Research Article

SCISET
www.sciset.com

Journal of Clinical Pulmonology

2023
Vol. 1 No. 1: 102

Microscopic Alveolar Lesions in Lungs of Stable Chronic Obstructive Pulmonary Disease Patients

Iwao Emura*, Hiroyuki Usuda

Departments of Surgical Pathology, Japanese Red Cross Nagaoka Hospital, Nagaoka City, Japan

Abstract

Objective: This study aims to uncover the mechanisms responsible for neutrophil recruitment in the lungs of patients with stable chronic obstructive pulmonary disease (COPD) and to explore the role of minute lesions of alveolar damage (MLADs) in the development of emphysema.

Methods: Immunohistochemical examination was conducted on 74 lobes from 74 patients with stable COPD and 78 lobes from 78 control patients without COPD.

Results: MLADs were identified as microscopic foci of inflammatory lung injury characterized by the fragmentation and disappearance of alveolar epithelial cells, along with the disappearance of ring-like or tubelike capillary structures from the alveolar septage. MLADs were detected in 11 out of 74 COPD patients. Tumor necrosis factor (TNF)-α+ macrophages and hypoxia-inducible factor (HIF)-2α+ macrophages were found in 100% of patients in the smoking group, with or without COPD, and in all COPD, patients exhibiting MLADs. The numbers of neutrophils in alveolar septae and spaces were significantly higher in the COPD smoking group and non-COPD smoking group compared to the non-COPD non-smoking group. Moreover, neutrophil numbers were notably larger in and around MLADs than in lung tissues away from MLADs in smoking patients with COPD. Masson body-like tissues, suggestive of exudate organizations, and mild interstitial fibrosis, indicative of the fibroproliferative phase of MLADs, were observed in patients with COPD and smoking patients without COPD.

Conclusion: These findings suggest that HIF-2α+ macrophages and TNF-α+ macrophages induced by smoking-related hypoxia play a crucial role in recruiting neutrophils. MLADs, developing in lungs with an increased number of recruited neutrophils, likely contribute significantly to the subsequent development of full-scale emphysema.

Keywords: Chronic obstructive pulmonary disease; Emphysema; Minute lesions of alveolar damage; Tumor necrosis factor-α; Hypoxia-inducible factor-2α; Hypoxia; Smoking.

Corresponding author:
Iwao Emura, Departments of Surgical Pathology, Japanese Red Cross Nagaoka Hospital, Nagaoka City, Japan. E-mail: emura_i@nagaoka.jrc.or.jp

Citation: Emura I, Usuda H. (2023) Microscopic Alveolar Lesions in Lungs of Stable Chronic Obstructive Pulmonary Disease Patients. J Clin Pulmonol. Vol 1(1): 102.

Received: February 04, 2023; **Accepted:** February 22, 2023; **Published:** February 28, 2023

Abbreviations

MLADs: Minute lesions of alveolar damage; COPD: Chronic obstructive pulmonary disease; TNF-α: Tumor necrosis factor-α; HIF-2α: Hypoxia-inducible factor-2α

Introduction

Chronic obstructive pulmonary disease (COPD) poses a significant global health burden, affecting approximately 10% of the general population, with prevalence soaring to 50% among heavy smokers. Cigarette smoking stands out as the primary cause of

COPD in developed nations, leading to a progressive, neutrophilic inflammatory airway disorder resulting from prolonged exposure to external stressors like tobacco smoke. While the recruitment of neutrophils in COPD is known to be mediated by various molecular signals, the specific histopathologic features underlying neutrophil-associated lung injury remain unexplored [1].

A prior study highlighted the existence of minute lesions of alveolar damage (MLADs) in lungs with stable idiopathic pulmonary fibrosis, initiated by hypoxia and involving HIF-2α+ macrophages, TNF-α+ macrophages, and neutrophils. Considering the hypoxic condition induced by smoking, this study investigates the potential roles of HIF-2α+ macrophages, TNF-α+ macrophages, and MLADs in recruiting neutrophils and contributing to the development of emphysema in patients with COPD [2-4].

