patients selected conform inclusion and exclusion criteria froAm hospitalized people. All patients has been investigated with a succession of investigations (X-ray,

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bronchoscopy, computed tomography, biopsy), up to histology and immunohistochemical determinations. Among the markers used in immunohistochemistry, we focused on p53 in order to observe to what extent it is useful to establish the histopathological type of lung cancer and to what extent is a predictor of the disease evolution.

Keywords: lung cancer, imunohistochemistry, p53

INTRODUCTION

The lung cancer is the leading cause of death determined by malignancies in the world, followed by breast, prostate and colon cancer. The malignant cells present a variety of genetic aberrations that can be grouped into six essential pathways: (1) the acquisition of self sufficient or autonomous growth signals; (2) insensitivity to growth inhibitory signals; (3) resistance to signals of apoptosis; (4) unlimited proliferation potential; (5) sustained angiogenesis; and (6) invasion and metastasis1.

The p53 protein is a protein with molecular mass of 53 kDa (from where its name derives). The gene p53 encoding the protein p53 is located on the short arm of chromosome 14. The protein p53 is involved in maintaining control cellular genome stability and its disruption can lead to the emergence of malignancies. In about 50% of human cancers, the mutant protein p53 was detected. At the cellular level it regulating the transcription of some genes involved in cell growth control and apoptosis. The gene p53 can be inactivated by punctiform mutations and protein p53 can be inactivated by the formation of complexes with the cellular proteins or by proteolysis.

The mutant p53 protein loses the ability to bind to the nucleus and to block the cells in the G1 phase of the cell cycle when DNA is damaged. The two variants arising normally from the cell blocking in G1 phase: DNA repair followed by division or inability to repair followed by apoptosis, can no longer take place, and the cell is pushed towards the formation of malignant cell clones2.

Description of the mutant p53 protein as being the most important molecular alteration during neoplasia was made by C. Caron and T. Souss Fromentel in 1990 in a large study3. Since 1992 over 150 articles on the role of p53 in carcinogenesis have been published (4).

In lung cancers, the mutant p53 protein appears in 33% of adenocarcinomas and in about 70% of small cell carcinomas (SCC). Most of the studies published so far have concluded that its presence is associated with a poor prognosis and resistance to chimiotherapy (5).

Purpose of the study

This study aims to analyze the presence of mutant p53 protein in tumoral cells in patients with non small cell carcinoma (NSCC). Also, in cases with positive p53, we try to establish correlations between the stage of the disease and the presence and this marker. Regarding the establishment of correlations between the presence/ absence of p53 in tumor cells and the duration of the patients' survival since the moment of diagnosis, further studies are needed.

MATERIAL AND METHODS

The study group comprised 40 patients, selected from a larger group, applying inclusion and exclusion criteria. The aim was to obtain tumoral material to be histologically and immunohistochemically processed.

The inclusion criteria were: male or female patients aged over 18 years (no upper limit); symptoms suggestive of pulmonary impairment; standard chest radiograph suggestive of tumor; computed tomography (CT) highly suggestive for lung cancer; histopathological confirmation of cancer by biopsy from bronchoscopy or surgical interventions. Exclusion criteria were: male or female patients aged under 18 year; patients with contraindication to bronchoscopy, patients with contraindication for administration of contrast material in CT; patients without histopathological confirmation or confirmation of small cell carcinoma (SCC).

Patients were studied starting from anamnesis, clinical examination, laboratory investigations, of which emphasis was placed on radiography, bronchoscopy and CT. Standard radiography is looking for the presence of opacities in the lung, mediastinal

enlargement suggestive of adenopathies and the presence of pleural effusions. Computerized tomography with contrast agent injection sought to describe the lesion, its relation to neighboring structures (hilum, pleura, chest wall), the presence of local invasion and distant determinations, TNM (tumor, nodes, metastasis) staging. Bronchoscopy searched the mobility of vocal cords, the aspect of trachea and bronchial tree; when pathological aspects (congestion, infiltration, vegetation, tumor) are present, biopsy was performed. Surgery is performed for diagnosis purpose, in patients without confirmation from bronchoscopy (mediastinoscopy, video-assisted thoracoscopy, classical thoracotomy) and/ or for curative purpose in resecable patients.

