Original Article

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TANAFFOS

Inflammatory Serum Biomarker Pattern in Emphysema and Chronic Bronchitis Phenotypes of Acute Exacerbation of Chronic Obstructive Pulmonary Disease

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Background: COPD exacerbation is characterized by both airway and systemic inflammation. The present study aimed to investigate the relationship between serum levels of some inflammatory biomarkers and the phenotypes of COPD exacerbation.

Materials and Methods: This study includes known COPD patients, presenting to a hospital with acute exacerbation of COPD. Serum levels of CRP, ESR, CBC, TNF-α, IL-8, and IL-6 were measured at the time of admission. According to the previously done HRCT, the patients were divided into two groups including emphysema and chronic bronchitis. Levels of serum biomarkers were compared in the two groups. The relationships between biomarkers and duration of hospitalization were assessed too.

Results: Comparison of quantitative CRP levels, WBC, and platelet counts did not show a statistically significant difference between emphysema and chronic bronchitis but it was significantly higher than control subjects. Although not statistically significant, ESR level was higher in emphysema. TNF-alpha was 6.0±1.5 ng / ml and 1.5 ng / ml in the emphysema and chronic bronchitis groups, respectively. TNF-α had no significant difference compared to the groups. Although higher than the control group, IL-6 and IL-8 did not show significant differences between emphysema and chronic bronchitis. The two groups did not statistically differ in terms of hospital stay but patients with higher serum TNF-α tended to have longer hospitalization and ICU admission.

Conclusion: The present study showed predictably higher inflammatory biomarkers in COPD exacerbation but no significant difference between the two phenotypes of COPD and these two entities could not be discriminated based on inflammatory bio-factors.

Keywords: Chronic obstructive pulmonary disease; Emphysema; Chronic bronchitis; Tumor necrosis factor- α (TNF-α)

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is globally defined as irreversible airflow limitation in the airways. The disease is often progressive. (1). COPD is a combination of airway obstruction and systemic inflammation pointing out the fact that more severe disease probably results in higher serum level of some inflammatory biomarkers during COPD exacerbations (2). Evidence shows also systemic inflammation even in stable COPD (3). Therefore, there is a theory to count COPD as a part of a chronic systemic inflammatory syndrome (4). It seems that C-reactive protein (CRP) and fibrinogen may be

the most known inflammatory markers in during both stable COPD and exacerbations (2, 5-7).

Some authors have attempted to study to know more about the disease and concluded that COPD patients with HRCT-confirmed emphysema are showed more severe lung function impairment, more intense airway inflammation, and possibly more serious systemic dysfunction(8). There is no established consensus for the assessment of inflammatory biomarkers in COPD, although identification of specific biomarkers of disease severity might potentially influence a targeted-therapy.

Considering that COPD is an inflammatory process, providing specific therapeutic protocols based on etiologic biomolecules, viruses, bacteria etc. may be possible and can potentially reduce unnecessary antibiotic therapy. Over 130 biomarkers have been studied from 2000 to 2015 including: CRP, interleukins, tumor necrosis factor (TNF) superfamily, chemokines, brain natriuretic peptide (BNP), molecules involved in collagen synthesis i.e. metalloproteinase, and many other (9-11). Technical differences in measurements of biomarkers (immunometric, immunoturbidimetric, latex agglutination, and ELISA) can cause a variation in results; thus, doing more research can increase statistical power for estimation of tests' sensitivity and specificity.

The aim of the present study was to assess IL8, IL6, TNF-α, leukocyte count, platelet count, ESR, and quantitative CRP in acute exacerbations of both COPD phenotypes (emphysema and chronic bronchitis) and comparison with normal population to help to gather detailed information about inflammatory biomarkers in COPD in Iranian population.

