Original Paper

Cytopathological Aspects in the Bronchoalveolar Lavage Fluid in Chronic Obstructive Pulmonary Disease

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ABSTRACT: Chronic Obstructive Pulmonary Disease (COPD) is a clinical syndrome characterised by a slow progressive decline in expiratory airflow [1], a process that has gradually developed over the years. Studies of patients with COPD show an inflammatory process in the small airways [2]. The aim of this paper is to identify the cytopathological aspects of the liquids present in the bronchoalveolar lavage in patients with COPD. We were performed a descriptive analytical case-control and prospective study on forty patients with COPD and ten asymptomatic smokers (healthy smokers or patients at risk). The percentage of marcophage, the type of the dominant inflamatory cell, in the bronchoalveolar lavage (BAL) liquid was significantly higher at patients with mild and moderate COPD as compared to patients with severe and very severe COPD. In the present work, the percentage of the neutrophil in the BAL liquid was significantly higher at patients with severe and very severe COPD, as compared to the patients with mild and moderate COPD and to aparently healthy smokers. In conclusion, we can say that COPD is characterized by an inflammatory process located in the small airways with predominant participation of macrophages, the procentage of macrophages in BAL fluid variyng inversely proportional to the severity of the disease.

***KEYWORDS**: COPD, BAL fluid, inflamatory cells.*

Introduction

The irreversible expiratory airflow obstruction is caused by specific anatomopathological lesions such as the loss of lung elastic recoil, fibrosis, and the narrowing of the small airways [3]. The partially reversible airway obstruction represents a consequence of the edema, bronchial hypersecretion, and the contraction of the smooth muscle in the smaller bronchi [4]. These histopathological lesions differ from one individual to another and can be responsible for the clinical heterogeneity of this syndrome. Studies of patients with COPD show an inflammatory process in the small airways. The factors that are characteristic of the inflammatory process in people who have a predisposition for the disease and who will develop the most severe forms of COPD, are still not known. Recent studies show that there exists an individual susceptibility to cigarette smoke exposure[5], whereas those patients who develop severe COPD will suffer from a longer and more intense inflammatory response than those who responded individually. Also, these studies prove that the mediators of the inflammatory cells of the inflammatory process caused by cigarette smoking can produce pulmonary structural alterations which are characteristic of COPD [6]. Smoking cessation in the early stages can result in a reversible inflammatory bronchial process [7], while smokers with advanced disease who continue smoking will experience a persistent inflammatory process even after quitting smoking [8].

Aim

The aim of this paper is to identify the cytopathological aspects of the liquids present in the bronchopulmonary lavage in patients with COPD and to compare them with the cytopathological aspects present in smokers who do not suffer from this disease (patients at risk for developing COPD).

Material and Method

This descriptive **analytical case-control** and **prospective** study was performed on forty patients with COPD and ten asymptomatic smokers (healthy smokers or patients at risk), who were admitted in hospital during an interval of 12 months, in Second Medical Clinic. After establishing the diagnosis and after considering the clinical history as well as the post-bronchodilator FEV1 values, I have identified the cytopathological aspects of the liquids present in the bronchopulmonary lavage in patients with COPD and in those at risk to develop this disease. Inclusion criteria for these patients were: familiar and unfamiliar patients with COPD, whose values were FEV1<80% out of FEV1 ideal, in correlation with FEV1/FVC <70% and with reduced reversibility (< 15% or 200 ml) of the FEV1 values after inhalation of β2 sympathomimetics; patients at risk for developing COPD: subjects who smoke over 10 packets per year (one packet per year represents 20 cigarettes per day for one year), extended exposure to bronchial irritators, first-degree relatives of warded patients with COPD (the control group). Patients with a past medical history of allergies and bronchial asthma, pregnant and lactating women, pulmonary neoplasm, pulmonary tuberculosis, myocardial infarction, and unstable angina pectoris were excluded. For patients enrolled in this clinical study, data were compiled using a study protocol that included anamnesis data, objective clinical examination, and the results obtained after paraclinical explorations. Bronchoscopy with bronchoalveolar lavage (BAL) is an important research tool for assessing airway inflammation in COPD [9]. The bronchopulmonary lavage was performed during a classical bronchoscopy and it consisted of the following procedure: 150 – 250 ml sterile, isotonic, saline solution, warmed to body temperature divided into quantities of 20-60 ml was infused through wide-bore tubing into a bronchi segment, followed by the collection of samples after each instillation through gentle aspiration, either with a syringe, or suction. After aspiration, we observed the volume of liquid recovered as well as its macroscopic aspect (clear, yellowish, hemorrhagic, lactescent). According to this volume, we approximated the amount and quality of the bronchoalveolar lavage. After fixation, the smears were stained with May-Grunwald-Giemsa method and analysed using microscopy.