Anatomo-pathological processing contained: macroscopic description of the piece, taking of fragments from all macroscopically identifiable lesions. The fragments were transformed into samples that were fixed, histologically processed, included in paraffin blocks, sectioned, displayed on the blades and colored6.

Immunohistochemistry - IHC - aims to visualize the antigenic properties of some substances from a tissue using monoclonal antibodies or poly-reactive sera. ABC method or the method of dextran7 is used for processing.

RESULTS

This retrospective study lasted 2 years, within the 3rd Department of “Marius Nasta" National Institute of Pneumology. It started from a total of about 2400 patients who addressed the Institute with various health disorders. After applying inclusion and exclusion criteria, out of 73 patients with suspected neoplasia, by the end of the study 40 patients were selected, who completed the entire protocol and were found to have NSCC type cancer (6 of patients who also completed the entire protocol, have been proven to have SCC).

The study group comprised 29 men (72.5%) and 11 women (27.5%) with NSCC type lung cancer, of which 34 were smokers (85%). They had the chest radiography suggestive of tumor (**Fig. 1**).

Bronchoscopy, as part of the study protocol, was designed to view the possible lesions and determine the operability of the tumor in selected patients (endobronchial lesion location, position in relation to the bifurcation of the trachea, presence or absence of extrinsic compression (through tumor mass or lymphadenopathy). Thus, in the patients of the above mentioned study we noticed: 29 cases with endobronchial lesions, out of which 24 (60%) with infiltration, 5 (12.5%) with burgeons; 11 (27.5%) had normal tracheobronchial tree 29 persons who underwent endobronchial biopsy, 2 left without confirmation, the histopathologic result being "without tumor material" (**Fig. 2**).

Computed tomography with contrast (**Fig. 3**) was performed for TNM staging purposes and in order to determine the extent to which patients can be included in a surgery protocol, or will be referred to the oncology network. In the 2 patients without histological confirmation of bronchoscopy, CT scan was useful to determine the best location for obtaining tumor material. After investigation, we noticed that no subject framed in the incipient disease stages I and IIA; in the operable stages IIB and IIIA framed 2 (5%) and respectively 13 (32.5%) patients, and in the surgically exceeded stages IIIB and IV, 7 (17.5%) and respectively 18 (45 %) patients to be met.

