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Comparison of Biomarkers in Blood and Urine According to Phenotypes in Chronic Obstructive Pulmonary Disease: Cross-Sectional Research

Kronik Obstrüktif Akciğer Hastalığında Fenotiplere Göre Kanda ve İdrarda Biyobelirteçlerin Karşılaştırılması: Kesitsel Araştırma

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ABSTRACT Objective: Chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity and mortality worldwide. Biomarkers have been recognized as an important tool in assessing COPD patients. In this study, we aimed to investigate the possible relationship between certain biomarkers and different COPD phenotypes. **Material and Methods:** Between January 1, 2017-December 31, 2017, a total of 85 patients who were admitted to the Çanakkale Onsekiz Mart University Faculty of Medicine Hospital Chest Diseases Outpatient Clinic with a diagnosis of COPD according to the Global Initiative for Chronic Obstructive Lung Disease 2017 Guidelines were included in this study. All patients filled in the COPD Assessment Test and modified Medical Research Council questionnaires. Patients were divided into 4 phenotypes: emphysema, chronic bronchitis, frequent exacerbation, and asthma COPD overlap syndrome (ACOS). Levels of C-reactive protein (CRP), desmosine, fibronectin, eotaxin, and interleukin (IL)-2 were measured and compared between the phenotypes. **Results:** The mean age was 64.56 years and 92.9% of the study population were males. Of 85 patients, 43 had emphysema, 13 had frequent exacerbation, 15 had chronic bronchitis, and 14 had ACOS phenotype. Blood fibronectin, eotaxin, and urine desmosine levels were significantly lower in patients with ACOS phenotype compared to the other phenotypes. In patients with the frequent exacerbation phenotype, the CRP level was significantly higher than that of the other three phenotypes. The IL-2 levels were similar in all phenotypes. **Conclusion:** Our study results suggest that these biomarkers may be useful in the differential diagnosis of COPD phenotypes.

Keywords: Pulmonary disease; chronic obstructive; phenotype; biomarkers

ÖZET Amaç: Kronik obstrüktif akciğer hastalığı (KOAH), ülkemiz ve tüm dünya ülkelerinde önemli mortalite ve morbidite nedeni olan bir hastalıktır. Biyomarker ölçümleri KOAH'ın değerlendirilmesinde giderek daha önemli bir hâl almaktadır. Çalışmamızın amacı, biyomarker düzeylerinin KOAH fenotipleri arasında anlamlı farklılık gösterip göstermediğini araştırmaktır. **Gereç ve Yöntemler:** Çalışmamıza, 1 Ocak 2017-31 Aralık 2017 tarihleri arasında, Çanakkale Onsekiz Mart Üniversitesi Tıp Fakültesi Hastanesi Göğüs Hastalıkları Polikliniğine başvuran, KOAH tanılı veya "Global Initiative for Chronic Obstructive Lung Disease" 2017 kılavuzuna göre KOAH tanısı konulan 85 hasta alınmıştır. Hastalar, KOAH değerlendirme Testi ve "modifiye Medical Research Council" anketleri doldurmuştur. Amfizem, sık alevlenenler, kronik bronşit ve astım-KOAH ortak sendromu (AKOS) olmak üzere 4 fenotipe ayrılmıştır. Hastalardan venöz kan örneği ve idrar örneği alınmıştır. C-reaktif protein (CRP), desmozin, eotaksin, fibronektin ve interlökin-2 (IL-2) biyobelirteç düzeyleri belirlenmiştir. **Bulgular:** Araştırmaya katılan hastaların yaş ortalaması 64,56'dır. Örneklemin %92,9'u erkektir. Toplam 85 hastanın 43'ü amfizem, 13'ü sık alevlenen, 15'i kronik bronşit, 14'ü AKOS fenotipindedir. Kan fibronektin, eotaksin ve idrar desmozin düzeyi AKOS fenotipindeki hastalarda diğer fenotiplerin tümünden anlamlı derecede düşüktür. Sık alevlenen fenotipindeki hastalarda CRP düzeyi diğer fenotiplerin tümünden anlamlı derecede yüksektir. Fenotipler arasında, IL-2 düzeyi ortalamaları açısından fark yoktur. **Sonuç:** Çalışmamız sonucunda, KOAH fenotipleri arasında biyobelirteç düzeyleri açısından anlamlı farklılıklar bulunmuş olup; çalışmamızın, bu konuda yapılacak daha kapsamlı araştırmalara ışık tutacağı kanaatindeyiz.

