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RESPIRATORY BRONCHIOLITIS AND BRONCHIOLITIS OBLITERANS: MORPHOLOGICAL ALTERATIONS

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Resumo

As doenças pulmonares intersticiais ainda cursam sem tratamento definido que previna a fibrose e a perda da função pulmonar, provavelmente devido à falta de conhecimento sobre a pato-fisiologia correcta. As bronquiolites podem ser fonte de estudos para entendimento de lesões iniciais decorrentes de alterações da transição epitélio-mesenquimatosa, importantes para prever a evolução clínica e o progóstico dos tumores e de alterações celulares pré-neoplásicas e intersticiais. Este estudo foi desenhado na tentativa de compreender as alterações de remodelação do epitélio e das células mesenquimatosas na Bronquiolite Respiratória e na Bronquiolite Obliterante.

Uma série de 45 casos de biopsias cirúrgicas foi dividida em dois grupos controlo (pulmão morfologicamente normal de doentes com pneumotórax espontâneo - 5 casos e com Bronquiolite Crónica assim designada por ausência de qualquer contexto clínico - 11 casos – sem alterações epiteliais relevantes e infiltrado linfo-plasmocitário parietal discreto) para comparar com casos de Bronquiolite Respiratória – 15 casos e Bronquiolite Obliterante – 14 casos. As alterações histopatológicas distintivas foram registadas para os quatro grupos, considerando as células epiteliais, basais e parietais, juntamente com o infiltrado inflamatório parietal e presente nos septos inter-alveolares adjacentes; a expressão de TTF-1, CK5.6.18 (LP34), Vimentina, CD10 and TGF-β foi registada para estudar a EMT – transição epitélio-mesênquima.

Nos casos de Bronquiolite Respiratória, a caracterização da fibrose foi estabelecida com a Vimentina, TGF- β e CD10, enquanto que a LP34 definiu a metaplasia brônquica do epitélio bronquiolar pela positividade das células basais e negatividade de células cilíndricas e ciliadas para TTF-1. Os casos de Bronquiolite Obliterante mostraram características idênticas, com proeminência de fibrose intersticial em alguns casos. Este último parâmetro teve relevância neste

estudo pela morfologia bi-linear em carril, determinada pela expressão de TGF- β e CD10, ainda não descrita até ao momento, nos septos inter-alveolares, adjacentes aos bronquíolos.

Embora dizendo respeito a uma pequena série, foi possivel compreender a lesão pré-neoplásica inicial que corresponde à metaplasia brônquica do epitélio respiratório, com células basias expressando LP34 e células cilíndricas ou ciliadas, sem expressão de TTF-1; juntamente com a expressão de TGF- β e Cd10 nas células basais e epiteliais, poder-se-á prever o desenvolvimento de adenocarcinomas brõnquicos periféricos, carcinomas adenoscamosos e carcinomas pleomórficos. O padrão septal de expressão bi-linear para os marcadores mesenquimatosos Vimentina, TGF- β e Cd10, requer estudos futuros que o relacionem com terapia precoce e adequada para prevenir a fibrose intersticial.

Palavras Chave: TGF-β; CD10; Bronquiolite Respiratória; Bronquiolite Obliterans; lesões pré-neoplásicas bronquiolares

Abstract

Interstitial lung diseases still lack definite treatment to preventing fibrosis evolution and respiratory function lost, probably due to unawareness of correct patho-physiology. Bronchiolitis may support early lesions studies concerning epithelial and mesenchymal alterations to preview clinical outcome and prognosis in both tumoral pulmonary diseases and induced pre-neoplastic epithelial and interstitial cellular alterations. This study was designed to understand both epithelial alterations and mesenchymal remodeling in Respiratory Bronchiolitis and Bronchiolitis Obliterans.

A series of 45 cases was divided in two control groups (normal looking pulmonary parenchyma obtained after surgical biopsy of patients with spontaneous pneumothorax - 5 cases and chronic bronchiolitis this way designated when scarce inflammatory infiltration was present without relevant epithelial alterations – 11 cases – without clear clinical interpretation) to be compared with Respiratory Bronchiolitis (15 cases) and Bronchiolitis Obliterans (14 cases) surgical biopsies. The morphological alterations were registered for epithelial, basal and parietal cells, together with adjacent septae enlargement and inflammatory infiltration; TTF-1, CK5.6.18 (LP34), Vimentin, CD10 and TGF-beta were applied to study EMT – epithelial-mesenchimal transition.

In respiratory bronchiolitis, fibrosis was high-lightened by Vimentin, TGF-beta and CD10, together with CK5.6.18 positive basal cells and cylindrical/ciliated epithelial metaplasia with lower TTF-1 expression. In Bronchiolitis Obliterans, the same characteristics were present with focal prominence of interstitial fibrosis. This last parameter was also demonstrated as bi-linear/railway pattern positivity in alveolar septae with TGF-beta and CD10 expression.

Although concerning a small series of cases it was possible to understand pre-neoplastic bronchial metaplasia of bronchiolar epithelium, after LP34 positive basal cells and TTF-1 negative cylindrical ciliated cells; together with TGF-beta and CD10 basal / epithelial positive cells, the development of bronchial peripheral adenocarcinomas, adenosquamous carcinomas and pleomorphic carcinomas may be previewed. The still not yet reported railway pattern expression of TGF-beta, Vimentin and CD10 in alveolar septae deserve further studies and understanding for early therapy implications to prevent interstitial fibrosis.

Key words: TGF-β; CD10; Respiratory Bronchiolitis; Bronchiolitis Obliterans; pre-neoplastic bronchiolar lesions

Introduction

Bronchiolitis encompass a spectrum of clinical and pathological entities concerning a non-tumoral pulmonary segment of diseases with complex ethiopathogeny resulting in idiopathic, primary or secondary types related or not to bronchial diseases and non-pulmonary diseases(1).

From insidious clinical symptoms till dyspnea with imagiological studies vary from minimal subtle lesions till interstitial diseases after restrictive or obstructive patterns. Bronchiolitis may be demanding interdisciplinary, being a challenge to clinicians, radiologists, surgeons and pathologists.

In the last classification of Idiopathic Interstitial Pneumonias (IIP) it becomes clear three types of interstitial lung diseases: airway centered, lobular centered and combined. In the first group respiratory bronchiolitis – interstitial lung disease (RB-ILD), airway centered interstitial fibrosis (ACIF) and cryptogenic organizing pneumonia (COP) are considered. Diseases mainly affecting the periphery of pulmonary lobules are interstitial pulmonary fibrosis/usual interstitial pneumonia (IPF/UIP), desquamative interstitial pneumonia (DIP) and acute interstitial pneumonia (AIP) (2).

In the combined interstitial lung diseases group, smoking related interstitial fibrosis (SRIF) and hypersensitivity pneumonitis (HP) form the largest group of interstitial lung diseases supporting chronic bronchiolar-pulmonary inflammation where epithelial remodeling occurs and might support carcinogenesis (3,4).

