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A profile of lung carcinomas: study on 364 cases

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Abstract

The authors have proposed to analyze retrospectively the clinical-morphological profile of a consistent group of lung carcinomas treated surgically. The studied material consisted of clinical and pathological medical records from 364 patients confirmed histopathologically with lung carcinoma after surgical intervention. Five main groups were defined based on the histopathological criteria and then compared. The assessment of clinical data, in spite of a wide range of clinical expressions, revealed some particular features for each of the defined groups. The morphological data outlined also different behavioral profiles for each of the histopathological types of lung carcinoma. These results showed that malignant epithelial tumors of the lung are still a major challenge from the detection until the therapeutic intervention and, therefore, the preoperative clinical-morphological investigation is crucial for a better adjustment of the therapeutic act according to the individual profile of each type of tumor.

Keywords: lung carcinoma, clinical-morphological profile.

Introduction

The lung cancer is one of the deadliest malignancies in the world, with a 1.5 million human tribute per year. Patients affected by lung cancer have a five-year survival rate varying from 13% to 21% in developed countries and from 7% to 10% in emerging countries [1].

It is known deep enough now the histopathology of lung cancer. Most histopathological classifications of lung cancer, including revised *WHO* classification, mention four main types: squamous cell carcinoma (SC), adenocarcinoma (ADK), large cell carcinoma (LCLC), and small cell carcinoma (SCLC). There are also described several histological subtypes and some rare forms.

The new techniques, immunohistochemistry (IHC) particularly, have provided a wide spectrum of information about histogenesis, tumor differentiation and tumor proliferation, widely used to classify these tumors.

In current practice, lung cancer is routinely classified as SCLC or Non Small Cell Lung Cancer (NSCLC) based on distinctive pathological and molecular features but also for appropriate therapeutic management [2].

NSCLC became from a rare malignancy 100 years ago the leading cause of cancer death in the Western world [3]. ADK accounts for 25 to 30% of NSCLC and is the most common histological type. It is typically classified as acinar, papillary, solid, and bronchioloalveolar [4, 5]. The incidence of ADK has increased in recent decades, while the incidence of SC has decreased [6].

Due to the emergence of modern chemotherapy, which has shown the relative sensitivity of SCLC and studies that have shown the frequency of early metastatic spread in this type of malignancy, the clinician is particularly concerned by the differentiation of lung cancer in SCLC and NSCLC. The same request comes from thoracic surgeons who need this distinction largely between SCLC and NSCLC for choosing therapeutic attitude, given that currently SCLC does not have surgical indication [7].

The aim of the study is to analyze a consistent group of lung carcinomas treated as a first step by surgical intervention, providing the latest diagnostic according to the established classification systems of pulmonary malignancies, correlating histopathological aspects with a number of clinical parameters and illustrating the most significant aspects found in the studied cases.

Materials and Methods

The study group consisted of 364 cases with lung tumors operated in the Department of Thoracic Surgery, Centre Hospitalier d'Avignon (France) and histologically diagnosed in the Department of Pathology of the same hospital. One of these tumors proved to be a metastatic tumor coming from a renal carcinoma.

The 363 remained primary tumors were divided, according to the histopathological appearance, in five main subgroups: (1) Carcinoid tumors; (2) SCLCs; (3) ADKs; (4) SCs; (5) Other types of NSCLC, represented mainly by LCLCs.

The inclusion criteria for patients in the study group and subgroups were: the existence of surgical procedure and the histopathological diagnosis of lung carcinoma.

The study material was represented by: patient's medical charts, surgical procedure records, and histopathologic diagnosis records.

The study was a retrospective one and was divided

into two sections: the clinical data study and the pathological study.

To collect data, "database" files were created on the computer where all the parameters to be studied were recorded. These parameters were divided into the following categories:

▪ Parameters defining the clinical profile (gender, age, clinical status, specific preoperative therapy) and

▪ Parameters defining the morphological profile (site, histopathological type, degree of differentiation, secretory phenotype, intratumoral necrosis, vascular intratumoral embolia, local invasion, lymph node involvement, distant spread and TNM stage).

For the assessment of microscopic parameters, tissue fragments from surgical excision samples were processed by the classical histological technique (fixation in 10% buffered formalin and paraffin embedding) and stained with classical techniques (Hematoxylin–Eosin and Alcian Blue) and IHC technique. A wide range of antibodies were used which were grouped as following:

▪ Markers to identify lung tumors: TTF-1, CK7, CK20, Anti-CA 15-3 antibody;

▪ Epithelial markers: CK AE1/AE3, CK5/6, BER-EP4, KL-1, ACE, PK EMA, CD56;

▪ Neuroendocrine (NE) markers: Chromogranin – Chromo, Synaptophysin – Syn, Neuron Specific Enolase – NSE;

▪ Rarely used markers: Anti-thyroglobulin antibody, BHCG, PLAP, PSA, Anti-thyrocalcitonin antibody, AFP, Panleuco-1, CD3, CD20, CD30, CD45, CD99, CD117, CD141, Vimentin, Desmin, Actin, E-cadherin, Calretinin.

Data processing was performed using the Microsoft Excel module from the Microsoft Office XP Professional software package.

Analysis of some of the parameters required a primary data filtering, consisting in definition of sets of specific categories:

▪ For age evaluation: adolescent (<25 years), young adult (25–44 years), adult (45–65 years), and old (>65 years);

▪ For clinical status evaluation: the classification recommended by *WHO* [8];

▪ For site assessment: left lung and right lung;

▪ For histopathological assessment: *WHO* classification of lung tumors [9];

For numerical parameters, the lowest value (VMIN), the highest value (VMAX), mean value (AV), and standard deviation (STDEV) were determined.

The graphs (charts) showing the evolution patterns of different assessed parameters and the comparisons between them were done with the "Graph" instrument included in the "Word" and "Excel" modules of the Microsoft Office XP Professional software package.

Results

Clinical data

Gender distribution

Overall, as expected, men were more affected than women were. However, SCs were usually encountered in men whereas carcinoid tumors were prevailing in women (Figure 1).

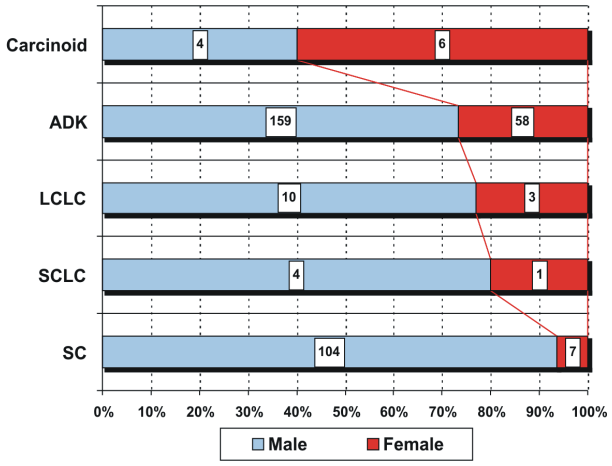

Figure 1 – ***Comparison between gender distribution of studied subgroups.***

Age distribution

Excepting the carcinoid subgroup that was encountered in younger patients, all the others subgroups had their average age in the seventh decade of life (Table 1).

Table 1 – ***Statistical parameters of age in studied groups***

Parameter	Carcinoid	LCLC	ADK	SCLC	SC
Lowest age [years]	16	39	34	49	43
Highest age [years]	77	78	84	80	82
AV [years]	47.9	61.8	61.9	62.6	66
STDEV	20.64	8.8	10.9	12.01	8.6

The distribution of cases by age group showed that lung carcinomas were mainly encountered after 45 years but with some slight differences between different types. Thus, whereas LCLCs were more frequent in adults (less than 65 years), more than half of SCs were discovered in old people (Figure 2).

