Obliterative bronchiolitis: varying presentations and clinicopathological correlation

K.D. Markopoulou^{*,§}, C.D. Cool^{*,+}, T.L. Elliot^{#,+}, D.A. Lynch^{#,+}, J.D. Newell Jr^{#,+}, V.A. Hale^{#,+},
K.K. Brown⁺, M.I. Schwarz^{†,+}, R.M. Tuder^{*}

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ABSTRACT: In obliterative bronchiolitis, inflammation and fibrosis lead to narrowing or occlusion of bronchiolar lumina.

To determine how bronchiolar structural alterations relate to lung physiology, 19 patients with a pathological diagnosis of obliterative bronchiolitis were studied. The bronchiolar inflammatory and fibrotic features were correlated to the clinical presentation, and lung function tests.

Eleven patients demonstrated airflow limitation, one had a restrictive pattern and one had a mixed pattern, two had isolated gas trapping, but four had normal spirometry. Mild-to-moderate bronchiolar inflammation was invariably present. It involved 60% of bronchioles subepithelially and 54% in the adventitia. Subepithelial fibrosis was observed in 15 patients and adventitial in 12. Adventitial bronchiolar inflammation correlated with forced expiratory volume in one second and forced vital capacity and inversely correlated with residual volume. Subepithelial fibrosis inversely correlated with subepithelial and adventitial inflammation. High-resolution computed tomography in 10 patients revealed inspiratory (five out of 10) and expiratory air trapping (five out of five), ground glass opacities (seven out of 10), bronchial wall thickening (five out of 10), bronchiectasis (two out of 10) and centrilobular nodules (two out of 10).

The present study suggests that inflammation and fibrosis occurs in bronchioles at different time points in the disease process, or that there is no transition between these types of pathology in the same patient. No correlation was observed between the degree of bronchiolar fibrosis and the degree of airflow limitation.

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Depts of ^{*}Pathology, [#]Radiology, and [†]Division of Respiratory Sciences and Critical Care Medicine, University of Colorado Health Sciences Center, Denver, Colorado, USA. ⁺National Jewish Medical and Research Center, Denver, Colorado, USA. [§]1st Pulmonology Dept, George Papanikolaou Hospital, Thessaloniki, Greece.

Correspondence: K. Markopoulou, Patriarchou Ioakeim 12, Thessaloniki, Greece.

Fax: 30 310277742

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Chronic inflammation and luminal compromise of the bronchioles has been recognized as a cause of respiratory disease since the beginning of the 20th century. However, the terminology and clinical presentation of bronchiolar diseases still causes confusion. LANGE [1], in 1901, introduced the term bronchiolitis obliterans to describe a case in which the bronchiolar lumens were obstructed by plugs of granulation tissue originating from the bronchiolar wall. In 1929, the term bronchiolitis fibrosa obliterans was used for similar findings [2], whereas in 1966 it referred to lesions consisting of concentric rings of fibrotic tissue in the wall of the airways [3]. MACKLEM *et al.* [4] in 1971, introduced the term chronic obstructive small airways disease describing seven patients with airflow limitation, who had chronic bronchiolar inflammation, with variable degrees of airway wall fibrosis. The term constrictive bronchiolitis was coined by GOSINK *et al.* [5] in 1973, to describe a peribronchiolar and mural infiltrate of mononuclear cells and granulation tissue, resulting in a compromise of the bronchiolar lumen. TURTON *et al.* [6] 8 yrs later, coined the term cryptogenic obliterative bronchiolitis

to characterize cases with otherwise unexplained progressive airflow obstruction. These patients had forced expiratory volume in one second (FEV₁) <60% predicted, no bronchodilator response or peak expiratory flow variability and no history of episodic wheezing, atopy, chronic bronchitis, emphysema or known causes of chronic airflow obstruction. In 1987, GUERRY-FORCE *et al.* [7] studied nine patients with histologically proven inflammation of small airways and described patients with both obstructive and restrictive spirometric patterns. Two years later, KINDT *et al.* [8] described bronchiolitis in 16 adult patients who had histological lesions of chronic bronchiolitis with spirometric patterns of obstruction, restriction or mixed obstruction and restriction. EDWARDS *et al.* [9] in 1992, used the term chronic transmural bronchiolitis to describe chronic bronchiolar inflammation in nine patients with different underlying diseases and obstructive or restrictive spirometric patterns. KRAFT *et al.* [10], in 1993, reported four females with a histological diagnosis of constrictive bronchiolitis, one of which had normal spirometry and one of which had a mixed obstructive

and restrictive pattern of disease. From these reports, it is clear that the disease spectrum associated with histological evidence of bronchiolar inflammation and fibrosis is varied, comprising cases with obstructive, restrictive or normal pattern of spirometry and diverse clinical and radiological presentation. Descriptive terminology is both evolving and continuously being revisited. The term chosen for use in this paper is obliterative bronchiolitis, because it evokes best the essence of luminal narrowing, which is caused by both inflammation and fibrosis. Obliterative bronchiolitis occurs in a number of clinical settings. These include infection, inhalational injury, bone-marrow transplantation, lung or heart-lung transplantation, drug toxicity, or in the context of a collagen-vascular disease. There are also idiopathic cases [11].