Anahtar Kelimeler: Akciğer hastalığı; kronik obstrüktif; fenotip; biyobelirteçler

Chronic obstructive pulmonary disease (COPD) usually occurs as a result of intense exposure to noxious particles or gases which leads to abnormalities in the airways and/or alveoli and is characterized with

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permanent airflow obstruction and respiratory symptoms.[1] It is a common, preventable, and treatable disease.

Currently, COPD is recognized as an umbrella definition which includes different clinical, physiopathological, and radiological phenotypes.[2] A phenotypic classification may provide important clues regarding the differential diagnosis among patients with separate features and identification of patients with similar prognosis and treatment response.[3]

Biomarkers may be helpful to gain a different view of the immunopathogenesis of COPD.

Although it is still unclear whether the blood C-reactive protein (CRP) level is an indicator of the intensity of inflammation or is a part of the inflammation itself in COPD, blood CRP levels, are correlation between airflow limitation and disease severity [Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages, BODE index (body mass index, obstruction, dyspnea, exercises capacity)] and has been shown to be associated with COPD mortality and morbidity.[4,5]

Fibronectin contributes to the continuation of tissue damage by increasing matrix metalloproteinase (MMP) activity and is a useful biomarker in identifying risk layers in COPD patients because of the inverse correlation of fibronectin with lung function in COPD patients.[6]

In COPD, interleukin (IL)-2 is thought to contribute to airflow limitation and cell damage.[7]

CXCL9 (MIG), CXCL10 and eotaxin1 are secreted from epithelium and macrophage stimulated by irritant inhalation. Proteases such as neutrophil elastase or MMP9 are secreted from the collected inflammatory cells. These molecules emphysema with elastin degradation and alveolar wall destruction; they also cause chronic bronchitis with increased secretion in goblet cells and submucosal glands.[8]

Previous research has demonstrated that urinary desmosine is elevated in all patients with COPD who are never smokers or who smoke without airway obstruction. Urine desmosine is a biomarker sensitive to lung elastin catabolism. Urinary desmosine can be used to identify individuals at risk of developing emphysema and to evaluate the efficacy of therapeutic interventions.[9]

Several studies have suggested that using a single biomarker does not provide adequate information about the disease.[5] In the present study, therefore, we intended to investigate the difference in blood fibronectin, CRP, eotaxin-1 and urine desmosine levels among four different COPD phenotypes and the role of these biomarkers in the pathophysiology and treatment response.

MATERIAL AND METHODS

Eighty five patients with established or newly diagnosed stable COPD according to the GOLD guidelines, who were admitted to the chest diseases outpatient clinic of our University, Faculty of Medicine Hospital between January 2017-December 2017, were included in this study. Patients with other major diseases were excluded. A written informed consent was obtained from each patient. Ethics committee approval was received from the Çanakkale Onsekiz Mart University Clinical Research Ethics Committee for approval of the study (date: February 15, 2017, no: 2011-KAEK-27/2016-E.146787). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Weight measurements for pulmonary function tests were performed while the patients were wearing light sportive clothes and the heights of the participants were measured for pulmonary function tests in the standing upright position with barefoot and during deep inspiration using a thin rod parallel to the ground and with a 0.5 cm sensitivity. The pulmonary function tests were performed in accordance with the American Thoracic Society criteria using a Viasys (VIASYS Healthcare, Höchberg, Germany) spirometer twice and the best measurement was recorded. For the bronchodilatation test, four puffs of salbutamol (400 μg) was administered by inhalation and percent forced expiratory volume in one second (FEV1%)/percent forced vital capacity (FVC%), FEV1, and FVC were measured at 15 min. According to the GOLD 2017 guidelines, COPD staging was performed. Modified Medical Research Council