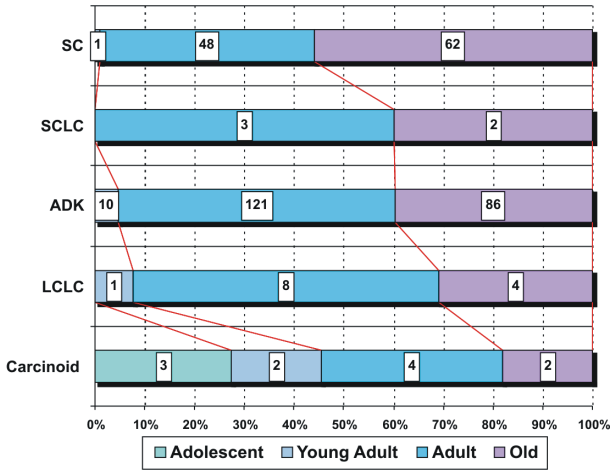

Figure 2 – ***Comparison between age distribution in studied groups.***

Clinical status

Most patients had a satisfactory clinical status, being included in *WHO* classes 1 and 2, which encouraged the radical surgery. However, the best clinical status was noticed for the carcinoid tumors and LCLCs and the worst for SCLCs (Figure 3).

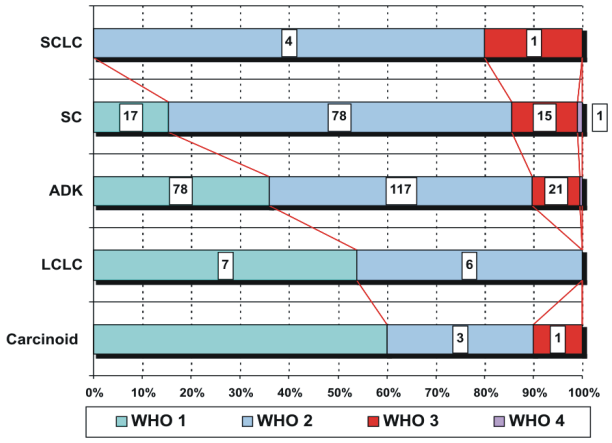

Figure 3 – ***Comparison between distribution based on clinical status in studied groups.***

Specific preoperative therapy

Preoperative adjuvant therapy was applied mostly in ADKs and SCs, confirming the usefulness of this approach in these types of tumors (Figure 4).

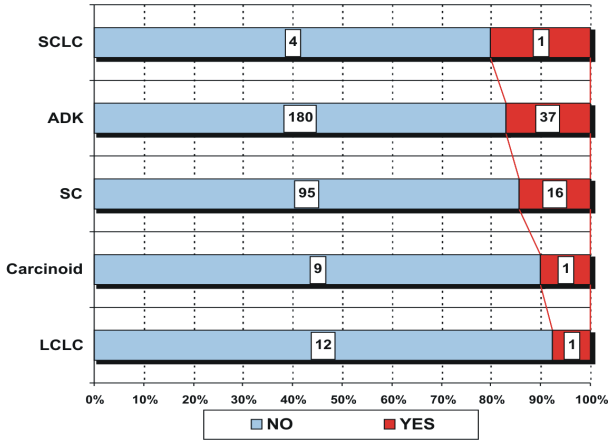

Figure 4 – ***Comparison between preoperative therapy distribution in studied subgroups.***

Morphological profile

Site – Lung

The analysis of tumor site revealed a slight predilection of carcinoids and ADKs, for the right lung whereas LCLCs and SCLCs were obviously more frequent in the left lung (Figure 5).

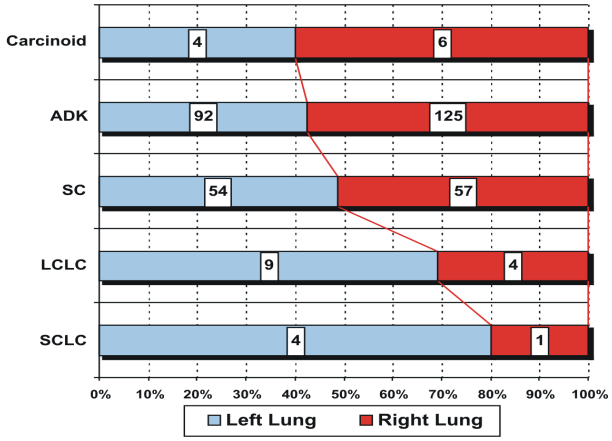

Figure 5 – ***Comparison between site distribution of tumors in studied subgroups.***

Histopathological types

In the study group, a net predominance of NSCLCs, a small percentage of SCLCs and a significant percentage of carcinoid tumors were observed. Also, worth to be mentioned the large proportion – almost one third – of SCs and the small percentage – 4.4% – of other types of non-small cell tumors (Table 2).

Table 2 – ***Distribution of histological types of LC***

Histopathological type		No. of cases		%	
NSCLC	*Adenocarcinoma*	*217*	348	*59.64*	95.64
	Squamous carcinoma	*111*		*30.5*	
	Other types	*20*		*5.48*	
SCLC		5		1.37	
Carcinoid tumor		10		2.74	
Secondary tumors		1		0.27	

Adenocarcinoma

The tissue architecture of this type of primary lung epithelial neoplasia is widely varied, with four major types: acinar, papillary, bronchioloalveolar and solid plus some rare variants. However, the lesions are, most of the times, composed of two or more different types or variants, which is why in the *WHO* classification has been introduced a special group called "mixed ADK" [9, 10].

The analysis of different histological types of ADK shares found in the studied cases, according to the 2004 *WHO* classification showed a clear prevalence of the acinar type (Table 2, Figure 6a).

Table 2 – ***Distribution of histological types of ADK***

Histopathological type		No. of cases		%	
Acinar		116		53.46	
Papillary		34		15.67	
Bronchioloalveolar	*Non-mucinous*	*5*	7	*2.3*	3.22
	Mucinous	*1*		*0.46*	
	Mixed	*1*		*0.46*	
Solid with mucus formation (S + MF)		27		12.44	
With mixed subtypes	*"Clear cell"*	*2*	4	*0.92*	1.84
	"Signet ring cell"	*1*		*0.46*	
	Fetal	*1*		*0.46*	
Unspecified		29		13.37	

The papillary form, less described in the literature [9, 11, 12] took second place followed by the group of solid tumors with mucus formation, which also had a proportion higher then 10%. Rare variants, commonly encountered in association with one of the major features, forming the so-called mixed subtypes have been identified only in four cases.

The bronchioloalveolar type, reported in the literature in the past with a great variability, ranging from 4% to 24% of all lung cancers [13], variability due to permanent changes over time of the morphological criteria definition [9, 14, 15], was less than 5% of the studied tumors, in accordance with current reporting data – 2 to 6% of all lung cancers [13, 16, 17] –, resulting from the restrictive change in the criteria defined by Travis WD *et al.* in 1999 [18].

The degree of differentiation

The degree of differentiation was assessed in all types except bronchioloalveolar forms, considered features of

in situ neoplasia. In the 174 cases with both the ADK type and the degree of differentiation recorded, moderately differentiated types were predominant (Figure 6b) but different histological subtypes of ADK showed different patterns of the differentiation degree. Thereby, while the mixed tumors were only poorly differentiated, half of the papillary tumors were well differentiated (Figure 7).