Bronchiolar changes in obliterative bronchiolitis may vary considerably. They can be subtle or show complete luminal obliteration. The histological abnormalities include submucosal and adventitial scarring, smooth-muscle hypertrophy, distortion of the airway lumen, bronchiolectasis, mucostasis and bronchiolarization of adjacent alveolar spaces. Inflammatory changes, submucosal, mural or peripheral (cellular bronchiolitis), are also common. In a given patient, any constellation of these findings may occur. The pathology does not always correlate with the functional status of the patients. Moreover, histological evidence for the disease may be present without physiological airflow limitation [12].

Presently, there is no clear understanding of how the diverse structural alterations relate to lung physiology, and whether a predominantly inflammatory component, compared to a dominant pattern of collagen deposition, impacts differently on the presentation and evolution of the bronchiolar disease.

This study was undertaken to test the hypothesis that the degree of inflammation or fibrosis in the small airways correlates with the clinical presentation in patients with the pathological diagnosis of obliterative/constrictive bronchiolitis.

Material and methods

Study subjects

A search of the "Pathology Database" from the University of Colorado Health Sciences Center and the "Interstitial Lung Disease Database" of the National Jewish Medical and Research Center was carried out. All cases involving a pathological diagnosis of obliterative or constrictive bronchiolitis/bronchiolitis obliterans/chronic bronchiolitis, proven by either open lung biopsy, video-assisted thoracoscopic biopsy, or examination of explanted lung, for the years 1993–2000, were reviewed. Inclusion criteria were the presence of luminal narrowing caused by chronic bronchiolar inflammation, increased bronchiolar collagen deposition, or both. Cases which developed following bone-marrow, lung or heart-lung transplantation were excluded. Patients with a histological diagnosis of respiratory bronchiolitis

interstitial lung disease and diffuse panbronchiolitis were also excluded.

Study design

Medical charts were abstracted and lung function tests prior to the biopsy were tabulated. High-resolution computed tomography (HRCT) scans of the chest, when available, were evaluated. Sections stained with haematoxylin and eosin were reviewed for a detailed description of findings, and scored for bronchiolar inflammation. Sections stained for collagen and smooth-muscle actin were examined for the presence of fibrosis. A control group consisted of 10 autopsy cases from the University of Colorado Hospital with normal lung histology.

Chart abstraction

Medical charts were abstracted for demographical information, smoking history, symptomatology, occupational or environmental exposure, past medical history, medications, and collagen vascular diseases serology. Nineteen patients (five male and 14 female) were included. All were Caucasian except for two Hispanics. Their median age was 45 yrs (confidence interval (CI) 36.5–54.3). Ten were nonsmokers, six were former smokers with a mean pack-yr history of 19.9 (range 2.5–60) and three were current smokers with a mean pack-yr history of 32 (range 6–14). Almost all of the patients had cough and dyspnoea prior to biopsy. Other symptoms included fever and chills in patient 14, myalgias and arthralgias in patient 17, anaemia, weight loss and arthralgias in patient 18 and haemoptysis in patient 19. Sputum production was minimal except for one individual, who was a smoker. The symptoms and their duration prior to biopsy are summarized in table 1.

Respiratory function

Eleven patients had an obstructive pattern by spirometric evaluation and nine of these had a FEV₁ <60% predicted. One patient had a restrictive pattern and one a mixed obstructive-restrictive pattern. Two patients had evidence of gas trapping, but without airflow limitation. Four patients had normal spirometry at the time of the biopsy. The spirometric values for the entire cohort are summarized in table 2 and for the subgroup with an obstructive pattern in table 3.