(mMRC) questionnaires and COPD Assessment Test (CAT) were filled. Using a four-detector Toshiba Asteion (Toshiba Medical Systems Corporation, Otawara, Japan) device, 1 mm thick sections were taken at every 10 mm and high resolution computed tomography examinations were performed. Demographic data including age, sex, residence, and smoking duration; anthropometric measurements including height and weight; hospital admission and hospital or intensive care unit stay during the last year were recorded. The patients were classified according to these data.

In our study, phenotype definitions were made as follows:

1. "Emphysema" phenotype; are patients with clinical, radiological and functional diagnosis of emphysema, the main complaints of which are shortness of breath and limitation of effort. The body mass index of patients with emphysema is low. There may be fewer exacerbations in the emphysema phenotype than in the chronic bronchitis phenotype, but patients with frequent exacerbations are seen in the advanced stages of the disease. The annual loss of FEV1 is higher in patients with severe emphysema and adversely affects the prognosis.[10]

2. Frequent exacerbations (>2 times a year), a second phenotype, cause decreased quality of life, increased mortality, and further reduction in lung function. On average, frequent exacerbations had significantly higher airway obstruction for the mMRC dyspnea scale and increased body mass index, Airway Obstruction, Dyspnea and Exercise (BODE) index compared to less frequent exacerbations.[2,11]

3. The "chronic bronchitis" phenotype defines patients who have sputum production and cough for 2 consecutive years, especially in the winter months, for 3 months or more, in accordance with the definition of chronic bronchitis. Mucus hypersecretion in COPD leads to more intense airway inflammation and increased respiratory tract infections. Therefore, more exacerbations are seen in the chronic bronchitis phenotype.[12]

4. The phenotype with "COPD-asthma overlap (ACO)" is defined as the presence of symptoms or signs of reversibility with airway obstruction that is not completely reversible.

In some guidelines, it is also defined as the presence of a pronounced asthma component in patients with COPD or as asthma complicated by COPD. For the definition of this phenotype, 3 major and 3 minor criteria were determined and 2 major criteria or 1 major and 2 minor criteria were recommended for diagnosis.[13]

Major criteria;

1. High positivity with bronchodilator test (>15% and >400 mL increase in FEV1),

2. Eosinophilia in sputum,

3. History of asthma.

Minor criteria;

1. High total immunoglobulin-E levels,

2. History of atopy,

3. Positive bronchodilator test performed at 2 different times (>12% and >200 mL increase in FEV1); however, the validity of these criteria should also be demonstrated.[13]

The measurement of CRP was performed using nephelometric method with the IMMAGE 800 protein chemistry analyzer (Beckman Coulter Diagnostics, CA, USA). The blood and urine samples were centrifuged [blood samples: for 15 min at 4,000 revolutions per minute (rpm), urine samples: for 20 min at 4,000 rpm] within 30 min after being collected and stored at -80ºC, until analysis. Blood IL-2, fibronectin, and eotaxin-1; and urine desmosine levels were measured using the enzyme-linked immunosorbent assay (ELISA) with a Biotek ELx808 device (BioTek Instruments, Winooski, VA, USA) and the results were obtained from a microplate reader.

STATISTICAL ANALYSIS

IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The normality of distribution was analyzed by the Shapiro-Wilk and Kolmogorov-Smirnov tests for continuous variables. Continuous data were presented in mean±standard deviation (SD) or median (minimum-maximum) values, while categorical data were expressed in number and frequency.

Continuous data were compared between 2 groups using the independent samples t or Mann-Whitney U tests according to the distribution of data. Fisher Freeman Halton test was used to compare qualitative data. A p value of <0.05 was considered statistically significant with 95% confidence interval.