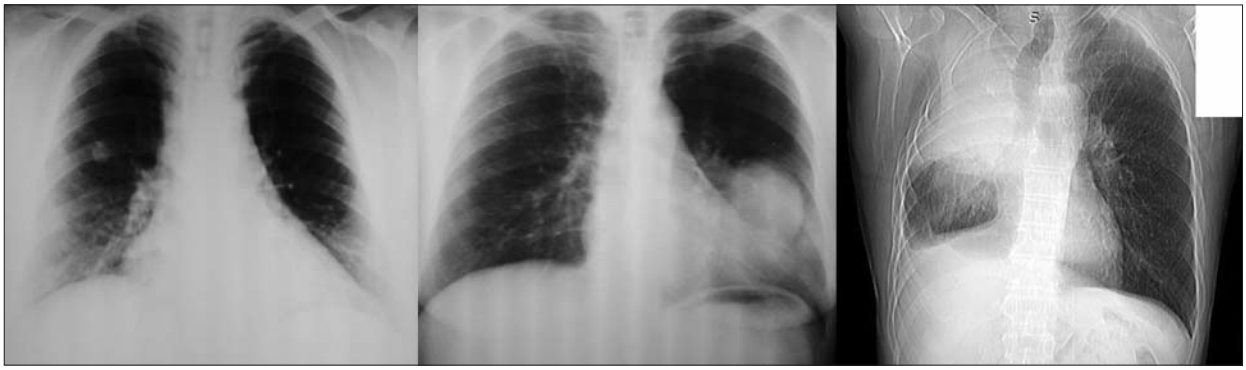

Figure 1. Chest Radiography: images suggestive for lung cancer

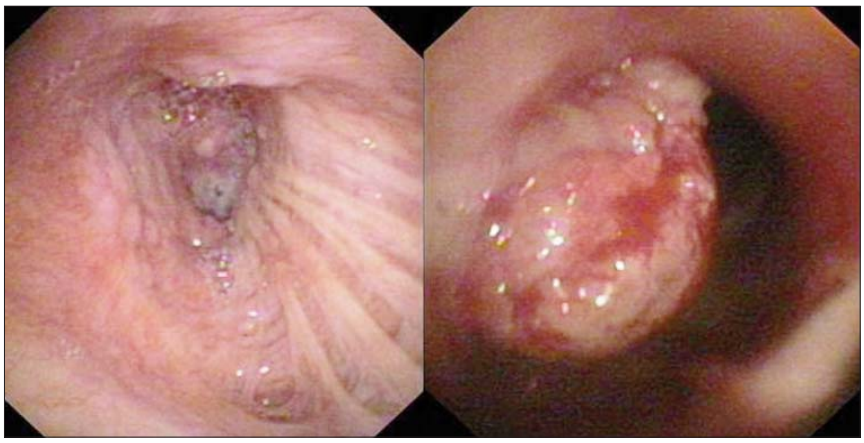

Figure 2. Bronchoscopy: infiltrative and burgeoning lesions

The latest investigation that has undergone in some patients was the surgical investigation, reserved to the cases without confirmation from bronchoscopy. The tumor material was taken from the bone (1 case), pleura (1), mediastinal lymph nodes (4) or from the tumor itself (7). Finally, the 40 patients who completed the investigational protocol presented the following histological types of neoplasm: adenocarcinoma 19 (47.5%), squamous cell carcinoma 17 (42.5%), adenosquamous carcinoma 1 (2.5 %), and 3 (7.5%) had poorly differentiated carcinoma (**Fig. 4,5**).

