

Histopathological Assessment of Non-Small Cell Lung Carcinomas

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ABSTRACT: Non-small cell lung carcinomas (NSCLC) are common lung tumors with numerous growth patterns associated with lesion types, which are frequently diagnosed late and are associated with a reserved prognosis. In this study, we analyzed histopathologically and statistically 52 cases of NSCLC, for which the growth types/patterns were described and compared with tumor grade, the presence of vessels invasion and stage of tumor. We observed the predominance of adenocarcinomas (ADK) and acinar, cribriform, solid and papillary growth patterns, frequent mixed transitional areas, while for squamous cell carcinomas (SCC) the non-keratinized aspect was dominant. Vascular invasion was identified in 38.5% of cases, most NSCLC being in advanced stages, respectively 57.7%. ADK with cribriform, solid, and micropapillary patterns, as well as nonkeratinized SCC (NKSCC) and large cell carcinoma (LCC) were significantly associated with high grade, vessels invasion, and advanced stage of tumors. Transitional areas of ADK and focal keratinizations in SCC suggest the presence of specific lung tumor histological lesions, which in evolution acquire aggressive patterns.

KEYWORDS: Tumor type, vascular invasion, tumor stage, non-small cell lung carcinoma.

Introduction

Non-small cell lung carcinomas (NSCLC) are the most common histopathological forms of lung cancer diagnosed worldwide [1].

While the epidemiology depends not only on tobacco exposure, but also on other environmental factors and genetic profile, global trends show that the incidence of NSCLC has started to decrease, with regional dependencies, as well as differences in education and economic status of patients [2].

With an incidence of over 85% and a high mortality, NSCLC includes adenocarcinomas (ADK- 40%), squamous cell carcinomas (SCC- 25-30%) and large cell carcinomas (LCC- 5-10%) [1,3].

Classical histological differentiation of tumor cells it is considered as independent predictor for survival in NSCLC cases regardless of tumor stage [4].

In general, tumor stage (pTNM) is a reliable indicator of NSCLC recurrence and prognosis, being a benchmark for the establishing of the therapeutic management.

However, there are heterogeneous tumor groups with different responses to treatment,

which are attributed to tumor purity, the presence of vascular or perineural invasion [4].

Also, numerous studies that have analyzed the prognosis of NSCLC growth types and patterns indicate that most lesions are diagnosed at advanced stages of the disease and a relatively high number of tumors present lymphovascular invasion at diagnosis [5].

On the other hand, the patients with small isolated lepidic ADK (L-ADK), they have an excellent prognosis, with survival approaching 100% at 5 years [6].

In this context, defining the relationship of growth patterns with histological parameters of aggressiveness of NSCLC may provide new information related to the biological behavior of tumor types and subtypes.

During the study, we descriptively analyzed histopathologically the subtypes of NSCLC in relation to tumor grade, vessels invasion, and stage of tumors.

Material and Methods

The study investigated 52 cases of non-small cell lung carcinomas (NSCLC), which came from patients investigated and operated on in the Craiova County Emergency Hospital during the period 2015-2022.

After the surgical intervention of total or partial pulmonary tumor resection, the biological material was fixed in 10% neutral formalin and analyzed macroscopically in the Pathology Department of the same hospital. The material was processed classically, with paraffin inclusion and Hematoxylin Eosin staining. The paraffin blocks were sectioned on semi-automatic microtomes, which produced 4µm sections, spread on slides, stained and permanently mounted.

Microscopic analysis of NSCLC followed the positive diagnosis and classification in compliance with the current criteria developed within the WHO (World Health Organization) [1].

In this study, we analyzed the tumor type and subtype, tumor grade, tumor stage and presence of vascular invasion. To establish the tumor subtype, in the case of tumors with mixed patterns, the quantitative percentage estimate of each pattern was performed, the lesion being classified according to the predominant one; in the case of mucinous adenocarcinomas and basaloid squamous carcinomas, a threshold of 50% of the tumor was used for classification [1].