MATERIALS AND METHODS

Subjects and study design

Through a cross-sectional study, a total number of 42 known COPD patients enrolled the current study among whom 22 were confirmed emphysematous cases by previously done HRCT, and the other 20 were diagnosed as chronic bronchitis without emphysema in HRCT. The sampling was done in the April-September interval to remove possible confounding effect of cold weather. Our inclusion criteria were known COPD patients with an increased intensity of dyspnea and sputum production according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (12), while comorbidities including coronary artery disease, pulmonary tuberculosis, primary and metastatic lung malignancies were meticulously excluded. No patients had been receiving any form of corticosteroids for 4 weeks prior to sample collection. Our control group included apparently healthy smokers with a normal spirometry. A blood sample was taken in the first 6 hours of admission from patients who had hospital admission indication according to the GOLD criteria (acute respiratory failure, onset of new physical signs (e.g., cyanosis), failure to respond to initial medical management, severe symptoms such as sudden worsening of resting dyspnea or tachypnea and/or decreased saturation) (12). IL8, IL6, and TNF-α were measured with commercial ELISA kits (Biosource Europe®, Nivelles, Belgium) and quantitative CRP was measured by immunonephelometry (Behring Nephelometer Analyzer II). The same measurements were done in the control group. The study protocol was approved by the local ethics committee at the coordinating center (Tehran University of Medical Science) and has been performed in accordance with the ethical standards laid down in the 2000 Declaration of Helsinki. All patients provided their written informed consent before sampling.

Statistical analysis

Data are expressed as mean ± SD or as median (interquartile ranges) for quantitative descriptive analysis of normally distributed and skewed data, respectively. Comparisons of biomarkers between COPD patients with and without emphysema were performed with Mann-Whitney U and independent Student's t-tests for skewed and normally distributed variables, respectively. Correlations were assessed using Spearman's and Pearson's correlation coefficients for skewed and normally distributed variables, respectively. Correlations between nominal variables were assessed using chi-square or fisher's exact tests. P values <0.05 were considered statistically significant. Analysis was performed with SPSS 20 (SPSS, Chicago, IL).

RESULTS

A total of 42 COPD patients were enrolled in the present study (22 and 20 in emphysema and chronic bronchitis groups, respectively). Females made up 9.1% of patients in emphysema group and 25% of patients in chronic bronchitis group. The mean age of the participants was 64.7 ± 11.74 years which ranged from 37 to 87 years of age. Kolmogorov-Smirnov test showed that age distribution among the two groups was normal. The mean ages of the patients in emphysema and chronic bronchitis group were 66.3 ± 2.3 and 63 ± 2.6, respectively, with no significant difference.

Biomarkers

Comparison of quantitative CRP levels did not show statistically significant difference between the groups (Table 1). Comparison of ESR levels between emphysema and chronic bronchitis groups showed that ESR levels were higher among emphysema patients but not statistically significant. White blood cell and platelet counts were approximately similar in two groups without a significant difference, although white blood cells were not categorized by their subtypes, which could provide useful information.

For determining normal levels of IL-6, IL-8, and TNF-α, we used previous studies, so that normal levels of IL-6, IL-8, and TNF-α in control groups were 2.5, 1.55 and 1.9 pg/ml, respectively (50). Our study showed that interleukins 6 and 8 levels did not significantly differ between emphysema and chronic bronchitis groups (Table 2).

Table 1. Mean levels of inflammatory biomarkers and their distribution.

Disease Feature		CRP mg/L	ESR mm/hr	WBC (1000/µl)	IL8 (pg/ml)	IL6 pg/ml	TNF (ng/ml)	PLT 1000/µl	Duration days
Emphysema	N	22	22	22	23	20	23	23	23
	Mean±SD	40.13±27.04	45.86±38.71	11.32±6.3	24.32±15.54	17.16±21.74	16.89±73.24	241.95±80.28	8.56±3.88
Chronic Bronchitis	N	21	19	20	21	19	21	21	21
	Mean±SD	25.8±23.34	41.94±38.59	9.6±3.19	21.36±14.22	19.3±26.2	50.91±168.61	215.33±77.81	11.28±2.14

Table 2. Comparison of biomarkers in COPD, emphysema, and chronic bronchitis against control group according to reference point