Materials and Methods

Patients

A total of 74 patients with COPD and 78 control patients without COPD, obtained from lobectomy procedures for lung cancer between 2012 and 2016, were retrospectively examined. Patients with severe heart failure, idiopathic pulmonary fibrosis, tuberculosis, and bronchiectasis were excluded.

Tissue Processing and Histopathological Examination

Lung tissues were fixed, embedded in paraffin, and subjected to various staining techniques, including hematoxylin and eosin, Gram stain, Grocott's stain, and elastic van Gieson stain.

Immunohistochemical Examination

Paraffin sections underwent immunohistochemical staining for various markers, including keratin, CD15 (neutrophil marker), CD34 (endothelial cell marker), TNF-α, and HIF-2α.

Clinical Diagnosis of COPD

COPD diagnosis followed international guidelines, with patients classified into COPD and non-COPD groups based on post-bronchodilator forced expiratory volume percentage (FEV1%).

Histopathological Examinations

MLADs were diagnosed based on immunohistochemistry, defined by specific features of lung injury. The study examined the presence or absence of MLADs, nodular granulation tissue, Masson body-like tissues, and alveolar septal fibrosis [5,6].

Statistical Analysis

Statistical analyses, including the Kruskal-Walli's test, t-test, chi-square test, and Fisher's exact test, were performed using SPSS statistics software.

Results

Patient Characteristics

The essential characteristics of the patients at the time of surgery are summarized in Table 1. Notably, none of the COPD patients had encountered exacerbation before the operation, and all individuals did not meet the diagnostic criteria for exacerbation at the surgical intervention [7-8].

Table 1: Results in patients with and without COPD.

	COPD			Non-COPD			
	S. (n=29)	S.C. (n=35)	No S. (n=10)	S. (n=24)	S.C. (n=25)	No S. (n=29)	*p*
Male	25(86%)	35(100%)	4(40%)	22(92%)	21(84)	2(7%)	<0.001
Age	66.0(61.0, 73.0)	71.0(65.0, 78.0)	77.0 (69.8, 81.0)	65.0(57.3, 71.0)	70.0 (65.8, 75.5)	68.0 (64.0, 74.5)	0.008
C.I.	810.0(600.0, 1200.0)	1100(700.0, 1520.0)	0.0(0.0, 0.0)	705.0(412.5, 990.0)	560.0 (340.0, 1000.0)	0.0(0.0, 0.0)	<0.001
LABA	7(24%)	7(20%)	1(10%)	0	0	0	<0.201
LAMA	1(3%)	3(9%)	0	0	0	0	0.002
LABA+LAMA	7(24%)	4(11%)	0	0	0	0	0.001
HIF-2α	29(100%)	21(60%)	2(20%)	24(100%)	5(20%)	1(3%)	<0.001
TNF-α	29(100%)	21(60%)	2(20%)	24(100%)	5(20%)	1(3%)	<0.001
N-s	128.0(86.5, 160.0)	90.0(54.0, 156.0)	50.0 (17.3, 90.8)	130.0 (109.0, 164.5)	71.0 (35.0, 119.5)	54.0(24.5, 100.0)	<0.001
N-a	42.0(24.5, 71.0)	32.0(54.0, 63.0)	7.5(4.8, 12.8)	36(18.8, 46.8)	5.0(3.0, 6.0)	5.0(3.0, 6.0)	<0.001
NGT	0	0	0	0	0	0	-
MBLT	14(48%)	12(34%)	1(10%)	4(17%)	0	0	<0.001
Fibrosis	29(97%)	19(54%)	4(40%)	9(38%)	7(28%)	1(3%)	<0.001
MLAD	6(21%)	3(9%)	2(20%)	0	0	0	0.005

Pathological Findings of MLADs

Mild extravasation of blood cells and intra-alveolar exudates were evident in architecturally normal lung tissue (Figure 1a). Microscopic foci of inflammatory lung injury, identified as MLADs, were present in these areas. MLADs exhibited the following features:

- Fragmentation and disappearance of alveolar epithelial cells, leaving cytokeratin-positive cell debris (Figure 1b).
- Disappearance of ring-like or tube-like capillary structures from the alveolar septae (Figure 1c).