Regarding the ADK grading, well-differentiated tumors (G1) were designated as lepidic, without/ with under 20% high-grade growth (solid/ micropapillary/ cribriform), moderately differentiated tumors (G2) were acinar/papillary without/ with under 20% high-grade growth, and G3 (poorly differentiated) tumors were designated as having any high-grade pattern in volume of ≥20%; for CCS, the classic criteria of the presence of keratinization, atypia and mitotic activity were used [1].

The statistical analysis used comparison tests (Fisher, chi square-χ²) within the Statistical Software for Social Sciences (SPSS 10), the obtained values being considered significant for p<0.05 values and at the limit of significance for p<0.1 values.

In carrying out the study, ethical rules in research were respected and the Local Ethics Commission approved the investigation proposal (no. 194/14.10.2022).

Results

The analysis of the 52 cases of NSCLC indicated the predominance of adenocarcinomas (ADK), identified in 51.9%, followed by squamous cell carcinoma (SCC) with 42.3% and large cell carcinoma (LCC) with 5.8% (Table 1).

ADK presented varied histological aspects, with numerous growth patterns, which were observed individually or in association (40.4%), sometimes suggesting an evolutionary lesional continuum of lesions.

In some cases there were areas of transition between the different growth patterns, most frequently between lepidic, papillary and acinar ones, but also areas of transition to more complex patterns, such as micropapillary or cribriform ones (Figure 1A).

Table 1. Cases distribution depending on the investigated parameters.

Parameter	No. cases	
Tumor type	Adenocarcinoma (ADK)	27
	Squamous cell carcinoma (SCC)	22
	Large cell carcinoma (LCC)	3
Tumor subtype	Acinar (A-ADK)	8
	Cribriform (C-ADK)	4
	Papillary (P-ADK)	4
	Solid (S-ADK)	4
	Micropapillary (MP-ADK)	3
	Mucinous (M-ADK)	2
	Lepidic (L-ADK)	2
	Basaloid squamous cell carcinoma (BSCC)	5
	Non-keratinizing squamous cell carcinoma (NKSCC)	17
Tumor grade	Well differentiated (G1)	2
	Moderate differentiated (G2)	22
	Poorly differentiated (G3)	28
Vascular invasion	Absent	32
	Present	20
Tumor stage	I	5
	II	17
	III	14
	IV	16

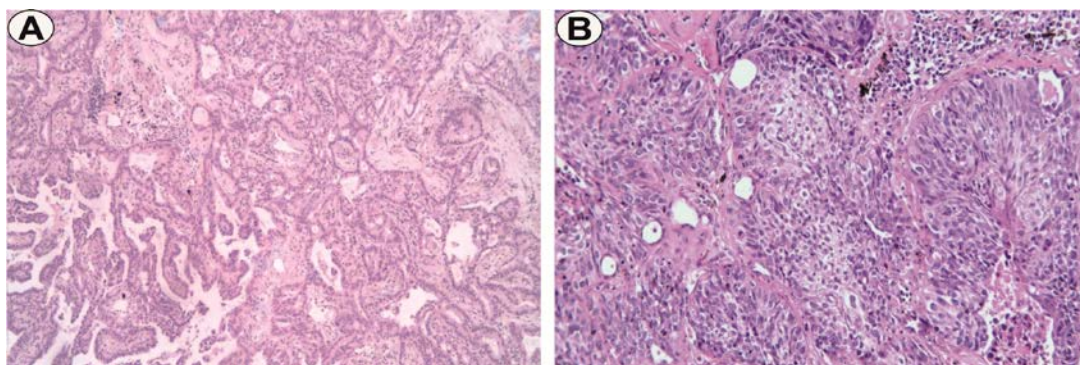


Figure 1. A. ADK-lepidic, papillary and cribriform transition, HE staining, x100; B. LCC, HE staining, x200.

SCC were characterized by a variable architecture, solid, insular or cordonal, with a predominance of squamous pattern without keratinization, or with focal keratinization, as well as a basaloid pattern, with peripheral palisading and sometimes with areas of necrosis. Cellular atypia and mitotic activity were variable, but generally increased.

LCC showed particular features, without glandular, squamous or small cellularity.

The tumors were composed of solid nests of round-polygonal cells, moderate/abundant cytoplasm, nuclei with visible nucleoli, with increased mitotic activity (Figure 1B).