RESULTS

The mean age of the patients was 64.56 years. The mean smoking pack year was 35.05. Of the patients 31.8%, 32.9%, 28.2%, and 7.1% were of COPD Stage 1, 2, 3, and 4, respectively. The mMRC stage was 0-1 in 83.5% and ≥2 in 16.5%. The CAT stage was <10 in 31.8% and ≥10 in 68.2% (Table 1).

The rates of the patients according to age and gender did not show a statistically significant difference according to the phenotypes (p>0.05) (Table 1).

The number of pack-years of cigarettes did not differ statistically significantly according to the phenotypes (p>0.05) (Table 1).

COPD stage rates do not differ statistically significantly according to the phenotypes (p>0.05) (Table 1).

mMRC stage rates differ statistically significantly according to the phenotypes (p<0.05); when the significance was examined, the rate of mMRC 2 and above in the Frequent exacerbation group was found to be statistically significantly higher than the other groups. No significant difference was found between the other phenotypes (Table 1).

CAT stage rates do not differ statistically significantly according to the groups (p>0.05) (Table 1).

Table 2 shows the comparison of biomarker levels between phenotypes. The mean eotaxin, fibronectin and desmosine levels were significantly lower than the other phenotypes in the asthma COPD overlap syndrome (ACOS) phenotype. However, the CRP level was significantly higher in the frequent exacerbation phenotype than the other phenotypes.

DISCUSSION

The CRP level increases in parallel with the increasing GOLD stage, CAT score, and mMRC dyspnea score.[14-17] Díaz et al. found a higher CRP level in COPD group compared to the control group, but did

TABLE 1: The descriptive characteristics of the patient.

	Phenotypes					
	Emphysema n=43	Frequent exacerbation n=13	Chronic bronchitis n=15	ACOS n=14	Total	p value
Age (year), mean±SD	63.44±7.42	68.15±11.13	65.20±9.01	64.00±9.10	64.56±8.62	[a]0.658
Sex, n (%)						
Male	40 (93)	12 (92.3)	14 (93.3)	13 (92.9)	79 (92.9)	[b]1.000
Female	3 (7)	1 (7.7)	1 (6.7)	1 (7.1)	6 (7.1)	
Smoking (pack/year)	37.67±12.27	35.00±14.14	29.40±10.27	33.07±15.55	35.05±12.99	[a]0.226
COPD stage						
Stage 1	14 (32.6)	1 (7.7)	5 (33.3)	7 (50)	27 (31.8)	[b]0.219
Stage 2	15 (34.9)	6 (46.2)	4 (26.7)	3 (21.4)	28 (32.9)	
Stage 3	11 (25.6)	3 (23.1)	6 (40)	4 (28.6)	24 (28.2)	
Stage 4	3 (7)	3 (23.1)	0 (0)	0 (0)	6 (7.1)	
mMRC stage						
mMRC 0-1	37 (86)	7 (53.8)	13 (86.7)	14 (100)	71 (83.5)	[b]0.014*
mMRC ≥2	6 (14)	6 (46.2)	2 (13.3)	0 (0)	14 (16.5)	
CAT stage						
<10	13 (30.2)	2 (15.4)	5 (33.3)	7 (50)	27 (31.8)	[b]0.297
≥10	30 (69.8)	11 (84.6)	10 (66.7)	7 (50)	58 (68.2)	

[a]Kruskal Wallis test; [b]Fisher Freeman Halton test; *p<0.05; ACOS: Asthma chronic obstructive pulmonary disease overlap syndrome; SD: Standard deviation; COPD: Chronic obstructive pulmonary disease; mMRC: Modified Medical Research Council; CAT: COPD Assessment Test.

TABLE 2: Biomarker levels according to the phenotypes.