HIF-2α+ macrophages (Figure 2a) and TNF-α+ macrophages (Figure 2b) were detected in and around the MLADs, accompanied by the accumulation of neutrophils in the alveolar septa and spaces (Figure 1d). Each MLAD involved up to seven alveoli, and hyaline membranes were notably absent. There were no indications of bacteria or inclusion bodies suggesting viral infection in or around the MLADs.

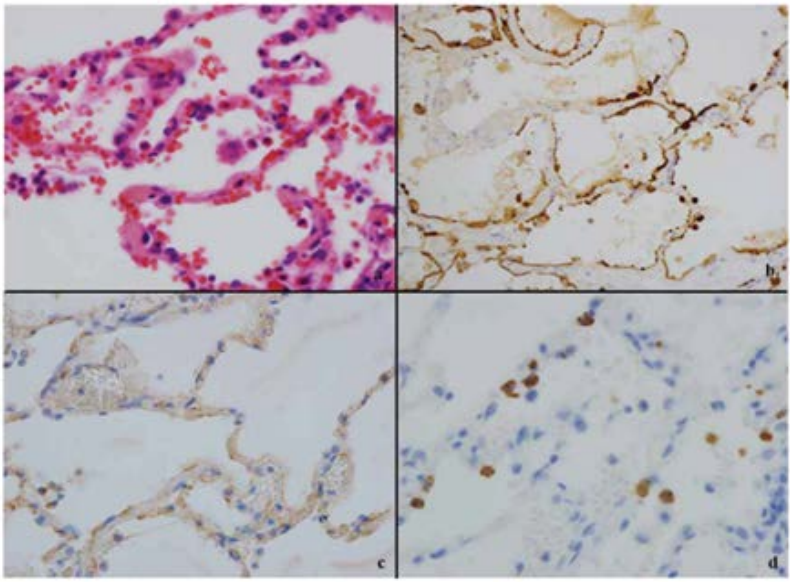

Figure 1: Pathological findings of minute lesions of alveolar damage. (a) Histopathological findings. Mild extravasation and intra-alveolar exudates were observed. (b) Alveolar epithelial cells were fragmented and had disappeared. (c) Tube-like or ring-like capillary structures in alveolar septae were destroyed. (d) Accumulation of CD15-positive neutrophils.