Onset of disease and exposure history

The time of disease onset could not be clearly identified in thirteen patients and symptoms gradually worsened. Three had well documented toxic-fume exposures prior to symptoms. Patient 15 was exposed to ammonia, nitrogen oxide (NO₂), carbon dioxide (CO₂) and carbon monoxide (CO) while fighting a fire at a photography processing laboratory, and patient 8

Table 1. – Patient characteristics, symptoms and their duration

Subject no.	Sex	Age yrs	Smoking	Pack- yrs	Years since quitting	Cough	Cough duration months	Sputum	Sputum duration months	Dyspnoea	Dyspnoea duration months	Wheeze	Wheeze duration months
1	M	57	FS	7	19					+	31		
2	F	21	NS			+	2			+	0.25		
3	F	45	NS			+	9	+		+	9		
4	F	50	FS	14	3	+	84	+	84	+	48	+	84
5	F	51	CS	20		+	4			+	4	+	4
6	F	36	NS			+	24			+	24		
7	F	44	CS	22		+	2	+		+	72		
8	M	38	CS	6		+	10			+	10	+	10
9	F	38	NS			+	15	+	15	+	15	+	15
10	F	58	FS	2.5	32					+	12		
11	M	50	FS	60	2					+	60		
12	F	52	NS			+	12			+	12		
13	M	36	FS	16	4	+	0.5			+	7		
14	F	57	NS			+	24						
15	M	34	NS			+	4			+	4	+	4
16	F	62	NS			+	6	+	4	+	6		
17	F	44	NS			+	12	+	12	+	16	+	12
18	F	35	NS			+	288	+	60	+	60	+	60
19	F	55	FS	20	8					+	72	+	12

M: male; F: female; FS: former smoker; NS: never-smoker; CS: current smoker; +: positive for given symptom.

was exposed to polymethylene polyphenol isocyanate (polymeric diphenylmethane diisocyanate product) for 3 h, without respiratory protection, while performing maintenance work at a plastics factory. Patient 6 had an unspecified fume exposure. Upper respiratory tract infection or flu-like syndrome preceded symptoms in patients 2 and 6. Patients 13 and 14 reported an acute onset of disease, but without any definite precipitating event.

A history of environmental exposure was obtained

from six patients. Patients 6 and 9 had pet birds in the home, patient 17 had worked in silos, and patient 13 had worked in a factory where ammonia was periodically used and had also worked and lived near silos. Patient 14 had possible mould exposure, secondary to water damage in the house, as well as talc inhalation. The talc was identified in the lung biopsy and was thought to originate from carpets and air ducts in a newly constructed home. Patient 11 had been a coal miner. Three patients were suspected of

Table 2. – Lung function tests

Patient no.	FEV ₁	FVC	FEV ₁ /FVC	TLC	RV	DL _{CO} /VA	MEF	B/D
1	14	32	35	105	200	100		
2	49	53	79					
3	17	49	38					
4	72	73	80	96	172	99	86	9
5	53	61	69			116		8
6	28	57	41	124	403	119	11	9
7	14	33	41	132	360	61	5	40
8	84	83	76	85	108	91	70	16
9	82	79	88	89	106	80	85	12
10	70	90	60	128	262	95	48	9
11	23	49	33	127	389	53	17	1
12	52	62	68	99	163	84		11
13	21	28	56	66	122		16	
14	63	64	76	97	165	109	82	14
15	84	92	72	98	76	100	91	7
16	76	61	92	98	98	87	113	
17	79	80	81	98	135	100		
18	23	66	30	135	349	61	6	
19	38	61	48	90	134	118	20	-1
Median	52	61	68	98	164	97	48	
CI	24–75	53–77	43–78	93–125	115–305	82–104	15–87	

All data is presented as % predicted. FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; TLC: total lung capacity; RV: residual volume; DL_{CO}/VA: Diffusing capacity for carbon monoxide corrected for alveolar volume; MEF: midexpiratory flow (between 25% and 75% of FVC); B/D: percentage change in FEV₁ after β_2 -agonist inhalation.

Table 3.—Lung function tests for the subgroup with obstructive pattern and forced expiratory volume in one second (FEV₁) <60% predicted

Vaible	Data
Subjects n	9
FEV ₁	28 (21.5–41.5)
FVC	57 (49–61.2)
FEV ₁ /FVC	41 (34.5–57.5)
TLC	124 (100.5–130.7)
RV	349 (172.2–381.7)
DL _{CO} /VA	92 (61–117)
MEF	11 (5.7–17.7)

Data are presented as % pred median (confidence interval). FVC: forced vital capacity; TLC: total lung capacity; RV: residual volume; DL_{CO}/VA: diffusing capacity for carbon monoxide corrected for alveolar volume; MEF: midexpiratory flow between 25% and 75% of FVC.

having hypersensitivity pneumonitis because of the presence of poorly formed granulomas (patient 9) or isolated giant cells (patients 6 and 10) on lung biopsy. As mentioned, patients 6 and 9 had pet birds at home. No exposure was identified in patient 10.

Serology

Antinuclear antibodies were measured in seven patients. One patient (patient 19) without systemic disease had a speckled pattern (1:160). Patient 18 was positive (1:80 speckled pattern), and also had positive antimyeloperoxidase titre (perinuclear antineutrophil cytoplasmic antibodies) of 1:10,240. Two patients had positive rheumatoid factors, patient 3 with active rheumatoid arthritis and patient 6 without joint disease.

