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Relation between airway cellular and bacterial findings and severity of COPD exacerbations: A multicentric study

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ABSTRACT

Aim: To evaluate the relationships between sputum and bronchoalveolar lavage (BAL) cellular and bacterial findings and severity of exacerbation of chronic obstructive pulmonary disease (ECOPD).

Patients & methods: A cross-section study was conducted on 307 patients with ECOPD. They underwent sputum and BAL inflammatory cell count and bacterial culture.

Results: Patients with severe ECOPD have significantly higher neutrophils percentage (neut.%), lower lymphocytes percentage (lymph.%), lower eosinophils percentage (eosin.%) and higher neutrophil/lymphocyte ratio (NLR) as compared to patients with mild ECOPD. It was also shown that patients with severe ECOPD had significantly higher BAL neut.%, lower lymph.%, lower eosin.%, and higher NLR as compared to the other two subgroups. Also, patients with severe ECOPD have significantly higher frequency of cases with monomicrobial (71.30% vs. 36.10%) and polymicrobial (21.25% vs. 2.10%) growths in comparison to patients with mild ECOPD.

Conclusions: Cellular and bacterial findings in sputum and BAL are related to severity of ECOPD.

Keywords: ECOPD, exacerbation severity, biomarkers, inflammation, sputum, bronchoalveolar lavage

INTRODUCTION

Exacerbation of chronic obstructive pulmonary disease (ECOPD) have significant impact on healthcare budgets, quality of life and death rates with above one-fifth of ECOPD hospitalized patients are dying within one year after discharge [1-3]. ECOPD are frequently caused by certain respiratory microorganisms even in the absence of proof of inflammation [4]. In this regard, the chief challenges are the exact identification of responsible bacteria and the appropriate use of antibiotics [5].

Usually, during development of ECOPD, the inflammatory cells such as neutrophils, eosinophils, macrophages, and lymphocytes are recruited by the pro-inflammatory stimuli causing damage and remodeling of lung tissues [1, 6]. The relation between the recruited inflammatory cells and the grades of functional damage of the lungs has not been elucidated in patients with ECOPD. Many research focus on the neutrophil and eosinophils percentage in blood but not the phlegm or bronchoalveolar lavage (BAL) [7].

Considering the significant influence of exacerbations on the ordinary course of ECOPD, it is of the greatest importance to identify the risk factors concomitant with exacerbations and its severity [8]. Therefore, we conducted this study to evaluate the relationships between sputum and BAL microbial and cellular findings and severity of ECOPD.

PATIENTS & METHODS

The present multicentric cross-sectional study was conducted during the period from April to August 2023. We obtained the approval from medical research ethics committee of Al-Azhar University Faculty of Medicine (Approval No. 1900). Informed consent was obtained from all participants. The study included 307 with ECOPD clinically, ECOPD was defined as a deterioration of pulmonary symptoms that direct the patients to ask health-care services. ECOPD severity was classified into mild, moderate, or severe based on the standard guidelines [9]. Patients were excluded if they had associated respiratory disease, acute or chronic infection, autoimmune diseases or malignant tumors.

Spirometry was performed for measurement of forced expiratory volume in the first second/forced vital capacity ratio and forced mid-expiratory flow 25.00%-75.00%. The sputum and BAL samples were collected at admission and before initiation of antimicrobial therapy. The sputum was collected

Table 1. Relation between severity of ECOPD & clinical data

Items	ECOPD severity			p-value
	Mild (n=94)	Moderate (n=133)	Severe (n=80)	
Age (years) mean±standard deviation	55.4±4.6	57.6±4.5	61.4±4.2*#	0.001
Sex (n [%])				
Male	72 (76.60)	109 (82.00)	65 (81.25)	0.663
Female	22 (23.40)	24 (18.00)	15 (18.75)	
BMI (kg/m^2) mean±standard deviation	25.4±3.8	27.2±4.1	28.8±4.6*	0.042
Smoking (n [%])				
Current smokers	62 (66.00)	112 (84.20)*	74 (92.50)*	0.001
Ex-smokers	23 (24.50)	11 (8.30)*	3 (3.75)*	
Non-smokers	9 (9.50)	10 (7.50)	3 (3.75)	
Smoking index (p/y) mean±standard deviation	23.8±16.2	26.8±18.9	28.8±13.6*	0.030
Pulmonary functions mean±standard deviation				
FEV$_1$/FVC ratio	65.2±2.8	62.3±4.6	59.1±5.0*	0.001
FEV1%	64.5±9.7	52.8 ±12.0*	44.4±6.9*#	0.011
FVC%	73.8±8.3	64.0 ±10.3*	57.0±5.7*#	0.002
FEF 25.00%-75.00%	52.9±7.5	44.3 ±10.8*	37.9±9.5*#	0.001