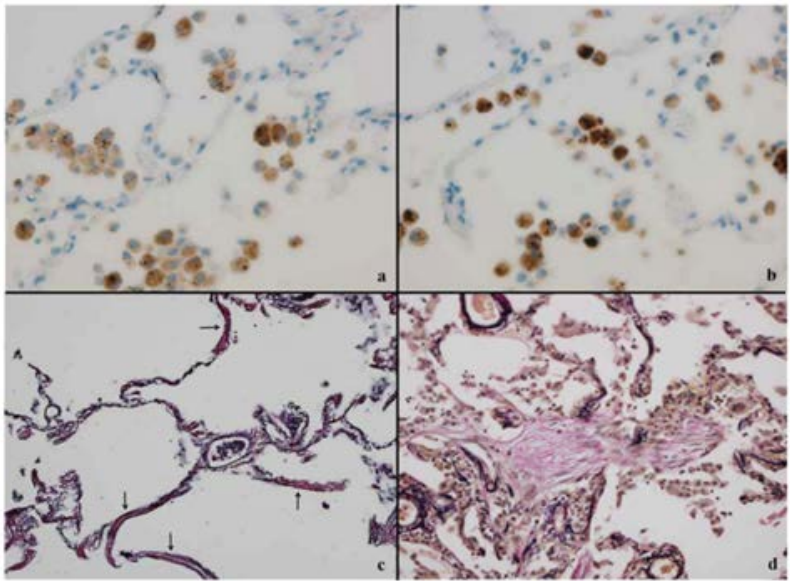

Figure 2: Pathological findings of lungs affected by chronic obstructive pulmonary disease. (a) Hypoxia-inducible factor 2α-positive alveolar macrophages. (b) Tumor necrosis factor α-positive alveolar macrophages. (c) Mild interstitial fibrosis (arrows). The alveolar structure was destroyed. (d) Masson-body-like tissue. Immunohistochemistry for (a) HIF-2α, (b) TNF-α. (c) Grocott's variation of Methenamine silver-nitrate, and (d) elastic van Gieson stain.

Frequency of Pathological Findings

Results are presented in Table 1. Key observations include:

TNF-α+ macrophages and HIF-2α+ macrophages were present in 100% of patients in the smoking group, irrespective of COPD status, and in 2 of 10 non-smoking COPD patients [9].

Significantly larger numbers of patients with HIF-2α+ macrophages and TNF-α+ macrophages were observed in both the COPD smoking and non-COPD smoking groups compared to the non-COPD non-smoking group (all P<0.001).

- Persistence of these macrophages post-smoking cessation was noted in 60% of COPD patients and 20% of non-COPD patients.
- The numbers of neutrophils in alveolar septae and spaces were significantly higher in both the COPD smoking and non-COPD smoking groups compared to the non-COPD non-smoking group (COPD smoking group vs. Non-COPD non-smoking group, P=0.001, others, P<0.001).
- Neutrophil numbers were slightly larger in and around MLADs than in lung tissues located away from MLADs.
- MLADs were detected in 11 COPD patients (smoking-6; smoking cessation-3; no smoking-2), with TNF-α+ macrophages and HIF-2α+ macrophages observed in all cases.
- Masson body-like tissues were observed in both COPD and non-COPD patients in the smoking group.
- Mild interstitial fibrosis was present across all groups.
- Nodular granulation tissue was not detected in patients with COPD.

These findings provide a detailed characterization of the pathological features associated with MLADs, emphasizing the role of macrophages and neutrophils in the context of smoking and COPD.

Discussion

The findings from this investigation shed light on crucial aspects of the pathological mechanisms associated with neutrophilic inflammatory lung injury in patients with Chronic Obstructive Pulmonary Disease (COPD). The following key points are highlighted [10-11]:

1. Role of HIF-2α+Macrophages and TNF-α+Macrophages:

- HIF-2α+macrophages and TNF-α+macrophages, induced by hypoxia resulting from smoking, were universally detected in all smoking patients, regardless of COPD status.
- The presence of these macrophages correlated with significantly larger numbers of neutrophils in both alveolar septae and spaces in the COPD smoking group and non-COPD smoking group compared to the non-COPD non-smoking group.
- Macrophages in ischemic disease sites accumulate both HIF-1α+ and HIF-2α+, and HIF activity stimulates the production and release of pro-inflammatory cytokines like TNF-α and interleukin-1.
- Proinflammatory cytokines released by macrophages in the alveolar lumen are believed to cause neutrophil adherence to capillaries and extravasation into the alveolar space.
- Hypoxia induced by smoking triggers an innate immune response, with HIF-2α+macrophages and TNF-α+macrophages playing crucial roles in the recruitment of neutrophils.

2. Importance of MLADs in Emphysema Development:

- MLADs were identified in lungs with stable COPD, and their presence was restricted to lungs where HIF-2α+ alveolar macrophages and TNF-α+ alveolar macrophages were present.
- Neutrophil numbers were tending to be larger in and around MLADs than in lung tissues located away from MLADs.
- MLADs exhibited features such as injury to alveolar epithelial cells, disappearance of capillary structures, and mild interstitial fibrosis in alveolar septae.
- These findings suggest that MLADs develop in lungs where large numbers of neutrophils have been recruited, possibly due to deteriorating hypoxia.
- The data supports the hypothesis that unopposed and increased elastolytic activity, facilitated by activated neutrophils, leads to elastic tissue destruction and, eventually, full-scale emphysema.