Bronchiolitis as an entity were not considered. The terminal respiratory unit (TRU) concept of Noguchi supports repair/remodeling in the adult lung, similar to development organogenesis,

where growth factors and extra-cellular matrix (ECM) intermingle epithelial mesenchymal transition (EMT), after the different types of bronchiolitis, support carcinogenesis (5).

Classically the last segment of small airways concerns the membranous bronchiole still with smooth muscle cell sheath, terminal bronchiole with fibroblast-epithelial support and respiratory bronchiole already with the same function as the alveolar duct. Smoking related diseases and hypersensivity pneumonitis induce chronic inflammation in this segment, still without clear clinical understanding in either pulmonary fibrotic diseases or carcinogenesis (6).

The idiopathic interstitial lung diseases histopathological patterns are the model applied to classify all non-tumoural pulmonary diseases due to the fact that these diseases follow one of those models and chronically end usually with a UIP – like pattern, with loss of the respiratory function. The early lesions might be common either to idiopathic and non-idiopathic pulmonary interstitial diseases but still lack correct identification. In the whole group of pulmonary non-tumoural diseases tobacco and inhaling diseases are the commonest in pulmonology routine practice. In both situations, multiple different histopathological patterns are identified: tobacco reflects several forms of pulmonary lobular distortion and hipersensitivty pneumonitis (HP) runs with malformed epithelioid granulomas, NSIP different patterns and COP like morphology (7).

In tobacco related morphology and HP evolution, bronchiolar disease seems to be the early trigger-point with RB and inflammatory myofibroblastic polyps in BO respectively, as the initial diseases still not identified or understood or even recognized as definite diseases because epithelial and adjacent interstitial remodeling has not yet been clarified.

By applying the commonly used routine antibodies in pathology an attempt can be made to stratify the early lesions of tobacco related diseases and early BO bronchiolar adaptions: CK5.6.18 (LP34) and TTF-1 identify bronchial basal cells and Clara cells / pneumocyte type II respectively being useful to quote bronchiolar epithelium where CK5.6.18 positive cells are usually absent and TTF-1 expression is relevant in bronchiolar-alveolar transition epithelial cells; vimentine as the commonest intermediary filament in the cytoskeleton of mesenchymal cells, is an easy way to identify fibroblasts, myofibroblasts, endothelial cells and macrophages (7).

Still not explored remain TGF-β and CD10 (CALLA) expression, either in mesenchymal and epithelial cells, but their role deserves interpretation for early therapeutic decisions, due to their function in epithelial-mesenchimal transition intermingling of epithelial, myoepithelial and myofibroblastic cells in persistent inflammatory stimulation. Great relevance has been given to TGF- β due to its importance in early stages of carcinogenesis and immunity functions related with fibrobasts proliferation, still without morphological understanding (8).

Objectives

This retrospective study was developed as an approach and attempt to comprehend interstitial and epithelial bronchiolar remodeling in smokers and organic particles inhalers using archived surgical biopsies of the Anatomical Pathology of the Centro Hospitalar e Universitário of Coimbra.

Ethical rules to retrospective studies were followed with patients' anonymity, concerning cases with the diagnosis of Respiratory Bronchiolitis and Bronchiolitis Obliterans with concordant clinical information.

The study was designed to stratify specific histopathological differential alterations to be supported through immunohistochemistry.

Materials and Methods

Materials

A group of 45 pulmonary surgical biopsies was selected, regularly collected from upper and middle lobes of patients whose clinical evolution demanded that behavior; 15 cases belonged to smokers with a histological pattern of Respiratory Bronchiolitis (RB) and 14 cases belonged to patients with inhaling history after contact with chickens/birds where Bronchiolitis Obliterans (BO) diagnosis was applied.

Two control groups were established with 11 cases of Chronic Bronchiolitis (CB) and 5 cases of normal lung of patients with spontaneous pneumothorax (with sub-pleural infancy infarct scars). Chronic Bronchiolitis, classically referred as a cellular bronchiolitis was considered when only a few lymphocytes and plamocytes infiltration was present in bronchioles walls without morphological alterations of either epithelium or surrounding alveolar septae in cases without other relevant morphologic alterations.

The 4 groups of study are concise in tables I to IV considering patients age and gender and surgical biopsy anatomical topography, according with each morphological diagnosis and in figure 1, the 4 morphological patterns are described according with classical histopathological criteria for each group of selected cases.

Table I: Spontaneous Pneumothorax

Case	Surgical Biopsy - topography	Age	Gender
1	Left upper lobe	87	Male
2	Sub-pleural biopsy not identified	16	Male
3	Right upper lobe	31	Female
4	Left upper lobe	30	Male
5	Right Upper, middle and lower lobes	38	

Table II: Chronic Bronchiolitis

Case	Surgical Biopsy - topography	Age	Gender
6	Right middle lobe	46	Female
7	Right middle lobe	58	Male
8	Lower left lobe	69	Female
9	Lower left lobe	43	Male
10	Lung	57	Female
11	Right middle lobe	52	Male
12	Right middle and lower lobes	70	Female
13	Left upper lobe	43	Male
14	Left lower lobe	62	Male
15	Right upper lobe	35	Female
16	Right middle and lower lobes	74	Female

Table III: Respiratory Bronchiolitis

Case	Surgical Biopsy - Topography	Age	Gender
17	Lung	41	Female
18	Left upper lobe	46	Male
19	Left upper lobe	33	Male
20	Right lung	26	Male
21	Upper lobe	25	Male
22	Left upper lobe	23	Male
23	Right lower lobe	54	Male
24	Left upper lobe	28	Male
25	Right upper lobe	31	Male
26	Left upper lobe	20	Male
27	Left apex	21	Male
28	Right upper lobe	16	Male
29	Left upper lobe	45	Male
30	Right lung	33	Female
31	Left upper lobe	34	Male

Table IV: Bronchiolitis Obliterans

Case	Surgical Biopsy – topography	Age	Gender
32	Left lower lobe	56	Male
33	Right lower lobe	67	Female
34	Right lower lobe	69	Male
35	Lower lobe	72	Female
36	Lung	44	Female
37	Right middle lobe	73	Female
38	Right lower lobe	67	Male
39	Right middle and lower lobe	54	Female
40	Right lung	68	Male
41	Right middle lobe	69	Female
42	Left lower lobe	72	Male
43	Right upper lobe	61	Female
44	Lung	53	Female
45	Right middle and lower lobes	55	Male

Methods

Morphological Study

The pulmonary tissue had been formalin fixed paraffin embedded and redundant tissue was selected to register morphological parameters and imunohistochemical studies. Morphological understanding of both interstitial and epithelial remodeling concerning bronchioles and adjacent alveolar septae were registered for each group of diseases.

Due to the different volume of tissue available for each case, a minimal number of twenty bronchiolar-vascular axes were considered to register morphological alterations.

Bronchiolar epithelium changes in RB and BO were scored after the following parameters: epithelial hyperplasia (flat, papillary incomplete and papillary complete) and ciliated cells presence, to be compared with either normal said bronchioles or Chronic Bronchiolitis alterations. When cylindrical and cylindrical ciliated cells were predominant in bronchiolar epithelium, bronchial metaplasia was considered.

Bronchiolar parietal fusiform cells were graded in less or more than 4 layers. The presence of sub-epithelial and bronchiolar parietal lymphocytes and plasmocytes was also registered.