Biomarker	Disease (Feature)	Reference	N	Observed prop.	Test prop.	Sig
IL-6	COPD	≤ 1.55	5	0.13	0.50	<0.001
		>1.55	34	0.87		
	Emphysema	≤ 1.55	2	0.10	0.50	<0.001
		>1.55	18	0.9		
	Chronic Bronchitis	≤ 3	3	0.16	0.50	0.004
		>3	16	0.84		
IL-8	COPD	≤2.5	0	0.0	0.50	<0.001
		>2.5	44	1.0		
	Emphysema	≤2.5	0	0.0	0.50	<0.001
		>2.5	23	1.0		
	Chronic Bronchitis	≤2.5	0	0.0	0.50	<0.001
		>2.5	21	1.0		
TNF-α	COPD	≤1.9	33	0.75	0.50	<0.001
		>1.9	1	0.25		
	Emphysema	≤1.9	18	0.78	0.50	0.011
		>1.9	5	0.22		
	Chronic Bronchitis	≤1.9	15	0.71	0.50	0.078
		>1.9	6	0.29		

The mean serum levels of TNF-α in emphysema and chronic bronchitis were 6.01±5 ng/ml and 1.5 ng/ml, respectively. Since TNF-α level was undetectable in some patients; this should be repeated with a sufficient sample size. TNF-α levels did not significantly differ between the chronic bronchitis group and controls [Exact Sig. (2-tailed) =0.78].

The mean hospital stay was 8.56±3.88 and 11.28±2.14 days in emphysema and chronic bronchitis groups, respectively. Although, there was not a significant difference in duration of hospitalization between the two groups, the patients with higher TNF-α levels tended to have longer hospital stay which was statistically significant with a spearman's correlation coefficient of 0.73.

DISCUSSION

Biomarkers with potential utility in the diagnosis, prognosis, and monitoring of the natural history of COPD and the effect of therapeutic interventions, are being widely researched in the last decade (13). Different methodologies including exhaled breath, sputum, nasal secretions, serum, bronchoalveolar lavage (BAL), and tissue has been used for obtaining and analyzing biofluid. Serum specimens are more accessible, easier to be analyzed by laboratories, and are obtained with less invasive techniques. In the present study, circulating levels of CRP, ESR, white blood cells, platelets, IL-6, IL-8, and TNF-α were measured in two phenotypes of COPD patients (chronic bronchitis and emphysema groups) and compared with the control group.

No differences were detected in quantitative CRP levels between emphysema and chronic bronchitis groups, although they were significantly higher than control group.

CRP was reported with a weak and inverse correlated biomarker with the forced expiratory volume in the first second (FEV1) (14, 15) while neither ESR, nor CRP were reliable markers of COPD severity (15). CRP seems to be potentially crucial not only in spirometry, but also in the fields of clinical and epidemiological aspects of COPD because of its reported values to predict mortality and cardiovascular diseases among stable COPD patients (5, 16). It is thought that increased CRP plasma level during acute exacerbations of COPD may associate with of vascular endothelial growth factor (VEGF) up-regulation, which may contribute to the raised risk of cardiovascular complications (17-20). Nevertheless, production of CRP in liver fluctuates according to genetic factors, so that selective single-nucleotide polymorphisms in CRP gene may be associated with higher and/or lower serum CRP levels (21, 22). In addition, different cytokines contribute to CRP synthesis via different molecular mechanisms and serum patterns of cytokines vary among COPD patients (23). Thus, CRP is a nonspecific inflammatory indicator and the question then arises: does CRP have a role in diagnosis and/or management of COPD in low-income countries?

Increased ESR is another event in response to much more acute phase proteins, fibrinogen, and immunoglobulins release as well as anemia (24). This may weaken the pure indirect inflammatory role of ESR to be assessed disregarding the fact that COPD, mainly if severe, is frequently associated with hyperfibrinogenemia and anemia (25, 26). This procedure may be beneficial then for low-income countries, where COPD prevalence is dramatically rising chiefly because of low costs to do (27). Since it is faster than ESR in response to respiratory infections such as pneumonia and exacerbated COPD and could fell down faster following antibiotic therapy, CRP seems more sensitive than ESR in this regard (28). Otherwise, delayed ESR plasma level decrease while decreasing CRP levels may lead us to think about a high risk condition of re-infection and poor health status (29). So, ESR and CRP could be complementary factors in the case of COPD severity assessment and not as alternative indexes in this matter

A statistically meaningful association between severity of peripheral blood leukocytosis and patients' ages in chronic bronchitis group was seen. As the age increased, leukocyte counts also increased in chronic bronchitis

group. Differentiation between cell lines was not performed. Some scientists point to the importance of eosinophil which further studies are needed to explore the contribution of eosinophil to the mechanism of disease in COPD (30). An association between leukocytosis and mortality rates has been proposed in COPD patients (31).