Past medical history

Six patients (patients 3, 5, 7, 9, 18, 19) had a prior clinical diagnosis of asthma with progressive deterioration of lung function, despite therapy with inhaled and systemic corticosteroids. Bronchodilator response was available for eight patients who did not have a clinical diagnosis of asthma. Three of these had positive responses (>10%). Patient 12 had chronic renal failure and had been on home dialysis for 25 yrs. Patient 18, during the course of her respiratory disease, had developed migratory arthralgias, anaemia, weight loss and an antineutrophil cytoplasmic antibody directed against myeloperoxidase (MPO-ANCA) titre of 1:10,240. Only one patient (patient 3) had rheumatoid arthritis, and had been treated with gold, plaquenil, methotrexate and sulphasalazine in the past. Five female patients (patients 5, 7, 10, 12, 19) had undergone hysterectomy, one (patient 5) for uterine neoplasm. Four patients (patients 9, 10, 15, 19) had a history of multiple pneumonias. Six patients (patients 4, 6, 7, 8, 15, 18) had received oral corticosteroids for variable periods of time prior to lung biopsy.

Pathology

All available sections from each case were reviewed three times (twice by pulmonary pathologists). If another significant histological finding, such as interstitial inflammation, prominent fibrosis or intra-alveolar exudates (organizing pneumonia, bronchiolitis obliterans organizing pneumonia, diffuse alveolar damage/acute interstitial pneumonia) was present, the case was excluded. Two slides from each of the final 19 cases, that had the greatest sample of bronchioles, were selected for further evaluation.

Additional serial sections from each case were prepared from the corresponding paraffin blocks. These slides were double-stained, first with an antibody directed against smooth muscle-specific actin to visualize the smooth muscle component of the bronchial wall, and second with picric acid-fast green stain (Pfalz and Bauer, Waterbury, CT, USA) for identification of the bronchial wall collagen.

Immunohistochemistry for smooth muscle actin

Sections were stained for smooth-muscle actin as described by COOL *et al.* [13].

Staining for collagen

After immunostaining for smooth muscle-specific actin, the sections were immersed for 30 min in a solution of 100 mL of saturated aqueous solution of picric acid with 0.1 g of fast-green FCF (Pfalz and Bauer, Waterbury, CT, USA) and 0.1 gm of Sirius-red F3BA (Pfalz and Bauer, Waterbury, CT, USA). The sections were then mounted with a medium and cover slipped.

Examination for bronchiolar inflammation

Two haematoxylin and eosin sections were scored for each case. Chronic inflammation was classified as either absent or present, with either a subepithelial, adventitial or both subepithelial and adventitial distribution (figs. 1–3). The degree of inflammation was scored semiquantitatively as minimal grade (1), moderate grade (2) or severe grade (3) for each bronchiole examined. The mean value for each anatomical type of inflammation, in each case, was derived by counting all bronchiolar structures present on both slides. A percentage was derived by counting inflamed bronchioles relative to the total number of bronchioles. An inflammation score (IS) was calculated by multiplying the degree of inflammation by the percentage of involved bronchioles. The maximum IS would be 300 for any patient (maximum inflammation score 3×100% of bronchioles).

Examination for airway wall fibrosis

The collagen stain indicated five patterns of bronchiolar wall fibrosis: 1) subepithelial deposition

Fig. 1. – Terminal bronchiole with marked subepithelial inflammation resulting in displacement of the lining epithelium towards the lumen (arrow and inset). Internal scale bar=82 microns.

(SF), usually in the form of thickening of the basement membrane; 2) adventitial deposition (AF), appearing as a cuff surrounding the bronchiole; 3) a combination of SF and AF; 4) minimal collagen deposition (MC), consisting of simple strands of collagen intermingled with inflammatory and resident cells; and 5) a thin layer of collagen in the basement membrane, which was indistinguishable from that present in the normal control bronchioles (figs. 4–8). Each bronchiole was examined for the presence or absence of one of the fibrotic patterns, and the percentage of bronchioles with SF, AF, MC was calculated.

High-resolution computed tomography

Three radiologists reviewed the scans, without knowledge of the patients' clinical histories, for the presence of bronchial-wall thickening, bronchiolectasis,

Fig. 2. – Example of adventitial bronchiolar inflammation, with a nodular clustering of lymphoid cells (arrow). Internal scale bar=82 microns.

Fig. 3. – Example of severe mural inflammation in terminal bronchiole involving both the subepithelial (long arrow and inset) and adventitial (short arrow) regions. Note the almost complete obliteration of the bronchiolar lumen by the thickened bronchial mucosa (b) (magnification). Internal scale bar=82 microns.

tree-in-bud, centrilobular nodules, bronchiectasis, ground-glass opacities, consolidation, emphysema, inspiratory and expiratory air-trapping. In cases of discrepancies, the scans were re-examined and a consensus decision was reached.