Luminal inflammatory myofibroblastic polyps were seen in BO and registered as its differential hallmark.

In bronchiolar adjacent septae, the presence of fusiform cells and lymphocytes/plamocytes was also registered as well as bronchial metaplasia (cilindrical and cilindrical ciliated cells).

Tables V to VIII, report the morphological parameters for each of the study groups.

Table V: Spontaneous pneumothorax – morphological registration

Histopathology			Bronchioles										Adjacent Alveolar Septae		
			Epithelial hyperplasia			Epithelial ciliated cells	Parietal fusiform cells		Luminal inflammatory myofibroblastic polyps	Parietal Lymphocytes and Plasmocytes			Lymphocytes and Plasmocytes	Fusiform cells	Bronchial metaplasia
Case	Diag	Age/G	Flat	Papillary incomplete	Papillary complete	0/1	< 4 layers	> 4 layers	0/1	Scarce	Concentric	Exuberant	0/1	0/1	0/1
1	BPM	87 M	1	0	0	0	1	0	0	1	0	0	0	0	0
2	BPM	16 M	1	1	0	0	1	0	0	0	0	0	0	0	0
3	BPM	31 F	1	0	0	0	1	0	0	1	0	0	0	0	0
4	BPM	30 M	1	1	0	0	1	0	0	1	0	0	0	0	0
5	BPM	38 F	1	0	0	0	1	0	0	1	0	0	0	0	0

0-absence; 1-presence.

Table VI: Chronic Bonchiolitis – morphological registration

Histopathology			Bronchioles										Adjacent Alveolar Septae		
			Epithelial hyperplasia			Epitelial ciliated cells	Parietal fusiform cells		Luminal inflammatory myofibroblastic polyps	Parietal Lymphocytes and Plasmocytes			Lymphocytes and Plasmocytes	Fusiform cells	Bronchial metaplasia
Case	Diag	Age/G	Flat	Papillary incomplete	Papillary complete	0/1	< 4 layers	> 4 layers	0/1	Scarce	Concentric	Exuberant	0/1	0/1	0/1
6	CB	46 F	1	1	0	1	1	0	0	0	1	0	0	0	0
7	CB	58 M	0	1	0	0	1	0	0	0	1	0	0	0	0
8	CB	69 F	1	1	0	0	1	0	0	0	1	0	1	1	1
9	CB	43 M	1	1	1	1	1	0	0	1	0	0	1	0	1
10	CB	57 F	1	0	0	1	1	0	0	1	1	0	1	0	0
11	CB	52 M	0	0	1	0	1	0	0	1	0	0	0	0	0
12	CB	70 F	0	1	0	0	1	0	0	0	0	1	1	0	0
13	CB	43 M	0	1	0	1	1	0	0	0	1	0	1	0	1
14	CB	62 M	1	1	0	1	1	0	0	0	1	0	1	0	0
15	CB	35 F	1	1	0	0	1	0	0	0	1	0	0	0	0
16	CB	74 F	1	1	0	1	1	0	0	1	0	0	0	0	0

0-absence; 1-presence.

Table VII: Respiratory bronchiolitis – morphological registration

Histopathology			Bronchioles										Adjacent Alveolar Septae		
			Epithelial hyperplasia			Epithelial ciliated cells	Parietal fusiform cells		Luminal inflammatory myofibroblastic polyps	Parietal Lymphocytes and Plasmocytes			Lymphocytes and Plasmocytes	Fusiform cells	Bronchial metaplasia
Case	Diag	IDD/G	Flat	Papillary incomplete	Papillary complete	0/1	< 4 layers	> 4 layers	0/1	Scarce	Concentric	Exuberant	0/1	0/1	0/1
17	RB	41 F	1	0	0	1	0	1	0	1	0	0	1	1	0
18	RB	46 M	0	0	1	0	0	1	0	1	0	0	1	1	0
19	RB	33 M	1	0	0	0	0	1	0	1	0	0	1	1	0
20	RB	26 M	0	1	1	1	1	0	0	1	0	0	1	1	0
21	RB	25 M	1	0	0	0	0	1	0	1	0	0	1	1	0
22	RB	23 M	1	0	1	0	0	1	0	1	0	0	1	1	0
23	RB	54 M	0	1	0	0	0	1	0	1	0	0	1	1	1
24	RB	28 M	0	1	0	1	1	0	0	1	0	0	1	1	1
25	RB	31 M	1	0	1	0	0	1	0	1	0	0	1	1	0
26	RB	20 M	0	1	0	0	1	0	0	1	0	0	1	1	0
27	RB	21 M	0	1	0	1	1	0	0	1	0	0	1	1	0
28	RB	16 M	0	1	0	0	1	0	0	1	0	0	1	1	0
29	RB	45 M	0	1	0	1	1	0	0	1	0	0	1	1	0
30	RB	33 F	1	0	0	0	1	0	0	1	0	0	1	1	0
31	RB	34 M	1	1	0	1	1	0	0	1	0	0	1	1	0

0-absence; 1-presence.

Table VIII: Bronchiolitis Obliterans cases– morphological registration

Histopathology			Bronchioles										Adjacent Alveolar Septae		
			Epithelial hyperplasia			Epithelial ciliated cells	Parietal fusiform cells		Luminal inflammatory myofibroblastic polyps*	Parietal Lymphocytes and Plasmocytes			Lymphocytes and Plasmocytes	Fusiform cells	Bronchial metaplasia
Case	Diag	IDD/G	Flat	Papillary incomplete	Papillary complete	0/1	< 4 layers	> 4 layers	0/1	Scarce	Concentric	Exuberant	0/1	0/1	0/1
32	BO	56 M	1	0	0	1	1	0	1	0	0	1	1	1	1
33	BO	67 F	1	0	0	0	1	0	1	0	0	1	1	1	1
34	BO	69 M	1	0	0	1	1	0	1	0	1	0	1	1	0
35	BO	72 F	1	0	0	1	1	0	1	0	1	0	1	1	1
36	BO	44 F	1	0	0	1	1	0	1	0	1	0	1	1	1
37	BO	73 F	1	0	0	1	1	0	1	0	1	0	1	1	1
38	BO	67 M	1	0	0	0	1	0	1	0	1	0	1	1	0
39	BO	54 F	1	0	0	0	1	0	1	0	1	0	1	0	0
40	BO	68 M	1	1	0	0	1	0	1	0	1	0	1	1	0
41	BO	69 F	0	1	0	0	1	0	1	0	1	0	1	1	0
42	BO	72 M	1	0	0	0	1	0	1	0	1	0	1	1	0
43	BO	61 F	1	0	0	0	1	0	1	0	0	1	1	1	1
44	BO	53 F	1	0	0	0	1	0	1	0	0	1	1	1	1
45	BO	55 M	1	0	0	0	1	0	1	0	1	0	1	1	0

0-absence; 1-presence.

Immunohistochemical Study

To complement interstitial and epithelial bronchiolar remodeling of the morphological registration, the followig panel of imunohistochemical anti-bodies was selected to register cellular adaptations at the bronchiolar – septal segment alterations- Table IX.