Secretory phenotype

The malignant cells of ADKs sometimes tend to secrete as they are deemed to have their origin in the mucus-producing glandular structures of the lung parenchyma. Secretory phenotype (Figure 6c) was revealed in 58% of cases.

Except in the cases included in the "solid mucus producing" type which is, by definition, a form of secretory tumor, the presence of secretory phenotype was most common in papillary tumors – 91% of cases and the rarest – 25% – in mixed tumors (Figure 8). In the bronchiolo-alveolar types prevailed the non-secretory subtype, with Clara cell/type II pneumocytes.

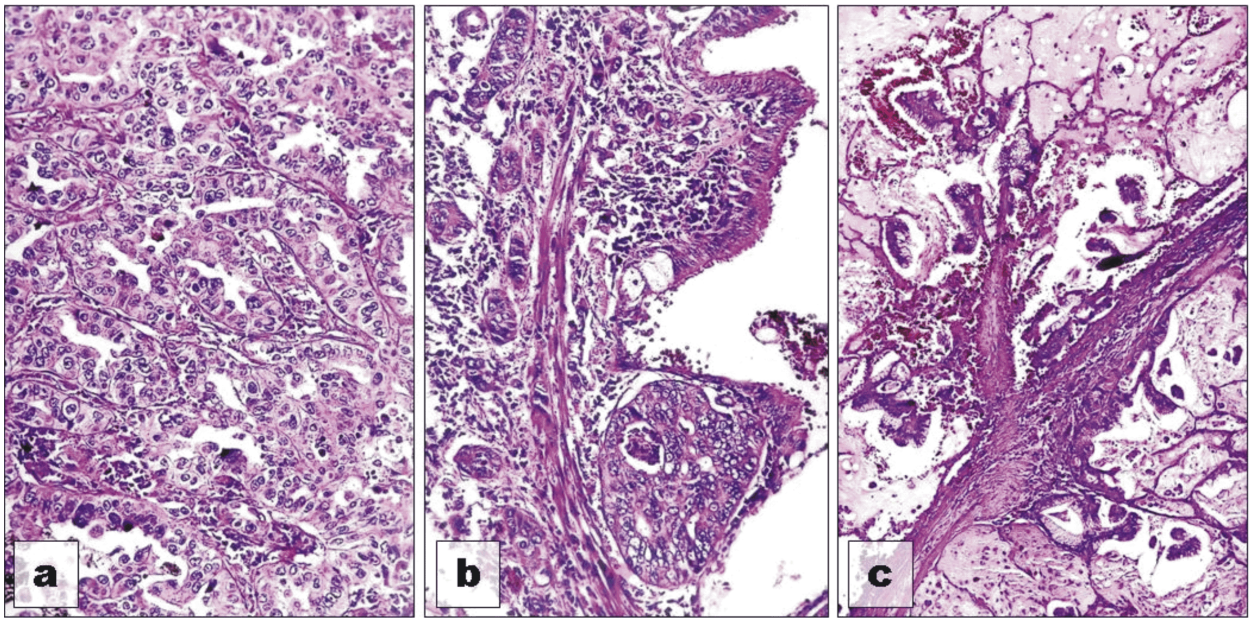

Figure 6 – ***Morphological features of adenocarcinoma: (a) Acinar pattern; (b) Moderate differentiation; (c) Secretory phenotype.***

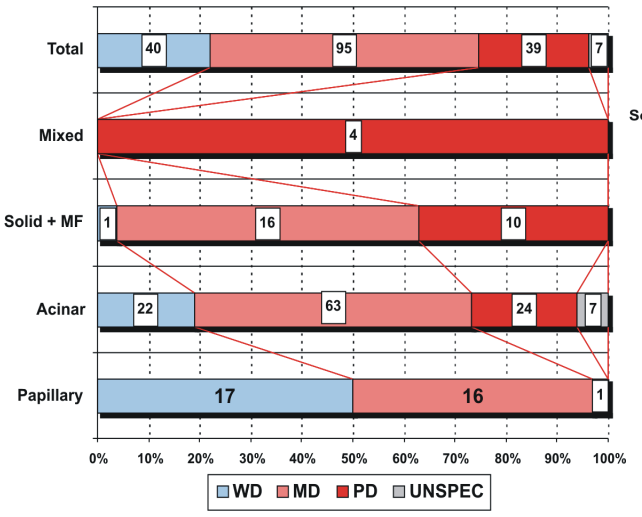

Figure 7 – ***Comparison between degrees of differentiation in ADK subtypes.***

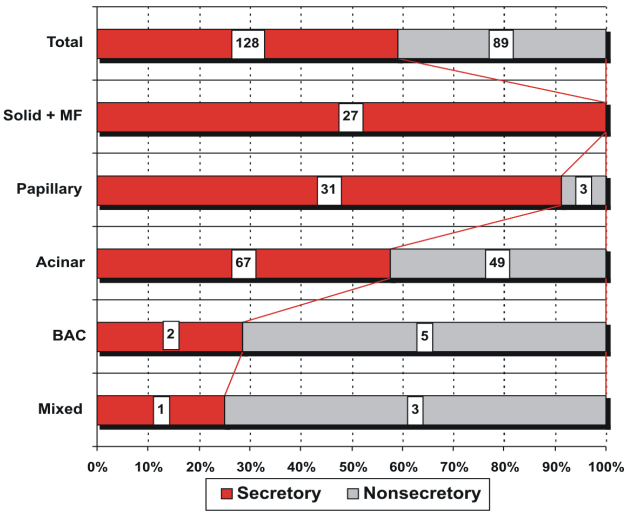

Figure 8 – ***Presence of secretory phenotype in ADK subtypes.***

Immunohistochemical assessment

The use of standard stain – HE and specific techniques for mucus – was enough to precise the diagnosis in only 60 cases, meaning less than one third of the 217 identified with ADK. Most of cases required the use of an IHC algorithm in two or, in rare cases, many steps (Table 3).

The first step was designed to demonstrate the pulmonary origin of the malignant proliferation. This first panel of antibodies included firstly, TTF-1, antibody with double diagnostic value, *i.e.*, to reveal not only the pulmonary origin of the tumor but also the membership in the lung ADKs group, then CK7, an antibody specific for primary lung epithelial malignant proliferations [19] and finally, CK20, an antibody useful both to differentiate colonic metastases and identify mucinous bronchiolo-alveolar tumors [20, 21]. This triad was used either completely (in most of the cases) or partially (Table 3).

Table 3 – ***Immunohistochemical algorithm used in the diagnostic of studied ADKs***

Main panel (TTF-1 / CK7 / CK20)			+ Other		
Antibodies combination		***No. of cases***	***Antibodies***	***No. of cases***	***Reason of use***
TTF-1+	Alone	3		–	
	+ CK7+	10	*CA 15-3+*	3	Precise secretory phenotype
	+ CK7+ / CK20 –	117	*CA 15-3+*	39	Precise secretory phenotype
			Ber-EP4+	11	Exclude mesothelioma
			CD56 –	4	Exclude SCLC
	+ CK7 – / CK20 –	2		–	
	+ CK7 – / CK20+	1		–	
TTF-1 –	+ CK7+ / CK20 –	15	*CA 15-3+*	4	Precise secretory phenotype
	+ CK7+	3	*CA 15-3+*	2	Precise secretory phenotype
			CK5/6 –	1	Exclude SC
	+ CK7+ / CK20+	2	*CD30 –, BHCG –, PLAP –, alpha-Phetoprotein –*	1	Exclude metastasis of testicular origin
			CA 15-3+	1	Precise secretory phenotype
	+ CK7 – / CK20 –	1	*Syn –, Chromo –, Ber-EP4+, CD56 – KL-1 –, CK AE1/AE3 – CA 15-3+*		Exclude NE tumor, mesothelioma, SCLC Exclude a SC Precise secretory phenotype
CK7+	+ CK20 –	3	*Syn +/–, Chromo +*	2	Exclude NE tumor

In the second step, usually one antibody was added either to precise the secretory phenotype of tumoral cells or to exclude other type of lung tumor. For the secretory phenotype, CA 15-3 was used because even is known as a tumoral marker of breast cancer [22], being derived from MUC1, may be used to identify the intracellular secretory feature of any type of secretory tumor [23]. Thus, the positivity of Ber-EP4 in ADKs was used to exclude the mesothelioma, the negativity of CD56 was used to exclude a SCLC and the negativity of CK5/6 was used to exclude the SC.

