



Revista Portuguesa de Pneumologia

ISSN: 0873-2159

sppneumologia@mail.telepac.pt

Sociedade Portuguesa de Pneumologia  
Portugal

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Revista Portuguesa de Pneumologia, vol. XV, núm. 6, novembro-diciembre, 2009, pp. 1101-1119

Sociedade Portuguesa de Pneumologia

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## Artigo de Revisão *Review Article*

Lina Carvalho<sup>1</sup>

**Reclassificação do carcinoma broncopulmonar:  
Diferenciação do tipo histológico em biópsias por  
imuno-histoquímica**

***Reclassifying bronchial-pulmonary carcinoma:  
Differentiating histological type in biopsies by  
immunohistochemistry***

Recebido para publicação/received for publication: 09.04.28  
Aceite para publicação/accepted for publication: 09.05.19

### **Resumo**

Os conhecimentos actuais da patologia molecular do cancro do pulmão requerem outra caracterização histológica, para além de carcinoma de células pequenas e carcinoma não pequenas células para suporte da terapia personalizada e entendimento do valor real dos fármacos actualmente disponíveis.

Como o diagnóstico e seguimento clínico da maioria dos casos de cancro do pulmão se baseia em produtos de biópsia e citologia, a classificação imunoistoquímica do carcinoma broncopulmonar (IBPCC) é necessária para suporte morfológico da classificação da WHO 1999/2004, clarificando as características celulares das neoplasias e o entendimento da carcinogénese.

### **Abstract**

The current state of molecular knowledge on lung cancer demands a histological classification which goes beyond small-cell and non-small-cell carcinoma to provide support for tailored therapy in aiding in understanding of the drugs currently available.

As diagnosis and follow-up in the vast majority of lung cancer cases is based on biopsies and cytology samples, Immunohistochemical Bronchial Pulmonary Carcinoma Classification (IBPCC) is necessary to reveal the raft of characteristics available. This provides morphological support for the WHO's 1999/2004 classification, in addition to an understanding of carcinogenesis.

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O painel imunoistoquímico reforça os tipos histológicos principais do carcinoma bronco – pulmonar: carcinoma epidermóide (queratinas de alto peso molecular – HWMC), carcinoma adenoscamoso (CK/ /TTF1, MWMC), carcinoma neuroendócrino (Chrg, Syn, CD56, TTF1, Ki67) e adenocarcinoma (CK7, Ck20, TTF1); as variantes do carcinoma de células grandes e do carcinoma sarcomatóide são englobados num único grupo de carcinomas pleomórficos (CK7, TTF1, HWMC, VMT, desmina, actina), onde cabe o polimorfismo e o pleomorfismo celular.

A quimioterapia do carcinoma broncopulmonar continuará baseada no platino e na gemcitabina no futuro próximo e a IBPCC será uma ferramenta simples e eficiente para o registo das características e tipos histológicos do carcinoma do pulmão presentes nas biópsias e amostras citológicas para suporte da evidência clínica e dos ensaios farmacêuticos.

**Rev Port Pneumol 2009; XV (6): 1101-1119**

**Palavras-chave:** Carcinoma broncopulmonar, imunoistoquímica.

The immunohistochemical panel clarifies the main morphology and cytology characteristics to maintain the leading histological types as squamous cell carcinoma (high weight molecular cytokeratins/HWMC), adenosquamous carcinoma (CK7, TTF1, HWMA), neuroendocrine carcinoma (Chrg, Syn, CD56, TTF1, Ki67), adenocarcinoma (CK7, CK20, TTF1) and bring the polymorphic and pleomorphic carcinomas under a single banner of pleomorphic carcinoma (Ck7, TTF1, HWMC, VMT, Desmin, Actin) which shelters large cell carcinomas and sarcomatoid carcinomas.

Lung cancer chemotherapy will still be based on platinum and gemcitabine for the near future and the IBPCC is a simple and efficient tool for streamlining the registration of lung cancer histological characteristics in biopsies and other reduced samples to support clinical evidence and trials.

**Rev Port Pneumol 2009; XV (6): 1101-1119**

**Key-words:** Bronchial-pulmonary carcinoma, immunohistochemistry.

### Introduction

Pathology is far from what it can be considered as to have been its beginning in the XVIII century by the first descriptions of Morgagni and further by Virchow with the use of the microscope. What is important to have in mind is the fact that the survival age was in the thirties years of age, even from the time of Hippocrates, Celsius and Galen. The Egyptians left their papyrus, namely Ebers' (Amenhotep Kingdom 1557 – 1501BC) with exhaustive reports of infections and tumours, although be-

nign, but related to the different parts of the body. Medicine began at Cos School in Greece (500BC), from where the Corpus Hipocraticus emanated, followed by the De Artibus of Aulus Cornelius Celsius from Verona – Italy. The Arabs had the possibility to ensemble the two knowledges while divagating around the Mediterranean during their struggles, making then a wealthy synthesis gathering also the Persian and Indian knowledge. Kanum is really a joint canon made then by Ibis Sina/ Avicena (980-1037). By then confidence

was achieved in recognizing malignant tumours and metastasizing<sup>1</sup>.

The actual known scientific method comes in the catholic/medieval time with Galileo (1564-1634) and Kepler (1571-1635), succeeding Copernicus (1473) and having as starting point the greec-italian-arab-indu knowledge since the Salerno School (XI century). The Magister Salernus as we get to know today was probably written by a Jew, an Arab, a Greek and a Christian. The *Tumori-bus Praeter Naturum* written by Gabriel Falopius (1523-1562) is the first pathology book and the microscope used by Malpighi (1562-1682) helped in rooting the anatomy bases. Histology encased in between by the eyes of Bichat (1771-1802) together with anatomy and pathology, reinforced by René Laennec (1781-1826). The expertise of Rudolf Virchow (1821-1826) with an acromatic microscope (1830) brought the zell/cell to the medical practice<sup>1-3</sup>.