Statistical analysis

All data were graded on a continuous scale. Spearman's rank correlation was used to examine the correlations between symptoms, spirometric values, pathological findings and HRCT findings. Kruskal-Wallis one-way analysis of variance was used for comparisons in spirometric values and pathological findings between current, former and never-smokers. The Mann-Whitney U test was used for comparisons in histological findings between

Fig. 4. – Example of subepithelial fibrosis (arrow) resulting in distortion of bronchiolar lumen. Internal scale bar=82 microns.

Fig. 5.—Example of adventitial fibrosis (top arrows). The bronchiolar smooth muscle layer is clearly discerned (bottom arrow) immunostained with antibody against smooth muscle actin. Internal scale bar=82 microns.

patients with normal and obstructive patterns of spirometry.

Results

Histological findings

All cases had evidence of luminal narrowing. The next most common finding was chronic inflammation of the bronchioles. Descriptions of the histological lesions are summarized in table 4, and the original pathological diagnoses in table 5.

Bronchiolar inflammation

A median of 10 (CI 9–12.5) bronchioles was analysed for each patient. Inflammation was generally

Fig. 6.—Advanced mural fibrosis (arrows) resulting in almost complete obliteration of bronchiolar lumen (b). Internal scale bar=82 microns.

Fig. 7.—Minimal collagen deposition in the wall of a terminal bronchiole as assessed by picrosirius red. Internal scale bar=82 microns.

mild to moderate and involved approximately half the bronchioles examined. The results are summarized in table 6.

Collagen deposition

A median of 16 bronchioles (CI 11.3–23) was studied for each patient. SF of collagen was observed in 15 of the 19 cases. AF was noted in 12 patients. In both subepithelial and adventitial bronchiolar regions, the collagen deposition affected a limited number of bronchioles (medians of 29% subepithelially and 16% in the adventitia). Results are summarized in table 7.

Inflammation and collagen deposition

SF inversely correlated with the extent of subepithelial inflammation (% SI) ($r_s=-0.55$, $p=0.01$), the

Fig. 8.—Example of a normal terminal bronchiole with a thin collagen layer stained with picrosirius red (arrow). Internal scale bar=51 microns.

Table 4. – Detailed histological findings

Lesion	Patients n	Patient nos
Bronchioles		
Acute inflammation	3	13, 14, 15
Chronic inflammation	19	
Eosinophils	2	2, 12
Goblet cell hyperplasia	4	2, 11, 13
Basement membrane thickening	1	8
Submucosal oedema	0	
Smooth muscle hyperplasia	7	2, 4, 5, 6, 11, 12, 15
Inspissated mucus	12	
Bronchiolodilatation	11	
Bronchiolar wall destruction	3	3, 13, 14
Luminal obliteration	2	1, 3
Lymphoid hyperplasia	7	4, 10, 11, 12, 13, 14, 19
Respiratory bronchiolitis	4	1, 3, 8, 11
Alveoli		
Bronchiolarization*	5	1, 4, 5, 6, 19
Alveolar wall fibrosis	3	2, 14, 19
Alveolar wall inflammation	5	2, 6, 9, 14, 19
Emphysema	10	
Alveolar macrophages	6	
Haemorrhage	2	8, 18
BOOP	3	6, 13, 14
Granulomas	1	9
Giant cells	2	6, 10

*: extension of bronchiolar type epithelium to centrilobular alveolar septae that have been modified by chronic inflammation with or without scarring. BOOP: bronchiolitis obliterans organizing pneumonia.

subepithelial inflammation score (SIS) ($r_s=-0.55$, $p=0.01$) and the grade of adventitial inflammation (AIG) ($r_s=-0.51$, $p=0.02$). The extent of minimal collagen deposition inversely correlated with AF ($r=-0.54$, $p=0.01$).

Histopathological findings and spirometric values

The adventitial inflammation score (AIS) correlated with FEV₁ % pred ($r_s=0.44$, $p=0.05$) and inversely correlated with the residual volume (RV) % pred ($r_s=-0.56$, $p=0.02$). The extent of adventitial inflammation (% AI) correlated with the forced vital capacity (FVC) % pred ($r_s=0.48$, $p=0.03$). In those patients whose FEV₁ was <60% pred ($n=9$), subepithelial fibrosis correlated with AF ($r_s=0.68$, $p=0.04$); AF correlated with FEV₁ ($r_s=0.77$, $p=0.01$) and with FEV₁/FVC ($r_s=0.75$, $p=0.02$).

When comparing histological differences between patients with a normal or an obstructive spirometric pattern, the AIS was higher in those with normal spirometry (Mann-Whitney U test, $p=0.04$).