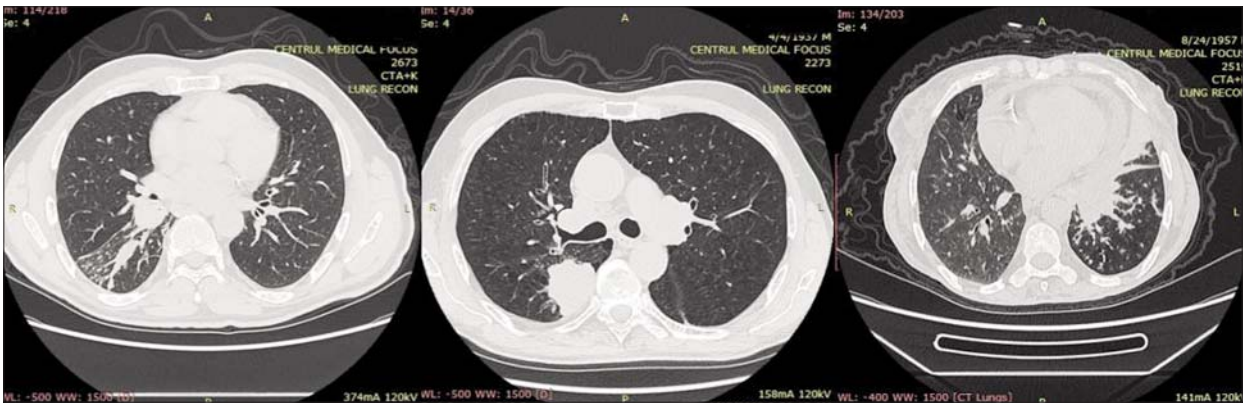

Figure 3. Computed tomography: aspects of lung tumors

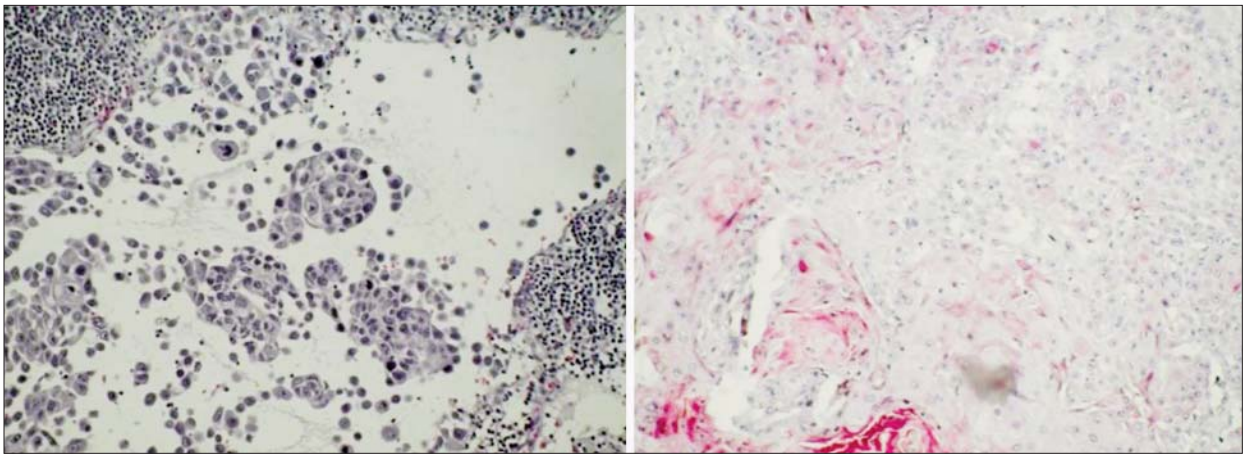

Figure 4. Optical Microscopy: adenocarcinoma and squamous cell carcinoma

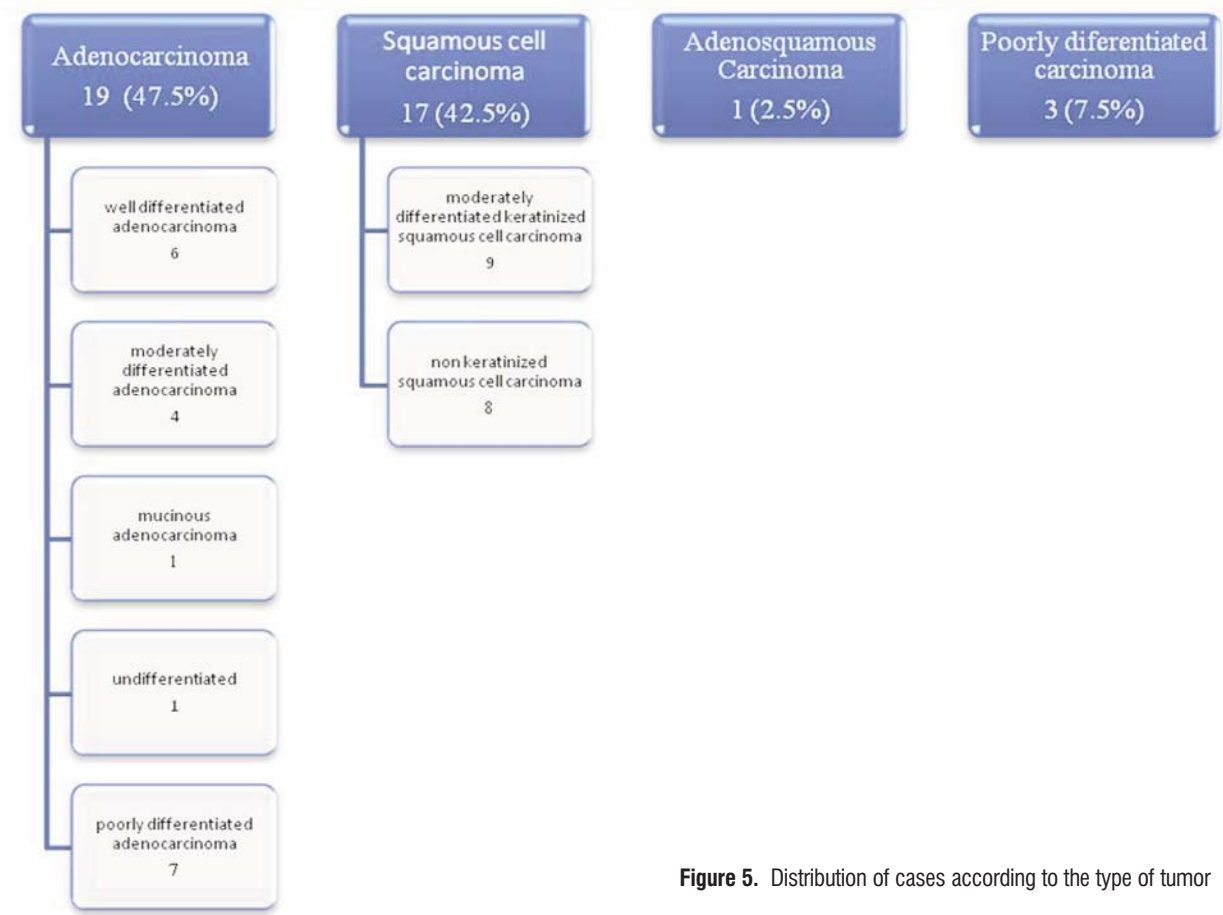

Figure 5. Distribution of cases according to the type of tumor

Of the patients with adenocarcinoma 6 (15%) had well differentiated adenocarcinoma, 4 (10%) - moderately differentiated adenocarcinoma, 1 (2.5%) - mucinous adenocarcinoma, 7 (17.5%) poorly differentiated adenocarcinoma and 1 (2.5%) - undifferentiated adenocarcinoma (**Fig. 4,5**).

Of the patients with squamous cell carcinoma 9 (22.5%) had moderately differentiated keratinized squamous cell carcinoma, and 8 (20%) non keratinized squamous cell carcinoma.

p53 is expressed as positive (in varying percentages) or negative in the tumor cells. (**Fig. 6**)

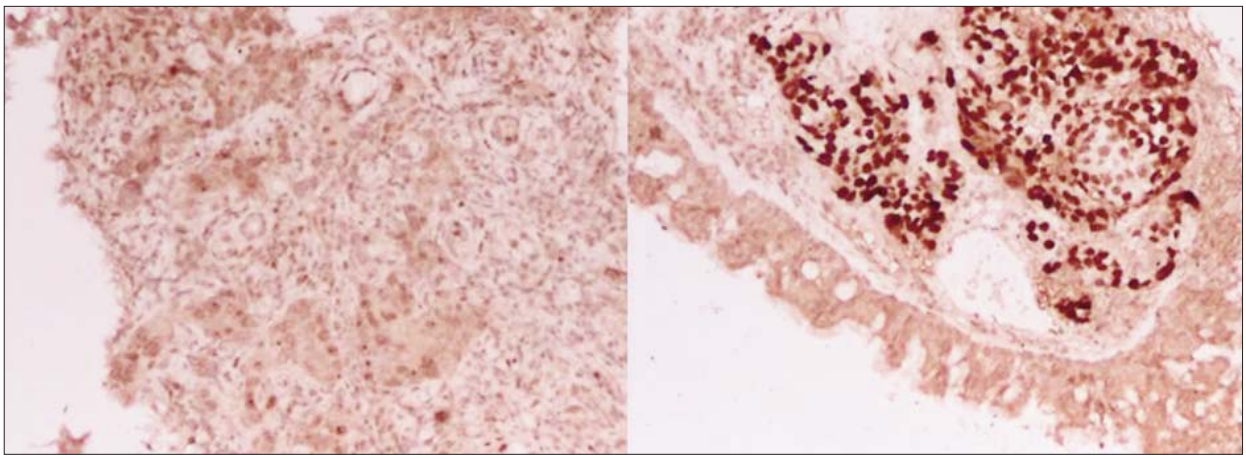

Figure 6. IHC image - p53 (adenocarcinoma, squamous cell carcinoma)

- Of the 19 patients with ADK, the distribution depending on type, was as follows **(Fig. 7)**:
  - o all the 10 (25%) patients with well or moderately differentiated ADK showed p53 positive
  - o the patient (2.5%) with mucinous ADK showed negative p53
  - o of the patients with poorly differentiated ADK, 4 (10%) had positive p53, and 3 (7.5%) had negative p53
  - o the patient (2.5%) with undifferentiated ADK had negative p53
- In the patients with squamous cell carcinoma, the following distribution of p53 was recorded (**Fig. 8**):
  - o of the patients with keratinized squamous cell carcinoma, 6 (15%) had positive p53, and 3 (7.5%) negative p53
  - o of the patients with squamous cell carcinoma without keratinization, 7 (17.5%) had positive p53, and 1 (2.5%) negative p53
- In the patient (2.5%) with adenosquamous carcinoma, p53 was positive in the tumor cells (**Fig. 9**)