There was not a statistically meaningful difference in platelet counts between emphysema and chronic bronchitis groups, although a positive correlation between platelet counts and ESR levels was seen in emphysema group, but not in chronic bronchitis group. Platelet activation may also contribute to increased cardiovascular risk in COPD. Some studies proposed platelet inhibition as a therapeutic target (32).

In contrast to control group, IL-6 was shown to be elevated in both COPD groups in our study. Ferrari et al., through a 3-year follow up study, introduced IL-6 as a useful inflammatory marker to predict poor exercise tolerance and higher rate and risk of mortality (33). Likely, ECLIPSE study endorsed that elevated IL-6 is a clinically useful marker predicting poor outcomes in COPD (31, 34, 35). Recent studies support a dissociation of IL-6 from inflammation in the lung and suggest that this cytokine plays an active role in the pathogenesis of COPD (36). These findings could actually represent HMG-CoA reductase inhibitors (statins) as beneficial therapies for COPD patients due to their powerful inhibitory effects on IL-6-mediated systemic inflammation (37-40).

Furthermore, CXCL8 (IL-8) was also shown to be at higher plasma levels in COPD patients when compared with control group in the current study. Often, increased numbers of macrophages and neutrophils occur in the lungs in COPD in association with abnormal innate immune response to raise interleukin-8 (CXCL8), growth-related oncogene (CXCL1), and regulated on activation normal T cell expression and secretion (CCL5-RANTES) as potent neutrophil chemoattractants (41) which is done by a variety of cells in the lung including macrophages and epithelial cells as well as neutrophils themselves (42). CXCL8 binds to chemokine receptors CXCR1 and CXCR2 to promote the influx of neutrophils into tissue sites of inflammation (43). Therefore, this may be a good try to consider both CXCR1 and CXCR2 as perfect targets in the case of COPD treatment (44, 45). Dual antagonists against CXCR1/2 may inhibit chemotaxis to a greater extent than CXCR2 alone (46). Corticosteroids partially suppress the production of neutrophil chemokines such as CXCL1 and CXCL8 by alveolar macrophages (47-49) and novel therapies are absolutely needed to inhibit the actions of neutrophil chemokines.

TNF-α was shown to be elevated in exacerbation of COPD in emphysema group, but considering that TNF-α values were undetectable in some patients, the study should be repeated to increase the statistical power. Our study showed that higher TNF-α levels may be associated with longer hospitalization (although the differences were not statistically meaningful).

Given the significant effect of smoking identified, any accurate interpretation of abnormal levels of inflammatory markers in COPD must take it into account. The fact that COPD patients were further categorized into emphysema and chronic bronchitis groups is the strength of our study, because as mentioned above, there may be a difference in pathogenesis and/or biomarker level between these two categories. The biomarkers we choose are investigated by majority of the previous studies and are often and easily measured in clinical practice. Our study had some potential limitations. First, we measured inflammatory biomarkers but not markers of tissue repair, and it is likely that the balance between inflammation and repair is important in COPD pathogenesis. Second, we had to exclude many patients because of comorbidities (e.g., heart failure, arrhythmias, severe hypertension) and this led to a less study population. Finally, TNF-α levels were undetectable in some patients and it needs to be repeated to validate the results.

CONCLUSION

The investigation of biomarkers in COPD has formed a new paradigm in diagnosis, risk stratification, and targeted

therapy which needs to be studied in different societies. Present study showed that inflammatory biomarkers are predictably higher in COPD exacerbations, in comparison with non-COPD smokers but possibly there is no significant difference in biomarker levels between emphysema and chronic bronchitis patients despite of worse lung function in emphysematous patients. These two entities could not be distinguished based on the inflammatory bio-factors. This is the first study of bio-inflammatory markers in emphysematous and chronic bronchitis COPD patients in Iranian population which needs to be continued with larger cohort studies to improve prognostic and/or therapeutic understanding about biomarkers in COPD.