3. Progressive Inflammatory Lung Injury:

- Masson body-like tissues and mild interstitial fibrosis, indicative of the fibroproliferative phase of MLADs, were observed in patients with COPD and smoking patients without COPD.
- These findings, although not specific hallmarks of COPD, suggest that inflammatory lung injury continued to progress despite treatment, eventually culminating in emphysema.

4. Comparison with Idiopathic Pulmonary Fibrosis:

- MLADs were detected in both patients with COPD and

idiopathic pulmonary fibrosis, but nodular granulation tissue (fibroproliferative phase of MLADs) was observed only in idiopathic pulmonary fibrosis.

- The absence of nodular granulation tissue in COPD patients may reflect differences in pulmonary function between COPD and idiopathic pulmonary fibrosis.

Limitations

The study acknowledges limitations, including a small sample size and the absence of comparative data from other reports. However, the detection of HIF-2α+macrophages and TNF-α+macrophages in all smoking patients suggests the relevance of the pathological findings to COPD.

Further research is deemed essential for a comprehensive understanding of the pathobiological mechanisms underlying inflammatory lung injury in COPD patients.

Conclusion

COPD, characterized by progressive neutrophilic inflammatory airway disorder, involves the recruitment of neutrophils mediated by HIF-2α+macrophages and TNF-α+macrophages induced by smoking-related hypoxia. MLADs play a crucial role in the development of subsequent full-scale emphysema, serving as microscopic foci of inflammatory lung injury associated with alveolar damage and neutrophil recruitment.

The study emphasizes the importance of elucidating the histopathologic features underlying neutrophil-associated lung injury for a comprehensive understanding of COPD pathogenesis.

References

1. Mannino DM (2002) COPD: Epidemiology, prevalence, morbidity and mortality, and disease heterogeneity. Chest 121: 121s-126s.
2. Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, et al. (2013) Global strategy for the diagnosis, management, and prevention of Chronic Obstructive Pulmonary Disease. Gold executive summary. Am J Respir Crit Care Med. 187: 347-65.
3. Stanescu D, Sanna A, Veriter C, Kostianev S, Calcagni PG, et al.(1996) Airways obstruction, Chronic expectoration, and rapid decline of FEV1 in smoker are associated with increased levels of sputum neutrophils. Thorax 51: 267-71.
4. O'Donnell RA, Peebles C, Ward JA, Daraker A, Angco G, et al. (2004) Relationship between peripheral airway dysfunction, airway obstruction, and neutrophilic inflammation in COPD. Thorax. 59: 837-42.
5. Grabcanovic-Musija F, Obermayer A, Stoiber W, et al. (2015) Neutrophil extracellular trap (NET) formation characterises stable and exacerbated COPD and correlates with air flow limitation. Respiratory Research 16: 59-70.
6. Cosio MG, Saetta M, Agusti A (2009) Immunologic aspects of chronic obstructive pulmonary disease. N Engl J Med 360: 2445-54.
7. Hunninghake GW, Crystal RG (1983) Cigarette smoking and lung destruction. Accumulation of neutrophils in the lungs of cigarette smokers. Am Rev Respir Dis 128: 833-38.
8. Emura I, Usuda H, Togashi K, Satou K (2015) Minute lesions of alveolar damage in lungs of patients with stable idiopathic pulmonary fibrosis. Histopathology 67: 90-95.
9. Burke B, Tang N, Corke KP (2002) Expression of HIF-1 alpha by human macrophages: Implications for the use of macrophages in hypoxia-regulated cancer gene therapy. J Pathol 196: 204-12.
10. Elzschig HK, Carmeliet P (2011) Hypoxia and inflammation. N Engl J Med 364: 656-65.
11. Nizet V, Johnson RS (2009) Interdependence of hypoxic and innate immune responses. Nat Rev Immunol 9: 609-17.