Analysis of tumor subtypes indicated the acinar pattern of ADK (A-ADK) as the most

frequent (15.4%), with round-oval or serrated tumor glands, which distorted the pulmonary architecture (Figure 2A).

Cribriform (C-ADK), papillary (P-ADK) and solid (S-ADK) types were present in 7.7% of cases each. C-ADK was characterized by the presence of invasive fused tumors back-to-back, giving the appearance of a more advanced A-ADK (Figure 2B).

P-ADK showed proliferation of glandular tumor cells along conjunctival-vascular axes, sometimes with secondary branches (Figure 2C).

S-ADK consisted of polygonal tumor cells arranged in islands/trabeculae sometimes anastomosed without other histological architecture (Figure 2D).

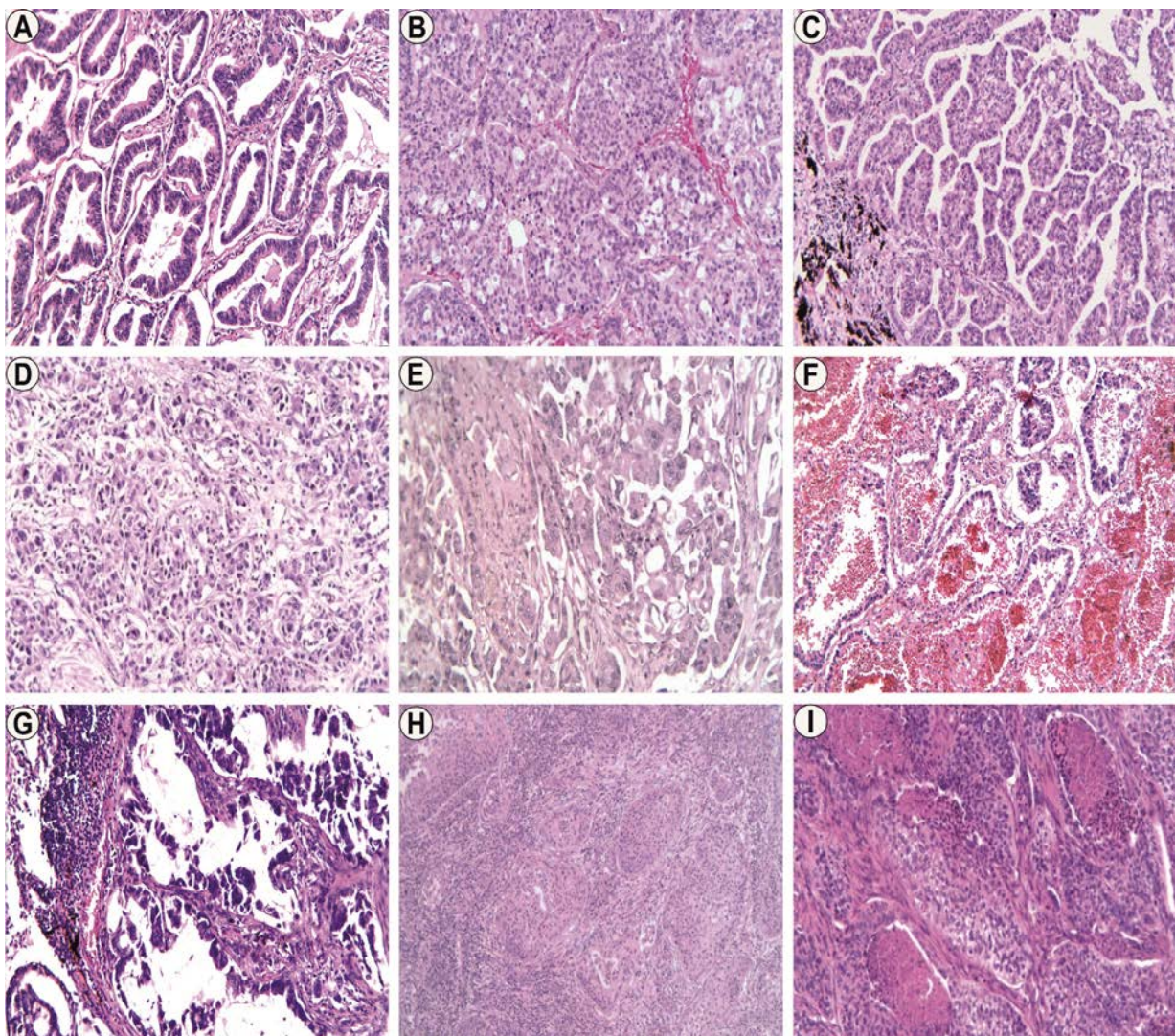


Figure 2. A. A-ADK, HE staining, x200; B. C-ADK, HE staining, x200; C. P-ADK, HE staining, x200; D. S-ADK, HE staining, x200; E. MP-ADK, HE staining, x200; F. L-ADK, HE staining, x200; G. M-ADK, HE staining, x200; H. NKSCC, HE staining, x100; I. BSCC, HE staining, x200.

Micropapillary adenocarcinoma (MP-ADK) was present in 5.8% of NSCLC and consisted of small, delicate papillae lacking a vascular axis with cuboidal cells with variable nuclear atypia and frequently with a stromal pattern with extensive infiltration of the stroma by tumor cell clusters (Figure 2E).

Lepidic adenocarcinoma (L-ADK) present in 3.8% of cases was characterized by minimal tumor architectural complexity, with proliferation of cells along the alveolar surface and invasive areas that met the diagnostic criteria (Figure 2F).