A third step was necessary in only two cases in order to clear up the pulmonary origin.

In one of these cases, the aspect of the neoplastic proliferation, with very poorly differentiated cells forming lobulated, compact areas with central necrosis, raised the suspicion of a tumor with neuroendocrine component, suspicion infirmed by the negativity of repeated immuno-marking with chromogranin and synaptophysin. The positivity for Ber-EP4 clone excluded the mesothelial origin, the negativity for CD6 excluded a small cell tumor, the positivity for CA 15-3 confirmed the secretory phenotype and the negativity for the KL-1 and AE1/AE3 cytokeratins excluded a squamous form so that the final diagnosis was of poorly differentiated ADK with secretory phenotype.

In the second case, the focal positivity for CD20, raised the suspicion of a secondary tumor of testicular origin, hypothesis excluded by the negativity of panel including CD30, BHCG, PLAP and alpha-fetoprotein. Anyway, the pulmonary origin of the tumor remained still uncertain due to the TTF-1 negativity.

Squamous carcinoma

Many classification systems have been proposed for the histological evaluation of this type of tumor. The *WHO* 2004 uses the criterion of dominant morphological neoplastic cells aspect, describing the following types: well-differentiated papillary type, small cell type, clear cell type and basaloid type [9].

In the current medical practice, the used system is the one based on cell differentiation criterion, relied on the presence, quantity and quality of the keratin production and on the evaluation of the intercellular junction degree, that is, in general, the tumoral degree of differentiation, system which describes: the well differentiated type, the moderately differentiated type and the poorly differentiated type [16, 24].

In the analysis of tumors, a combined classification was used, dividing them into "conventional" carcinomas, described in the past as "spinocellular carcinomas", basaloid carcinomas, SCs after preoperative therapy and carcinomas on which the morphology was not specified in the histopathology report.

The overwhelming majority of squamous tumors were of "conventional" or spinocellular type. Basaloid carcinomas represented a very small percentage – 5% of all identified SCs.

In an equally rare number of cases, the cell morphology and the tumor architecture were significantly altered by preoperative therapy usually performed in order to allow radical surgery, the diagnosis being based on a previous biopsy result (Figures 9 and 11).

The evaluation of the differentiation degree was possible only in spinocellular and basaloid tumors.

Whereas all cases of basaloid type were poorly differentiated, the spinocellular tumors were mainly moderately differentiated and well differentiated and only rarely poorly differentiated (Figure 10).

Immunohistochemical assessment

In spite of the fact that the use of standard stain (HE) was enough to precise the diagnosis of ADKs in 3/4 of cases (*i.e.*, 83 cases), the IHC tool had to be used in 28 cases in order to clarify the diagnosis (Table 4).

Thus, in three cases the specific ADK triad (TTF-1, CK7 and CK20) was not necessary to exclude the poorly differentiated form of an ADK, but NE markers were used in all these cases to exclude a NE tumor, CD56 was used in two cases to exclude a SCLC and KL1 was used in two cases to confirm the squamous phenotype.

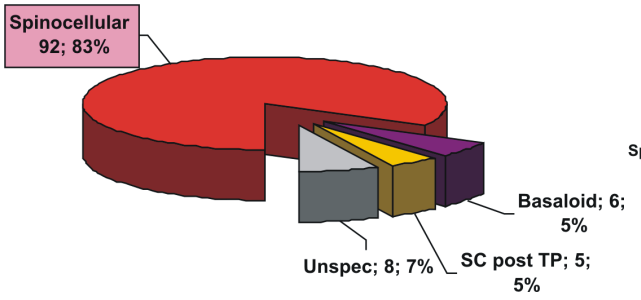

Figure 9 – *Types of squamous carcinomas.*

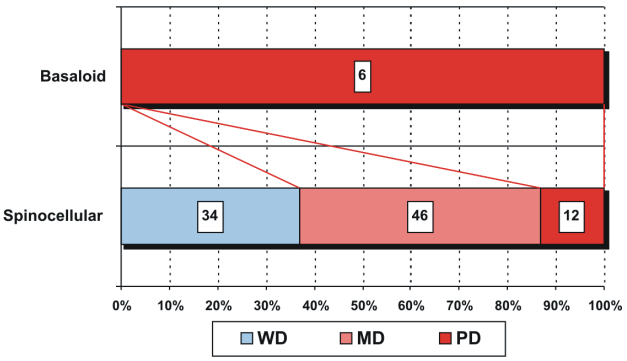

Figure 10 – *Differentiation grade in studied SC types.*

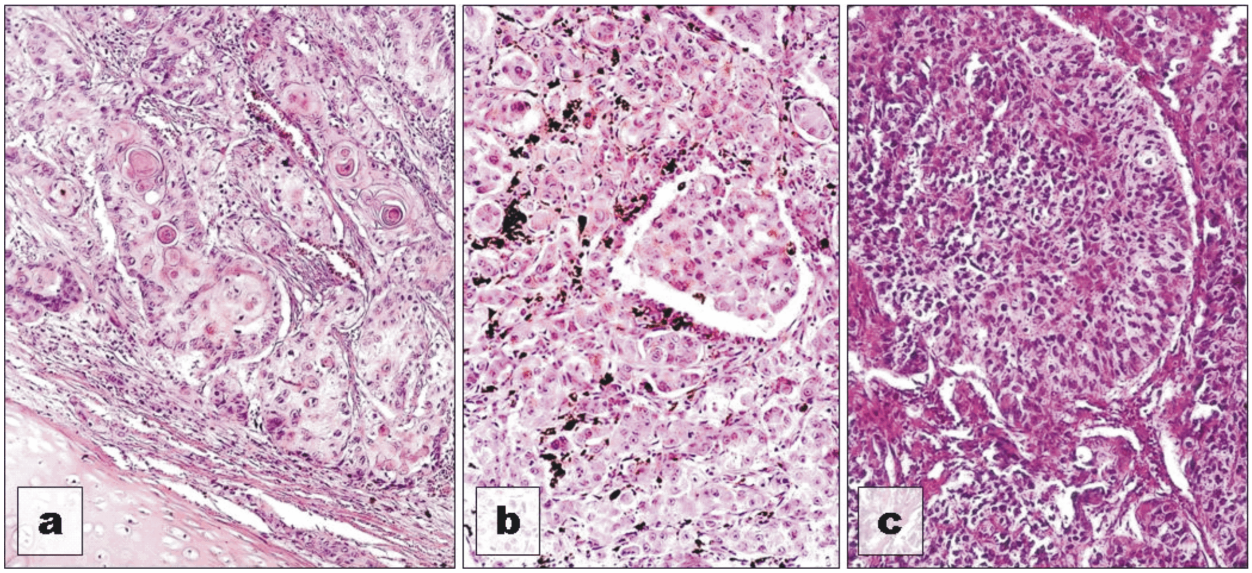

Figure 11 – *Morphological features of squamous carcinoma: (a) "Conventional" pattern; (b) Moderate differentiation; (c) Basaloid pattern.*