The data from the clinical examination and the subsequent results obtained after the paraclinical explorations were stored in a database which enabled us to perform common **descriptive statistics** and calculate confidence intervals as well as other basic indicators (mean or average scores, standard deviations, coefficients of variation, median). The comparison of the average levels was performed using the parametric tests for comparison (t-Student), while the correlation relationships existing among the determinant parametres were performed by determining the correlation coefficients (Pearson's correlation coefficient), using correlation straight lines or curves and adjusting correlation curves.

Results

1. The structure of the group of patients

This study was conducted on forty patients with COPD, 27 men and 13 women, aged between 42 and 74 (59,5 ± 13,8 years), who smoked between 26 and 52 packets of cigarettes per year (37,6 ± 11,2 packages per year), came from urban areas (26 patients) and rural areas (12 patients), suffered from acute exacerbations during the last year, from 2 to 6 per year (3,2 ± 0,8 acute exacerbations per year); from these only 9 patients had a family history of chronic pulmonary disease.

2. Epidemiological and anthropometric parameters

According to the FEV1 values the patients were grouped as follows: one group with mild and moderate COPD (FEV1 ≥ 50%) and the other group with severe and very severe COPD (FEV1 ≤ 50%).

Tabel 1.Anthropological and epidemiological characteristics of the patients studied divided into two groups according to the degree of the severity of COPD.

	Mild and moderate COPD	**Severe and very severe COPD**
Men	17	10
Women	5	8

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Patients from urban areas	16	12
Patients from rural areas	6	6
Average age (years)	57,4 ± 13,2	62 ±14,6
Age limit (years)	42 - 68	52 - 74
Average number of cigarettes smoked (packets per year)	31,6 ± 12,2	43,6 ± 14,8
Cigarettes smoked (packets per year)	26 - 50	28 - 57
Average number of acute exacerbations of COPD	1,2 ± 0,2	4,2 ± 0,6
Acute exacerbations of COPD during the past year	1 - 4	2 - 6
Family history of COPD (%)	3 / 22	6 / 18
Recurrent bouts of infectious bronchitis in childhood (%)	7 / 22	9/18

3. Clinical parameters

The patients included in this research study showed the following clinical features: coughing (27/40; 67,5%), expectoration (27/40; 67,5%), breathlessness (22/40; 55,5%), and wheezing (20/49; 50%). The **clinical symptoms** observed among these patients were: cyanosis (18/40; 45%), orthopnea (17/40; 42,5%), and bronchial rales (21/40; 52,5%). Body mass index (BMI) values observed among the selected patients ranged from 18,1 to 27,9 kg/mp (22,6±2,9 kg/mp).

Patients with **mild or moderate COPD** experienced symptoms such as coughing (15/22; 68,18%), expectoration (14/22; 63,6%), breathlessness (7/22; 31,8%) and wheezing (6/22; 27,2%). The objective features that these patients described were: orthopnea (4/22; 18,18%) and bronchial rales (3/22; 13,6%). Body mass index (BMI) values ranged from 19 to 31 kg/mp, with an average value of 27,25 ± 2,94 kg/mp.

Among the patients with **severe or very severe COPD**, the following symptoms were observed: coughing (12/18; 66,6%), expectoration (13/18; 72,7%), breathlessness (15/18; 83,3%) and wheezing (14/18; 77,7%). The clinical signs that the patients described were cyanosis (18/18; 100%), orthopnea (13/18; 72,2%), bronchial rales (18/18; 100%), and edema (6/18; 33,3%). BMI values ranged from 18 to 25 kg/mp, with an average value of 20,91 ± 1,97 kg/mp.

4. Functional Parametres

The post-bronchodilation absolute or percentual values of forced expiratory volume in one second (FEV1) were between 0,8 and 3,1 l (2,17 ± 0,46 l), respectively 32 and 79% (57,3 ± 7,6%). Post-bronchodilation, the values of the bronchial permeability index (BPI) were between 53 and 70%, with an average value of 55 ± 7,8%.

Tabel 2.Functional parameters of the patients studied divided into two groups according to the degree of the severity of COPD.

