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## Review Article

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### CYTOKINES IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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#### ABSTRACT

COPD is a complex disease with multiple pathological components, which we unfortunately tend to ignore when spirometry is used as the only method to evaluate the disorder. Additional measures are needed to allow a more complete and clinically relevant assessment of COPD. The earliest potential risk factors of disease in COPD are variations in the genetic background. Genetic variations are present from conception and can determine lifelong changes in enzyme activities and protein concentrations. In contrast, measurements in blood, sputum, exhaled breath, broncho-alveolar lavage, and lung biopsies may vary substantially over time. The identification of inflammatory mediators and understanding their interactions is important for the development of anti-inflammatory treatments for this important disease. Chronic lung inflammation appears to contribute to the pathogenesis of COPD, and markers of this process have promising predictive value in COPD. To implement markers for COPD in clinical practice, besides those already established for the  $\alpha$ -1-antitrypsin gene, further research and validation studies are needed. This review explores potential markers of early disease and prognosis in COPD by examining genetic markers in the  $\alpha$ -1-antitrypsin, cystic fibrosis transmembrane conductance regulator (CFTR), and MBL-2 genes, and by examining the biochemical markers fibrinogen and C-reactive protein (CRP), which correlate with degree of pulmonary inflammation during stable conditions of COPD.

**Keywords:** chronic obstructive pulmonary disease, biomarker, pathogenesis, prognosis, genetics.

#### INTRODUCTION

Chronic obstructive pulmonary disease (COPD), also known as chronic obstructive lung disease (COLD), and chronic obstructive airway disease (COAD), among others, is a type of obstructive lung disease characterized by chronically poor airflow. It typically worsens over time. COPD is nowadays a common cause of death throughout the world and an estimated 64 million people had COPD worldwide in 2004 (27).

According to WHO Golden burden of disease study "COPD, A common preventable and treatable disease, is characterised by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lungs to

noxious particle or gases. Exacerbations and comorbidities continue to the overall severity in individual patients".

The main symptoms include shortness of breath, cough, and sputum production (1). (COPD) is a chronic inflammatory disease of the airways and lung parenchyma, estimated to affect 9–10% of adults aged 40 yrs (12). COPD causes both significant mortality (13) and morbidity (14).

Current disease staging systems are mainly based on the Forced Expired Volume in one second (FEV<sub>1</sub>), the most commonly used spirometric measure for diagnosis and evaluation of treatment effect in COPD (54). Most people with chronic bronchitis have COPD (2). In the developing world, one of the common sources of air pollution is from poorly vented cooking and heating fires. Long-term exposure

to these irritants causes an inflammatory response in the lungs resulting in narrowing of the small airways and breakdown of lung tissue known as emphysema (4). Worldwide, COPD affects 329 million people or nearly 5% of the population. In 2011, it ranked as the fourth leading cause of death, killing over 3 million people (5). The number of deaths is projected to increase due to higher smoking rates and an aging population in many countries (6). It resulted in an estimated economic cost of \$2.1 trillion in 2010 (7).

### Signs and symptoms

The most common symptoms of COPD are sputum production, shortness of breath and a productive cough (8). COPD patients with abnormally low lean body mass are at an increased risk of death compared to those with normal lean body mass (5). The term cachexia is often used to describe the state of pathologically low lean body mass. However, some authors define cachexia as a state in which protein is lost specifically due to proinflammatory processes, such as those driven by tumour necrosis factor (TNF)- and interleukin (IL)-6 (6). It has also been suggested that elevated IgE play a role in chronic airway obstruction. The association of IL-1 gene with COPD has been studied in recent times and its polymorphisms at the position -511 have been found to be associated with susceptibility to COPD (70).