	Phenotypes				
	Emphysema n=43	Frequent exacerbation n=13	Chronic bronchitis n=15	ACOS n=14	
	mean±SD	mean±SD	mean±SD	mean±SD	p value
Fibronectin	52.93±14.97	55.15±10.53	53.80±6.81	42.07±3.73†	0.001
CRP	0.59±0.41	3.23±3.41‡	0.51±0.40	1.04±1.23	<0.001
IL-2	14.23±3.49	15.78±8.62	15.13±4.66	10.94±4.47	0.069
Eotaxin	587.40±147.96	594.46±102.61	1205.73±102.19	468.79±53.96†	<0.001
Desmosine	1278.14±351.13	1290.23±235.63	1205.73±102.19	1053.07±59.33†	0.015

†Significantly lower as compared with the other groups; ‡significantly higher as compared with the other groups; ACOS: Asthma chronic obstructive pulmonary disease overlap syndrome; SD: Standard deviation; CRP: C-reactive protein; IL-2: Interleukin-2.

not find an association between smoking and CRP levels.[18] The CRP level was positively correlated with fat free mass, body mass index, and number of exacerbations during the last year and negatively correlated with FEV1 and the partial pressure of oxygen. In consistent with the aforementioned study, the CRP level was positively correlated with number of exacerbations during the last year in our study. Furthermore, the frequent exacerbation phenotype's mean CRP level was significantly higher than in the other phenotypes. Therefore, this close relationship may be useful to identify patients with this phenotype in whom history taking is troublesome.

Several studies showed significantly higher urine desmosine levels in patients with COPD compared to controls.[9,19,20] A previous study investigated the role of plasma and urine desmosine levels as a biomarker in COPD and compared with healthy individuals, patients with stable asthma, and patients with stable COPD, and those with exacerbation. While blood and urine desmosine levels were higher in COPD patients compared to healthful non-smokers, they were similar in healthful non-smokers, asthma patients, and healthful smokers. These findings suggest that desmosine may be a specific marker for COPD. Urine desmosine levels were associated with COPD exacerbation and blood desmosine levels were elevated in 40% of COPD patients, irrespective of the exacerbation status. The authors speculated that the cause of the positive association between carbon monoxide diffusion test and desmosine levels suggested increased elastin degradation. There was a positive and strong association between blood desmosine levels and age and a negative correlation between carbon monoxide diffusion capacity and desmosine levels. Their findings also suggested that urine desmosine levels increased during exacerbation in COPD and blood desmosine levels increased in stable COPD patients with reduced lung capacity.[21] In the aforementioned study, desmosine levels increased in COPD patients, but there was no significant difference between COPD and asthma patients in this regard.[21] In consistent with these results, in the present study, we found lower urine desmosine levels in patients with ACOS phenotype which shows similar pathophysiological features with asthma.

In a large-scale, prospective cohort study among mild-to-moderate COPD patients with more than 7 years of follow-up, fibronectin/CRP ratio was associated with mortality.[22]

However, it is evident that neither CRP nor fibronectin is specific to the lungs and, therefore, their blood levels may not be correlated with the pathology in the lungs.[22] Kicic et al. investigated the role of abnormal extracellular matrix production and storage by airway epithelial cells in the pathogenesis of asthma.[23] The epithelial cells were obtained by bronchial brushing and were grown in culture. The ability of these cells to close mechanical wounds was examined via microarray, gene expression and silencing, and transcript regulation analysis and the only extracellular component with reduced gene expression was fibronectin in patients with asthma.[23] In the aforementioned study, the airway cells failed to produce fibronectin in response to wounding or cytokine/growth factor stimulation. The authors, hence,

concluded that the reduced capacity of airway cells to produce fibronectin was an important contributor to the dysregulated airway epithelial cell repair in asthma.[23] In our present study, fibronectin levels of patients with ACOS phenotype were significantly lower than the other phenotypes. Among the COPD phenotypes, ACOS is relatively different from other phenotypes given its clinical and pathophysiological similarities to asthma which may be the cause of reduced fibronectin levels in our study.