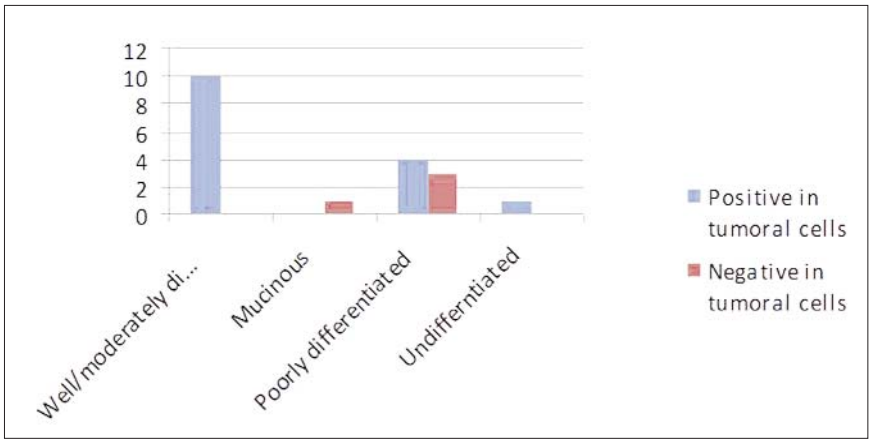

Figure 7. Presence of p53 in tumor cells in patients with ADK

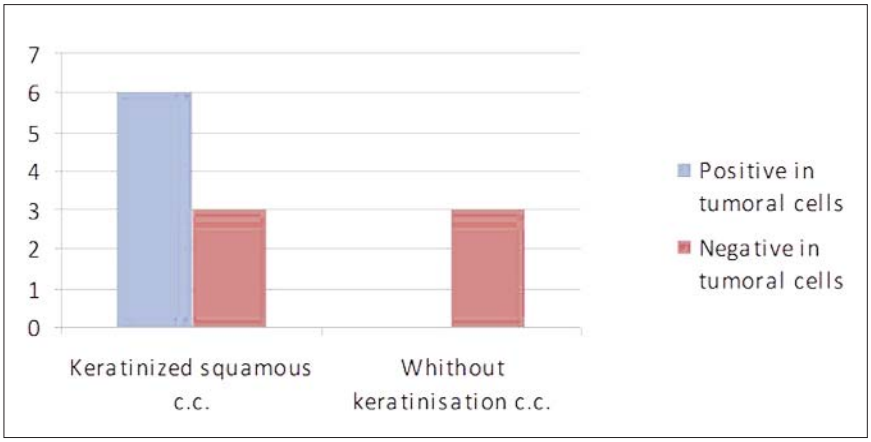

Figure 8. Presence of p53 in tumor cells in patients with squamous cell carcinoma

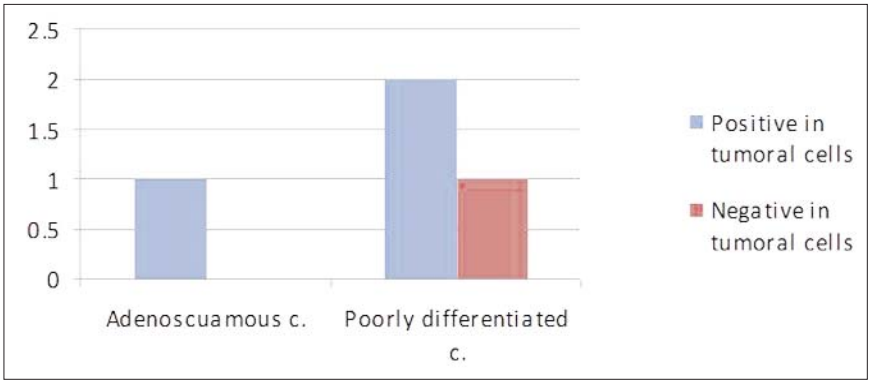

Figure 9. Presence of p53 in tumor cells in patients with poorly differentiated carcinoma and adenosquamous carcinoma