No significant differences in pathological patterns were found between the patients who did or did not require lung transplantation.

Imaging findings

HRCT studies were available for 10 patients. Of those, eight had been performed within 1 yr of the

Table 5. – Original pathological diagnoses of the cases in the study

Patient no.	Original diagnosis
1	Obliterative bronchiolitis
2	Mild chronic bronchiolitis
3	Obliterative destructive bronchiolitis consistent with rheumatoid arthritis
4	Mild chronic bronchiolitis
5	Mild chronic bronchiolitis, pulmonary vascular remodelling with intimal thickening, normal lung parenchyma
6	Multifocal interstitial fibrosis consistent with bronchiolitis obliterans. Giant cells and cholesterol clefts can be the result of a hypersensitivity pneumonitis
7	Mild chronic bronchiolitis with centrilobular emphysema
8	Chronic bronchiolitis, intraalveolar haemorrhage
9	Patchy nonspecific lymphocytic pneumonitis with lymphocytic bronchiolitis. Poorly formed granulomas. Differential diagnosis includes hypersensitivity pneumonitis, post-viral infection, collagen vascular disease.
10	Concentric, partially obliterative, active bronchiolitis
11	Constrictive bronchiolitis with focal bronchiolar fibrous obliteration, lymphocytic infiltrate and peripheral emphysema
12	Chronic obliterating bronchiolitis
13	Bronchiolitis obliterans with mucoid impaction. Many neutrophils inside the mucus plugs. Multifocal ulceration of bronchial epithelium, with formation of granulation tissue polyps within the lumens of muscular bronchioles. Consistent with previous viral infection.
14	Destructive obliterative bronchiolitis with focal areas of mucus plugging
15	Mild chronic bronchiolitis with smooth muscle hypertrophy and irregular fibrosis with luminal distortion of respiratory and terminal bronchioles.
16	Obliterative bronchiolitis
17	Chronic bronchiolitis of the constrictive type (sometimes called small airways disease)
18	Proliferative obstructive bronchiolitis, pulmonary haemosiderosis
19	Mild chronic bronchiolitis

biopsy. In five cases, expiratory images were also available. Bronchial-wall thickening was present in five cases, bronchiectasis in two cases, and bronchiolectasis in one case. No patient had tree-in-bud findings. Centrilobular nodules were observed in two patients. A ground-glass pattern was relatively common, being observed in seven patients. Inspiratory air-trapping was present in seven cases, whereas expiratory air-trapping was found in all five cases where expiratory scans were available. Because of the small number of available studies, no statistical analysis was performed.

Analysis according to smoking status

No differences related to smoking status (smoker, former-smoker, never-smoker) were found in spirometry

Table 6. – Bronchiolar inflammation

Site of inflammation	No of patients	Grade of inflammation	Bronchioles involved %	Total inflammation score
Subepithelial	19/19	2 (1–2)	60 (22.5–71.5)	86.8 (27.8–140.3)
Adventitial	19/19	1.5 (1.2–1.8)	54 (46.3–90.5)	89.6 (58.3–123.2)

Data are presented as median (confidence interval).

or pathology (Kruskal-Wallis one-way analysis of variance).

Symptom duration and spirometry

The duration of dyspnoea was inversely correlated with FEV₁/FVC ($r_s = -0.49$, $p = 0.04$) and mid-expiratory flow (MEF) % pred ($r_s = -0.69$, $p = 0.03$), and positively correlated with RV % pred ($r_s = 0.61$, $p = 0.01$).

Follow-up

After lung biopsy, patients were treated with prednisone, and/or cyclophosphamide or azathioprine or colchicine or clarithromycin. Four (patients 1, 3, 6, 11) underwent lung transplantation, and remain alive. Six improved symptomatically and spirometrically on medical therapy (patients 5, 8, 9, 13, 14, 15), one (patient 12) is stable and one (patient 16) died shortly after consultation at the National Jewish Center. Six are alive but there was no available clinical follow-up. Patient 2 was lost to follow-up. HRCT follow-up was not available for most of the patients.

Discussion

The cases studied were selected on the basis of pathological criteria of bronchiolar inflammation and scarring. Despite the common pathology, there was a marked heterogeneity in their clinical presentation and radiological and functional features.