Table IX: bronchiolar mesenchymal and epithelial remodeling selected imunohistochemical anti-bodies

	Antibody, Clone and manufacturer	Diluition	Antigenic recuperation	Incubation	Method	Positive Control
Mesenchimal adaptation	**Vimentine** (clone VIM 3B4, DAKO)	1/200	Citrate pH6 MW 480W 20’	30 min	LSAB	Colon
	TGFβ (clone TGFB17, Leica)	1/40	Citrate pH6 MW 480W 20’	30 min	LSAB	Ductal invasive breast cancer
	CD10	RTU	Tris-EDTA pH9 52 min	48 min	Bond-Max	Acute apendicitis
Epithelial adaptation	**CK 5.6** (clone LP34,Leica)	1/100	Pronase 10’	60’ min	LSAB	Epithelial cells
	TTF-1 (8G7G3/1, DAKO)	1/100	EDTA pH8 MW 480W 40’	60 min	LSAB	Small cell Carcinoma

Vimentine was selected to identify fibroblasts and CD10 to characterize early progenitor/myoepithelial cells and TGF – beta, mainly produced by macrophages, maybe present in either fusiform or epithelial cells during carcinogenesis.

The two epithelial markers follow bronchial basal cell metaplasia in bronchioles – CK5.6.18 and type II pneumocytes hyperplasia in either bronchiolar or septal epithelium – TTF-1.

Over the morphological registration applied, immunohistochemistry was scored in the following cell types: bronchiolar epithelial cell – BEC; bronchiolar ciliated cell – BCC; bronchiolar basal cell – BBC; bronchiolar fibroblastic cell – BFiC; bronchiolar fusiform/ myofibroblast cell – BFuC; one surface of bronchial adjacent alveolar septae – S1 and two surfaces of alveolar septae – S2.

Statistical Analisys

The immunohistochemical antibodies frequence was analized according with the different cell types positivity by applying STATISTICA 9.1 (StatSoft, Inc., 2009) based in the Qi-Square Test (2x3 and 2x4) with $p \leq 0,05$ significance.

Results

Histopathological Distinction

In normal looking bronchioles parietal lymphocytes and plasmocytes may be present in the bronchioles wall where fusiform cells appear in less than 4 layers; in general the epithelium is flat concerning simple cuboidal epithelium.

In the cases of chronic bronchiolitis, inflammatory infiltration became relevant either by forming a rim in the walls of the bronchioles and being present in the adjacent septae. In general the epithelium became papillary (8 cases) with ciliated cells (7 cases), representing bronchial metaplasia.

In the cases of respiratory bronchiolitis the characteristics of inflammation registered for normal looking bronchioles were present as well as the epithelial characteristics of chronic bronchiolitis. Fusiform cells showed their relevance in alveolar septae concerning all cases and in bronchiolar walls (7/15 cases).

In the cases of bronchiolitis obliterans, inflammatory miofibroblastic polyps were present in all cases. Epithelial ciliated cells were seen in 5/14 and fusiform cells were present in 4 or less layers, similar to normal looking bronchioles; the inflammatory infiltration was similar to what was seen in chronic bronchiolitis. Concerning the alveolar septae, only one case did not show fusiform cells and all the cases presented lymphocytes and plasmocytes.

In figure 1 morphological specific characteristics of each study group are summarized.