This way we get to the contemporary medicine we can consider to have its beginning in 1953 with the DNA double helix of Watson and Creek and in fifty years we jumped into the molecular pathology and modern medicine with a huge advance in disease therapy, gathering all technical methodologies now available. At this point is the demanding diagnosis of bronco-pulmonary carcinoma made on small biopsies (or a couple of cells) submitted to the WHO 1999/2004 classification<sup>4-5</sup>.

We owe to the Sweden doctor Leiv Kreyberg the first histological characterization of bronchial-pulmonary carcinomas done in 1954 after studying carbon mines workers. These patients exhibited the illnesses dependent on mines environment: carbon lung and silicosis together with tuberculosis and developing

lung cancer very frequently. Smoking accelerated the incidence of lung cancer as we know today. In 1967, the Organisation Mondiale de la Santé supported the first work directed to the standardization of tumoural nomenclature adapted to each organ to discipline Pathologists that frequently renamed differently the same lesion. *Types Histologiques des Tumeurs du Poumon* was the blue book number 1 of the WHO blue book series directed to the histological classifications of neoplasias in each organ<sup>2,6</sup>.

The incidence of lung cancer is far from diminishing its incidence although the habit of smoking is clearly decreasing and still 60 to 70% of new lung cancer cases are diagnosed in non surgical stages with 10 to 12 months probability of survival. This situation and the poverty of survival rates achieved with the possible therapy conducting to a high mortality raised the general histological reference of small cell carcinoma and non small cell carcinoma in Pathologists' reports as the first histological type meant non surgical behaviour because of very poor prognosis compared with the other types. As molecular pathology is developing towards predictive therapy which means personalized choice dependent on carcinoma histological type and quite defined particularities, the diagnosis has to be made with approximate precision in the small biopsies obtained by endobronchial examination, transthoracic biopsies and even in groups of small number of neoplastic cells of cytological methods<sup>7-10</sup>.

The use of immunohistochemical antibodies and the experience acquired in reporting lung cancer in surgical specimens based in total inclusion in paraffin of tumours, have conducting either to WHO classification and to the

recognition of the possible different patterns of bronchial-pulmonary carcinomas that can be explored in small biopsies<sup>11,12</sup>.

### Lung cancer classifications till WHO 1999/2004

The morphological descriptions of lung carcinomas have been refined since Kreyberg and the **OMS/1967** classification with maintenance of the histological types, firstly described in five groups, revealing a large heterogeneity recognized *ab initio*:

1. adenocarcinomas and epidermoid *epitheliomas*;
2. carcinoids;
3. solid tumours with or without mucus and giant cell and clear cell *epitheliomas*;
4. Small cell anaplastic *epitheliomas* and subtypes (and mixed tumours where combined carcinomas and neuroendocrine large cell tumours were reported);
5. Non-classifiable tumours neither by pattern nor by cell type.

The following reorganizations of criteria reported in 1976 and 1981 preserved the initial nomenclature and the **OMS/1981** written in French reports the actual histological types for malignant epithelial pulmonary tumours:

Epidermoid carcinoma  
Small cell carcinoma  
Adenocarcinoma  
Large cell carcinoma  
Adenosquamous carcinoma  
Carcinoid  
Bronchial glands carcinoma

In a special group – IV. *Tumeurs Divers* – carcinosarcoma and pulmonary blastoma were recognized. Spindle cell carcinoma and other neuroendocrine tumours were included in the groups of epidermoid carcinoma and small cell carcinoma respectively<sup>6</sup>.

The WHO 1999/2004 classifications maintained the same histological groups and basic knowledge in genetics was added to the new **Blue Book** format of the in English written WHO editions<sup>5</sup>.

Beyond establishing criteria for recognition of patterns and histological types, the actual classification has also commitment with prognosis and predictive studies of molecular pathology have definitely shown a correlation between morphology and prognosis outcome directing the choice of therapy<sup>13,14</sup>. As the available molecular studies can not be forgotten when reporting small biopsies and cytological smears, the morphological criteria have to be applied as sharply as possible to neoplastic cells to be reliable and exclusive as this tumoural representation is kept in 60 to 70% of cases in non-surgical stages at the time of diagnosis, when therapy has to be decided<sup>15</sup>.

Small cell carcinomas, epidermoid carcinomas and adenocarcinomas offer no problems to be classified even in cases where differential diagnosis between primary and secondary adenocarcinomas has to be. The last situation is reasonably solved by applying morphological criteria and the currently used immunohistochemical antibodies CK 20 (digestive adenocarcinomas) and CK7 and TTF1 (pulmonary adenocarcinomas). Meanwhile the recognition of more variants to be included in the WHO recognised groups reinforce the need of recurring to embryology and cellular type knowledge to

decide rigorous classification and differential diagnosis.

Still in the actual classification, repetitions of cellular types under different histological types and the recognition of large cells, giant cells, spindle cells and inflammatory cells in small biopsies raise the utility of a new future classification with synthesis of language and grouping.

### Differentiating histological types in biopsies by immunohistochemistry

#### Squamous cell carcinoma

The variants of epidermoid carcinoma actually recognized include papillary, clear cell, small cell and basaloid morphologies. Only the first one can be recognized in small biopsies without the use of immunohistochemistry (IHC) when clear basal membrane invasion is obvious together with the well differentiated pattern with extensive keratinisation<sup>16</sup>.

The other patterns and poorly differentiated epidermoid carcinoma – not exhibiting keratinisation and justifying the title of epidermoid carcinoma instead of squamous cell carcinoma – require use of IHC<sup>17,18</sup>.

