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Original Research Paper

Sputum and hematic cell profile in exacerbations of chronic obstructive pulmonary disease (AECOPD)

Medicine

KEYWORDS: -

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ABSTRACT

Inflammation increases during exacerbations of COPD and there are changes in sputum and hematic cells profile. Better identification of these changes will increase our knowledge and potentially guide therapy. The aim of this study was to investigate sputum and hematic cell profile in EACOPD. In this study, 56 AECOPD patients have been evaluated at first consultation, and after 21 days in relation to the number of sputum inflammatory and hematic cells. In AECOPD there is significant growth of cellular sputum elements, with predominance of macrophages and neutrophils, compared with their content 21 days after. Cellular stratification in the sputum of patients with AECOPD resulted predominantly of neutrophilic and paucigranulocytic type. Leukocytosis in the blood coincides with AECOPD and the number has decreased with the return in stable condition. Neutrophil/lymphocyte ratio (NLR) results a significant indicator to detect acute exacerbations of COPD, and to evaluate recovery. Diagnosis of AECOPD is supported by increased sputum inflammation as proxy of airways inflammation, and increased systemic inflammation as demonstrated by increased number of blood cells. Background: Inflammation increases during exacerbations of COPD and there are changes in sputum and hematic cells profile. Better identification of these changes will increase our knowledge and potentially guide therapy. Aim: The aim of this study was to investigate sputum and hematic cell profile in AECOPD. Methods: There is a prospective study conducted in Regional Hospital, Fier. There are enrolled patients with stage III and IV of COPD in AECOPD condition. In this study, 56 AECOPD patients have been evaluated at first consultation, and after 21days in relation to the number of sputum inflammatory and hematic cells. All of the AECOPD patients were stratified, according to the number of neutrophils (>61%) and eosinophils (>2.5%) in the sputum samples. Individual patients were classified into the eosinophilic (EO) with sputum eosinophils >2.5% of total cells, the neutrophilic (NE) with neutrophils >61%, the paucigranulocytic COPD (PA) with eosinophils ≤2.5% and neutrophils ≤61%. The examination specimen was an early-morning spontaneously produced sputum, after the deep breathing followed by strong cough. Depending on the cells, which are seen in the sputum judged whether it is received or not satisfactory specimen. If there are sufficient quantities of macrophages is understood that we are dealing with sputum, not with saliva. If the cellular elements that dominate are squamous cells, or Candida and ciliated epithelial cells, we are dealing with oral or sinonasal elements. Hematological examination was conducted with Sysmex XS-100i Automated Hematology Analyzer, by fluorescent cytometry.

Statistical analisys

All data collected into Microsoft Excel program were exported to SPSS (Statistical Package for Social Sciences) 20.0, by which was performed statistical analysis. For all categoric variables absolute numbers and corresponding percentages were calculated. For all numeric variables, where the data are with normal distribution, mean values ± std. deviation were calculated. Presentation of data was conducted by simple and composed tables, as well as through graphs. It was considered significant p values ≤0.05.

Characteristics data of patients are presented in Tab.1.

TAB. 1 CHARACTERISTICS DATA OF PATIENTS

Variable	Mean values ± Std. deviation	Min. value	Max. value
Age	69.3± 7.06	52.00	82.00
Age of smoking initiation	18.3±9.8	.00	60.00
Cigarette/day	28.3±14.3	.00	80.00
Smoking years	38.6±14.8	.00	60.00
Age of smoking initiation	59.3±39.2	.00	224.00
Duration of cough (yrs.)	9.4±5.8	2.00	30.00
Duration of sputum (yrs.)	7.96±5.52	1.00	30.00
Duration of dyspnea (yrs.)	7.2±5.26	1.00	35.00
Recuperative time (days)	7.05±1.86	5.00	12.00
BMI index	25.47±5.09	16.50	48.40
FVC (% predicted)	56.89±11.84	30.00	91.10
FEV1 (% predicted)	36.87±8.57	20.00	50.00
Tiffeneau Index (%)	51.44±9.18	25.40	67.10
SaO2	90.34±3.78	84.00	97.00
CAT score	26.54± 6.8	9.00	35.00
MRC dyspnea scale	3.52± .87	2.00	5.00
Gender	Male	54 (96.4%)	
	Female	2 (3.6%)	
COPD GOLD stage	Stage III	27 (48%)	
	Stage IV	29 (52%)	
COPD GOLD category	C3	2 (3%)	
	D3	43 (77%)	
	D4	11 (20%)	
Exacerbation	Type I	32 (57.2%)	
	Type II	12 (21.4%)	
	Type III	12 (21.4%)	