Note. *Significant results vs. mild ECOPD; #Significant results vs.s moderate ECOPD; BMI: Body mass index; ECOPD: Exacerbation of chronic obstructive pulmonary disease; FEF: Forced expiratory flow; FEV$_1$: Forced expiratory volume in 1st second; & FVC: Forced vital capacity

according to recommendations in [10]. The flexible bronchoscopy was used for BAL collection. BAL was performed by instillation of sterilized isotonic saline solution (70 ml) with direct suction into a hygienic sterilized polypropylene bottle. Both BAL and sputum samples were transported immediately to the laboratory and processed within one hour. Then, liquefied sputum and BAL were centrifuged at an appropriate speed, suspended, and examined using automated cell counter and hematological analyzer. The neut.%, lymph.% and eosin.% were reported. Gram stain was done to evaluate quality of the sputum samples; samples containing ≥10 leucocytes and <25 squamous epithelial cells per low-power field (<10/LPF) were considered of good quality [11]. The slides were examined underneath oil immersion (1,000X) amplification for microorganisms followed by culturing. The sputum and BAL cultures were made on usual media designed for culturing and identification of respiratory bacteria including blood agar, chocolate agar, and MacConkey agar. We did quantitative cultures by the calibrated loop method. The specimen (0.1 ml) was overlaid onto solid media and colony forming unit (CFU) were calculated after 24 hours of incubation [12]. To avoid overestimation of bacterial etiology, we considered that specimens with CFU<10^4/ml as colonization and omitted from the research, while specimens with CFU≥10^4/ml considered as an infection. As regards gram-positive bacteria: catalase, mannitol fermentation and DNase tests were used, and for gram-negative bacteria: triple sugar iron agar), motility indole ornithine medium, indole, citrate, urease and oxidase tests were used [13].

Data was statistically analyzed by SPSS program version 17.0 (SPSS Inc., Chicago, USA). We used Shapiro-Wilk test for analysis normality of the considered variables. Chi-square (X^2) test was used to assess the comparisons between the groups as regards qualitative data while one-way ANOVA was used for comparison of quantitative data. Statistical significance was set at p-value<0.05.

RESULTS

The present study included 94 patients (30.60%) with mild ECOPD, 133 patients (43.30%) with moderate ECOPD and 80 patients (26.10%) with severe ECOPD. Patients with severe ECOPD are significantly older with higher body mass index as compared to those with mild ECOPD. In addition, they comprised significantly higher frequency of current smokers and higher smoking index in comparison to patients with mild ECOPD. Moreover, they have more deteriorated pulmonary functions as compared to other subgroups (**Table 1**).

Comparison between the studied subgroups regarding sputum cellular findings revealed that patients with severe ECOPD have significantly higher neut.%, lower lymph.%, lower eosin.% and higher neutrophil/lymphocyte ratio (NLR) as compared to patients with mild ECOPD. It was also shown that patients with severe ECOPD had significantly higher BAL neut.%, lower lymph.%, lower eosin.% and higher NLR as compared to the other two subgroups (**Table 2**).

Regarding sputum bacterial isolates, it was found that patients with severe ECOPD has significantly higher frequency of cases with monomicrobial (71.30% vs. 36.10%) and polymicrobial (21.25% vs. 2.10%) growths in comparison to patients with mild ECOPD (**Table 3**).

In respect to bacterial isolates from BAL culture, it was also found that patients with severe ECOPD expressed significantly higher frequency of polymicrobial growth in comparison to the other two subgroups (**Table 4**).