High-weight-molecularcytokeratins(HWMC – LP34/34βE12/...) are of daily use as adenocarcinomas of the bronchus or of the lung do not express those keratins that are expressed by metaplastic pavementoid cells in respiratory epithelium, where normally a clear basal cell positive layer is distinctly defined till the epithelium of the bronchioles where it is absent (Fig. 1).

Also TTF1 (Thyroid Transcription Factor 1) and CK7 are consistently negative in epidermoid carcinomas and if positive, the he-

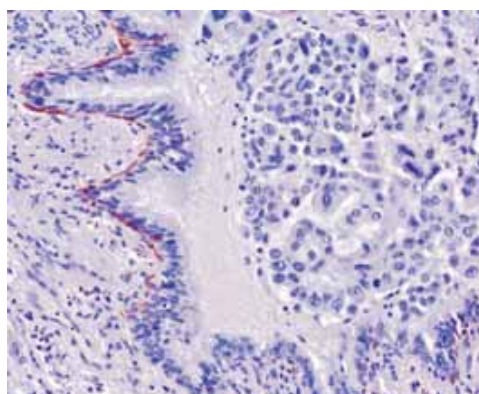


Fig. 1 – High weight molecular cytokeratins in basal bronchial cells. Mixed type adenocarcinoma. 34βE12 X 200

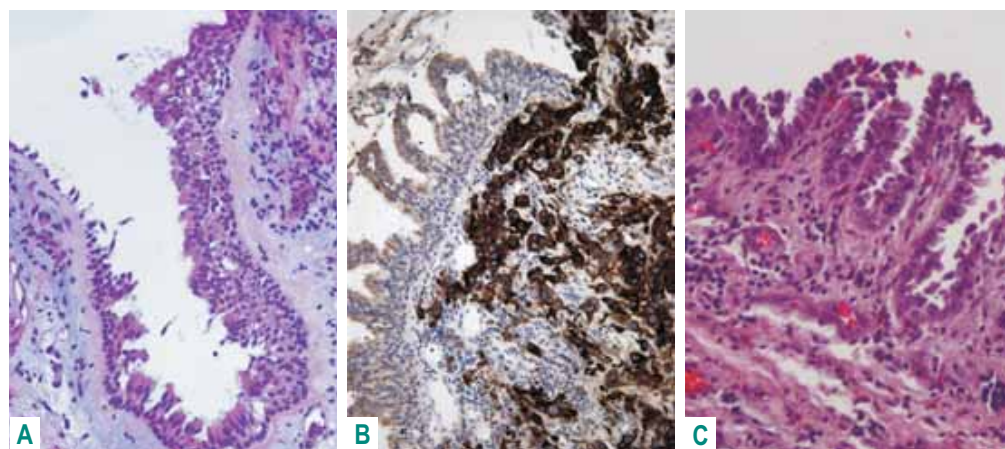
terogeneity of cell types has to be considered and reported to make the diagnosis of combined small cell carcinoma or adenocarcinoma and then validate therapy and prognosis<sup>19</sup>.

The basaloid variant can only be reported as a pattern if no keratinisation is present as the basaloid carcinoma included in the large cell carcinoma group has poorer prognosis. CK7 may be expressed together with HWMC in these carcinomas and their rarity has also to be kept in mind.

Small cell variant of epidermoid carcinoma has become easily recognisable when TTF1 and Ki67 proliferation marker are searched together with HWMC. A Ki67 proliferation index higher than 80% together with clear expression of HWMC indicates a combined small cell carcinoma and epidermoid carcinoma; TTF1 may show the same quantity of nuclear expression or lower in this situation.

The WHO classification does not embrace the cellular capacities determined by IHC in small pieces of tissue as it has been done considering surgical specimens where poor-





**Fig. 2** – Bronchial preinvasive lesions: basal cell hyperplasia HE X 100; papillary hyperplasia CK20 X 100; papillary metaplasia HE X 100

ly differentiated areas of carcinomas were not considered to be reported and not explored as it would have made a much more complex classification – the 10% law<sup>20</sup>.

### Adenocarcinoma

Heterogeneity of primary bronchial-pulmonary adenocarcinomas and also of sarcomatoid and large cell carcinomas of the lung is related to embryology and multiple bronchial – pulmonary cell types with different functions are now under the explanation of the epithelial mesenchymal transition (EMT) theory<sup>21</sup>.

As referred for squamous cell carcinoma, HWMCs delineate the basal cell bronchial layer till bronchioles where ciliated cells continue to express CK7 and alveolar cells, CK7 and TTF1 focally. Cell types vary from cuboidal to columnar, with either variable mucinous cytoplasm and basal or hobnail nuclei<sup>22-24</sup>.

The expression of CK20 by adenocarcinomas has to be related with the common em-

bryological origin of upper and lower airways together with anterior intestine as it does not occur in mature respiratory epithelium. These primary carcinomas when expressing CK7 without TTF1, define intestinal carcinomas and having histogenetical dependence from respiratory epithelium might well be called bronchial adenocarcinomas<sup>25,26</sup>.

The histogenesis of bronchial-pulmonary adenocarcinomas has long taken the attention of Pathologists and Researchers as firstly mature cells were not supposed to give origin to malignant cells in respiratory epithelium where epidermoid carcinoma develops from epidermoid metaplasia and small cell carcinoma, from one or various types of related cells with neuroendocrine variable expression not yet clearly defined beyond the high proliferation rate<sup>27,28</sup>.

Meanwhile bronchial and bronchiolar preinvasive lesions can be demonstrated for adenocarcinoma as: basal cell hyperplasia, papillary hiperplasia, papillary metaplasia and bronchiolar columnar cell dysplasia (BCCD) (Fig. 2)<sup>29,30</sup>.