Table 4 – *Immunohistochemical algorithm used in the diagnostic of studied SCs*

Main panel (exclude ADK)		+ Other		
Antibodies combination	***No. of cases***	***Antibodies***	***No. of cases***	***Reason of use***
No ADK triad	3	KL-1+ [redacted] Neuroendocrine marker(s) –	1	Confirm squamous type
		CD56 –	1	Exclude SCLC
		[redacted]	1	Exclude neuroendocrine tumor
CK7 – / CK20 –	1	No additional marker		Exclude ADK
ADK triad –	9			
	8	CK AE1/AE3+	4	Confirm squamous type
		KL-1+	1	Confirm squamous type
		Ber-EP4+	2	Exclude mesothelioma
		CA 15-3 –	1	Exclude secretory phenotype
TTF-1 –, CK7+	1	CK AE1/AE3+		Confirm squamous type
CD20 –	1			
TTF-1 –, CK7+, CD20 –	4	Chromo –, Syn –	1	Exclude neuroendocrine tumor
		CK AE1/AE3+	1	Confirm squamous type
		Squamous epithelial marker + and CD56 –, Syn –, Chromo –	2	Confirm squamous type Exclude SCLC and neuroendocrine tumor
TTF-1+, CK7 –, CD20 –	1	CK AE1/AE3+, Ber-EP4+		Confirm squamous type, exclude mesothelioma

In the remaining 25 cases, the ADK triad was used either entirely or partially in order to exclude the ADK phenotype. In 10 of these cases, no additional marker was necessary to elucidate the diagnosis. In another 12 cases, the IHC investigation had to be continued with a second step, using either an epithelial marker in order to confirm the squamous phenotype (eight cases), either a marker to exclude the secretory phenotype (one case) or the mesothelioma (two cases) and finally, NE markers to exclude a NE tumor (one case). In the last three cases, two additional IHC steps were necessary: one to confirm the squamous phenotype (in all three cases) and another one to exclude SCLC and NE phenotype in two of the cases or the mesothelioma in the third case (Table 4).

Large cell lung carcinoma

If in the past the weight of this tumor type was about 10% of all lung cancers, today the incidence of "classic" LCLC decreased in favor of very poorly differentiated forms of SC or ADK due to the new techniques of morphological identification such as IHC marking and molecular determinations [16, 25]. The share of this type of tumor in the studied cases – 3.5% of the 364 lung tumors, is part of this trend.

The *WHO* classification includes the next morphological variants: basaloid, with clear cells, lymphoepithelioma–like, with rhabdoid phenotype and neuroendocrine [9, 16]. The 11 tumors encountered in the studied group were divided in two subgroups:

(a) Undifferentiated LCLC without NE component: seven cases. In one case, HE stain was enough to establish the diagnosis. In the other six cases, IHC marking was necessary for elucidation (Table 5).

Table 5 – ***Immunohistochemical algorithm used in the diagnostic of studied undifferentiated LCLCs***

Main panel (exclude ADK)		+ Other			
Antibodies combination	***No. of cases***	***Epithelial markers***	***No. of cases***	***Neuroendocrine markers***	***No. of cases***
No ADK triad	1	CA 15-3+	1	No	1
CK7 +/-	1	CK AE1/AE3+	1		1
ADK triad –	2	CK AE1/AE3+	1	Syn –, Chromo –	1
		KL-1+, EMA+	1		1
TTF-1 –, CK7 –	1	CK AE1/AE3+, CA 15-3+	1		1
TTF-1 –, CK7+, CD20 –	1	EMA+, BER-EP4+	1		1

Thus, in two cases the use in a first step of an epithelial marker was enough to establish the diagnosis of undifferentiated large cell tumor. For the remaining four tumors, the morphological aspect required the use, in a second step of the NE markers, whose negativity excluded the NE variant of large cells tumor.

(b) LCLC with NE component: six cases. In one case, the diagnosis could be established using only the classical HE stain. For the other five cases, the use of NE markers, usually applied in a combination of at least two was crucial for the diagnosis.

Other rare types of NSCLC

The adenosquamous carcinoma

The adenosquamous carcinoma is a rare form of LC (<10% of all LCs), with a biphasic pattern, consisting of both a component with glandular differentiation and another one with squamous differentiation, usually almost in equal proportions but never less than 10% any of them [9, 12, 16]. Four such cases were identified in the studied group. In three of these cases, the HE stain allowed the identification of the two distinct types of differentiation: squamous and glandular. In the fourth case, the tumor had and overall poorly differentiated pattern which imposed the use of IHC tool. The obtained profile was: "TTF-1+, CK7+ and CK20 –" in areas with sketches of glandular differentiation and „KL-1+ and EMA+" in areas with squamous differentiation.

Mucoepidermoid carcinoma

This variant of salivary gland-type carcinoma was identified in two patients. In one patient, the tumor aspect on HE alone, consisting of a close intermingling between a glandular component made of tubular features, variable in size, sometimes very large, and containing a mucoid material, and a squamous component allowed to establish the diagnostic of low-grade mucoepidermoid carcinoma.

In the other case, the tumor consisted, on one hand, of compact areas made of polygonal or spindle cells with eosinophilic cytoplasm, irregular and nucleolated nuclei, with numerous mitoses, areas usually centered by necrosis and, on the other hand, of glandular pseudolumina which, in some areas, contained mucosecretory cells, which imposed the use of IHC tool.

The negativity for TTF-1 and positivity for CK7 in glandular areas and intense positivity for CK5/6 in compact areas, helped to establish the final diagnosis of high-grade mucoepidermoid carcinoma.

Sarcomatoid form

The sarcomatoid form was observed in only one case. The tumor showed, around an extended area of central necrosis, viable tumoral zones, formed by atypical spindle cells, with large, nucleolated nucleus, unique or multiple. The diagnosis of poorly differentiated carcinoma of sarcomatoid type was sustained by the negativity of tumor cells for EMA, CK7 and CK20 and the positivity of some tumor cells for KL-1 and Vimentin.

Small cell lung carcinoma

Although various statistics accredits the percentage of SCLCs between 10 and 20% of all lung carcinomas, only five such cases were identified in the study group, with a percentage of 1.37%. With conventional standard HE staining and using an IHC algorithm that included mandatory the neuroendocrine markers chromogranin and synaptophysin, the cases were divided into two categories: SCLC and neuroendocrine cell origin SCLC. The latter was the most common, being observed in four cases.

Carcinoid tumors

Only 10 carcinoid tumors were identified in the study group, representing 2.74% of all tumors, percentage that fits in the data reported in the literature. Seven of these tumors were typical carcinoid tumors. IHC was necessary in seven of the 10 cases and the positivity for neuro-endocrine markers in all these cases established the final diagnosis.

Intratumoral necrosis

The intratumoral necrosis (Figure 12) was present in more than half of the LCLC, SC, SCLC subgroups, less frequently in ADKs and never in the carcinoid tumors (Figure 13).