Table 2. Relation between severity of ECOPD & cellular findings

Items	ECOPD severity			p-value
	Mild (n=94)	Moderate (n=133)	Severe (n=80)	
Sputum mean±standard deviation				
Neutrophils %	79.8±3.4	83.3±9.7	87.9±2.7*	0.001
Lymphocytes %	3.5±0.9	2.4±1.1	1.5±0.6*	0.001
Eosinophils %	3.9±1.4	2.8±1.7	1.7±0.6*	0.001
NLR	25.0±12.0	45.0±25.2*	69.0±24.2*#	0.001

Note. *Significant results vs. mild ECOPD; #Significant results vs.s moderate ECOPD; BAL: Bronchoalveolar lavage; ECOPD: Exacerbation of chronic obstructive pulmonary disease; & NLR: Neutrophil/lymphocyte ratio

Table 2 (continued). Relation between severity of ECOPD & cellular findings

Items	ECOPD severity			p-value
	Mild (n=94)	Moderate (n=133)	Severe (n=80)	
BAL mean±standard deviation				
Neutrophils %	24.5±10.6	38.0±17.1*	54.6±15.0*#	0.022
Lymphocytes %	19.9±4.4	13.6±5.9*	8.2±3.2*#	0.011
Eosinophils %	1.2±0.9	0.6±0.6*	0.3±0.2*#	0.001
NLR	1.5±1.6	4.2±4.0*	8.5±5.3*#	0.002

Note. *Significant results vs. mild ECOPD; #Significant results vs.s moderate ECOPD; BAL: Bronchoalveolar lavage; ECOPD: Exacerbation of chronic obstructive pulmonary disease; & NLR: Neutrophil/lymphocyte ratio

Table 3. Relation between severity of ECOPD & bacterial organisms isolated from sputum culture

Items	ECOPD severity			p-value
	Mild (n=94)	Moderate (n=133)	Severe (n=80)	
Negative bacterial growth n (%)	**58 (70.20)**	**19 (14.30)***	**9 (11.25)***	**0.019**
Monomicrobial growth n (%)	**34 (36.10)**	**84 (63.20)***	**57 (71.30)***	**0.014**
Strept.pneumoniae	12 (12.70)	14 (11.20)	3 (3.70)	
Staph.aureus	10 (10.60)	25 (18.80)	12 (15.00)	
K.pneumoniae	6 (5.30)	22 (16.50)*	14 (17.50)*	0.045
E.coli	1 (1.00)	10 (7.50)*	16 (20.00)*#	0.002
Acinetobacter	1 (1.00)	-	5 (6.25)	
P.aeruginosa	-	-	3 (3.70)	
H.influenzae	4 (4.20)	12 (9.00)	4 (5.60)	
Enterobacter	-	1 (0.75)	-	0.518
Polymicrobial growth n (%)	**2 (2.10)**	**12 (9.00)***	**17 (21.25)***#	**0.031**
Haemophilus influenzae+Staph.aureus	-	-	5 (6.25)*#	0.013
Haemophilus influenzae+Strept.pneumoniae	1 (1.00)	7 (5.20)	6 (7.50)	0.304
P.aeruginosa+ K.pneumoniae	-	1 (0.75)	1 (1.25)	0.579
K.pneumoniae+Staph.aureus	1 (1.00)	4 (3.00)	1 (1.25)	0.743
K.pneumoniae+E.coli	-	-	4 (5.00)*#	0.013

Note. *Significant results vs. mild ECOPD & #Significant results vs.s moderate ECOPD

Table 4. Relation between severity of ECOPD & bacterial organisms isolated from BAL culture