Results:

AECOPD patients have significantly increased total of sputum cells in 39 (69.6%), macrophage -55 (98.2%), neutrophils -51 (91.1%), lymphocytes -37(66.1%), eosinophils -19(33.9%) and epithelial cells -9(16.1%). After 21 days of treatment have remained increased total cells in 13 (23.2%), totally normal or decreased macrophages, neutrophils -13(23.2%), lymphocytes -45(80%), eosinophilis -6(10.7%), and epithelial cells -26(46.1%). Sputum cell comparison at

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AECOPD and 21 days after are presented in Tab. 2.

Tab. 2 Sputum cell comparison at AECOPD and 21 days after

Cell structure	Means ± Std. Deviation AECOPD	Means ± Std. Deviation 21 days after	Comparison of means (t-test)
Nr of sputum cells	14.4±4.51	8.71±3.52	P<0.0001
% eosinophils	.83±1.32	.28±.59	P<0.0001
% neutrophils	51.63±10.22	31.52±7.44	P<0.0001
% macrophage	33.13±7.12	43.03±7.98	P<0.0001
% lymphocytes	6.71±2.76	9.70±4.50	P<0.0001
% epithelial cells	7.45±3.73	10.34±3.91	P<0.0001

Cell sputum stratification resulted: eosinophilic 9(16.1%), neutrophilic 16(28.6%) and paucigranulocytic 31(55.4%).

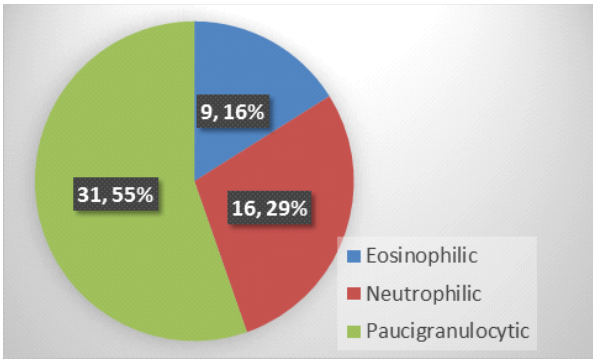

Fig. 1 Sputum cell stratification in AECOPD patients

Sputum cell comparison at AECOPD according the stage of diseases are seen in Tab. 3. and Fig. 2.

Tab. 3 Sputum cell comparison at AECOPD according the stage of diseases

Cell structure	Means ± Std. Deviation stage IV	Means ± Std. Deviation stage III	comparisons of means (t-test)
Nr of sputum cells	16.77±4.18	12.2±3.63	P=0.0001
% eosinophils	0.62±1.23	1.02±1.4	P=0.26
% neutrophils	54.77±10.10	48.7±9.59	P=0.024
% macrophage	31.62±1.17	34.51±6.9	P=0.66
% lymphocytes	6.28±2.52	7.1±2.95	P=0.27
% epithelial cells	7.31±3.95	7.57±3.23	P=0.79

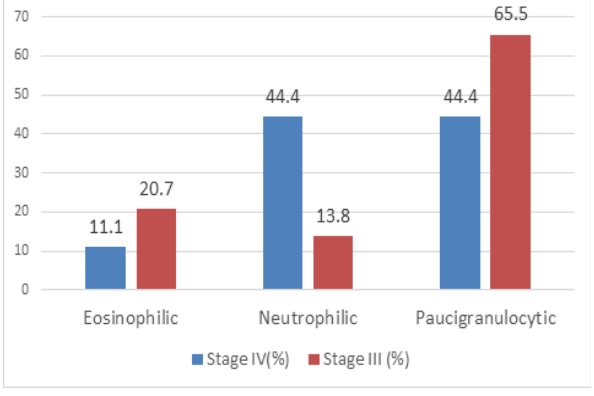

Fig. 2 Sputum cell stratification according the stage of diseases

The average initial blood leukocytosis was 11777± 5233, after 21 days in 2630± 8593 (P<0.0001). AECOPD leukocyte formula (%) and after 21 days resulted respectively: rod nuclear 6.63±3.68 and 2.79±2.51 (P<0.0001), neutrophils 12.38±72.41 and 60.68±10.12 (P<0.0001), eosinophilis 2.1±2.69 and 3.81±3.49 (P=0.0045), basophils 0.21±0.27 and 0.22±0.28 (P=0.8478), monocytes 8.15±4.53 and 7.49±3.15 (P=0.3727), lymphocytes 17.07±8.80 and 27.62±8.19 (P<0.0001). (Tab. 4) There was increased level of leukocytes in 35 (62.5%) patients, rod nuclear 26 (46.4%), neutrophils 28 (50%), eosinophilis 7(12.5), basophils 1(1.8%), monocytes 21(37.5%), and lymphocytes 1(1.8%).