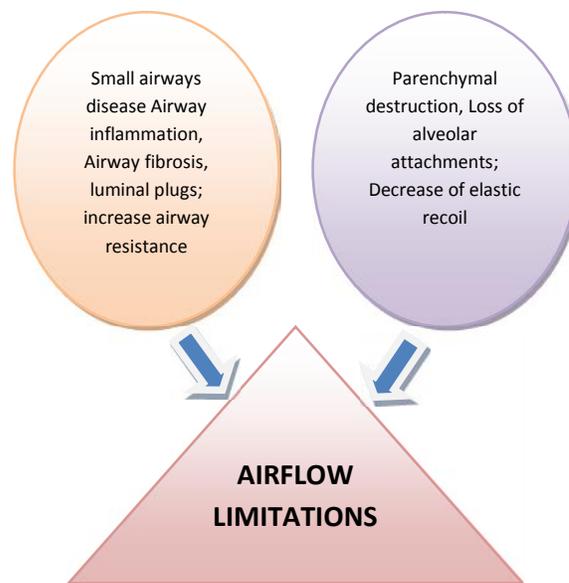
Support for the role of systemic inflammation comes from cross-sectional studies in which higher blood levels of TNF- (17-20), IL-6 (19, 20) and C-reactive protein (CRP) (19) were seen in underweight COPD patients compared with normal weight patients. However, in earlier studies (17-19), body mass index (BMI) was used to stratify patients. This is imperfect, since patients can have significant muscle wasting without a significant decrease in total body weight, if there is a corresponding increase in fat mass (21).

These symptoms are present for a prolonged period of time (2) and typically worsen over time (4). It is unclear if different types of COPD exist (3). While previously divided into emphysema and chronic bronchitis, emphysema is only a description of lung changes rather than a disease itself, and chronic bronchitis is simply a descriptor of symptoms that may or may not occur with COPD (1).

### Causes

The primary cause of COPD is tobacco smoke, with occupational exposure and pollution from indoor fires being

significant causes in some countries (1). However, airway obstruction develops only in a minority of smokers (23) although most of them develop an inflammatory reaction in the bronchioles (22).



It therefore seems obvious that other factors besides smoking must be involved in the pathogenesis of COPD.

- Smoking
- Air pollution
- Age and gender
- Occupational exposures
- Genes
- Lung growth and development
- Exposure to particles
- Asthma/Bronchial hyperactivity
- Chronic bronchitis
- Miscellaneous (poverty, malnutrition, low birth weight, and infectious diseases including HIV/AIDS and tuberculosis).

### Pathology, pathogenesis and pathophysiology

Inhaled cigarette smoke and other noxious particles, such as smoke from biomass fuels cause lung inflammation, a normal response that appears to be modified in patient who develops COPD. This chronic inflammatory response may induce parenchymal tissue destruction (emphysema) and disrupt normal repair and defence mechanisms (resulting in

small airway fibrosis). These pathological changes lead to air trapping and progressive airflow limitations. A brief overview follows of the pathological changes in COPD, their cellular and molecular mechanism, and how these underlie physiologic abnormalities and symptoms characteristic of the disease (28).

### **Pathology**

Pathologic changes characteristic of COPD are found in the airways, lung parenchyma, and pulmonary vasculature (29). The pathological changes include chronic inflammation, with increased numbers of specific inflammatory cell types in different parts of the lung, and structural changes resulting from repeated injury and repair. In general, the inflammatory and structural changes in the airways increase with disease severity and persist on smoking cessation.

### **Pathogenesis**

The inflammation in the respiratory tract of COPD patients appears to be a modification of the inflammatory response of the respiratory tract to chronic irritants such as cigarette smoke. The mechanism of this amplified inflammation is not yet understood but may be genetically determined. Patients can clearly develop COPD without smoking, but the nature of the inflammatory response in these patients is unknown. Oxidative stress and an excess in proteinases in the lung further modify lung inflammation. Together, these mechanisms lead to the characteristic pathological changes in COPD. Lung inflammation persists after smoking cessation through unknown mechanism, although autoantigens and persistent microorganisms may play a role (30).