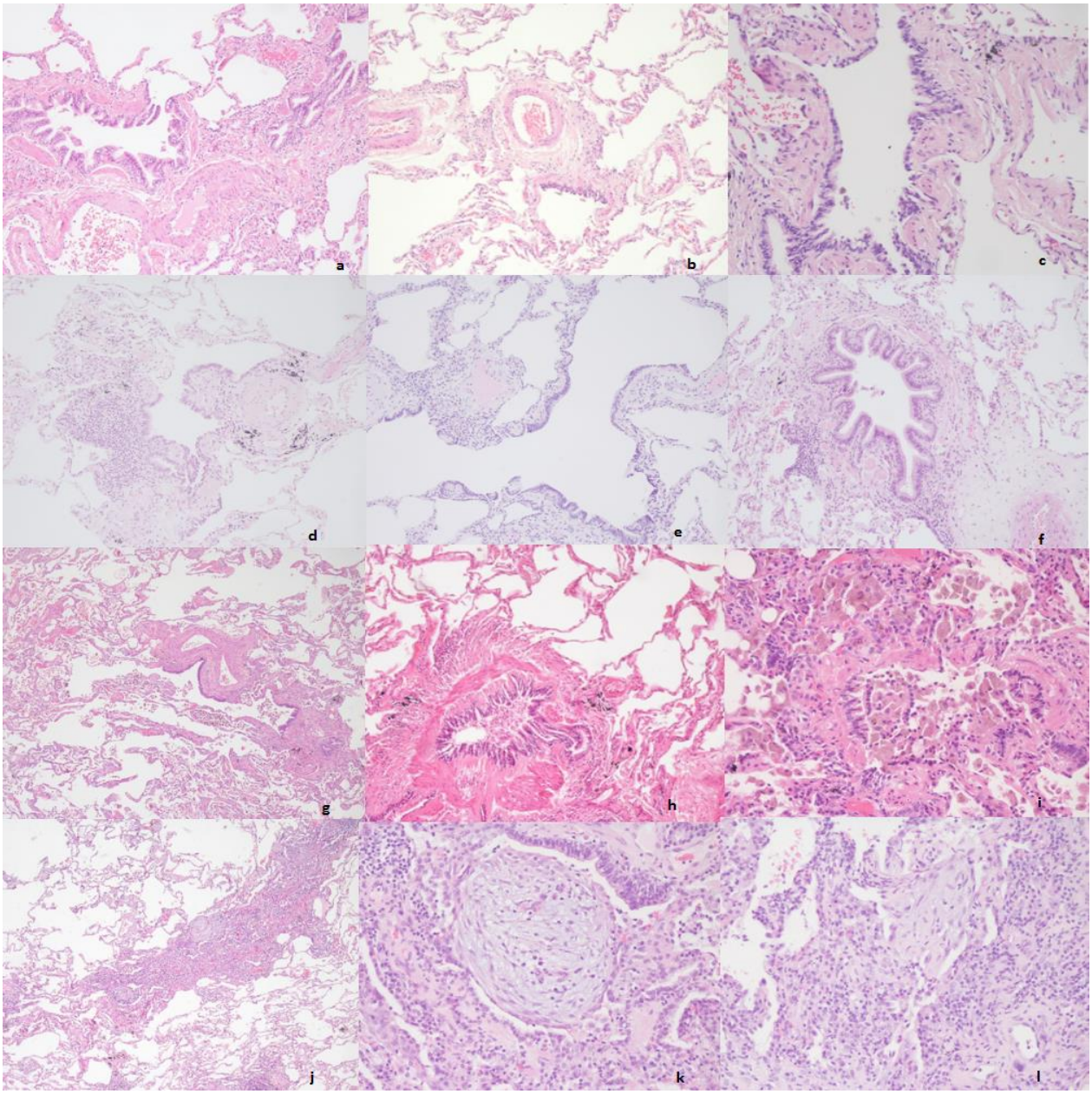

Fig. 1: Normal looking bronchioles - (**a**) pappilary bronchiolar epithelium and parietal fusiform cells under less than four layers (HEX100); (**b**) flat bronchiolar epithelium in bronchiolar – septal transition (HEX200); and (**c**) predominantly papillary epithelium in TRU (HEX200). **Chronic Bronchiolitis** criteria supported by scarce inflammatory infiltration in (**d**) (HEX100) and (**e**) (HEX100) with subepithelial fibroblasts inducing complete papillary epithelium in (**f**)(HEX100). **Respiratory Bronchiolitis** with luminal smoking pigmented macrophages (**g**) (HEX200) and peri-bronchiolar fibrosis and fusiform cells hyperplasia over 4 layers (**h**) (HEX40), also with luminal pigmented macrophages (**i**) (HEX100). **Bronchiolitis Obliterans** characterized by luminal inflammatory myofibroblastic polyps without relevant septal alterations (**j**) (HEX40), inducing inflammatory parietal infiltration under bronchiolar epithelium (**k**)(HEX200)/(**l**)(HEX200).

Immunohistochemical Results

Tables X to XIII report the immunohistochemical parameters for each of the study groups.

Table X: Spontaneous pneumothorax – immunohistochemical results

Case	Diag	Age/G	LP34								TTF1								Vimentina								TGFβ								CD10							
			BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2
1	BPM	87 M	-		+	-	-		-	-	+		+	-	-		+	-	-		-	-	-		+	-	-		-	-	-		-	-	-		+	-	-		+	-
2	BPM	16 M	-		-	-	-		-	-	+		+	-	-		+	-	-		-	-	-		+	-	-		-	-	-		-	-	A		-	-	-		+	-
3	BPM	31 F	-		-	-	-		-	-	+		+	-	-		+	-	-		-	-	+		+	-	+		-	-	+		+	-	+		-	-	-		+	-
4	BPM	30 M	-		-	-	-		-	-	+		+	-	-		+	-	-		-	-	+		+	-	+		-	-	-		-	-	A		-	-	-		+	-
5	BPM	38 F	-		-	-	-		-	-	+		+	-	-		+	-	-		-	-	-		-	-	+		-	-	-		-	-	A		-	-	-		+	-

\+ presence; - absence; A-apical epithelial staining

Table XI: Chronic Bonchiolitis – immunohistochemical results

Case	Diag	Age/G	LP34								TTF1								Vimentina								TGFβ								CD10							
			BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2
6	CB	46 F	-		-	-	-		-	-	-		+	-			+	-	-		+	-			+	-	-	-	-	+	+		+	+	A		-	-	-		+	-
7	CB	58 M	-		-	-	-		-	-	+		+	-	-		+	-	-		+	-	-		+	-	A		-	-	-		+	-	A		-	-	-		+	-
8	CB	69 F	-		-	-	-	+	-	-	+		+	-	-	+	+	-	-		-	-	+	+	+	-	A		-	-	-	-	+	-	A		-	-	+	A	+	-
9	CB	43 M	-	-	+	-	-	-	-	-	+	-	+	-	-	+	+	-	-	-	-	-	+	-	-	-	A	-	-	-	-	-	+	+	A	A	-	-	+	A	+	+
10	CB	57 F	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	-	-	-	-	+	+	-	+	-	A	-	-	-	-	-	+	-	A	A	-	-	-	-	+	+
11	CB	52 M	-	-	+	-	-	-	-	-	+	-	+	-	-	+	+	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	+	+	A	A	-	-	-	A	+	+
12	CB	70 F	-		-	-	-		-	-	+		+	-	-		+	-	-		-	-	+		+	-	-		-	-	-		-	-	A		-	-	-		+	+
13	CB	43 M	-	-	-	-	-	+	-	-	+	+	+	-	-	+	+	-	-	-	+	-	+	+	+	-	A	+	-	-	-	-	+	+	A	A	-	-	-	-	+	+
14	CB	62 M	-	-	+	-	-	+	-	-	+	-	+	-	-	+	+	-	-	-	-	-	-	-	+	-	A	-	-	-	+	-	+	-	A	A	-	-	-	A	+	+
15	CB	35 F	-		+	-	-		-	-	+		+	-	-		+	-	-		-	+	-		+	+	-		-	-	-		+	+	A	A		-	-		+	+
16	CB	74 F	-	-	+	-	-		+	-	+	-	+	-	-		+	+	-	-	+	-	+		+	+	+	-	-	-	-		+	-	A	A	-	-	-		+	+

\+ presence; - absence; A-apical epithelial staining

Tabel XII: Respiratory bronchiolitis – immunohistochemical results

Case	Diag	Age/G	LP34								TTF1								Vimentina								TGFβ								CD10							
			BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2
17	RB	41 F	-		-	-	-		-	-	+		+	-	-		+	-	-		-	+	+		+	-	+		+	-	+		+	+	A		-	-	-		+	-
18	RB	46 M	-		+	-	-		-	-	+		+	-	-		+	-	-		-	-	+		+	-	+		+	+	+		+	+	A		-	-	-		+	-
19	RB	33 M	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	A	A	-	-	-	-	+	-
20	RB	26 M	-	-	-	-	-	-	-	-	+	-	+	-	-		+	+	-	-	-	-	-		+	+	A	A	-	+	+		+	+	-	-	-	-	-		+	+
21	RB	25 M	-		-	-	-		-	-	+		+	-	-		+	-	-		-	-	-		+	+	A		-	-	-		+	+	-		-	-	-		+	+
22	RB	23 M	-		-	-	-		-	-	+		+	-	-		+	-	-		-	-	+		+	-	-		-	-	-		-	-	-		-	-	-		+	-
23	RB	54 M	-		-	-	-	-	-	-	+		+	-	-	+	+	+	-		+	-	+	-	+	+	-		-	-	-	+	+	+	-		-	-	-	-	+	+
24	RB	28 M	-	-	+	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	+	-	+	-	+	-	+	-	-	-	+	-	A	A	-	-	-	-	+	+
25	RB	31 M	-	-	+	-	-		-	-	+	-	+	-	-		+	-	+	+	+	+	+		+	+	+	+	+	+	+		+	+	A	-	+	-	+		+	+
26	RB	20 M	-		+	-	-		-	-	+		+	-	-		+	-	-		-	-	-		+	-	-		-	-	-		+	-	-		-	-	-		+	+
27	RB	21 M	-	-	-	-	-		-	-	+	+	-	-	-		+	+	-	-	-	-	+		+	-	+	+	+	-	+		+	-	-	-	-	-	+		+	-
28	RB	16 M	-		-	-	-		-	-	+		+	-	-		+	-	-		-	-	+		+	-	+		+	-	+		+	-	+		-	+	-		+	+
29	RB	45 M	+		+	-	-		-	-	+		+	-	-		+	-	-		-	+	+		+	+	+		+	+	+		+	+	A		+	-	+		+	+
30	RB	33 F	-		+	-	-		-	-	+		+	-	-		+	-	-		-	-	+		+	-	+		+	+	-		+	-	A		-	-	-		+	+
31	RB	34 M	-		-	-	-		-	-	+		-	-	-		+	-	-		-	-	+		-	+	+		-	-	+		-	+	A		-	-	-		+	+