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Conflicts of interest

All authors declare that they have no conflicts of interest.

REFERENCES

1. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. ***Am J Respir Crit Care Med*** 2007;176(6):532-55.
2. Donaldson GC, Seemungal TA, Patel IS, Bhowmik A, Wilkinson TM, Hurst JR, Maccallum PK, Wedzicha JA. Airway and systemic inflammation and decline in lung function in patients with COPD. ***Chest*** 2005;128(4):1995-2004.
3. Sin DD, Man SF. Skeletal muscle weakness, reduced exercise tolerance, and COPD: is systemic inflammation the missing link? ***Thorax*** 2006;61(1):1-3.
4. Fabbri LM, Rabe KF. From COPD to chronic systemic inflammatory syndrome? ***Lancet*** 2007;370(9589):797-9.
5. Dahl M, Vestbo J, Lange P, Bojesen SE, Tybjaerg-Hansen A, Nordestgaard BG. C-reactive protein as a predictor of prognosis in chronic obstructive pulmonary disease. ***Am J Respir Crit Care Med*** 2007;175(3):250-5.
6. Hurst JR, Perera WR, Wilkinson TM, Donaldson GC, Wedzicha JA. Systemic and upper and lower airway inflammation at exacerbation of chronic obstructive pulmonary disease. ***Am J Respir Crit Care Med*** 2006;173(1): 71-8.
7. Koutsokera A, Kiropoulos TS, Nikoulis DJ, Daniil ZD, Tsolaki V, Tanou K, et al. Clinical, functional and biochemical changes during recovery from COPD exacerbations. ***Respir Med*** 2009;103(6):919-26.
8. Boschetto P, Quintavalle S, Zeni E, Leprotti S, Potena A, Ballerin L, et al. Association between markers of emphysema and more severe chronic obstructive pulmonary disease. ***Thorax*** 2006;61(12):1037-42.
9. Franciosi LG, Page CP, Celli BR, Cazzola M, Walker MJ, Danhof M, et al. Markers of disease severity in chronic obstructive pulmonary disease. ***Pulm Pharmacol Ther*** 2006;19(3):189-99.
10. Takahashi T, Kobayashi S, Fujino N, Suzuki T, Ota C, He M, et al. Increased circulating endothelial microparticles in COPD patients: a potential biomarker for COPD exacerbation susceptibility. ***Thorax*** 2012;67(12):1067-74.
11. Takahashi T, Kubo H. The role of microparticles in chronic obstructive pulmonary disease. ***Int J Chron Obstruct Pulmon Dis*** 2014;9:303-14.
12. Vestbo J, Hurd SS, Agustí AG, Jones PW, Vogelmeier C, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. ***Am J Respir Crit Care Med*** 2013;187(4):347-65.
13. Snell N, Newbold P. The clinical utility of biomarkers in asthma and COPD. ***Curr Opin Pharmacol*** 2008;8(3):222-35.
14. Aronson D, Roterman I, Yigla M, Kerner A, Avizohar O, Sella R, et al. Inverse association between pulmonary function and C-reactive protein in apparently healthy subjects. ***Am J Respir Crit Care Med*** 2006;174(6):626-32.