### **Pathophysiology**

There is now a good understanding of how the underlying disease process in COPD leads to the characteristic physiological abnormalities and symptoms. For example, inflammation and narrowing of peripheral airways leads to decrease forced expired volume (FEV1). Parenchymal destruction due to the emphysema also contributes to the airflow limitation and leads to decreased gas transfer. Other complications are as:

- Airflow limitations and air trapping
- Gas exchange abnormalities
- Mucus hypersecretion
- Pulmonary hypertension
- Exacerbations

### **Prevention**

Most cases of COPD are potentially preventable through decreasing exposure to smoke and improving air quality. (9) Usual care for COPD patients includes smoking cessation, prevention and treatment of acute exacerbations, airway and secretion management, nutritional interventions and pulmonary rehabilitation programs, usually with physical therapy and exercise. Education in self-management is also included, as it results in fewer hospitalizations/days of hospitalization (24), reduced morbidity (25), improved outcomes and reduced costs during 12-month follow-up (26). An annual influenza vaccination in those with COPD reduces exacerbations, hospitalizations and death. Pneumococcal vaccination may also be beneficial (10, 11).

### **Cytokines**

Cytokines are a group of proteins that bring about communication between different cell types involved in immunity. They are low molecular weight glycoproteins and are produced by lymphoid and non-lymphoid cells during the course of immune response. Cytokines may be regarded as soluble messenger molecules of immune system (45).

White blood cells (leukocytes) possess the attributes of diversity, specificity, memory, and self/nonself recognition, the hallmarks of an adaptive immune response. Some leukocytes, especially T lymphocytes, secrete various protein molecules called cytokines. These molecules act as immunoregulatory hormones and play important roles in the regulation of immune responses (46).

They can act as short messengers between the cells or long range messengers by circulating in the blood and affecting cells at far off sites. The latter function is comparable to that of hormones. The term interleukin (IL) is frequently used to represent cytokines. There are more than a dozen interleukins (IL1.....IL12), produced by different cells with wide range of functions. The main function (directly or indirectly) of cytokines is to amplify immune responses and inflammatory response (45).

### **Therapeutic uses of cytokines**

Cytokines secreted by helper-T (TH) cells can activate various phagocytic cells; enabling them to phagocytose and kill microorganisms more effectively. The two major subpopulations of T lymphocytes are the CD4 T helper (TH)

cells and CD8 T cytotoxic (TC) cells. TH cells secrete cytokines that regulate immune response upon recognizing antigen combined with class II MHC. TC cells recognize antigen combined with class I MHC and give rise to cytotoxic T cells (CTLs), which display cytotoxic ability. Functions of cytokines are as follows (Table-1):

It is now possible to produce cytokines in vitro. Some of the cytokines have potential applications in the practice of medicine. For instance, IL-2 is used in cancer immunotherapy, and in the treatment of immunodeficiency diseases. IL-2 induces the proliferation and differentiation

**Table-1: Functional groups of selected cytokines**

Cytokine	Secreted by	Targets and effects
<b>Some cytokines of innate immunity</b>		
Interleukin 1(IL-1)	Monocytes, macrophages, endothelial cells, epithelial cells	Vasculature (inflammation); hypothalamus (fever); liver (induction of acute phase proteins)
Tumor Necrosis Factor (TNF- )	Macrophages	Vasculature (inflammation); liver (induction of acute phase proteins); loss of muscle, body fat (cachexia); induction of death in many cell types; neutrophils activation
Interleukin 12 (IL-12)	Macrophages, dendritic cells	NK cells; influences adaptive immunity (promotes T <sub>H</sub> 1 subset)
Interleukin 6 (IL-6)	Macrophages, endothelial cells	Liver (induces acute phase proteins); influences adaptive immunity (proliferation and antibody secretion of B cell lineage)
Interferon (IFN- )	Macrophages	Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells
Interferon (IFN- )	Fibroblasts	Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells
<b>Some cytokines of adaptive immunity</b>		
Interleukin 2(IL-2)	T cells	T-cell proliferation; can promote AICD. NK cell activation and proliferation; B-cell proliferation
Interleukin 4(IL-4)	T <sub>H</sub> 2 cells; mast cells	Promotes T <sub>H</sub> 2 differentiation; isotype switch to IgE
Interleukin 5(IL-5)	T <sub>H</sub> 2 cells	Eosinophil activation and generation
Interleukin 25(IL-25)	Unknown	Induces secretion of T <sub>H</sub> 2 cytokine profile
Transforming growth factor (TGF- )	T cells, macrophages, other cell types	Inhibits T-cell proliferation and effector functions; inhibits B-cell proliferation; promotes isotype switch to IgE; inhibits macrophages
Interferon (IFN- )	T <sub>H</sub> 1 cells; CD8 <sup>+</sup> cells; NK cells	Activates macrophages; increases expression MHC class I and class II molecules; increases antigen presentation