	Mild and moderate COPD	**Severe and very severe COPD**
Post-PD-FEV1 % predicted	68,64 ± 22,22	35,25 ± 8,58
FEV1 decrease (ml)	156,7 ± 24	503,66 ± 78
SaO_2 (%)	91,5 ± 3,54	79,78 ± 7,03 %

Accordingly with the FEV1 values recorded at spirometry, we classified the patients between two level groups depending on the gravity: patients with mild and moderate COPD (22/40; 55%), who have been recorded with an average FEV1 value of 68,64 ± 22,22% and an average value of decreasing FEV1 of 156,7ml ± 24ml, and patients with severe and very severe COPD (18/40; 45%) who recorded a percentual average FEV1 value of 35,25 ± 8,58 % and an average value of decreasing FEV1 of 503,66ml ± 78ml.

5. Puls-oximetric parameters

This method recorded average SaO_2 values of 91,5 ± 3,54% at patients with mild or moderate *COPD* and an average SaO_2 value of 79,78 ± 7,03% at patients with severe and very severe *COPD* . If patients with mild and moderate COPD have shown only mixted and obstructive pulmonary dysfunctions, the patients with severe and very severe COPD have shown the phenomenon of respiratory failure.

Tabel 3. Macroscopic and microscopic aspects of the recovered BAL fluid in the patients studied

	Control group	**Mild and moderate COPD**	**Severe and very severe COPD**
Volumes of recovered bronchoalveolar lavage fluids	126,2 ± 21,4 ml	90,5 ± 13,4 ml	54,1 ± 9,2 ml
% volume recovered BAL from volume BAL instiled	66,3%	48%	30%
Nonsquamous cells (cells/ml)	6400±120cells/ml	8800 ± 160 cells /ml	12800 ± 285 cells/ml)
Macrophages (%)	90,25 ± 8,63%	60,5 ± 4,03%	74,46 ± 4,2%
Neutrophiles (%)	5,33 ± 1,8%	22,1 ± 6,69%	30,85 ±6,8%
Eosinophiles (%)	0,54 ± 0,3%	0,23 ± 0,04%	0,71±0,8%
Lymphocytes (%)	0,7 ± 0,3%	4,46 ± 1,31%	2,92 ± 0,8%

6. The exam of the recovered bronchoalveolar lavage (BAL) fluid

The measurement of the volumes of recovered bronchoalveolar lavage fluid (in ml) has enabled us to observe the following results. If from smokers without clinical signs of disease have been extracted volumes of bronchoalveolar lavage fluids with values between 105 and 168 ml, with an average value of 126,2 ± 21,4 ml, which in relative value represents 66,3% from instilled liquid.

From patients with COPD have been extracted **volumes** of bronchoalveolar lavage fluids with values between 45 şi 98 ml, with an average value of 72,6 ±15,4 ml, which represents 34,2% of instilled liquid. The volumes of recovered bronchoalveolar lavage fluids have been significantly higher at patients with mild and moderate COPD *(*90,5 ± 13,4 m*l),* than the volumes of broncho-alveolar lavage fluids extracted from patients with severe and very severe COPD (54,1 ± 9,2 ml). In relative value, from patients with mild and moderate COPD have been recovered 48% from the value of the volumes of instilled lavage liquid, while from patients with severe and very severe COPD have been extracted an average 30% from the value of volumes of instilled lavage liquids.

The **cytological** examination of the recovered bronchoalveolar lavage fluid offered important information about the inflamatory process at the level of small airways.

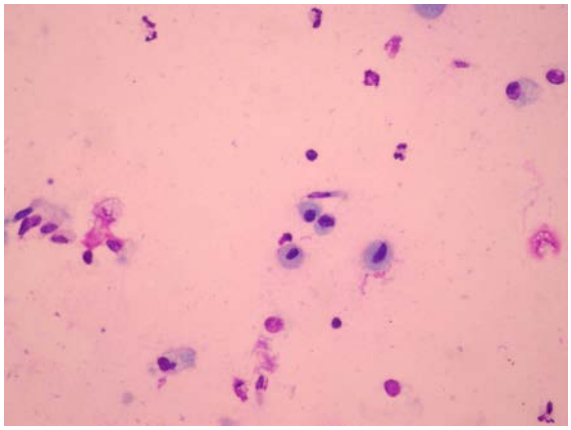

Fig 1.Cytologic aspects in mild COPD patient, May-Grunwald-Giemsa staining 20x, cylindrical epithelium rare, rare macrophages, rare lymphocytes, rare neutrophils

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The average value of the number of nonsquamous cells shown in the BAL lavage liquid was higher at patients with COPD (10083,43 ± 1560 cel/ml), than the average value recorded at the control-group of patients (6208,33± 900,42 cel/ml). The average number of nonsquamous cells found in the BAL of the patients with severe and very severe COPD was significantly statistic higher (12800 ± 285 cells/ml) than the average values shown by patients with mild and moderate COPD (8800 ± 160 cells /ml) and by control-group (6400 ±120 cells/ml).