\+ presence; - absence; A-apical epithelial staining

Table XIII: Bronchiolitis Obliterans – immunohistochemical results

Case	Diag	Age/G	LP34								TTF1								Vimentina								TGFβ								CD10							
			BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2	BEC	BCC	BBC	BFuC	BFiC	BM	S1	S2
32	BO	56 M	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	+	+	-	+	+	-	-	+	-	+	A	+	-	-	-	+	+	-	A	-	-	-	-	+	-
33	BO	67 F	-		+	-	-	-	-	-	+		-	-	-	-	+	+	+		+	+	-	-	+	-	-		-	-	-	-	+	+	-		-	-	-	-	+	-
34	BO	69 M	-	-	+	-	-		-	-	-	-	+	-	-		+	-	-	-	-	-	+		+	-	+	A	+	-	-		+	+	-	A	-	-	-		+	-
35	BO	72 F	-	-	+	-	-	+	+	-	+	-	+	-	-	+	+	+	-	-	-	-	+	-	+	+	-	+	+	-	-	+	+	+	-	A	-	-	-	+	+	+
36	BO	44 F	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-
37	BO	73 F	-	-	+	-	-	+	-	-	+	-	+	-	-	+	+	-	-	-	-	-	+	+	+	-	+	+	-	+	+	+	+	-	-	A	-	-	-	-	+	-
38	BO	67 M	-		+	-	-		-	-	+		+	-	-		+	-	-		+	-	+		+	-	-		-	-	-		+	-	-		-	-	-		+	-
39	BO	54 F	-		-	-	-		-	-	-		+	-	-		+	-	-		-	-	+		+	-	A		-	-	+		+	+	-		-	-	-		+	-
40	BO	68 M	-	-	+	-	-		-	-	+	+	+	-	-		+	-	-	-	+	+	+		+	+	A	-	+	+	+		+	+	A	-	-	-	+		+	-
41	BO	69 F	-		-	-	-		-	-	+		+	-	-		+	-	-		+	-	+		+	+	-		+	-	-		+	+	A		-	-	-		+	+
42	BO	72 M	-		+	-	-		-	-	-		+	-	-		+	-	-		+	-	+		+	-	-		-	-	-		+	-	-		-	-	-		+	-
43	BO	61 F	-		-	-	-	-	-	-	+		+	-	-	-	+	+	-		+	+	+	-	+	+	A		+	+	+	+	+	+	A		-	-	+	+	+	-
44	BO	53 F	-	-	-	-	-	+	-	-	-	-	+	-	-	+	+	+	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	+	A	A	-	-	-	-	+	+
45	BO	55 M	-		-	-	-		-	-	+		+	-	-		+	-	-		-	-	+		+	+	-		-	-	-		+	+	+		-	-	-		+	-

+ presence; - absence; A-apical epithelial staining

Integrated Immunohistochemical Results

Bronchiolar epithelial and basal cells were TTF1 positive in all group cases. Bronchial type basal cells identified with LP34 were relevant in chronic bronchiolitis (5/11 cases), respiratory bronchiolitis (7/15 cases) and bronchiolitis obliterans (8/14 cases).

The expression of TGF-beta was seen in RB (10/15 cases) and BO (4/14 cases) epithelial cells; basal and ciliated cells had a lower expression, still relevant when compared with CB.

Vimentine helped in the distinction between fibroblasts and bronchiolar parietal fusiform cells where Vimentine-negative cells were considered smooth muscle cells. In normal looking bronchioles, Vimentine was expressed in two cases, identifying fibroblasts in comparison with the presence of the well-formed rim of less than 4 layers Vimentin-negative fusiform cells considered smooth muscle cells. This morphological and immunohistochemical pattern was considered the standard for the other study groups. For TGF-beta and CD10 scoring, Vimentine standard was subjacent to distinguish fusiform cell types.

Fibroblasts identified with Vimentine became relevant in all cases mainly in BO. Fusiform cells and fibroblasts expression of TGF-beta was irrelevant in normal looking bronchioles and in CO cases. For RB and BO the expression of TGF-beta was observed in BFiC and BFuC in parallel in the same positive cases representing 5/15 cases for RB and 4/14 cases for BO; some cases had TGF-beta BFiC positive cases but BFuC positive cells were not identified alone.

The linear expression of Vimentine, TGF-beta and CD10 in inter-alveolar septae was common for the 3 anti-bodies. Concerning double-linear railway type expression, 8/11 cases in BO and 10/15 cases in RB presented this particular morphology.

In figure 2, the relevant immunohistochemical results are illustrated.