of T-and B-cells, besides increasing the cytotoxic capacity of natural killer cells. A group of cytokines namely interferons can combat viral infection by inhibiting their replication (45).

**Secretion of factors**

A number of important proteins central to development of immune responses are secreted by activated macrophages (Table-2). These include a collection of cytokines, such as interleukin 1 (IL-1), TNF- and interleukin 6 (IL-6), that promote inflammatory responses. Typically, each of these agents has a variety of effects. For example, IL-1 activates lymphocytes; and IL-1, IL-6, and TNF- promote fever by affecting the thermoregulatory centre in the hypothalamus (46).

contribute to tumour destruction by macrophages. Finally, as mentioned earlier, activated macrophages secrete a number of cytokines that stimulate inducible haematopoiesis (46).

**Potential marker of COPD severity**

A marker is a measurement known to be associated with a clinical outcome. Thus, exercise capacity, as tested in a laboratory, is a marker of the patient’s exercise intolerance in daily life, and health status scores provide a marker of the patient’s health-related quality of life. In the absence of other widely accepted and validated markers, lung function measurement and, specifically, the forced expiratory volume in one second (FEV1), has been used as a global marker for all the pathophysiological changes in COPD.

**Table-2: Some factors secreted by activated macrophages**

Factor	Function
Interleukin 1 (IL-1)	Promotes inflammatory responses and fever
Interleukin 6 (IL-6) TNF-	Promote innate immunity and elimination of pathogens
Complement proteins	Promote inflammatory response and elimination of pathogens
Hydrolytic enzymes	Promote inflammatory response
Interferon alpha (IFN- )	Activates cellular genes, resulting in the production of proteins that confer an antiviral state on the cell
Tumor necrosis factor (TNF- )	Kills tumor cells
GM-CSF	
G-CSF	Promote inducible haematopoiesis
M-CSF	

Activated macrophages secrete a variety of factors involved in the development of an inflammatory response. The complement proteins are a group of proteins that assist in eliminating foreign pathogens and in promoting the ensuing inflammatory reaction. The major site of synthesis of complement proteins is the liver, although these proteins are also produced in macrophages. The hydrolytic enzymes contained within the lysosomes of macrophages also can be secreted when the cells are activated. The buildup of these enzymes within the tissues contributes to the inflammatory response and can, in some cases, contribute to extensive tissue damage. Activated macrophages also secrete soluble factors, such as TNF- , that can kill a variety of cells. The secretion of these cytotoxic factors has been shown to

Whilst there are currently very few well-validated markers, there are a large number of candidate markers that could potentially be valuable for the assessment of COPD. A number of these are described in Table 3 & 4.