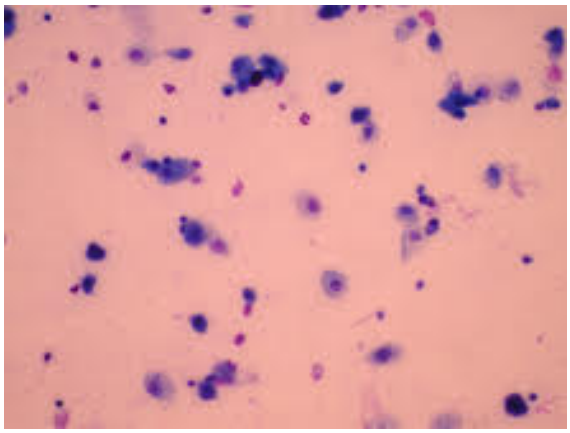

Fig 2. Cytologic aspects in moderate COPD patient May-Grunwald-Giemsa staining (20x), rare cylindrical epithelium, macrophages relatively common, relatively rare lymphocytes, neutrophils rare, very rare eosinophils

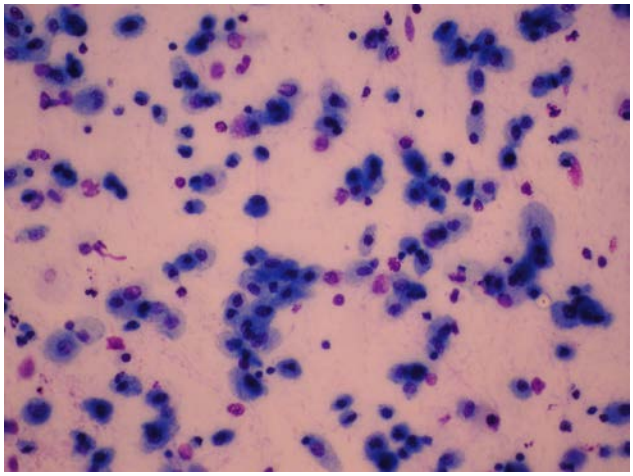

Fig 3. Cytologic aspects in severe COPD patient, May-Grunwald-Giemsa staining (20x), frequently macrophages, lymphocytes relatively common, rare neutrophils, eosinophils relatively common

The average value of the percentage of macrophages in the bronchoalveolar lavage liquid was lower at patients with COPD (70,16 ± 7,86%), as compared to the average value of the macrophages percentage shown by the control group (90,25 ± 8,63%). The average value of the macrophages percentage from BAL was significantly lower at patients with severe and very severy COPD (60,5 ± 4,03%) as compared to the patients with mild or moderate *COPD* (74,46 ± 4,2%).

The percentage of neutrophils has recorded higher average values at patients with COPD (25,58± 8,4%), in comparison with patients from the control-group (5,33 ± 1,8%). The average value of the neutrophils percentage in recovered BAL was significantly statistically higher (30,85 ±6,8%) at patients with severe and very severe COPD, in comparison with patients with mild and moderate COPD (22,1 ± 6,69%) and with the patients from the control-group (5,33 ± 1,8%).

The lymphocytes from the recovered BAL liquid have recorded significantly higher average values at patients with COPD (4 ±1,71%), as compared to those recorded at the patients from the control-group (0,7 ± 0,3%). The Lymphocytes in the BAL liquid have recorded higher average values at patients with mild and moderate COPD (4,46 ± 1,31%) as compared to the patients with severe and very severe COPD (2,92 ± 0,8%) and to the patients from the control-group (0,7 ± 0,3%).

The average value of the percentage of eosinophils in the BAL liquid was more reduced at patients with COPD (0,24 ± 0,01%) than at patients from the control-group (0,6 ± 0,03%). The percentage of eosinophils in the recovered BAL liquid has recorded higher average values at patients with severe and very severe COPD (0,71±0,8%), as compared to patients with mild and moderate COPD (0,23 ± 0,04%) and to patients from control-group (0,54 ± 0,3%).