15. Corsonello A, Pedone C, Battaglia S, Paglino G, Bellia V, Incalzi RA. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) as inflammation markers in elderly patients with stable chronic obstructive pulmonary disease (COPD). ***Arch Gerontol Geriatr*** 2011;53(2):190-5.
16. Sin DD, Man SF. Why are patients with chronic obstructive pulmonary disease at increased risk of cardiovascular diseases? The potential role of systemic inflammation in chronic obstructive pulmonary disease. ***Circulation*** 2003;107(11):1514-9.
17. Bello G, Cailotto F, Hanriot D, Kolopp-Sarda MN, Latger-Cannard V, Hess K, et al. C-reactive protein (CRP) increases VEGF-A expression in monocytic cells via a PI3-kinase and ERK 1/2 signaling dependent pathway. ***Atherosclerosis*** 2008;200(2):286-93.
18. Teramoto S, Akashi S. Chronic respiratory diseases and atherosclerosis. ***Nihon Rinsho*** 2011;69(1):158-62.
19. Sala E, Villena C, Balaguer C, Ríos A, Fernández-Palomeque C, Cosío BG, et al. Abnormal levels of circulating endothelial progenitor cells during exacerbations of COPD. ***Lung*** 2010;188(4):331-8.
20. Valipour A, Schreder M, Wolzt M, Saliba S, Kapiotis S, Eickhoff P, et al. Circulating vascular endothelial growth factor and systemic inflammatory markers in patients with stable and exacerbated chronic obstructive pulmonary disease. ***Clin Sci (Lond)*** 2008;115(7):225-32.
21. Szalai AJ, Wu J, Lange EM, McCrory MA, Langefeld CD, Williams A, et al. Single-nucleotide polymorphisms in the C-reactive protein (CRP) gene promoter that affect transcription factor binding, alter transcriptional activity, and associate with differences in baseline serum CRP level. ***J Mol Med (Berl)*** 2005;83(6):440-7.
22. Crawford DC, Sanders CL, Qin X, Smith JD, Shephard C, Wong M, et al. Genetic variation is associated with C-reactive protein levels in the Third National Health and Nutrition Examination Survey. ***Circulation*** 2006;114(23):2458-65.
23. Packard RR, Libby P. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. ***Clin Chem*** 2008;54(1):24-38.
24. Reinhart WH. Die Blutsenkungsreaktion--mehr als ein alter Zopf? [Erythrocyte sedimentation rate--more than an old fashion?]. ***Ther Umsch*** 2006;63(1):108-12.
25. Eickhoff P, Valipour A, Kiss D, Schreder M, Cekici L, Geyer K, et al. Determinants of systemic vascular function in patients with stable chronic obstructive pulmonary disease. ***Am J Respir Crit Care Med*** 2008;178(12):1211-8.
26. Guidelines on selection of laboratory tests for monitoring the acute phase response. International Committee for Standardization in Haematology (expert panel on blood rheology). ***J Clin Pathol*** 1988;41(11):1203-12.
27. Buist AS, Vollmer WM, McBurnie MA. Worldwide burden of COPD in high- and low-income countries. Part I. The burden of obstructive lung disease (BOLD) initiative. ***Int J Tuberc Lung Dis*** 2008;12(7):703-8.
28. Buess T, Ludwig C. Diagnostische Wertigkeit des C-reaktiven Proteins im Vergleich zur Senkungsreaktion als Eintrittsroutine [Diagnostic value of C-reactive protein in comparison with erythrocyte sedimentation as routine admission diagnostic test]. ***Schweiz Med Wochenschr*** 1995;125(4):120-4.
29. Osei-Bimpong A, Meek JH, Lewis SM. ESR or CRP? A comparison of their clinical utility. ***Hematology*** 2007;12(4):353-7.
30. Bafadhel M, Pavord ID, Russell REK. Eosinophils in COPD: just another biomarker? ***Lancet Respir Med*** 2017;5(9):747-59.
31. Celli BR, Locantore N, Yates J, Tal-Singer R, Miller BE, Bakke P, et al. Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease. ***Am J Respir Crit Care Med*** 2012;185(10):1065-72.
32. Maclay JD, McAllister DA, Johnston S, Raftis J, McGuinnes C, Deans A, et al. Increased platelet activation in patients with stable and acute exacerbation of COPD. ***Thorax*** 2011;66(9):769-74.
33. Ferrari R, Tanni SE, Caram LM, Corrêa C, Corrêa CR, Godoy I. Three-year follow-up of Interleukin 6 and C-reactive protein in chronic obstructive pulmonary disease. ***Respir Res*** 2013;14(1):24.
34. Pinto-Plata V, Casanova C, Müllerova H, de Torres JP, Corado H, Varo N, et al. Inflammatory and repair serum biomarker

pattern: association to clinical outcomes in COPD. ***Respir Res*** 2012;13(1):71.