Tab. 4 Leukocytic formula at AECOPD and 21 days after

Leukocytic formula	Means± Std. Deviation AECOPD	Means± Std. Deviation 21 days after	comparisons of means (t-test)
Nr of leukocytes	11.777±5.233	8.593±2.630	P<0.0001
% Rod nuclear	6.63±3.68	2.79±2.51	P<0.0001
% Neutrophils	72.41±12.38	60.68±10.12	P<0.0001
% Eosinophilis	2.1±2.69	3.81±3.49	P=0.0045
% Basophils	.21±.27	.22±.28	P=0.8478
% Monocytes	8.15±4.53	7.49± 3.15	P=0.3727
% Lymphocytes	17.07±8.80	27.62±8.19	P<0.0001

In the Table 5 is presented leukocytic formula at AECOPD according to the stage of diseases.

Tab. 5 Leukocytic formula at AECOPD according to the stage of diseases

Leukocytic formula (103)	Means± Std. Deviation stage IV	Means± Std. Deviation Stage III	comparisons of means (t-test)
Nr of leukocytes	13.862±6.594	9.855±2.340	P=0.0033
Rod nuclear	1.11±0.97	1.1±0.93	P= 0.2970
Neutrophils	1.18±0.96	0.96±0.98	P=0.96
Eosinophilis	0.22±0.64	0.27±0.7	P=0.1494
Basophils	7.4±0.38	0	-
Monocytes	0.7±0.95	0.82±1.0	P=0.64
Lymphocytes	0.85±0.36	0.72±0.52	P=0.28

Initial neutrophil/lymphocyte ratio (NLR) and 21 days after AECOPD have resulted respectively, 7.464±12.922 (1.04 - 97.9) and 2.509±1.18 (0.71 - 7.09) (p= 0.004). (Fig. 3)

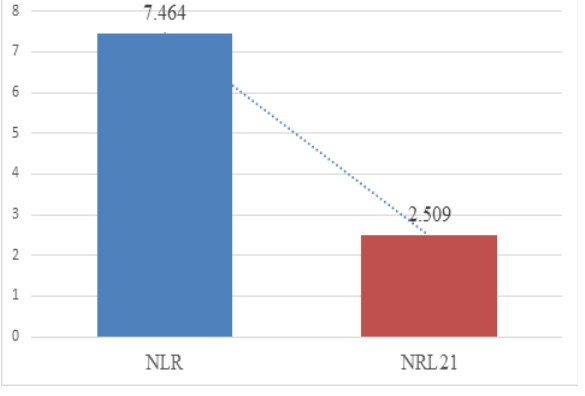

Fig. 3. Initial neutrophil/lymphocyte ratio and 21 days after

Discussion:

Studies of bronchial inflammation of COPD patients have provided contradictory results. A study with bronchial biopsy resulted in a 30 fold increase of eosinophils, small increases in neutrophils and T-lymphocytes (Saetta et al., 1994), whereas in another study with bronchoalveolar lavage, liquid resulted with the neutrophils and eosinophils, but with a more pronounced increase in the neutrophils (Balbi et al., 1997). Noninvasive studies of sputum are more easily conducted, but then again results are contradictory: without changes in cell count (Bhowmik et al. 2000) or increase in lymphocytes, neutrophils and eosinophils (Fujimoto et al., 2005). According to authors, neutrophils results to be connected with the gravity of exacerbations independent of etiology, whereas eosinophilia as an indicator of viral exacerbations (Papi et al., 2006). Mixed pattern of cell elements reflects the heterogeneity of the exacerbations, and partially explains the variability of different inhaler steroid response in COPD. (Footitt J.et al., 2009)

In our study it results that in 33.9% of cases, there has been an increase of eosinophils and according to the stratification of sputum the eosinophilic type has resulted in 16.1% of the patients. The main presentation during exacerbation of bronchial secretion is increased neutrophils (Balbi et al., 1997), which resulted even in our study, where the cellular profile of sputum together with the neutrophilic (28.6%) and paucigranulocitic (55.4%) results in 84% of the cases, which is connected even with the appearance of change of purulence in sputum (Stockley et al., 2001).