Several studies showed increased bronchoalveloar lavage, sputum, and blood eotaxin levels in patients with COPD.[24-27] In a study, increased eotaxin levels were observed in patients with stable COPD patients, compared to healthful individuals, and a negative correlation between eotaxin levels and FEV1 was found.[28] D'Armiento et al. also found an association between rapid progression in COPD and high eotaxin levels.[29] Another study showed higher eotaxin levels in COPD patients with exacerbation compared to stable COPD patients and healthy controls.[30] In the aforementioned study, stable COPD patients had also higher eotaxin levels than healthy controls. In the present study, different from previous studies in the literature, eotaxin levels were lower in patients with ACOS phenotypes. In our study, the patients continued their inhaled corticosteroid and beta-agonist treatment; therefore, inhaled corticosteroid might have suppressed eotaxin levels and caused the discrepancy between our study and the others.

Furthermore, it has been suggested that T lymphocytes are associated with the pathogenesis, and progression of COPD, although the exact relationship between T lymphocytes and COPD still remains to be elucidated.[31-34] One study found higher IL-2 levels in stable COPD patients compared to those with rapidly progressing COPD.[29] However, stable COPD patients in the aforementioned study had lower eotaxin levels, compared to healthy controls.[29] Increased IL-2 levels may enhance T cell response and prevent bacterial and viral lung infections, thereby affecting the disease course positively.[35] In the present study, we found similar IL-2 levels in the COPD phenotypes. This can be attributed to the involvement of similar IL-2 pathways in all of these phenotypes.

Since there is no research on biomarker levels among COPD phenotypes in the literature, our study brings a new perspective. However, the limited number of patients and the predominance of male sex is the limitation of our study.

Further studies to determine the same biomarker levels among COPD phenotypes in larger patient groups will contribute to the generalization of our results.

CONCLUSION

Several biomarkers have been used as an adjunct to identify, diagnose and treat diseases in clinical practice. Different phenotypes of COPD are associated with distinct pathophysiological and prognostic features. In this study, we compared the levels of some biomarkers that may play a role in the pathophysiology of COPD, in different phenotypes and found significantly lower blood eotaxin, blood fibronectin and urinary desmosine levels in ACOS patients, compared to other phenotypes. In addition, the CRP levels were significantly higher in the frequent exacerbation phenotype compared to the others, while the mean IL-2 levels were similar in all of the phenotypes. Although further large-scale studies are needed to establish a definite conclusion, we believe our study will improve our understanding of the role of biomarkers in clinical practice.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

This study is entirely author's own work and no other author contribution.