Noguchi (Cancer 1995) called attention for small adenocarcinomas while in Europe still large tumours were diagnosed. Those small adenocarcinomas are of mixed type, with or without predominance of bronchioloalveolar (BAC) non-mucinous pattern with different prognosis, better when predominant non-mucinous BAC is present. Neoplastic cells express CK7 and TTF1, independently of the present patterns, even when concerning a pure acinar or papillary adenocarcinoma, this histological type long known to have the poorest prognosis among adenocarcinomas<sup>31-33</sup>.

Terminal respiratory unit adenocarcinoma described by Noguchi do not overtake 3cm diameter, has central desmoplastic stroma with tumoural acinar pattern, surrounded by papillary, micropapillary, acinar or non-mucinous BAC, keeping fidelity to CK7 and TTF1 nuclear expression and negativity to the other IHC markers (Fig. 3)<sup>34-35</sup>.

The above referred theory is extended to larger adenocarcinomas where less organized patterns are present and then the TTF1 nuclear expression may be lost while CK7 is constant and this event has to be taken as very important when reporting biopsies as the less differentiated patterns determine a poorer prognosis and are often gathered in lymphatic vessels of bronchial biopsies<sup>36,37</sup>.

Differential diagnosis with mesothelioma keeps being a challenge when clinicians forget to refer pleural expansion without the presence of a definite mass and after reporting adenocarcinoma CAT does not show the tumour. The classical pseudo-mesotheliomatous adenocarcinoma has become rare. Nowadays calretinin keeps being the most reliable antibody for malignant mesothelial cells together with cytokeratins (CK5/6 or

CK7) to mark spindle mesotheliomatous cells. The use of anti-glicoproteins antibodies as CEA and Ber-EP4 is needed to discard adenocarcinoma and cytoplasmic membrane reinforcement by EMA in malignant mesothelial cells is reliable<sup>38-41</sup>.

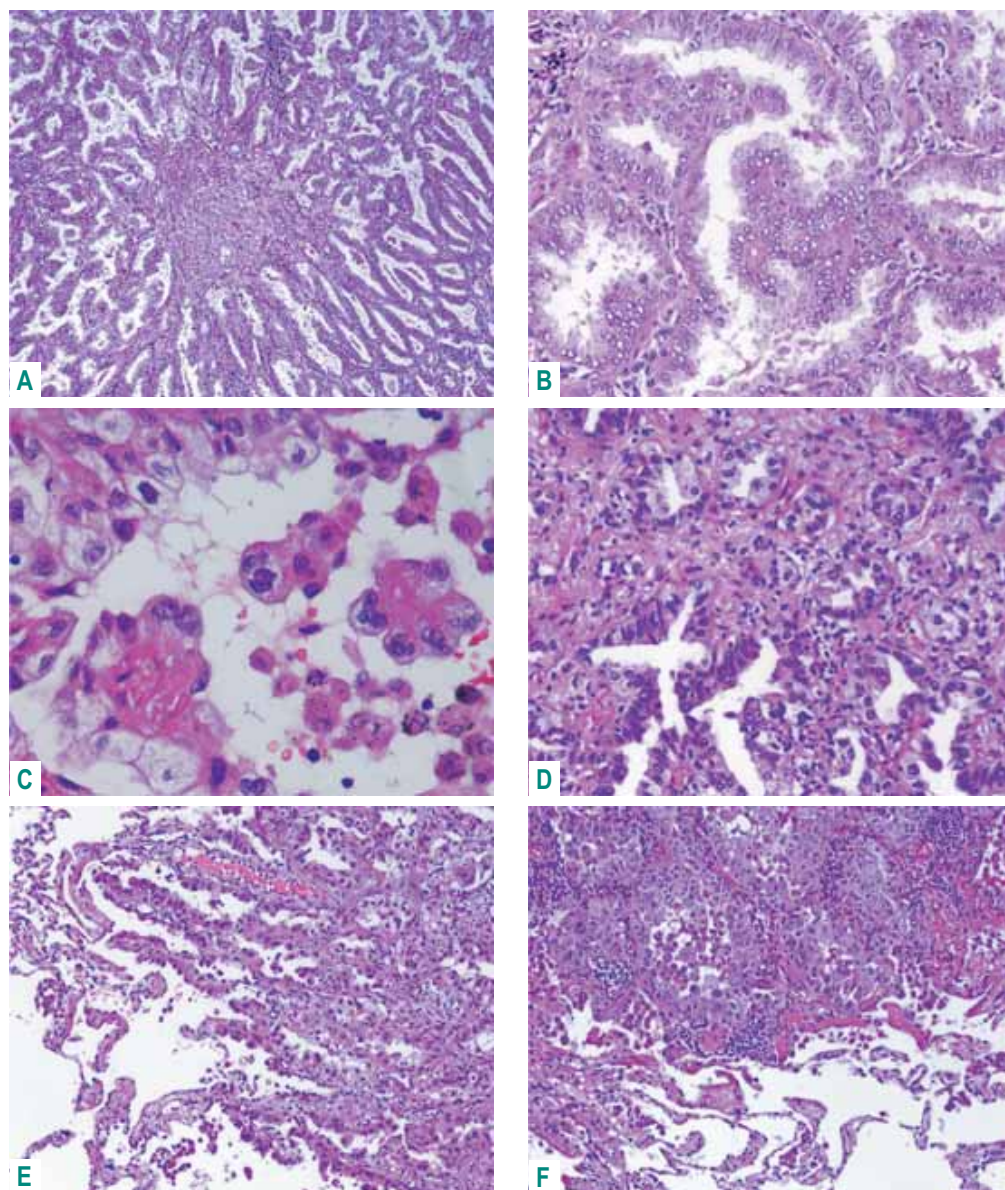
BAC keeps being controversial as considered an in situ carcinoma in WHO classification and then reported with metastases in some series. Nonetheless atypical adenomatous hyperplasia is the preinvasive lesion of non-mucinous type that is also the pattern found in mixed type adenocarcinoma while pure mucinous BAC is multifocal and often bilateral. The mixed non-mucinous and mucinous type is seen in larger lesions when desmoplastic reaction is present with acinar invasion and then related to lymph node metastases. This last situation is no more a BAC in WHO classification but a mixed type adenocarcinoma<sup>42</sup>.