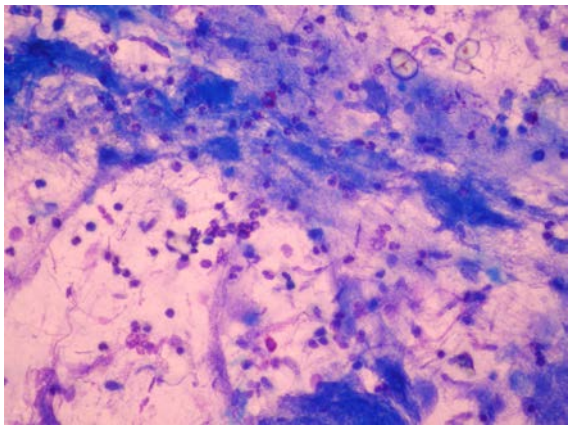

Fig 4. Cytologic aspects in very severe COPD patient, May-Grunwald-Giemsa staining (20x), mass of mucus, rare macrophages, lymphocytes common, neutrophils, relatively rare eosinophils

Discussions

The measurement of the volumes of bronchoalveolar lavage liquid (expressed in ml) has shown a significant decrease of the volumes of liquids recovered at the bronchoalveolar lavage at patients with severe and very severe COPD, as compared to the patients with mild and illness and asimptomatic smokers moderate

The lung structural cells, the epitelial and mesenchimal cells, are involved in the adjusting mechanisms of the inflamatory process within the lungs of the patients with COPD through the inflamation mediators they produce [1**0**]**.**

The inferior respiratory tract presents an infiltration with inflamatory cells both to the patients with COPD and to smokers [1**1**]. In the case of the inflamatory process from the small airways, the highest values of the number of nonsquamous cells have been recorded at patients with severe and very severe COPD highlighting their role in the bronchiolar inflamatory process.

In the stable phase of COPD, the inflamatory cells dominant at the bronchioles and alveoli level are the hyperpigmented macrophages. Actually, these cells are the most numerous inflamatory cells which are present both in the normal lungs and in the lungs of the patients with COPD. The macrophagic mediators are known as key pathogene mediators in producing COPD. The type of the dominant inflamatory cell in the BAL liquid was the marcophage which recorded the highest percentage at the healthy smokers. The average value of the percentage of marcophage in the bronchoalveolar lavage (BAL) liquid was significantly higher at patients with mild and moderate COPD as compared to patients with severe and very severe COPD.

Similarly, the neutrophils are identified in the lumen of the small airways [1**2**], in the bronchial glands and they progressively acumulate in the lung tissue [1**3**]**.** In the present work, the average value of the neutrophil percentage in the bronchoalveolar lavage liquid BAL was significantly higher at patients with severe and very severe COPD, as compared to the patients with mild and moderate COPD and to aparently healthy smokers.

The lymphocytes, especially the T-CD_8^+, are found in a high number in the small airways [1**4**] alveolar structures alveolare, vessels and lymph nodes [1**5**]**.** Usually, the increasing in the concentration of lymphocytes is associated with severe forms of illness [**16,**17]. A series of clinical studies showed that T lymphocytes which are present in the lungs of the patients with COPD express receptors for CCR5 și CxCR3, considered to be receptors of T_h1 lymphocyte [1**8**]. This fact makes the difference between BPCO and the bronchial asthma where LTh2 are predominantly found . In current study, the average levels of the lymphocytes percentage in the BAL have shown sensibly equal values at patients with mild and moderate COPD as well as at patients with severe and very severe forms of COPD or at patients from the control-group.

The presence of eosinophils in the airway wall [1**9**] as well as the presence of the eosinophil mediators in the bronchoalveolar lavage liquid BAL [**20**] and in COPD patients' sputum show an eosinophilic activation at these patients. There has been described in an interesting way a subtype of COPD, responsive to corticoids [**21**]; it is characterised by eosinophily – the presence of eosinophils in the patients' sputum and by the presence of the mediators of the mastocitary activation in the patients' sputum and in the bronchoalveolar lavage liquid BAL. In our study, the eosynophils percentage was aproximately the same, both at patients with mild and moderate COPD and at patients with severe and very severe COPD.

Conclusions

The type of the dominant inflamatory cell in the BAL liquid was the marcophage which recorded the highest percentage at the healthy smokers. The average value of the percentage of marcophage in the bronchoalveolar lavage (BAL) liquid was significantly higher at patients with mild and moderate COPD as compared to patients with severe and very severe COPD.

The average value of the neutrophil percentage in the bronchoalveolar lavage liquid BAL was significantly higher at patients with severe and very severe COPD, as compared to the patients with mild and moderate COPD and to aparently healthy smokers.

We can say that COPD is characterized by an inflammatory process located in the small airways with predominant participation of macrophages, the percentage of macrophages in BAL fluid variyng inversely proportional to the severity of the disease.

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