Inflammatory characteristic changes, with high levels of macrophages and lymphocytes, are reported in the airways of smokers. These are more pronounced in patients with COPD and represents normal exaggerated response to inhaled toxins. Inflammatory cellular pattern changes in stable and exacerbated condition of asthma and COPD. Neutrophils and macrophages predominate in COPD and their levels correlate with the gravity of the disease and still more during exacerbation. This is in contrast to asthma, where macrophages are thought not to play a role. Lack of neutrophils and presence of eosinophilia in sputum may be an indicator of viral infection during exacerbation of COPD. (Papi et al., 2006) Taken together, these data indicate that non-invasive tests can be used in clinical practice to provide clinical information regarding etiology. Eosinophilic inflammation is generally not associated with COPD. In the stable situation, there is little evidence for the role of eosinophils, except to a specific phenotype of COPD, indicating less expressed emphysema and thickening of the bronchial walls in CT, and a good reaction to corticosteroids (Kitaguchi Y et al., 2006; Brightling CE et al. 2005). During exacerbation of COPD it is recognized that there may be an inflammatory pattern of "asthma-like" with increased number of eosinophils (Barnes PJ, 2000). Increased eosinophils during exacerbation of COPD, at least partly, is associated with viral infections (Papi A., 2000; Rohde G et al., 2004). Although neutrophils are related to the presence of bacteria, neutrophils also increased during exacerbation associated with viral infections and those without demonstrable pathogens. (Papi et al., 2006)

From our data, after 21 days of exacerbation, in the sputum there is a decrease in the number of total cells, a decrease in the neutrophils and eosinophils percentage, as well as an increase in the percentage of lymphocytes. The cell count in sputum as well as their structure expressed in percentage in AECOPD has significant differences with the results after 21 days (P<0.0001). As it results even from our data, at the time of the exacerbation remission, there is a decrease in the number of neutrophils, which is related to the eradication of the bacteria from the sputum (White et al., 2003). During a COPD exacerbation, great observing studies haven't shown a significant increase of macrophages in sputum, or the bronchial tissues, even as a percentage of the total cell and neither as an absolute increment of cells (Bhowmik et al., 2002; Papi et al., 2006). In our study the result is different – an increase of macrophages in the exacerbation.

There has been a significant difference in the average number of blood leucocytes in AECOPD and 21 days after (P<0.0001). Just so, the leucocyte formula (%) in AECOPD and after 21 days results in a significant drop of rod nuclear (P<0.0001), neutrophils (P<0.0001), an increase of eosinophils (P=0.0045) and of lymphocytes (P<0.0001). With the improvement of the exacerbations there is a significant decrease of the leucocyte numbers, mainly as a consequence of the neutrophils dropping. The number of neutrophils in blood increases with the systemic inflammation. The increased number of neutrophils is connected to the progression of COPD (Sinden et al., 2010). Our data matches the ones from the studies where the leucocyte levels have resulted with significant statistically increase in the patients with COPD exacerbations, compared to the ones in remission.

A high number of leukocytes does not necessarily indicate infection; the number of leukocytes can be increased by physiological stress, the use of steroids or beta-agonists. One indicator used in the evaluation of inflammation in COPD is the neutrophil/lymphocyte ratio (NLR). NLR value is found significantly higher in the COPD patients with stable and exacerbated than the controls (respectively, p <0.001, p <0.05 level). (n et al., 2016)

As the physiological response of leukocytes in the blood to stressful factors is the increase in number of neutrophils and decrease of lymphocytes, the ratio of these two elements with each other is used in intensive care practice. (Zahorec R, 2001) As the physiological response of leukocytes in the blood to stressful factors is the increase in number of neutrophils and decrease of lymphocytes, the ratio of these two elements with each other is used in intensive care practice. In various studies NLR was evaluated for a possible role in periods of chronic inflammatory diseases.

Our results suggest that matching the literature, the NLR can be considered like a reliable indicator and simple in the determining of inflammation growth in patients with COPD. Furthermore, NLR can be useful for discovering early acute exacerbations that are possible in COPD patients (n et al., 2016).

Conclusion: Diagnosis of AECOPD is supported by increased sputum inflammation as proxy of airways inflammation, and increased systemic inflammation as demonstrated by increased number of blood cells. AECOPD inflammation is more evident in stage IV of the diseases.

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