35. Agustí A, Edwards LD, Rennard SI, MacNee W, Tal-Singer R, Miller BE, et al. Persistent systemic inflammation is associated with poor clinical outcomes in COPD: a novel phenotype. ***PLoS One*** 2012;7(5):e37483.
36. Rincon M, Irvin CG. Role of IL-6 in asthma and other inflammatory pulmonary diseases. ***Int J Biol Sci*** 2012;8(9):1281-90.
37. Young RP, Hopkins R, Eaton TE. Pharmacological actions of statins: potential utility in COPD. ***Eur Respir Rev*** 2009;18(114):222-32.
38. Neukamm A, Høiseth AD, Einvik G, Lehmann S, Hagve TA, Søyseth V, et al. Rosuvastatin treatment in stable chronic obstructive pulmonary disease (RODEO): a randomized controlled trial. ***J Intern Med*** 2015;278(1):59-67.
39. Lee TM, Lin MS, Chang NC. Usefulness of C-reactive protein and interleukin-6 as predictors of outcomes in patients with chronic obstructive pulmonary disease receiving pravastatin. ***Am J Cardiol*** 2008;101(4):530-5.
40. Young RP, Hopkins RJ. Effect of statins on cancer in chronic obstructive pulmonary disease. ***Thorax*** 2011;66(4):354-5; author reply 355-6.
41. de Boer WI, Sont JK, van Schadewijk A, Stolk J, van Krieken JH, Hiemstra PS. Monocyte chemoattractant protein 1, interleukin 8, and chronic airways inflammation in COPD. ***J Pathol*** 2000;190(5):619-26.
42. Scapini P, Lapinet-Vera JA, Gasperini S, Calzetti F, Bazzoni F, Cassatella MA. The neutrophil as a cellular source of chemokines. ***Immunol Rev*** 2000;177:195-203.
43. Ludwig A, Petersen F, Zahn S, Götze O, Schröder JM, Flad HD, et al. The CXC-chemokine neutrophil-activating peptide-2 induces two distinct optima of neutrophil chemotaxis by differential interaction with interleukin-8 receptors CXCR-1 and CXCR-2. ***Blood*** 1997;90(11):4588-97.
44. Holz O, Khalilieh S, Ludwig-Sengpiel A, Watz H, Stryszak P, Soni P, et al. SCH527123, a novel CXCR2 antagonist, inhibits ozone-induced neutrophilia in healthy subjects. ***Eur Respir J*** 2010;35(3):564-70.
45. Lazaar AL, Sweeney LE, MacDonald AJ, Alexis NE, Chen C, Tal-Singer R. SB-656933, a novel CXCR2 selective antagonist, inhibits ex vivo neutrophil activation and ozone-induced airway inflammation in humans. ***Br J Clin Pharmacol*** 2011;72(2):282-93.
46. Kaur M, Singh D. Neutrophil chemotaxis caused by chronic obstructive pulmonary disease alveolar macrophages: the role of CXCL8 and the receptors CXCR1/CXCR2. ***J Pharmacol Exp Ther*** 2013;347(1):173-80.
47. Standiford TJ, Kunkel SL, Rolfe MW, Evanoff HL, Allen RM, Strieter RM. Regulation of human alveolar macrophage- and blood monocyte-derived interleukin-8 by prostaglandin E2 and dexamethasone. ***Am J Respir Cell Mol Biol*** 1992;6(1):75-81.
48. Armstrong J, Sargent C, Singh D. Glucocorticoid sensitivity of lipopolysaccharide-stimulated chronic obstructive pulmonary disease alveolar macrophages. ***Clin Exp Immunol*** 2009;158(1):74-83.
49. Armstrong J, Harbron C, Lea S, Booth G, Cadden P, Wreggett KA, et al. Synergistic effects of p38 mitogen-activated protein kinase inhibition with a corticosteroid in alveolar macrophages from patients with chronic obstructive pulmonary disease. ***J Pharmacol Exp Ther*** 2011;338(3):732-40.