Mucinous adenocarcinoma (M-ADK) represented 3.8% of NSCLC analyzed and consisted of the presence of tumor or columnar cells with intracytoplasmic mucin and small nuclei, with discrete nuclear atypia and adjacent alveolar spaces with mucin (Figure 2G).

Nonkeratinizing squamous cell carcinomas (NKSCC) were present in 32.7% of cases and were characterized by islands of tumor cells with varying degrees of nuclear atypia, sometimes with focal keratinization especially in the periphery of the islands, sometimes with a

confluent trabecular/reticular appearance, with areas of acantholysis and focal necrosis (Figure 2H).

Basaloid squamous cell carcinoma (BSCC) was identified in 9.6% of cases, characterized by small to intermediate-sized cells, with lobular organoid architecture and peripheral palisading with increased mitotic activity, sometimes with focal keratinization and central necrosis (Figure 2I).

The tumor grade of NSCLC indicated the predominance of poorly differentiated high-grade lesions (G3-3.8%), most of the tumors having solid, micropapillary, cribriform growth patterns in increased quantity. In a smaller number of cases, the lepidic pattern (G1-3.8%) or the acinar/papillary (G2-42.3%) pattern predominated; in the case of SCC, cellular pleomorphism and increased mitotic activity were associated with G3 lesions (Table 1).

Lymphovascular invasion was present in a significant number of NSCLCs (38.5%), although the tumors in which this parameter was not present predominated (Table 1) (Figure 3A).