Items	ECOPD severity			p-value
	Mild (n=94)	Moderate (n=133)	Severe (n=80)	
Negative bacterial growth n (%)	**32 (34.00)**	**18 (13.50)***	**7 (8.75)***	**0.021**
Monomicrobial growth n (%)	**35 (37.20)**	**73 (54.90)***	**31 (38.75)**	**0.042**
Strept.pneumoniae	18 (19.10)	21 (15.70)	1 (1.25)*#	0.015
Staph.aureus	12 (12.70)	17 (12.70)	6 (7.50)	0.609
K.pneumoniae	1 (1.00)	15 (11.20)	9 (11.20)	0.068
E.coli	-	6 (4.50)	9 (11.20)*#	0.019
Acinetobacter	1 (1.00)	-	1 (1.20)	0.457
P.aeruginosa	-	1 (0.75)	-	0.518
H.influenzae	3 (3.10)	12 (9.00)	5 (6.20)	0.336
Enterobacter	-	1 (0.75)	-	0.518
Polymicrobial growth n (%)	**27 (28.70)**	**42 (31.60)**	**42 (52.50)***#	**0.002**
H.influenzae+Staph.aureus	-	1 (0.75)	3 (3.70)	0.229
H.influenzae+Strept.pneumoniae	14 (14.80)	23 (17.30)	5 (2.20)	0.082
K.pneumoniae+Staph.aureus	7 (7.40)	5 (3.70)	5 (6.20)	0.470
K.pneumoniae+Acinetobacter spp.	-	1 (0.75)	3 (3.70)	0.229
E.coli+Acinetobacter spp.	-	1 (0.75)	-	0.518
H.influenza+K.pneumoniae+Staph.aureus	-	3 (2.20)	10 (12.50)*#	0.001
E. coli+P.aeruginosa+K.pneumoniae	6 (6.30)	6 (4.50)	9 (11.20)	0.298
Acinetobacter+K.pneumoniae+Strept.pneumoniae	-	2 (1.50)	7 (8.70)*#	0.015

Note. *Significant results vs. mild ECOPD & #Significant results vs.s moderate ECOPD

DISCUSSION & CONCLUSIONS

The present study revealed that severe ECOPD patients have significantly higher neutr.% and NLR with significantly lower lymph.% in sputum and BAL. These findings indicate that severe ECOPD is based on neutrophil-dominated innate immunity. One possible explanation of our findings is that the decreased lymphocytes indicate impaired adaptive immunity in the respiratory tract, which made patients more susceptible to infections. In line with these conclusions, many other studies reported that predominantly neutrophilic ECOPD patients tend to present by more severe exacerbation [1, 7, 14, 15].

Moreover, the study in [7], with follow-up of COPD patients, reported that after the first year of follow-up, patients with high sputum neutrophil proportions had increased risk of severe exacerbation. Likewise, it was found that patients with severe ECOPD have increased NLR in BAL [15].

On contrast, it was documented that higher neutrophils count in the airways is one of the main contributors against bacterial infection and other microorganisms [16, 17]. The double-sword edge of neutrophils in ECOPD is not fully clarified and still warrant future studies to be explored.

In harmony with our findings, it was reported that ECOPD patients with low lymphocyte had more severe exacerbation that requires longer hospital stays, longer ventilation times, and higher in-hospital mortality [18]. In addition, a study analyzing ECOPD patients admitted to ICU found that the non-survivor patients had significantly lower peripheral lymphocyte count than surviving patients. In addition, the present study found significantly lower eosinophils count in patients with severe ECOPD. Previous studies agree with us as the presence of the peripheral eosinophilic endotype was found to be higher in the mild-to-moderate ECOPD [1, 19].

The present study found that in patients with severe exacerbations, 88.75% and 91.25% of patients have significant bacterial growth in sputum and BAL, respectively. *K.pneumoniae* and *E.voli* were the most common isolates. These findings point out that there was a major shift of bacterial infection in severe ECOPD from less pathogenic species identified in mild exacerbation to a more virulent species, which are difficult to treat. Accordingly, we suggest that severe ECOPD patients might need a different antibiotic therapy than those with mild or moderate exacerbations. This bacterial shift may be attributed to the fact that severe ECOPD patients have significant impairment of lung defense mechanisms, which enhance colonization and proliferation of pathogenic bacteria in the airways. In accordance with these findings, a Korean study analyzed patients with severe ECOPD and found a direct correlation between severity of airway obstruction and bacterial identification rate, with *P.aeruginosa* and *strept.pneumoniae* showing strongest association [20]. Also, it was reported that a highly significant relationship was detected between severity of ECOPD and *K.pneumoniae* isolation [21]. Other investigations of the relationship between bacteriologic etiology and lung function in patients with ECOPD demonstrated that *P.aeruginosa* and *enterobacteriaceae* were predominant in patients with severely impaired lung function [22].

In conclusion, cellular and bacterial findings in sputum and BAL are related to severity of ECOPD.

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Declaration of interest: No conflict of interest is declared by authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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