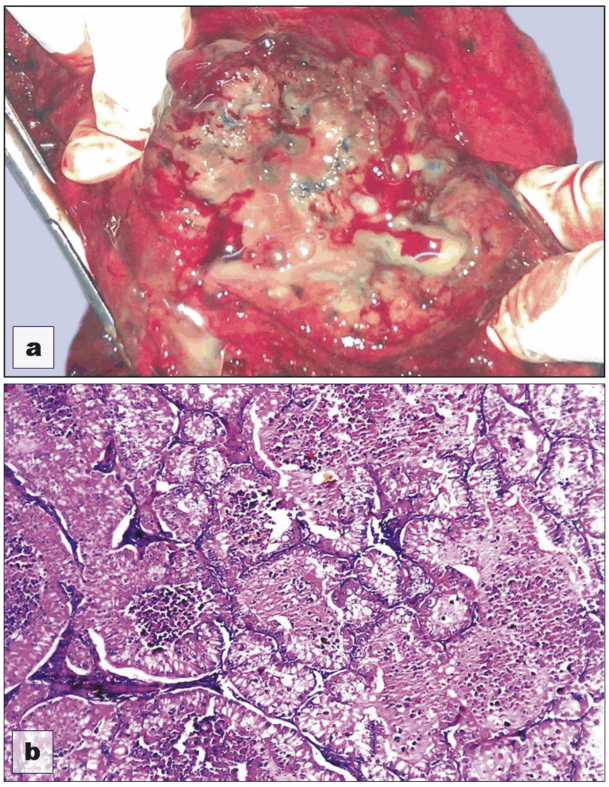

Figure 12 – *(a) Suppurative necrosis. (b) Foci of necrosis in the tumor masses.*

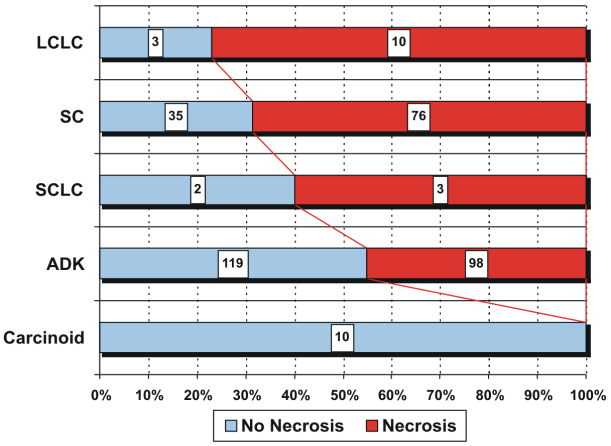

Figure 13 – *The presence of intratumoral necrosis.*

Vascular intratumoral embolism

The vascular embolism (Figure 14) was not a common finding. It was noticed mostly in the SCLC subgroup, never in the carcinoid tumors and in less than one quarter of the others types of lung cancer (Figure 15).

Local invasion – T

The local invasion was minor for the carcinoid tumors, moderate for ADK, SC and LCLC subgroups and significant for the SCLC, correlating with the known aggressiveness of each type of tumor (Figure 16).

Lymph node involvement – N

It has been found that more than half of the tumors were confined to the lung, without lymph node involvement, allowing curative treatment. Carcinoid tumors had the less aggressive behavior and whereas ADKs had the most aggressive one (Figure 17).

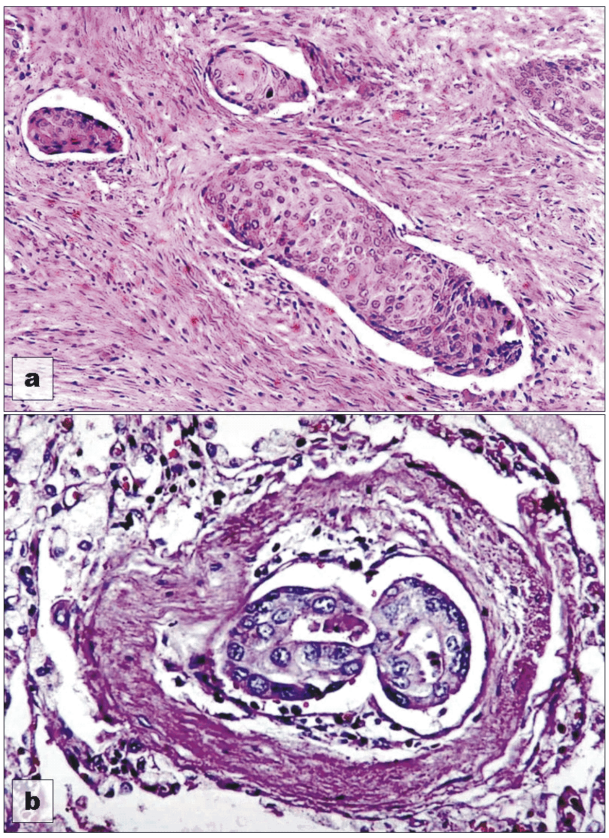

Figure 14 – *(a) Emboli in lymphatic vessels. (b) Arteriolar emboli.*

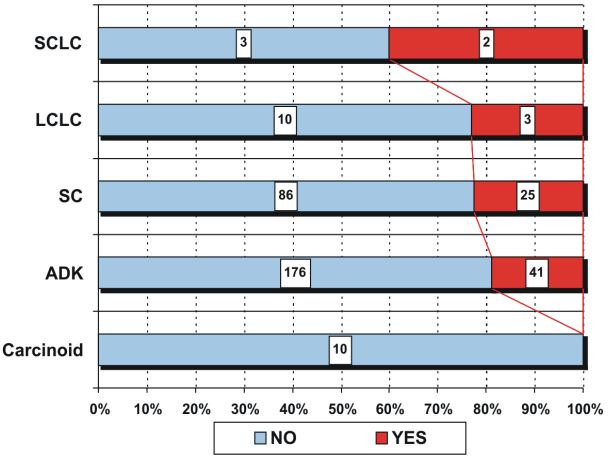

Figure 15 – *The presence of intratumoral embolism.*

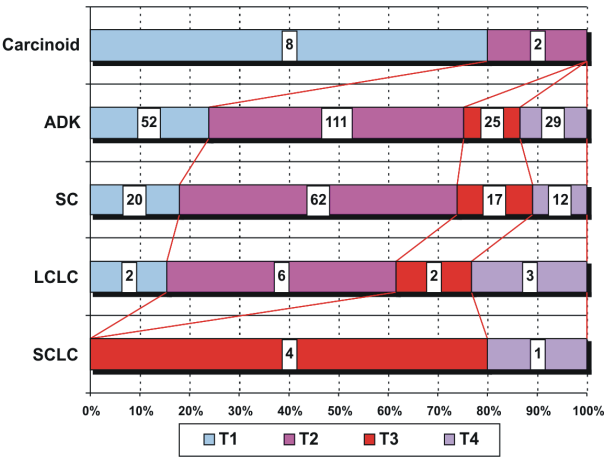

Figure 16 – *Distribution of local invasion (T).*

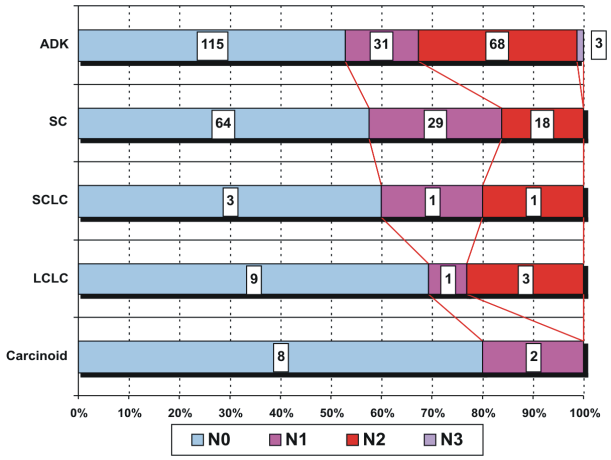

Figure 17 – ***Distribution of lymph nodes invasion (N).***

Distant spread – M

The metastatic disease was found in a quarter of the ADKs. More than 90% of all other subgroups were metastasis free, allowing radical surgery (Figure 18).