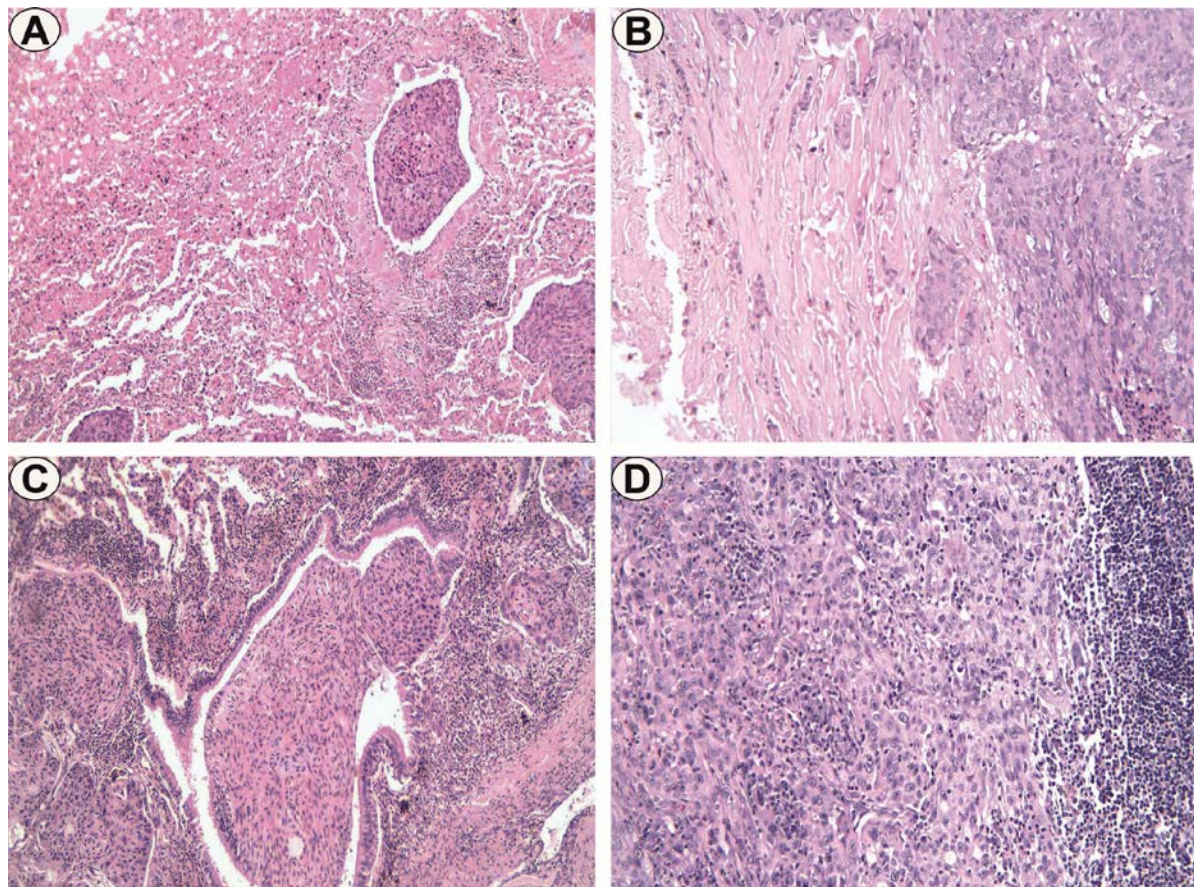


Figure 3. A. Vascular invasion, HE staining, x100; B. Invasion of the visceral pleura, HE staining, x200; C. Bronchial invasion, HE staining, x100; D. Lymph node metastasis, HE staining, x200.

Analysis of tumor stage of NSCLC indicated the predominance of stages III-IV (57.7%).

In addition to the specific size range, some of the NSCLC classified as T2 had associated invasion of the visceral pleura or showed invasion of the main bronchus or extensive atelectasis, while some T3 tumors showed invasion of the parietal pleura or the presence of separate nodules in the same lobe as the tumor (Figure 3B-C).

Tumor metastases were present at the lymph node level (pN), in the contralateral lobes (pM1a) or distant, in the bone, brain, liver and/or adrenal level (pM1b-c) (Figure 3D).

Although only ADK were classified in stage I, and LCC only in stages III-IV, the aspects were without statistical significance ($p=0.102$, χ^2 test).

The subtypes C-ADK, S-ADK and MP-ADK, but also LCC and NKSCC were significantly associated with vascular invasion ($p=0.004$, χ^2 test) (Figure 4A).

Vascular invasion was significantly or at the limit of significance associated with G3 NSCLC ($p=0.010$, χ^2 test) (Figure 4B), with the pT3 category ($p=0.077$, χ^2 test), with the metastases in lymph node ($p<0.001$, χ^2 test), distant metastases ($p<0.001$, χ^2 test) and with advanced stages III-IV ($p<0.001$, χ^2 test) (Figure 4C).

While stage I was exclusively associated with L-ADK and A-ADK, stages III-IV were associated with NKSCC, BSCC, MP-ADK, S-ADK and C-ADK, statistically significant differences ($p<0.001$, χ^2 test) (Figure 4D).