The IHC expression of BAC is also variable as limited to CK7 and TTF1 in non-mucinous type and revealing CK20 and scarcity of CK7 and no TTF1 in some cases of mucinous BAC. This IHC pattern can be related to an intestinal mucinous BAC that can be multifocal and distinct from bronchial adenocarcinoma (Fig. 4)<sup>43</sup>.

No descriptions are available till now to distinguish gender patterns and it is well stated that pulmonary adenocarcinomas in women have a better prognosis. The feminine adenocarcinomas may show particular patterns as hepatoid pattern and oestrogen, progesterone and variable cytoplasmic membrane glicoproteins IHC expression. When in small biopsies an endometrioid pattern is suggested (Fig. 5).

The immunohistochemical patterns of adenocarcinomas raise the possibility of defi-

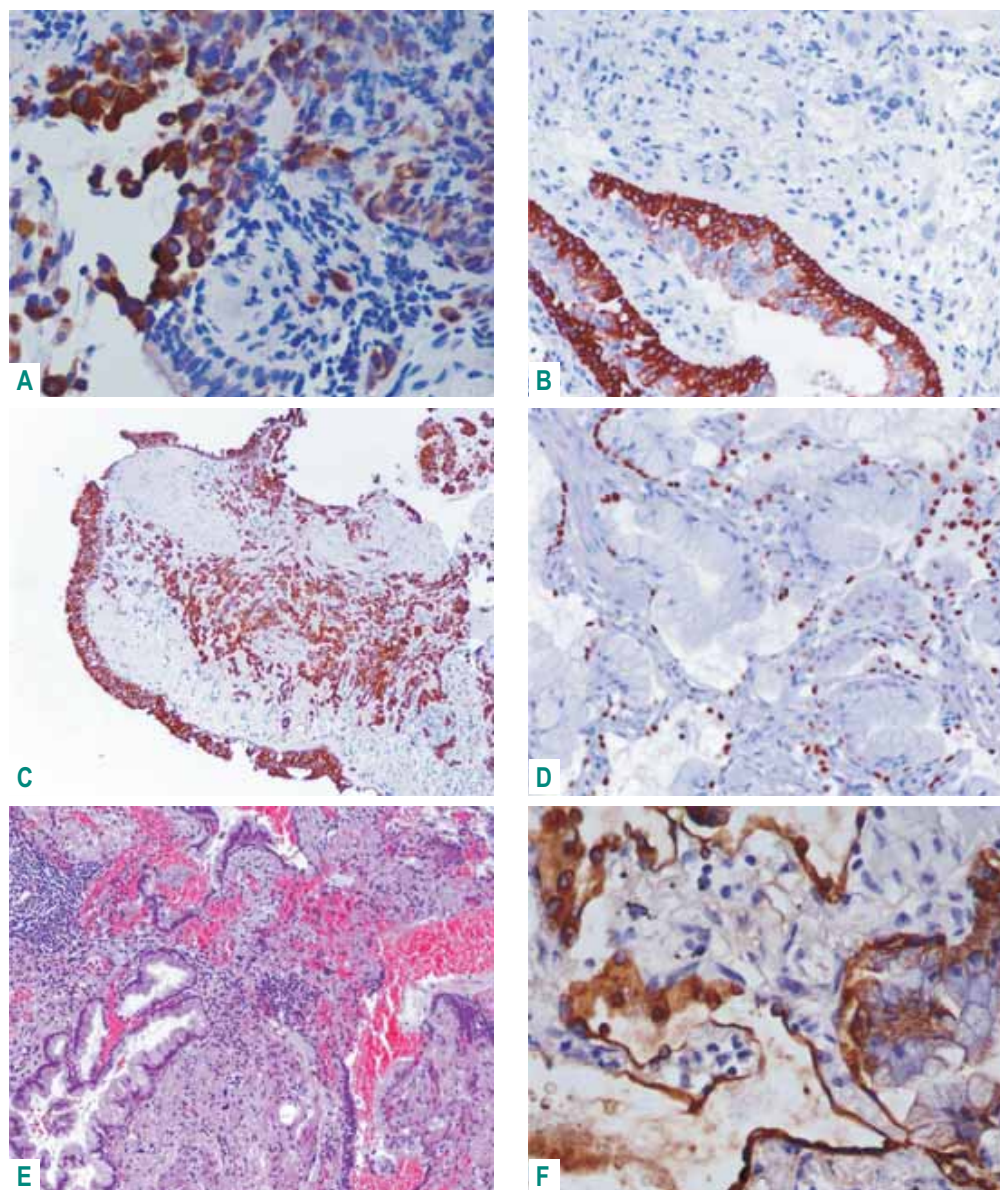




**Fig. 3** – Terminal respiratory unit adenocarcinoma. A: central desmoplasia; B: true papillae; C: micropapillae; D: acinar pattern; E – F: peripheral acinar and papillary growth

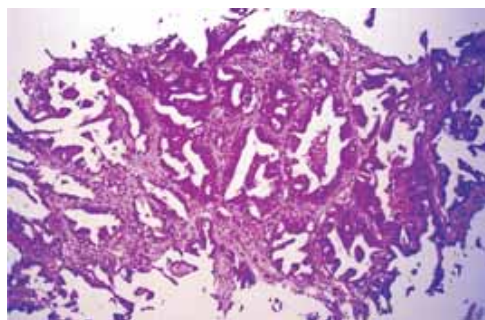
# RECLASSIFICAÇÃO DO CARCINOMA BRONCOPULMONAR: DIFERENCIAÇÃO DO TIPO HISTOLÓGICO EM BIÓPSIAS POR IMUNO-HISTOQUÍMICA

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**Fig. 4** – Bronchial adenocarcinoma – A: positive CK20 in malignant cells, not expressed in respiratory epithelium; B: CK 34βE12 in current basal cells; C: CK7 revealing microacinar pattern (TTF1-). BAC – mucinous type – D: TTF1 -; E: intestinal glandular type; F: CK20 scarce positivity (CK7+)





**Fig. 5** – Adenocarcinoma - «endometrioid» acinar pattern. Female 62 years old; peripheral left lower lobe tumour (CAT) and bone metastases. HE X 100

ning a classification as simple as the categories of IHC antibodies expressed, assumed in a small panel useful to define the platform for predictive therapy<sup>44-48</sup>.