- In the 3 patients with poorly differentiated carcinoma, the following situations were recorded: one patient (2.5%) showed positive p53, one patient (2.5%) p53 negative, and one patient (2.5%) had inconclusive result (**Fig. 9**)

DISCUSSION

In this study, 29 (72.5%) patients recorded positive p53 in tumor cells (8 had negative p53 and one patient had an inconclusive result). Among the 19 patients with adenocarcinoma, 14 had positive p53 (> 30%) in tumor cells, and of the 17 patients with squamous cell carcinoma, 13 had positive p53 (> 30%); the same is valid for the patient with adenosquamous carcinoma and for one of the patients with poorly differentiated carcinoma. Among the patients with distant determinations, p53 was positive in 10 of 17 cases. On the other hand, among those with p53 positive, we noted that a small number had p53 values less than 30% (% patients), while in 18 patients the said values were between 30-59%, and in 6 patients the same were close to 90%. Dividing the patients in the group without secondary determinations (staged M) and those with metastases (M1), we noted the following situations: In the group of patients staged M0 (23 patients = 57.5%) the positive values of the marker prevail, most of them being framed in the range 30-60%. In this group, only two showed negative p53. In patients staged M1 (17 = 42.5%), nearly half (eight individuals = 47%) recorded negative p53, the rest having positive values , prevalently also in the range 30-59%.

From these data we can concluded that the marker has no predilection for one of histological types of NSCC, it being positive in 73.68% of patients with adenocarcinoma and in 76.47% of patients with squamous cell carcinoma, but shows a positive correlation with NSCC group, 72.5% positive results being recorded in the whole. In contrast, it negatively correlates with disease stage in patients staged Ml, nearly half of them having negative p53.

On the other hand, in terms of patients' mortality, there were: 9 were deceased by the end of the study, 10 were alive and in 10 patients no records were available.

We are able to appreciate that the positive marker p53 in tumor cells indicated a poor prognosis in 47.37% of the cases.

CONCLUSIONS

1. p53 is a marker present in NSCC tumor cells, without it to be able, alone, to define the histopathological type. For this, combinations of markers must be tested, which to include it.
2. p53 was not found to be an indicator of disease progression, the patients staged Ml having moderately elevated levels or absence of the marker in tumor cells.
3. The presence of p53 indicates a poor prognosis for survival.
4. It is necessary the present study to be followed by other research to see to what extent the combinations of markers (including p53) have a diagnostic or prognostic value for various NSCC cancers.

REFERENCES

1. Zhu C-Q., Shih W., Ling C-H., Tsao M-S. Immunohistochemical markers of prognosis in non-small cell lung cancer: a review and proposal for a multiphase approach to marker evaluation. J Clin Pathol 2006;59:790-800 doi:10.1136/jcp.2005.031351
2. Moldoveanu E., Popescu L.M. Apoptoza. Mecanisme moleculare. Editura Universitara „Carol Davila" - Bucuresti 1999, 165 pg.(A4), ISBN:973-98145-4-7
3. Caron de Fromentel C., Soussi T. TP53 tumor suppressor gene: a model for investigating human mutagenesis. Genes Chromosomes Cancer. 1992;4(1):1-15.
4. Soussi T. p53 Antibodies in the Sera of Patients with Various Types of Cancer: A Review. Cancer Research 2000;60(7):1777-88.
5. Vaughan C.A., Singh S., Windle W. et al. Gain-of-Function Activity of Mutant p53 in Lung Cancer through Up-Regulation of Receptor Protein Tyrosine Kinase Axl. Genes & Cancer 2012, http://ganc.sagepub.com, doi: 10.1177/1947601912462719
6. Ordinul nr. 1217/ 2010 al Ministerului Sanatatii, privind aprobarea ghidurilor de practica medicala pentru specialitatea anatomie patologica. online
7. ePatology, last update may 2010. online