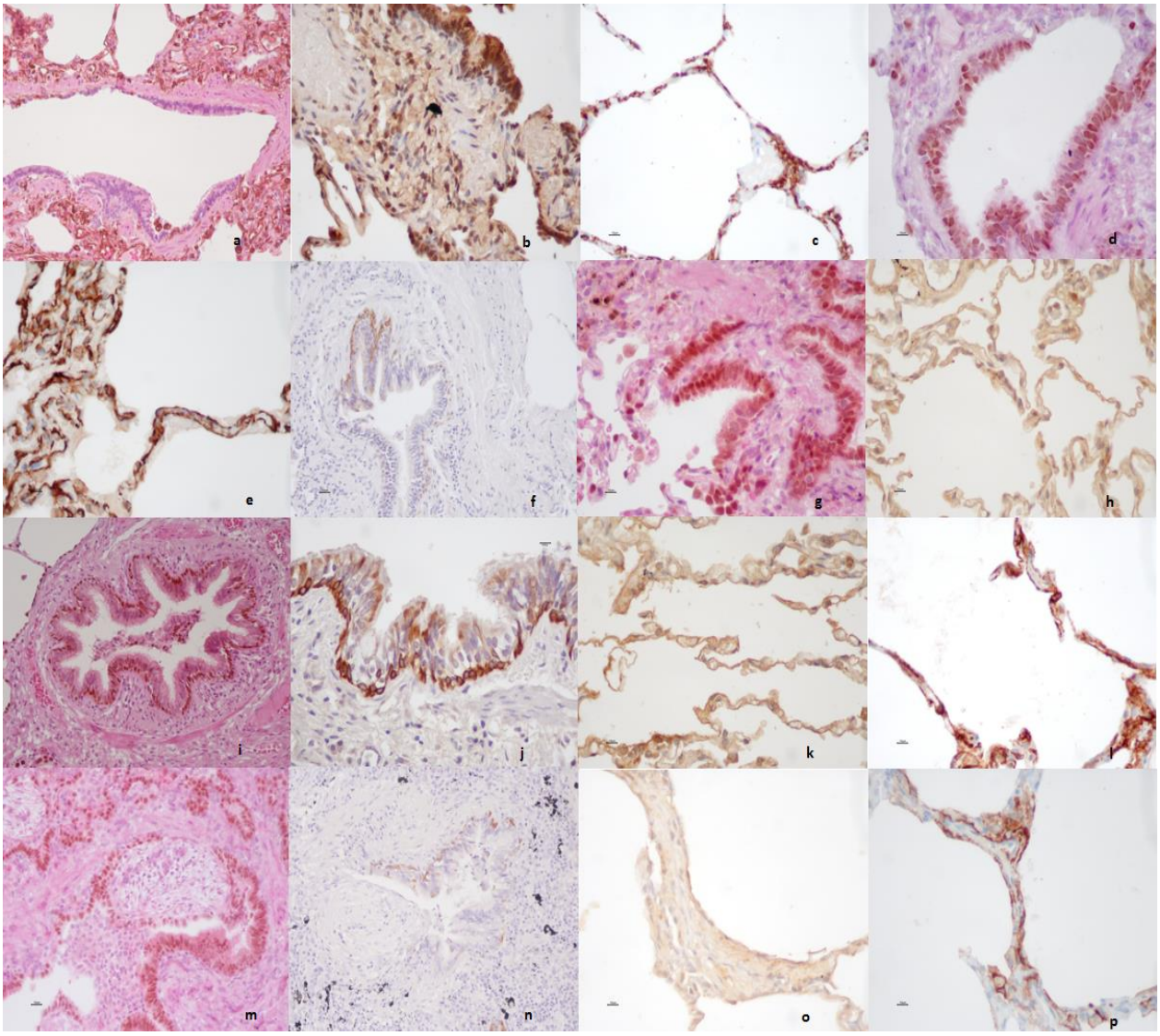

Fig.2: Normal looking bronchioles: vimentin emphasized vimentin-negative smooth-muscle cells and fibroblasts/myofibroblasts intermingled under bronchiolar epithelium (**a**)(vimentinX200); (**b**) TGF-betaX400 expression in bronchiolar epithelial cells and fibroblasts with cytoplasmic and nuclear positivity; (**c**) CD10X400 expression in bronchiolar adjacent septal as linear pattern; (**d**) TTF-1X400 in basal and cylindrical bronchiolar epithelial cells. **Chronic Bronchiolitis**: (**e**) TGF-betaX400 expression in railway pattern in alveolar septae and (**f**) LP34X200 basal cells positivity; (**g**) TTF-1X400 positive cells hyperplasia and (**h**) CD10X100 with similar TGF-beta railway pattern staining. **Respiratory Bronchiolitis**: (**i**) TTF-1X200 positive basal cells and negative cylindrical ciliated bronchial metaplastic cells in papillary epithelium with sub-epithelial scarce inflammatory infiltration; (**j**) LP34X200 emphasized bronchial type basal cells; (**k**) TGF -betaX200 and (**l**) CD10X400 under railway pattern positivity**. Bronchiolitis Obliterans**: (**m**) TTF-1X200 and (**n**) LP34X200 expression in bronchial metaplasia of bronchiolar epithelium; (**o**) TGF-betaX400 and (**p**) CD10X400 delineating interstitial fibrosis and endothelial cells.

Statistical Cluster Analysis

Table XIV: Comparison between frequencies of positive markers, types of cells and patologies (NA: not applicable; NS: non-statistical relevance (p>0.005))

		BPM		CB		RB		BO		P
		N=5	(%)	N=11	(%)	N=15	(%)	N=14	(%)	
BEC	CK 5.6.18	0	0	0	0	1	6,67	0	0	NS
	TTF-1	5	100	10	90,91	15	100	10	71,43	NS
	VIM	0	0	1	9,09	1	6,67	2	14,29	NS
	TGFβ	3	60	7	63,64	12	80	7	50	NS
	CD10	4	80	11	100	9	60	5	35,71	0,008
BBC	CK 5.6.18	1	20	5	45,46	7	46,67	8	57,14	NS
	TTF-1	5	100	11	100	13	86,67	12	85,71	0,16
	VIM	0	0	4	36,36	2	13,33	8	57,14	0,029
	TGFβ	0	0	0	0	9	60	7	50	0,003
	CD10	1	20	1	9,09	3	20	0	0	NS
BFuC	CK 5.6.18	0	0	0	0	0	0	0	0	NS
	TTF-1	0	0	0	0	0	0	0	0	NS
	VIM	0	0	2	18,18	3	20	4	28,57	NS
	TGFβ	0	0	1	9,09	6	40	4	28,57	0,163
	CD10	0	0	0	0	0	0	0	0	NS
BFiC	CK 5.6.18	0	0	0	0	0	0	0	0	NS
	TTF-1	0	0	0	0	0	0	0	0	NS
	VIM	2	40	7	63,64	12	80	12	85,71	0,032
	TGFβ	1	20	2	18,18	9	60	6	42,86	0,135
	CD10	0	0	2	18,18	4	26,67	2	14,29	NS
BCC	CK 5.6.18	NA	NA	0	0	1	6,67	0	0	NS
	TTF-1	NA	NA	1	9,09	1	6,67	1	7,14	NS
	VIM	NA	NA	0	0	1	6,67	0	0	NS
	TGFβ	NA	NA	1	9,09	4	26,67	5	35,71	NS
	CD10	NA	NA	7	63,64	2	13,33	5	35,71	0,029
SBM	CK 5.6.18	NA	NA	3	27,27	1	6,67	3	21,43	NS
	TTF-1	NA	NA	6	54,55	3	20	4	28,57	0,165
	VIM	NA	NA	2	18,18	0	0	2	14,29	NS
	TGFβ	NA	NA	0	0	2	13,33	4	28,57	0,136
	CD10	NA	NA	4	36,36	0	0	2	14,29	0,037
S1	CK 5.6.18	0	0	1	9,09	0	0	1	7,14	NS
	TTF-1	5	100	11	100	14	93,33	14	100	NS
	VIM	4	80	10	9,09	14	93,33	14	100	NS
	TGFβ	1	20	10	9,09	13	86,67	14	100	0
	CD10	5	100	11	100	15	100	14	100	NS
S2	CK 5.6.18	0	0	0	0	0	0	0	0	NS
	TTF-1	0	0	1	9,09	3	20	5	35,71	NS
	VIM	0	0	2	18,18	6	40	5	35,71	NS
	TGFβ	0	0	5	45,45	9	60	11	78,57	0,02
	CD10	0	0	8	72,73	10	66,67	3	21,43	0,004

When considering TGF-beta and CD10, they were expressed randomly in cytoplasm and apical surface of epithelial cells.

In RB and BO, TGF-beta has proved to be a bronchiolar basal cell marker with statistical value of p=0,003.

For TGF-beta and CD10 railway pattern expression in alveolar septae, statistical relevance was p=0.02 and p=0.004 respectively in RB and BO.

Discussion and Conclusions

Since 2010 Anna-Luise Kazenstein has been giving relevance to “severe interstitial fibrosis in cigarette smokers with no evidence of interstitial lung disease” referring to the absence of the commonly recognized histopathological patterns that characterize IIP. Instead she proposed a list of morphological distortions of bronchial-pulmonary normal morphology for smokers: interstitial fibrosis, fibroblast foci, peribronchiolar metaplasia, honey-comb change, emphysema and RB (3,9).