REFERENCES

1. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease 2022 Report. Global Initiative for Chronic Obstructive Lung Disease Inc; 2022. [Link]
2. Corlateanu A, Mendez Y, Wang Y, Garnica RJA, Botnaru V, Siafakas N. "Chronic obstructive pulmonary disease and phenotypes: a state-of-the-art." Pulmonology. 2020;26(2):95-100. [Crossref] [PubMed]
3. Hernández Vázquez J, Ali García I, Jiménez-García R, Álvaro Meca A, López de Andrés A, Matesanz Ruiz C, et al. COPD phenotypes: differences in survival. Int J Chron Obstruct Pulmon Dis. 2018;13:2245-51. [Crossref] [PubMed] [PMC]
4. Leung JM, Sin DD. Biomarkers in airway diseases. Can Respir J. 2013;20(3):180-2. [Crossref] [PubMed] [PMC]
5. Agustí A, Edwards LD, Rennard SI, MacNee W, Tal-Singer R, Miller BE, et al; Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) Investigators. Persistent systemic inflammation is associated with poor clinical outcomes in COPD: a novel phenotype. PLoS One. 2012;7(5):e37483. [Crossref] [PubMed] [PMC]
6. Annoni R, Lanças T, Yukimatsu Tanigawa R, de Medeiros Matsushita M, de Morais Fernezlian S, Bruno A, et al. Extracellular matrix composition in COPD. Eur Respir J. 2012;40(6):1362-73. [Crossref] [PubMed]
7. Zhang Y, Ren L, Sun J, Han F, Guo X. Increased serum soluble interleukin-2 receptor associated with severity of acute exacerbation of chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2021;16:2561-73. [Crossref] [PubMed] [PMC]
8. Hao W, Li M, Zhang Y, Zhang C, Wang P. Severity of chronic obstructive pulmonary disease with 'exacerbator with emphysema phenotype' is associated with potential biomarkers. Postgrad Med J. 2020;96(1131):28-32. [Crossref] [PubMed]
9. Kim C, Ko Y, Kim SH, Yoo HJ, Lee JS, Rhee CK, et al. Urinary desmosine is associated with emphysema severity and frequent exacerbation in patients with COPD. Respirology. 2018;23(2):176-81. [Crossref] [PubMed]
10. Miravitlles M, Soler-Catalu-a JJ, Calle M, Molina J, Almagro P, Quintano JA, et al. A new approach to grading and treating COPD based on clinical phenotypes: summary of the Spanish COPD guidelines (GesEPOC). Prim Care Respir J. 2013;22(1):117-21. [Crossref] [PubMed] [PMC]
11. Wu JJ, Xu HR, Zhang YX, Li YX, Yu HY, Jiang LD, et al. The characteristics of the frequent exacerbator with chronic bronchitis phenotype and non-exacerbator phenotype in patients with chronic obstructive pulmonary disease: a meta-analysis and system review. BMC Pulm Med. 2020;20(1):103. [Crossref] [PubMed] [PMC]
12. Kania A, Krenke R, Kuziemski K, Czajkowska-Malinowska M, Celejewska-Wójcik N, Kuźnar-Kamińska B, et al. Distribution and characteristics of COPD phenotypes-results from the Polish sub-cohort of the POPE study. Int J Chron Obstruct Pulmon Dis. 2018;13:1613-21. [Crossref] [PubMed] [PMC]
13. Bujarski S, Parulekar AD, Sharafkhaneh A, Hanania NA. The asthma COPD overlap syndrome (ACOS). Curr Allergy Asthma Rep. 2015;15(3):509. [Crossref] [PubMed]
14. Mackay AJ, Donaldson GC, Patel AR, Jones PW, Hurst JR, Wedzicha JA. Usefulness of the Chronic Obstructive Pulmonary Disease Assessment Test to evaluate severity of COPD exacerbations. Am J Respir Crit Care Med. 2012;185(11):1218-24. [Crossref] [PubMed]
15. Weiss DJ, Segal K, Casaburi R, Hayes J, Tashkin D. Effect of mesenchymal stromal cell infusions on lung function in COPD patients with high CRP levels. Respir Res. 2021;22(1):142. [Crossref] [PubMed] [PMC]
16. Munuswamy R, De Brandt J, Burtin C, Derave W, Aumann J, Spruit MA, et al. Monomeric CRP is elevated in patients with COPD compared to non-COPD control persons. J Inflamm Res. 2021;14:4503-7. [Crossref] [PubMed] [PMC]
17. Julike K, Tarigan AP, Ganie RA. The relationship between forced expiratory volume 1 (FEV 1) with 25(OH) vitamin D level and hs-CRP in COPD in H. Adam Malik General Hospital Medan. Indonesia Journal of Biomedical Science. 2019;13(1):16-21. [Crossref]
18. Díaz O, Parada A, Ramos C, Klaassen J, Díaz JC, Andresen M, et al. Proteína C Reactiva en la EPOC y su relación con la gravedad de la enfermedad, las exacerbaciones y las comorbilidades [C-Reactive protein levels in patients with chronic obstructive pulmonary disease]. Rev Med Chile. 2012;140:569-78. [Crossref] [PubMed]