Studies addressing whether there is a close relationship between the histological lesions in obliterative bronchiolitis and the patients' physiological impairment are still lacking. Prior to the present study, the authors hypothesized that indices of disease severity and airflow limitation would correlate with bronchiolar fibrosis. It was found that the cases of obliterative bronchiolitis presented in this study fitted the description provided by COLBY and MYERS [12], where small airways disease were divided into two groups: 1) cases with acute and chronic cellular bronchiolitis with less conspicuous scarring; and 2) cases with constrictive

bronchiolitis, varying from fibrotic and inflammatory lesions to complete airway obliteration. COLBY and MYERS [12] commented that the most common findings were mild chronic inflammatory infiltrates and mural scarring. In the present study, it was found that subepithelial fibrosis inversely correlated with subepithelial and adventitial inflammation. These findings indicate that there is a temporal compartmentalization between the inflammatory and fibrotic bronchiolar changes. In other words, the subepithelial bronchiolar lesions were either primarily fibrotic or inflammatory. This implies that inflammation and fibrosis occur at different time points in the course of the disease, or alternatively, that patients with one type of presentation usually do not show transition to another type of bronchiolar pathology.

Surprisingly, no correlation was found between the degree of subepithelial collagen deposition and airflow indices, for the entire group, or the obstructive subgroup. The lack of significant correlation between morphological parameters and airway physiology in the present study may have occurred because chronic bronchiolitis presents as a heterogeneous disease at the lung tissue level. Inflammation was mild or moderate and involved only half of the bronchioles. Moreover, SF was present in only one-third of the bronchioles examined. Importantly, four patients did not have morphological evidence of SF, despite the clinical evidence of airflow-limitation. AF affected a much smaller number of airways.

A clinical diagnosis of asthma was common in the patient group. Pathological studies in asthma usually focus on bronchial alterations, including smooth muscle cell hyperplasia, mucus plugging, thickening of the epithelial basement membrane, submucosal oedema, infiltration of eosinophils, mast cells and lymphocytes, and epithelial cell shedding. Most of the papers published on the morphology of small airways in asthma reveal an increased thickness of the basement membrane, the smooth muscle layer, the adventitial area and the submucosa. Distal-airway inflammation is also present. In addition, SAETTA *et al.* [14] observed luminal obstruction by mucus in 50% of the bronchioles examined in six patients who died suddenly during an asthma attack. However, evidence of luminal narrowing is scarce. In most of the studies, irreversible scarring of small airways has not been specifically addressed. In a study of six elderly non-smoking asthmatics who died of causes other than asthma, SOBONYA [15] found two cases with reduced small airways diameter, with histological evidence of inflammation or fibrosis. Moreover, there have been no detailed pathological studies of lungs with refractory asthma including a component of irreversible airway remodelling [16]. Despite the bronchodilator

Table 7. – Bronchiolar collagen deposition

Type of collagen deposition	No of patients	Bronchioles involved %
Subepithelial deposition	15/19	29 (5.3–55)
Adventitial deposition	12/19	16 (0–33.3)
Minimal collagen deposition	16/19	21 (6.5–29.6)

Data are presented as median (confidence interval).

response in several of the present study's patients, their pathology was not consistent with this disease. It is possible that some cases of severe, progressive asthma actually represent obliterative bronchiolitis. Occupational exposure to isocyanate compounds can cause asthma or hypersensitivity pneumonitis [17]. Biopsies of patients with isocyanate-induced asthma have similar findings as those described above [18]. Patient 8, who was exposed to isocyanates, had a clinical disease consistent with asthma, with considerable bronchodilator response. Biopsy revealed chronic bronchiolitis with basement membrane thickening and peribronchiolar scarring, as well as haemosiderin-laden macrophages. Smooth-muscle hyperplasia and eosinophils were not noted. This patient's lung function had improved, but oral corticosteroid therapy is still required.

Exposures to either nitrous oxides [11], sulphurous oxides or ammonia have been associated with the development of obliterative bronchiolitis [19]. The typical clinical course following toxic fume exposure consists of three phases: 1) an acute onset, with upper respiratory symptoms and, sometimes, pulmonary oedema; 2) a latent period; and 3) an irreversible obstructive, mixed or restrictive physiological picture. Patient 15 had continuous symptoms of dyspnoea and cough after his fume exposure. Physiological tests at the time of lung biopsy, 4 months after exposure, indicated normal airflow parameters with a decreased RV and marked arterial oxygen desaturation during exercise. Although the patient did not have obstructive physiology, pathological findings demonstrated obliterative bronchiolitis. After 4 yrs of treatment with combinations of prednisone, cyclophosphamide and colchicine, the patient now has normal spirometry, except for increased RV and a normal diffusing capacity and exercise capacity.

Constrictive bronchiolitis is a well known complication of rheumatoid arthritis [20]. Gold and penicillamine therapy have been implicated in some patients [21]. The precipitating factor is unclear in patient 3, who had received both of these treatments prior to onset of symptoms.