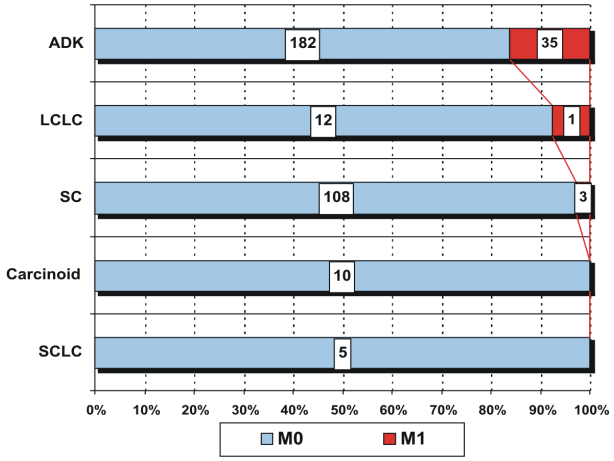

Figure 18 – ***Distribution of distant metastases (M).***

TNM stage

More than 3/4 of the tumors were diagnosed in curable stages (including IIIA), the most advanced stages being found in ADK subgroup (Figure 19).

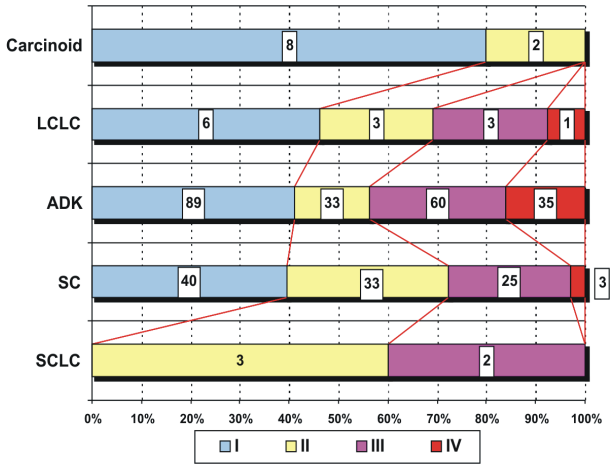

Figure 19 – ***Differentiation grade in studied SC types.***

Discussion

Five major groups of histopathological types of lung epithelial malignancies have been defined in the studied cases and ranked in descending order of their weight as follows: ADKs group, SC group, LCLCs group, SCLCs group and carcinoid tumors group.

Clinical data

The clinical examination is one of the important stages in the evaluation strategy of a patient with lung tumor. Various types of lung tumors included in the study exhibited a wide range of clinical expressions, which are resumed in Table 6.

Table 6 – ***Comparison between clinical profiles of tumor groups***

Parameter	Carcinoid	SCLC	ADK	SC	LCLC
Gender ratio	F/M: 1.5	M/F: 4	M/F: 2.7	M/F: 14.8	M/F: 3.3
Age [years]	40% A + YAD	60% AD	55% AD	55% O	61% AD
Clinical status	*WHO* 1,2	*WHO* 2	*WHO* 1,2	*WHO* 2,3	*WHO* 1,2
Specific preoperative therapy	10%	25%	17%	14%	7.7%

F – Female; M – Male; A – Adolescent; YAD – Young adult; AD – Adult; O – Old; *WHO* – World Health Organization.

Adenocarcinomas

Patients were generally men with an average age of almost 62 years with mild to moderate impairment of performance status (*WHO* grades 1 or 2) and which followed a preoperatively specific treatment in a significant percentage of cases (17%), most often chemo- and radio-therapy.

Squamous cell carcinomas

Patients were almost exclusively elderly male patients, with a mean age of 66 years, with moderate to severe impairment of performance status (*WHO* grades 2 and 3) and which followed in a relatively significant percentage of cases (14%) a preoperatively specific treatment, most often chemo- and radio-therapy.

Large cell carcinomas

Patients were generally men, with an average age of almost 62 years with mild to moderate impairment of performance status (*WHO* grades 1 or 2) which, with one exception, did not follow a preoperative specific treatment.

Small cell carcinomas

Patients were generally men, with an average age of almost 63 years with moderate impairment of performance status (*WHO* grade 2), and which, with one exception did not follow a preoperative specific treatment.

Carcinoid tumors

Patients were mostly women, with an average age of almost 48 years, with mild to moderate impairment of

performance status (*WHO* grades 1 or 2) and which, with one exception did not follow a preoperative specific treatment.

Morphological profile

The morphological diversity of the studied lung tumors has overlapped to a large extent over the distribution recorded and accepted in the literature [26], where SC is credited with an incidence nearly two times lower, and SCLC represent almost a quarter of lung carcinomas (Figure 20).

There was also another difference, namely the reduced incidence of small cell tumors. This "deviation" from the current reports, however, could be explained by the particularity of the study group, *i.e.*, patients diagnosed with lung tumor and hospitalized in a surgical clinic to be operated, knowing that small cell tumors primarily benefit from other components of the oncological therapeutic strategy, their discovery after surgery being usually a surprise. The morphological profile was, as well as the clinical one extremely diverse (Table 7).

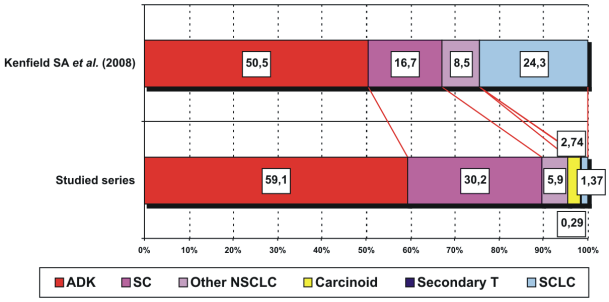

Figure 20 – *Comparison with other studies.*

Table 7 – *Comparison between morphological profiles of tumor groups*

Parameter	Carcinoid	SCLC	ADK	SC	LCLC
Site (lung)	R: 60%	L: 75%	R: 57%	R: 51%	L: 69%
Histological type	–	NE	Acinar	Conventional	UD
Differentiation	Typical	UD	MD	MD	UD
Secretion	–	–	59%	–	–
IHC	70%	100%	72%	25%	84%
Necrosis	No	60%	45%	68%	76%
Embolia	No	40%	19%	22%	23%
pT	T1: 80%	T3: 80%	T3,4: 25%	T3,4: 26%	T3,4: 38%
pN	N1: 20%	N1,2: 40%	N1,2: 45%	N1,2: 42%	N1,2: 30%
pM	No	No	16%	7.6%	2.7%
TNM	I: 80%	TNM ≥ IIA – 100%	TNM ≥ IIIB – 27%	TNM ≥ IIIB – 12%	TNM ≥ IIIB – 23%

IHC – Immunohistochemistry; L – Left; NE – Neuroendocrine; MD – Moderately differentiated; R – Right; UD – Undifferentiated.

Adenocarcinomas

ADK is one of the eight major groups of lung carcinoma described by the 2004 *WHO* classification, representing from 30% to 50% of lung cancers and probably 30% of the invasive types [9, 16, 27].

ADKs included in the study were more frequently located in the right lung, with predilection for the upper lobe. The architectural pattern was generally acinar, with moderate degree of differentiation, disposition in compact masses of tumor cells in almost a quarter of cases and secretory phenotype in over half of the cases.