19. Devenport NA, Reynolds JC, Parkash V, Cook J, Weston DJ, Creaser CS. Determination of free desmosine and isodesmosine as urinary biomarkers of lung disorder using ultra performance liquid chromatography-ion mobility-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2011;879(32):3797-801. [Crossref] [PubMed]
20. Cocci F, Miniati M, Monti S, Cavarra E, Gambelli F, Battolla L, et al. Urinary desmosine excretion is inversely correlated with the extent of emphysema in patients with chronic obstructive pulmonary disease. Int J Biochem Cell Biol. 2002;34(6):594-604. [Crossref] [PubMed]
21. Huang JT, Chaudhuri R, Albarbarawi O, Barton A, Grierson C, Rauchhaus P, et al. Clinical validity of plasma and urinary desmosine as biomarkers for chronic obstructive pulmonary disease. Thorax. 2012;67(6):502-8. [Crossref] [PubMed] [PMC]
22. Man SF, Xing L, Connett JE, Anthonisen NR, Wise RA, Tashkin DP, et al. Circulating fibronectin to C-reactive protein ratio and mortality: a biomarker in COPD? Eur Respir J. 2008;32(6):1451-7. [Crossref] [PubMed]
23. Kicic A, Hallstrand TS, Sutanto EN, Stevens PT, Kobor MS, Taplin C, et al. Decreased fibronectin production significantly contributes to dysregulated repair of asthmatic epithelium. Am J Respir Crit Care Med. 2010;181(9):889-98. [Crossref] [PubMed] [PMC]
24. Tateno H, Nakamura H, Minematsu N, Nakajima T, Takahashi S, Nakamura M, et al. Plasma eotaxin level and severity of asthma treated with corticosteroid. Respir Med. 2004;98(8):782-90. [Crossref] [PubMed]
25. Paplińska M, Hermanowicz-Salamon J, Nejman-Gryz P, Białek-Gosk K, Rubinsztajn R, Arcimowicz M, et al. Expression of eotaxins in the material from nasal brushing in asthma, allergic rhinitis and COPD patients. Cytokine. 2012;60(2):393-9. [Crossref] [PubMed]
26. Ghosh N, Choudhury P, Kaushik SR, Arya R, Nanda R, Bhattacharyya P, et al. Metabolomic fingerprinting and systemic inflammatory profiling of asthma COPD overlap (ACO). Respir Res. 2020;21(1):126. [Crossref] [PubMed] [PMC]
27. Lababidi R, Cane J, Bafadhel M. P54 Eosinophil migration is enhanced towards IL-5 and eotaxin in COPD. Thorax. 2017;72:A111-2. [Crossref]
28. Bade G, Khan MA, Srivastava AK, Khare P, Solaiappan KK, Guleria R, et al. Serum cytokine profiling and enrichment analysis reveal the involvement of immunological and inflammatory pathways in stable patients with chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2014;9:759-73. [Crossref] [PubMed] [PMC]
29. D'Armiento JM, Scharf SM, Roth MD, Connett JE, Ghio A, Sternberg D, et al. Eosinophil and T cell markers predict functional decline in COPD patients. Respir Res. 2009;10(1):113. [Crossref] [PubMed] [PMC]
30. Adnan AM, Ammar AZ, Khalil K. Role of eotaxin in chronic obstructive pulmonary disease. International Journal of Pharmaceutical Sciences Review and Research. 2013;21(1):10-4. [Link]

31. Tzanakis N, Chrysofakis G, Tsoumakidou M, Kyriakou D, Tsiligianni J, Bouros D, et al. Induced sputum CD8+ T-lymphocyte subpopulations in chronic obstructive pulmonary disease. Respir Med. 2004;98(1):57-65. [Crossref] [PubMed]

32. Saetta M, Baraldo S, Corbino L, Turato G, Braccioni F, Rea F, et al. CD8+ve cells in the lungs of smokers with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1999;160(2):711-7. [Crossref] [PubMed]

33. O'Shaughnessy TC, Ansari TW, Barnes NC, Jeffery PK. Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of CD8+ T lymphocytes with FEV1. Am J Respir Crit Care Med. 1997;155(3):852-7. [Crossref] [PubMed]

34. Saetta M, Di Stefano A, Turato G, Facchini FM, Corbino L, Mapp CE, et al. CD8+ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1998;157(3 Pt 1):822-6. [Crossref] [PubMed]

35. Robbins CS, Franco F, Mouded M, Cernadas M, Shapiro SD. Cigarette smoke exposure impairs dendritic cell maturation and T cell proliferation in thoracic lymph nodes of mice. J Immunol. 2008;180(10):6623-8. [Crossref] [PubMed] [PMC]