In clinical routine, both smoking related diseases and HP cause the largest number of complex cases, with relevance to the development of squamous cell carcinoma in peripheral regions of the lung, often with a smoking history (4, 19).

Bronchiolitis is a large designation for inflammatory and potentially fibrosing diseases affecting mainly the lobular respiratory and transitional small airways. Generally correspond to inflammatory processes of the small airways and of the surrounding duct - alveolar septae as early lesions of usually known etiology, and occurs commonly in clinical practice (11, 12).

Then secondary bronchiolitis are commoner and regards infectious acute bronchiolitis mainly in infancy, hypersensitivity implications, inclusion in the spectrum of smoking-related disorders, particles, fumes and gas inhalation, chronic aspiration, drug bronchiolar toxicities, and granulomatous morphology as observed in sarcoidosis and neoplasia; bronchiolitis obliterans is common in chronic lung allograft dysfunction together with constrictive clinical manifestations and morphology (13).

Primary or idiopathic bronchiolitis may be considered. This group includes clinicopathologic entities that have to be sufficiently distinct as separate entities without known etiology under the morphological patterns of diffuse pan bronchiolitis, constrictive bronchiolitis, bronchiolitis obliterans with pathological descriptions used as clinical diagnosis. The morphological designation of cellular bronchiolitis due to the presence of parietal inflammation (lymphocytes and plasmocytes) with fibrosis, may be designated as chronic bronchiolitis, with particular clinical implications (14).

In our study, RB showed underestimated inflammatory infiltration scored as sub-epithelial lymphocytes and plasmocytes while it showed up to be a constant marker for BO and CB. In BO, the intra luminal inflammatory myofibroblastic polyps were constantly observed. Also the bronchiolar-adjacent septae kept the inflammatory infiltration.

Vimentin was as easy marker applied to characterize the fusiform cells presence, either in bronchioles walls and the septae, allowing the distinction between smooth muscle cells under or over 4 layers/ myofibroblasts and interstitial fibroblasts.
These observations can be understood as early mesenchymal adaptation related with inhaling tobacco and organic particles preceding chronic lobular remodeling.

Prevention of bronchiolitis evolution to chronical fibrosing pulmonary interstitial diseases with adequate treatment may be crucial and the newest drug pirfenidone is an available small molecule with anti-fibrotic, anti-inflammatory, and antioxidative activities, by modulating cytokines and growth factors, including TGF-β1, TNF-α, bFGF, IFN-γ, IL-1β, and IL-18 in animal models while

the mechanism of action is not currently clear, it is considered to exert inhibitory effects in the pathogenesis of IPF (15,17).

Any etiology of chronic bronchiolitis course contributes to airway narrowing and distortion or complete obliteration of small airways also adjacent lobular septum, without relationship with etiology.

Patients are mostly asymptomatic in the clinical course of smoking-related interstitial fibrosis (SRIF) in smokers must be distinguished from the idiopathic interstitial pneumonias and other chronic interstitial fibrosing lesions. It is characterised by heterogeneous thickening of alveolar septa by thick hyalinised collagen bundles admixed and hyperplasia of smooth muscle cells with minimal accompanying inflammation. These alterations predominate in subpleural and centrilobular parenchyma, but can also accompany emphysema and respiratory bronchiolitis (18).

Still needing clear practical studies, chest CT imaging in medical practice raised the likelihood of cases of interstitial lung disease where respiratory bronchiolitis, respiratory bronchiolitis-associated interstitial lung disease, and desquamative interstitial pneumonia, viewed as a continuum of smoking-induced diseases, rather than as distinct entities, have to be dealt with to define the epidemiology, clinical features, prognosis, and treatment options.

Bronchiolitis obliterans in transplanted lungs may be a model to understand epithelial to mesenchymal transition activated by macrophages. Their secretory products might be therapeutic targets to limit inflammation: TNFα, IL-1β and IL-8 that raises TGF-β1 epithelial to mesenchymal transition implications in bronchial epithelial cells isolated from lung transplant patients. Blocking

TNFα, but not IL-1β, seems to inhibit epithelial to mesenchymal transition as anti-TNFα treatment improved forced expiratory volume in 1 second and 6-min walk distances in four patients included in a pilot study (19).

With TGF-beta and CD10, epithelial-mesenchymal transition was evident to be triggered in epithelial cells of both RB and BO, together with IHC (ImunoHistoChemical) expression in fibroblasts and smooth muscle cells/myofibroblasts.

The railway type positivity still not reported in literature for Vimentine , TGF-beta and CD10 may become an histopathological marker to preview septae fibrosis, a distinctive way of double inearity of alveolar septae, whose significance needs further studies, and in our study seems to be the septal EMT activation.

In smoking lungs, are identified subpleural foci of interstitial fibrosis associated with emphysema and this smoking-related interstitial fibrosis and respiratory bronchiolitis-interstitial lung disease with fibrosis, or airspace enlargement with fibrosis were observed in biopsies. Respective HRCT features consisted of subpleural upper - mid zone emphysematous changes with reticulation and variable ground-glass alteration. Morphology showed circumscribed hyalinized interstitial fibrosis and emphysema; macrophages were present in the airspaces. Respiratory bronchiolitis with fibrosis was suggested to avoid confusion with other forms of ILD. RB probably accounts for some of the cases of interstitial lung diseases as AL Katzenstein refers asymptomatical evolution of SRIF (9,20).

In organizing pneumonia, fibrin may appear as a marker of acute microvascular lesion and be seen in alveoli with unclear clinical significance. Biopsies and radiographic evidence of COP with that feature may help to identify patients who may benefit from more intensive steroid therapy (21).

Atypical adenomatous hyperplasia and bronchioloalveolar pattern in pulmonary adenocarcinoma relate with terminal respiratory unit but observations of transitions from normal ciliated columnar cells to adenocarcinoma via dysplastic mucous columnar cells were retrieved in pulmonary adenocarcinomas; terminal respiratory unit type adenocarcinomas were defined as adenocarcinomas with type II pneumocytes, Clara cells or with bronchiolar cell morphology, consistently TTF-1 positive. Non-terminal respiratory unit type adenocarcinomas have mucous columnar cell changes continuous with bronchial ciliated columnar cells that became dysplastic with loss of cilia and are consistently TTF-1 negative; acute inflammation and honeycombing changes were present in surrounding lung. These mucous columnar cell change belong to non-terminal respiratory unit type adenocarcinoma, and mucous columnar cell change is a precursor lesion of pulmonary adenocarcinoma (22).

We observed that bronchiolar epithelium acquired bronchial characteristics in RB cases by adopting a predominant papillary morphology with ciliated cells; TTF-1 remained the classical marker of TRU while LP34 allowed to relevate epidermoid metaplasia in bronchiolar basal cells, where TTF-1 was constantly positive. Also in alveolar sepatae, where TTF-1 positive cells were increased in RB and BO.

Still without clinical application but remaining as a point for further studies, TGF-beta and CD10 positive epithelial cells stand for the understanding of pleomorphic carcinomas development after EMT.

Our study results raise the commitment of pathology with the identification of early lesions that support bronchiolar-raising bronchial type adenocarcinoma/adenosquamous carcinoma, needing further studies.

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