Our data are consistent with those of the literature. Thus, in China, the country with the largest number of smokers and the highest number of cases of lung cancer in the world, the acinar form represent almost 40% of all ADKs and its incidence is increasing [28, 29], and in Europe is dominant with between 50% and 60% of all ADKs [30].

Intratumoral necrosis was a frequent phenomenon in almost half of the cases but with the lowest frequency among NSCLCs. Although only a quarter of the tumors were classified into stages T3 and T4, ADKs had the largest share of regional lymph nodes invasion and distant metastasis of all tumors included in the study while, somehow paradoxically, the intratumoral vascular invasion or embolism had the lowest rate.

Overall, these defining elements of aggressive biological behavior have made a significant proportion of ADKs to be classified in stages recognized as inoperable, being thus the most aggressive of all NSCLCs. Diagnosis required in three quarters of the cases IHC investigation.

Squamous cell carcinomas

The SC is the second histological type of primary epithelial malignant tumors of the lung, representing from 30% to 40% of them [31, 32] although there are still geographical areas where it holds the supremacy [16].

SCs followed almost the same pattern of localization as ADKs, being identified more frequently in the right lung and with predilection for the right upper lobe.

The tumor architectural pattern was clearly dominated by spinocellular forms, with a moderate degree of differentiation. Intratumoral necrosis was 1.5 times more frequent than in ADKs. As well as the intravascular invasion phenomenon, the occurrence of tumor emboli was more common.

Tumor development and lymph nodes invasion were comparable to those of the ADK but dissemination was two times lower, so finally the inoperability of those tumors was over two times lower than that of ADKs.

It should be noted that squamous tumors raised the smallest problems of histopathological diagnosis of all tumors included in the study, IHC being used to elucidate the diagnosis in only a quarter of cases.

Large cell carcinomas

LCLCs showed a clear preference for locating the left lung, usually in its upper lobe, the undifferentiated histological pattern prevailing at the expense of the neuroendocrine differentiation.

Tumor necrosis was almost a rule, slightly more than three quarters of this type of tumors showing intratumoral

necrotic foci of less or greater extent, making it the most necrotizing pulmonary tumor type.

Vascular invasion phenomenon with intravascular embolism formation had the highest frequency of all non-small cell tumors.

Tumor extension also was the largest of non-small cell tumors. Instead, invasion of the lymph nodes was the lowest among the tumors of the same family. Overall, however, the tumor extension assessed by the TNM system placed a quarter of LCLCs in the inoperability area, between ADKs and SCs.

Poor or no cellular differentiation and the need for precise discrimination between forms with and without neuroendocrine differentiation resulted in the use in most of the cases of some more or less extensive antibody panels, the use of IHC techniques being the most frequent of all non-small cell tumors.

Small cell carcinomas

The distinction between SCLCs and NSCLCs is a crucial step in the diagnosis of lung cancer [33]. On one hand, SCLC includes the following subtypes: small cell carcinoma, combined small cell carcinoma (SCLC combined with glandular or squamous component) and neuroendocrine cell origin SCLC [34]. On the other hand, the latter subset of SCLC is one of the extremes of the spectrum of the lung neuroendocrine tumors, including typical carcinoid (low malignancy), atypical carcinoid (intermediate malignancy) and neuroendocrine tumors (high malignancy) which include, in their turn, large cell neuroendocrine carcinoma and SCLC. Due to the differences in the clinical behavior the therapeutic strategy and epidemiology, these tumors are classified separately by the 2004 *WHO* classification [35].

Small cell tumors were found usually in the left lung showing a pattern of neuroendocrine differentiation in most cases, a marked necrotizing potential, almost equal to that of SCs and with the highest vascular invasion and intravascular embolism potential of all tumor types.

The tumor extension classified as T3 in 80% of cases, accompanied by a significant invasion of the lymph nodes, placed between those of ADKs and SCs, caused the TNM staging of these tumors to start from IIA. But, the lack of distant metastasis made only one case to be inoperable.

The identification of the SCLC is greatly facilitated by its IHC profile. Thus, almost all SCLC are immunoreactive to cytokeratins, TTF-1 and EMA. Neuroendocrine and neural differentiation is underlined by the expression of DOPA decarboxylase, calcitonin, NSE, chromogranin A, CD56, gastrin releasing peptide and insulin 1-like growth factor. One or more of these markers of neuroendocrine differentiation can be identified in about 75% of SCLC [36].

In this group of tumors, IHC staining was a compulsory element in the histopathological examination algorithm.

Carcinoid tumors

In the last *WHO* classification [9], the carcinoid tumors are divided into two groups: typical carcinoid and atypical carcinoid tumors. The typical carcinoid, called the well-differentiated neuroendocrine carcinoma or low malignancy [35], represents less than 5% of all primitive lung tumors, the characteristic of this form being the positivity for TTF-1 in a proportion ranging between 43% and 53% [37, 38]. For the atypical carcinoid, also called the moderately differentiated neuroendocrine carcinoma with intermediate malignancy [35], the characteristic is the more intense positivity of neuroendocrine markers than for the SCLC.

The carcinoid tumors, although generally located in the right lung as well as the ADKs and SCs, showed a lobar predilection for the left lower lobe. Most of the times were typical or low malignancy forms, without intratumoral necrosis or vascular invasion.

The tumor extension was low, the vast majority being small localized tumors.

The phenomenon of lymph nodes invasion was reduced and the distant metastasis absent so broadly the TNM staging was "I" in most of the cases.

IHC staining proved to be an almost indispensable tool for clarifying the histopathological diagnosis of those tumors.

Conclusions

Clinical aspects can take sometimes special features: SCs were found mostly in elderly men accompanied by a evident change of the condition of the patient, compared to a significant predominance of carcinoid tumors in women, usually less than 50 years, accompanied by a very good survival, or the reduced survival of patients with ADKs, despite a lower impairment of the general condition of the patient until the discovery of the tumor.

From morphological point of view, ADKs showed the most aggressive biologic behavior, displaying the most frequently lymph nodes extension and distant metastasis. LCLCs showed a predilection for the left lung and the almost pathognomonic presence of the phenomenon of necrosis. The few detected SCLC also showed a "tropism" for the left lung and biological behavior comparable to that of ADKs, but emphasized particularly during local invasion and somewhat explainable by the highest rate of intratumoral vascular embolism. SCs, although showed a significant phenomenon of intratumoral vascular embolism had the lowest overall rate of extension of all non-small cell tumors. Finally, the carcinoid tumors have been shown to be the most "benign" regarding the morphological aspect of the cell population as well as the biological behavior.

IHC marking represents, within the histopathological investigation, one of the almost indispensable tools for the diagnosis of lung tumors. On one hand, due to the "privilege" of the lung of being an almost mandatory stop on the various routes of metastasis of the different malignant tumors, the IHC marking alone can discriminate between a primary and a secondary lung tumor. On the other hand, it contributes decisively to the proper classification of each primitive tumor type, a strong argument in favor of this last statement being our observations regarding the morphological profile of carcinoid tumors but especially of the large cell tumors and in particular of the small cell tumors. The only tumor group on which the IHC marking was not a rule was the SCs group.

Finally, we could add also that the malignant epithelial lung tumors are still a major challenge from the detection

point until the therapeutic intervention, challenge that can be solved only by the assumption of a collective and integrated responsibility of a complex medical team that gathers together both clinicians specialized in pulmonary pathology, namely pneumologists, radiologists, pathologists, oncologists and thoracic surgeons but also the family physician, the first faithful watchman of the community health.

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