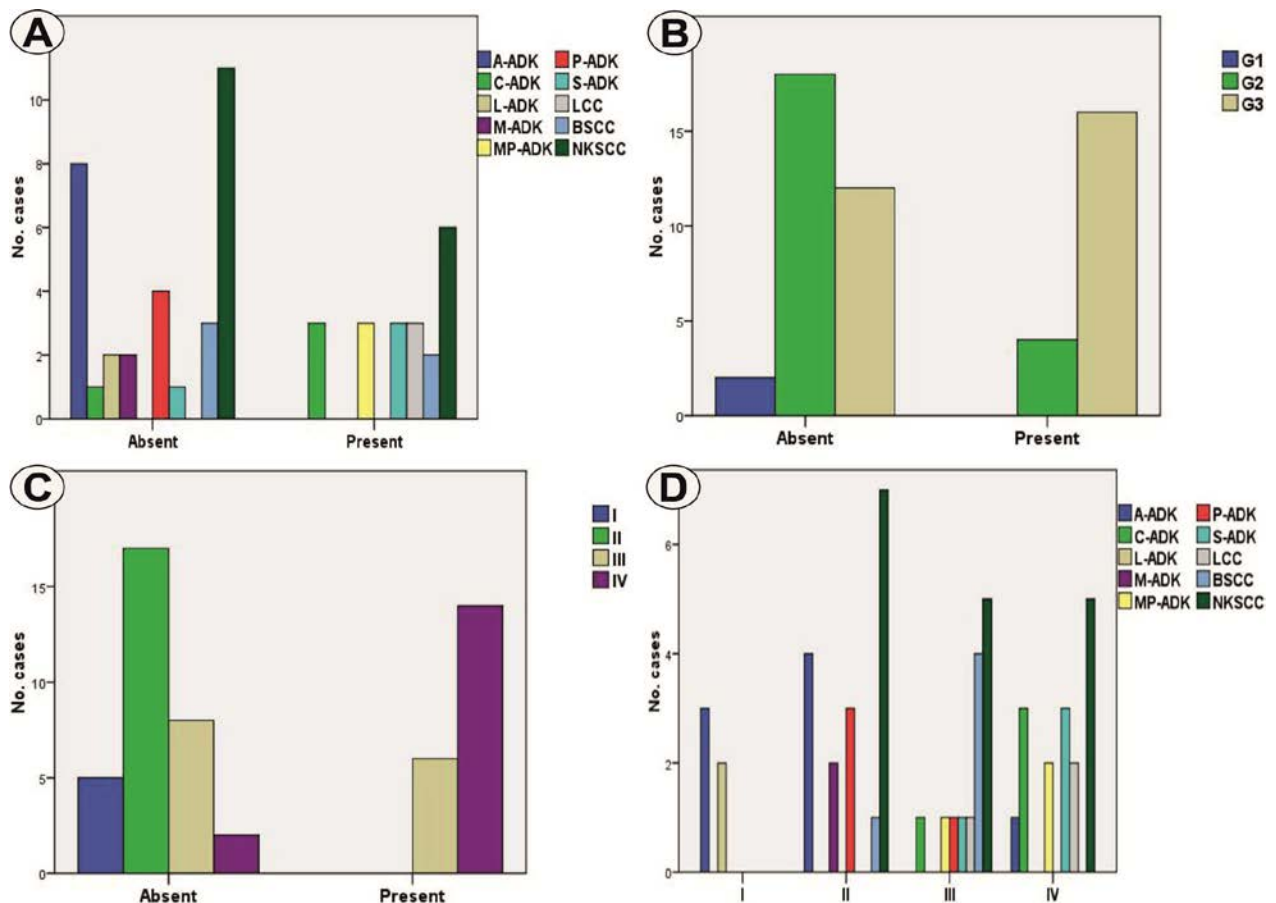


Figure 4. A. NSCLC distribution depending on tumor type/pattern and vascular invasion; B. NSCLC distribution depending on tumor grade and vascular invasion; C. NSCLC distribution depending on tumor stage and vascular invasion; D. NSCLC distribution depending on tumor type/ pattern and tumor stage.

Discussions

Over time, the incidence of NSCLC has varied so that SCC, which used to be the most common lung tumor, has been surpassed in many regions of the world by ADK [1].