Based on the lung biopsy, three patients might have had some of the histological findings of hypersensitivity pneumonitis. This disease is histologically characterized by a uniform chronic interstitial pneumonia with peribronchiolar accentuation, non-necrotizing granulomas in the peribronchiolar interstitium, and foci of bronchiolitis obliterans with organizing pneumonia [22]. None of the present cases met all these diagnostic criteria. Since not all cases exhibit the full range of histological findings, the possibility of hypersensitivity pneumonitis cannot definitely be excluded in these three patients. Moreover, constrictive bronchiolitis has been described in association with hypersensitivity pneumonitis [12]. However, overt severe airflow obstruction is considered rare [23].

Patient 18 had a p-ANCA titre of 1:10,240 and had had a cough, without dyspnoea, since early childhood. Eight years prior to biopsy, this patient developed exertional dyspnoea and was diagnosed as having asthma, and treated with inhaled bronchodilators and corticosteroids, as well as short bursts

of oral corticosteroids. Lung function deteriorated rapidly, demonstrating airflow limitation. Prior to lung biopsy, the patient experienced migratory arthralgias, anorexia, weight loss and anaemia. The renal function remained normal. The patient was treated with prednisone and trimethoprim-sulphamethoxazole for exacerbation of respiratory symptoms, and showed improvement in both symptoms and spirometry. The biopsy showed inflammation and destruction of the respiratory bronchioles with fibrotic changes extending from them. Haemosiderin-laden macrophages were present in the alveolar spaces. Intra- and extracellular deposition of immunoglobulin (Ig)A, IgM and C₃ was demonstrated by immunofluorescence. The patient was placed on cyclophosphamide, with resolution of her systemic symptoms. Respiratory function stabilized, but did not return to normal. In a report of histological changes in the lung in 14 patients with MPO-ANCA, no patients were found with nongranulomatous airway inflammation [24]. A recent study described MPO-ANCA positivity in four of 30 patients with diffuse panbronchiolitis [25]. The patient's history of bronchitic symptoms since childhood is suggestive of diffuse panbronchiolitis, however the patient never had sinusitis. Furthermore, biopsy did not reveal the typical foamy interstitial macrophages seen in diffuse panbronchiolitis. Conversely, SCHWARZ *et al.* [26] have reported two cases of microscopic polyarteritis with recurrent pulmonary capillaritis and diffuse alveolar haemorrhage, which developed progressive irreversible airflow limitation 10 yrs after disease onset.

Chest radiographical findings in obliterative bronchiolitis consist of decreased vascular markings and hyperinflation. The HRCT scan shows areas of decreased lung attenuation (air trapping) interspersed with areas of normal attenuation during expiration and often during inspiration. Bronchiectasis, bronchial-wall thickening or bronchiolar dilatation may also be present [27]. The same constellation of findings was present in the present patients. However, seven of 10 patients also had areas of ground-glass attenuation, which was relatively mild in extent. The present authors have previously identified ground glass attenuation in 50% of patients with constrictive bronchiolitis [28]. The appearance of ground glass attenuation may be related to relative over-perfusion of more normal areas of lung.

The classification of inflammation as submucosal or adventitial was based on the description of MYERS and COLBY [29] of the inflammation in constrictive bronchiolitis as being intraluminal, mucosal, submucosal and peribronchiolar. Conversely, GOSINK *et al.* [5], in an earlier report, had classified bronchiolar collagen deposition in a similar manner, characterizing the fibrosis found in constrictive bronchiolitis as submucosal and peribronchiolar. To the best of the authors' knowledge, the classification proposed in the present study has not appeared in any research papers on bronchiolitis. However, HALEY *et al.* [30] have utilized a similar approach in a morphometric study of the distribution of CD45⁺ leukocytes and eosinophils in patients who died with severe asthma. The airway wall was studied as two separate

compartments, an "inner area" (between basement membrane and smooth muscle) and an "outer area" (between smooth muscle and alveolar attachments). The inner and outer areas correspond to the subepithelial and adventitial partitioning respectively in the present study.

Approximately one-half of the patients in the present study were current or former smokers. Comparing the three groups (never-smokers, former smokers, current smokers), there were no significant differences in lung function or pathology between them. In addition, pack-yrs of smoking and age could not explain either the degree of airflow limitation or patients' symptoms. This implies that the bronchiolar lesions in the current smoking group were probably not related to tobacco use. Diffusing capacity for CO, corrected for alveolar volume, was normal in the present patient group, excluding any extensive interstitial involvement. Surprisingly, there were no significant differences in the pathological findings between the four patients who required lung transplantation and those that did not. The small number of patients requiring transplantation might account for this finding.

In summary, nineteen patients with chronic bronchiolitis, with or without luminal obliteration, were studied. It appears that there is temporal and spatial compartmentalization of the bronchiolar inflammation and collagen deposition in these patients, particularly between the subepithelial and adventitial region of the bronchioles. Only adventitial inflammation was clearly associated with preserved lung function. Further studies are needed to clarify the complex histological category of obliterative bronchiolitis.

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