### Neuroendocrine tumours

Depending on patient age different small cell tumours raise differential diagnosis solved by IHC and complementary analysis to distinguish from small cell carcinoma. For this histological type, after nuclear crushing artefact, Ki67 proliferation index is the most reliable marker revealing more than 80% positive malignant nuclei. The alveolar cell maker TTF1 is also expressed by these tumours but not consistently. While chromogranin and synaptophysin expression are constant in carcinoids, CD56 is more prone to small cell carcinoma<sup>49,50</sup>.

Small cell carcinoma is keratin temperamental varying the expression from null till high expression of HWMC or CK7. In these cases a combined small cell carcinoma and squamous cell carcinoma or adenocarcinoma has to be taken in mind in small biopsies and careful attention given to bron-

chial epithelium that may reveal also positive TTF1 and Ki 67 dysplasia (Fig. 6)<sup>51,52</sup>. Neuroendocrine carcinomas in the lung concern typical and atypical carcinoids and large cell neuroendocrine carcinoma beyond small cell carcinoma and histological criteria are clearly defined in the WHO 2004 classification. It is important to recognize typical carcinoid lymph node metastases as this may occur till 14% of the cases<sup>53,54</sup>.

The thyroid transcription factor 1 was also found in type II pneumocytes and connects an adenocarcinoma to the lung but also characterizes small cell carcinoma. Usually carcinoids do not express that factor that has also been understood as a proliferation marker. The proliferation index validated by Ki67 in small and artefactual biopsies is mandatory to distinguish typical and atypical carcinoid (<4-10%), large cell neuroendocrine carcinoma (20-50%) and small cell carcinoma (>80%) together with neuroendocrine markers usually<sup>55,56</sup>.

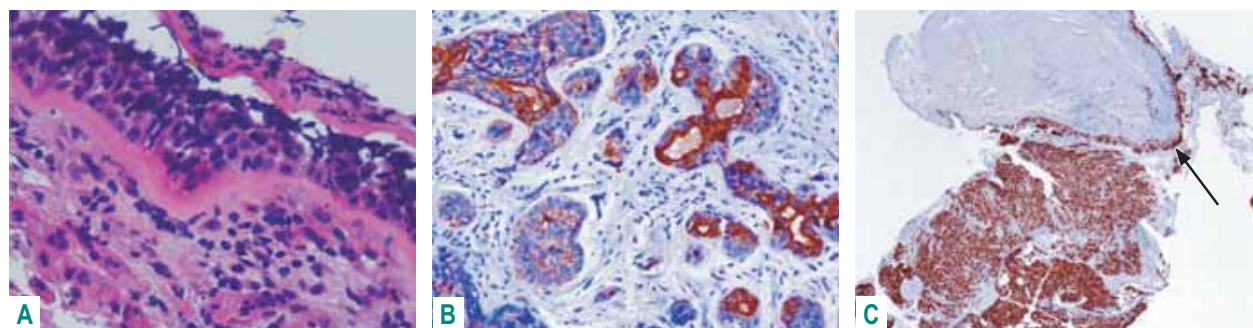
### Adenosquamous carcinoma

Not many concern has been given to this pulmonary carcinoma but in small biopsies it is common to have HWMC and CK7 expression in neoplastic cells. When carefully observed it is possible to see in the pavementous/epidermoid cellular clusterings, luminal drafts with both different expression and patterns of those cytokeratins.

This way adenosquamous carcinoma may present in a spectrum from a well differentiated type to moderately differentiated and poorly differentiated types keeping the expression of CK7 and HWMC in separate and/or in the same cells, with combined patterns. Usually TTF1 is absent but if pre-

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**Fig. 6** – Combined small cell carcinoma and adenocarcinoma – A: neuroendocrine epithelial dysplasia; B: CK7 revealing acinar pattern; C: Ki67 100% positive malignant nuclei and characterizing neuroendocrine epithelial dysplasia (arrow)

sent the cell type has to be considered to raise the possibility of a combined small cell or a TRU adenosquamous carcinoma, depending on Ki67 nuclear rate<sup>57</sup>.

It has to be kept in mind that salivary gland tumours have epidermoid and glandular patterns that are not mistaken for adenosquamous carcinomas. Also the cam5.2 marker and androgen and oestrogen receptors are expressed in those tumours.

### Pleomorphic carcinomas

The confidence in IHC to classify bronchial – pulmonary carcinomas is defined in Table I (empty spaces mean negativity) based in the WHO 2004 classification. Then large cell carcinomas and sarcomatoid carcinomas have to be joined under the designation of pleomorphic carcinomas because of three reasons: the present approach is a result of working mostly in biopsies beyond surgical specimens; pleomorphism (different cell types) and polymorphism (one cell type with various forms) interface each other in the two groups of large cell and sarcomatoid carcinomas; lastly and to reinforce the two mentioned reasons, large cells and clear cells

are seen everywhere in the previous histological types and need IHC (and mucinous stains PAS and Alcian blue) to allocate them.