In this study ADK were the most frequent tumor type, with the acinar pattern (A-ADK) being the most common, more than double compared with other architectural subtype (cribriform, solid, papillary, lepidic, micropapillary, mucinous); in case of SCC, the non-keratinized (NKSCC) tumors predominated.

In general, tumors with a predominantly lepidic pattern have a prognosis which is better compared with predominantly acinar (A-ADK) or papillary adenocarcinoma (P-ADK), while micropapillary (MP-ADK) and predominantly solid (S-ADK) tumors have a worse prognosis [7].

In ADK cases, numerous growth patterns are described, and the lesion is classified according to the predominant one, so that the prognosis is also dependent on the histological aspect; at the same time, no such predictive relationship for evolution is described in the case of SCC [1].

In the study we took into account the thresholds and criteria required for the classification of NSCLC and the establishment of tumor grade.

In our study, G2/G3 (high-grade, moderately and poorly differentiated) NSCLC accounted for over 95%. In the study by Sun Z et al. conducted on NSCLC cases, histological grade was statistical associate with survival after regulating for age groups, gender, smoking status, stage of tumor, histological pattern and therapeutic options [8].

Patients with high grade carcinomas, if compared with low grade (well differentiated) ones, had a superior risk for death (80%) [8].

Some studies suggest that poorly differentiated pulmonary ADK (solid patterns) may present a focal keratinized histopathological appearance, which thus cannot be distinguished from squamous cell carcinoma, indicating the importance of immunohistochemistry (TTF-1 and p40) in their differential diagnosis [3].

The prevalence of lymphovascular invasion in NSCLC cases varies between 5% and 40% [5].

In a study conducted on NSCLC cases diagnosed at tumor stage T1-2N0, Sung SY et al. included 381 patients, for whom a significant decrease of disease-free interval during 5 years

was found in cases with lymphovascular invasion, along with an increased risk of lymph node and distant metastasis [5].

The presence of lymphovascular invasion is a worse factor for prognosis for disease-free period and general survival [9].

Another study which investigated the significance of prognosis of vascular invasion in lung carcinoma and compared patients with stage IA NSCLC with lymphovascular invasion with those with stage IB NSCLC without invasion, concluding that the prognostic outcomes for both cohorts were comparable [10].

The TNM staging system redefined the T staging factor for patients with vascular invasion, raising it from T1 to T2a and advancing the tumor stage from IA to IB [9].

In our study, lymphovascular invasion was statistically significantly associated with ADK (solid, cribriform, micropapillary), LCC, and NKSCC, with poorly differentiated G3 tumors and advanced stages.

Recent studies have shown that tumor size is important. In this context, it has been observed that tumors over 5cm present a poor prognosis when compared to older TNM systems, and the larger tumors than 7cm have a similar prognosis [11].

A similar aspect is seen in the case of atelectasis/pneumonitis, which did not matter whether it was partial or total in NSCLC [11].

In contrast, when invasion at the level of the diaphragm was established (parameter T3 or T4 in different editions), the prognosis was reserved when compared with other T3/T3 descriptors. [11].

In this study, tumors in stages III-IV predominated; while stage I was exclusively associated with ADKL and ADKA, stages III-IV were associated with NLSCC, BSCC, MP-ADK, S-ADK, and C-ADK.

In the case of NSCLC, 30% of patients can benefit from surgical therapy, provided that the diagnosis is made in stages I-IIIa of the disease [12].

In this context, there are observations indicating that the 5-year survival of patients with NSCLC varies from over 70% in stage IA to under 10% in stage IIIB [12].

Conclusions

LCC and SCC are generally associated with high grade, but also vascular invasion and stages III-IV, despite areas of focal tumor keratinization.

Cribriform (C-ADK), micropapillary (MP-ADK) and solid (S-ADK) patterns are related with vascular invasion and advanced stages, being the most aggressive growth patterns of ADK, compared to lepidic and acinar ones that seem to associate milder histological features.

However, the relatively large number of ADK cases with transitional areas makes the prognostic classification of NSCLC difficult and suggests the existence of initial lesions with differentiated growth patterns that subsequently evolve architecturally and cytologically, with the acquisition of a biological behavior adapted to the particularities of the lung histology.

Conflict of interests

None to declare.

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