The bronchial-pulmonary carcinoma grouping is designed in Tabel II where morphology and adequate IHC panel are ensembled in order to be used in cytology, biopsies and surgical specimens reporting. The utility of this classification can be tested in a biopsy of a poorly differentiated squamous cell carcinoma exhibiting large cells that keep expressing only HWMC confirming that grading carcinomas is not important. Also, as large cells may represent pleomorphic adenocarcinomas, only CK7 expression is expected; on the other hand, the expression of CK7 and HWMC together point to poorly differentiated adenosquamous carcinoma. The TTF1 nuclear positive staining keeps a good marker to recognize pulmonary carcinomas with variable patterns.

It is important to verify that the columnar and/or cuboidal cells of an adenocarcinoma component in a pleomorphic carcinoma also express vimentin in an irregular way. This may be important to recognize prog-

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**Table I** – Bronchial – Pulmonary carcinoma: Histological typing and immunohistochemistry

		Ck7	Ck20 CDX2 MUC2	TTF1	34βE12 LP34 p63	VMT Desmin Actin	CD56 Synapto	Chromog	Ki67
<b>SQC</b>					+				
<b>SCC</b>				+/-			+		>90%
<b>ADC</b>	<b>Bronchial/intestinal</b>	+	+	+/-					
	<b>Mixed</b>	+		+					
	<b>Acinar &amp; BAC / TRU</b>	+		+					
	<b>Papillary</b>	+		+					
	<b>BAC mucinous</b>	+	+/-	+/-					
	<b>Nonmucinous</b>	+		+					
	<b>Mixed</b>	+	+/-	+/-					
	<b>Solid carcinoma</b>	+		+/-	+/-				
<b>LCC</b>	<b>LCNEC</b>	+		+			+		50-80%
	<b>Other variants</b>	+		+/-	+/-		+/-		
<b>ADSQ</b>	<b>Well differentiated</b>	+		+/-	+				
	<b>Moderately differentiated</b>	+		+/-	+				
	<b>Poorly differentiated</b>	+			+				
<b>Sarcom</b>	<b>Pleomorphic ADC</b>	+		+		+/-			
	<b>SQC</b>	+			+	+/-			
	<b>SCC</b>	+			+/-	+/-	+		
	<b>LCNEC</b>	+			+/-	+/-	+		
	<b>Spindle Cell C</b>	+			+	+			
	<b>Giant Cell C</b>	+			+/-	+			
	<b>Carcinosarcoma</b>	+			+	+			
<b>Carcinoid</b>	<b>Typical / atypical</b>	+/-		-/+	+/-			+	<10%

SQC – squamous cell carcinoma

ADC – adenocarcinoma

TRU – terminal respiratory unit

LCNEC – large cell neuroendocrine carcinoma

C – carcinoma

SCC – small cell carcinoma

BAC – bronchioloalveolar carcinoma

LCC – large cell carcinoma

ADSC – adenosquamous carcinoma

VMT – vimentin

nosis and histogenesis in future. By now, vimentin, desmin and smooth muscle actin have to be expressed (one positive antibody is sufficient) to define a pleomorphic carcinoma independently of the observed histological patterns<sup>58</sup>.

## Discussion

The Pathologist concern is beyond the diagnosis of non-small cell or small cell carcino-

ma when reporting small biopsies or cytology specimens and IHC became an easy and useful tool. Cell morphology and histological types and patterns are leaders of reporting and rationalize the choice of limited IHC panel. This concern goes further than diagnosis as important therapeutic drugs are revealing prognostic value based in bronchial and pulmonary carcinoma histology<sup>59-62</sup>.

The IHC panel defined in Table I and quoted in the proposed immunohistochemical

bronchial-pulmonary carcinoma grouping/ /classification – IBPCC – in Table II is widely used as the applied antibodies are reliable and easily validated. The usefulness of the presenting IBPCC relies in its simplicity of understanding cell types and cellular potentialities either in bronchial cylindrical and ciliated epithelium and also in carcinomas arising in the TRU unit or when presenting as solid tumours of heterogeneous patterns and cellularity. This work may be a refining of the attempt made by Dr Edwards in 1987 when ICM was not so accessible<sup>63</sup>. The IBPCC raises from the knowledge acquired in reporting surgical specimens after observing loads of slides of the whole sectioned tumour. An adenocarcinoma of the lung does not express HWMC and even in the hilum where intestinal adenocarcinomas develop more frequently, CK20 and CK 7 are exclusive with a variability of the nuclear presence of TTF1. Also when acinar and/or papillary patterns are present in a small biopsy the expression of TTF1 and CK20 persist in the surgical specimen where complementing patterns of a mixed adenocarcinoma show up. BAC is a challenging group of tumours whose macroscopy and histology has not yet been definitely corroborated. The mucinous type expressing CK20 and non-mucinous type with hobnail and Clara cells expressing CK7 and TTF1, with mixed types in between, as prone intestinal type with mucinous papillae or as gathering CK20, CK7 and TTF1 expression, have different therapeutical, staging and prognostic effects. The first group known to be multifocal and with bilateral potentiality and the other types more often an unique mass, with or without central desmoplasia, defy the WHO criteria.

The above supposed purity changes when HWMC or other markers need to be included according with morphology beyond well differentiated squamous cell carcinoma. When neoplastic cells express CK7 uniformly and TTF1 and HWMC in clusters, adenosquamous carcinoma is a correct diagnosis. Signet ring cells can be seen in this context. Whether scales or prickles are absent squamous cell carcinoma keeps being well characterized by expressing only HWMC.

Differential diagnosis of lymphoma, PNET, synovial sarcoma ... have to be taken in account when dealing with small cell tumours and consider small cell carcinoma in the group of pulmonary neuroendocrine carcinomas leaving tumorlets behind as a preinvasive lesion in the development of carcinoid. Again TTF1 plays an important role together with Ki67 as proliferation predictors in small cell carcinoma and large cell neuroendocrine carcinoma. Some small cell carcinomas present with longer survival and still no explanation is adequate. It is fantasy admit that these cases might be poorly combined small cell carcinomas where CK7 and HWMC are expressed at cellular level without pertinent patterns as understood for adenosquamous carcinomas<sup>64,65</sup>.

The mesenchimal antibodies more often used are vimentin, actin and desmin that are not expressed in adenocarcinomas, neuroendocrine carcinomas and epidermoid differentiating carcinomas. Better than considering two independent groups of large cell carcinoma and sarcomatoid carcinoma, still subdivided, or quote them together as poorly differentiated carcinomas, an unique group of pleomorphic carcinoma (Table II) shows to be either comprehensive and fea-



**Table II** – Bronchial-pulmonary carcinoma – Immunohistochemical classification

**Squamous cell carcinoma** – HWMC

Variants: papillary; clear cell; small cell; basaloid

**Adenosquamous carcinoma** – CK7/ TTF1/HWMC

Variants: well, moderately, poorly differentiated

**Neuroendocrine carcinoma** – chromogranin/synaptophysin/CD56/TTF1/ Ki67

Histological types: Carcinoid – typical / atypical  
Small cell carcinoma / Combined  
Large cell neuroendocrine carcinoma / Combined

**Adenocarcinoma** – CK7/TTF1/CK20

Histological types: Acinar adenocarcinoma  
Papillary adenocarcinoma  
Mixed Acinar / BAC - TRU  
Mixed adenocarcinoma  
Intestinal adenocarcinoma  
Intestinal bronchioalveolar carcinoma  
Pulmonary bronchioalveolar carcinoma  
Mixed bronchioalveolar carcinoma  
Mucinous carcinoma and Cystadenocarcinoma  
Feminine adenocarcinoma

**Pleomorphic carcinoma** – CK7/TTF1/HWMC/MT/Desmin/Actin

Histological types: Pleomorphic squamous cell carcinoma  
Pleomorphic adenocarcinoma  
Clear cell adenocarcinoma  
Solid adenocarcinoma with or without mucin production  
Fetal adenocarcinoma  
Signet ring cell carcinoma  
Basaloid carcinoma  
Lymphoepithelioma-like carcinoma  
Large cell carcinoma with rhabdoid phenotype  
Spindle cell carcinoma  
Giant cell carcinoma  
Carcinosarcoma  
Pulmonary blastoma

**Salivary gland tumours**

Mucoepidermoid carcinoma  
Adenoid cystic carcinoma  
Epithelial-myoepithelial carcinoma

sible as IHC is rationalized to cover all the listed histological types.

Grouping these carcinomas as poorly differentiated carcinomas might be minimalist as in these rare histological types may reside knowledge concerned with histogenesis and allowing the understanding of the actual theories of carcinogenesis meaning specific differentiation.

Pathologists fight with understanding where and why started a metaplasia or dysplasia to help in Preventive Medicine. In bronchial – pulmonary carcinoma some architectural alterations are coming to attention and being reported to help clinicians to follow patients with more expertise. In Table III a potential group of preinvasive lesions is recognized and data has to be gathered to recognize them in small biopsies.

Embriogenesis may conduct our research to understand polarized epithelial phenotype together with highly motile mesenchimal phenotype as is observed in pleomorphic carcinomas, together when carcinomatous patterns are evident or independently by recognizing solid or sarcomatoid patterns that should not be called poorly differentiated. This understanding can be again translated to cellular level and revealed by immunohistochemistry. Two good examples for illustrating this spectrum are lymphoepitelioma-like carcinoma and solid adenocarcinoma. The first case might be considered a poorly differentiated squamous cell carcinoma because there is HWMC cellular expression and is known to have a better prognosis than the other histological subtypes included in the large cell carcinoma. The second example fluctuates between a solid adenocarcinoma with mucin production and solid adenocarcinoma expressing TTF1 focally beside dis-

crete large cells or small spindle cells with cytoplasmic vimentin<sup>39</sup>.

At the bottom of the group carcinosarcoma and blastoma polarize epithelial to mesenchimal transition theory for carcinogenesis and embryogenesis potentiality respectively. Spindle cell and giant cell patterns reinforce the cellular level capacities and the IBPCC already discussed<sup>67,68</sup>.

The IBPCC is a useful tool as molecular pathology is coming to the pathologist bench demanding accurate morphology recognition based in small tissue or cell samples dependent on an IHC panel that has to be reported having in mind reliable prognosis outcome<sup>69,70</sup>.

Predictive therapy is already a reality in some pulmonary pathology centres and it is a result of trials that gather therapy, gene expression and/or mutations and prognosis. Still the commoner applied language is small cell carcinoma and non-small cell carcinoma with already cellular morphological and immunohistochemical bias. In the application and distinction between clinical outcomes from the applied therapies, first and second lines, a second biopsy may become mandatory to revalidate cellular characteristics to predict therapy answer<sup>39, 71, 72</sup>.

Still an enormous effort has to be done in order to understand the multidirectionality importance of each morphological pattern in each histological type of lung cancer when observing surgical specimens by descending to molecular level. After this laborious task more knowledge will be brought to cellular level characterization when diagnosing in small samples or neoplastic cells<sup>73-76</sup>.

The complementary or primordial approach may reside in the neoplastic cells

**Table III** – Bronchial-pulmonary pre-invasive lesions

Squamous metaplastic carcinoma in situ
Basal cell hyperplasia with atypia
Bronchial papillary hyperplasia
Bronchial papillary metaplasia
Neuroendocrine dysplasia
Tumorlet
Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia
Bronchiolar columnar cell dysplasia
Atypical adenomatous hyperplasia

entourage concerning immune cells and mesenchymal cells wondering for stem cells and predictive therapy for these tar-

gets is already on the route by blocking angiogenesis and growing factors that make a wide net<sup>77-80</sup>.

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