**Biological markers:**

Validation of new biological markers is a difficult and time consuming process. Given that airway inflammation is a central component in the pathogenesis of COPD. Inflammatory cells and mediators would present a logical target as potential markers for disease monitoring and the assessment of therapeutic interventions. COPD is characterised by neutrophilia (34) and increased levels of inflammatory mediators, including IL-6 (31), IL-8 (32, 33), TNF- (31, 33) and leukotriene B4 (35, 36). In a comparison

**Table-3: Potential marker of COPD severity**

<b>Demographics</b>	Age; gender; height; weight; pack-years; disease years
<b>Spirometry</b>	FEV <sub>1</sub> (litres and % predicted); FVC (litres and % predicted); FEV <sub>1</sub> /FVC ratio
<b>Clinical variables</b>	Arterial oxygen tension; arterial carbon dioxide tension; $\beta_2$ -agonist reversibility; methacholine, histamine or AMP challenge; body mass index; baseline dyspnoea index; 6 minute walking distance
<b>Cytological variables</b>	
Local (Sputum, BAL, Biopsy)	Neutrophils; macrophages; eosinophils; CD8 <sup>+</sup> lymphocytes
Systemic (Plasma or Serum)	Neutrophils; macrophages; eosinophils; CD8 <sup>+</sup> lymphocytes
<b>Biochemical variables</b>	
Local (Sputum, BAL, Biopsy)	Interleukin-6; interleukin-8; TNF- $\alpha$ ; fibrinogen; C-reactive protein
Systemic (Plasma or Serum)	Interleukin-6; interleukin-8; TNF- $\alpha$ ; fibrinogen; C-reactive protein
Exhaled breath	Nitric oxide; carbon monoxide

FEV<sub>1</sub>, forced expired volume in one second; FVC, forced vital capacity; BAL, broncho-alveolar lavage; TNF- $\alpha$ , tumour necrosis factor .

**Table-4: Potential markers for the assessment of chronic obstructive pulmonary disease**

Biological markers	Physiological markers	Symptomatic markers
<b>Expectorated cellular markers of inflammation</b>	<b>Markers of lung function</b>	<b>Symptoms</b>
Neutrophils, macrophages, eosinophils, mast cells, lymphocytes	Aspiratory capacity, D <sub>L,CO</sub>	MRC Respiratory Questionnaire
<b>Expired gases</b>	<b>Physiological tests of small airway obstruction</b>	<b>Breathlessness</b>
NO, CO, H <sub>2</sub> O <sub>2</sub>	Lung hyperinflation	MRC Dyspnoea scale, Borg scale, BDI/TDI, UCSD dyspnoea scale
<b>Expired air condensate</b>	<b>Rate of decline of lung function</b>	<b>Disease-specific health status (health related quality of life)</b>
LTB <sub>4</sub> , cytokines, aldehydes	<b>Exercise testing</b>	CRDQ, SGRQ, BPQ, PFSDQ, PFSS, CCQ
<b>Peripheral blood markers</b>	6-min walk test	<b>Generic health status</b>
Activated neutrophils, TNF- $\alpha$ , soluble TNF receptors, IL-6, IL-8, CRP	<b>Bronchial hyperreactivity</b>	SF-36, NHP, EQ-5D
<b>Sputum protease/anti-protease levels</b>	<b>Skeletal muscle function</b>	<b>Cognitive function</b>
HNE, MMPs, $\alpha_1$ -AT, SLPI, TIMPs	<b>Lean body mass</b>	
<b>Urine markers</b>	<b>Imaging</b>	
	CT scan, PET, hyperpolarised gas MR	
	<b>Exacerbations</b>	
Markers of matrix degradation, e.g.desmosine	Rate, type	
	<b>Quantification of luminal airway mucus</b>	
	<b>Measures of pulmonary hypertension</b>	

TNF- $\alpha$ : tumour necrosis factor- $\alpha$ ; IL: interleukin; ECP: eosinophilic cationic protein; MPO: myeloperoxidase; NO: nitric oxide; CO: carbon monoxide; H<sub>2</sub>O<sub>2</sub>: hydrogen peroxide; LTB<sub>4</sub>: leukotriene B<sub>4</sub>; CRP: C-reactive protein; HNE: human neutrophil elastase; MMPs: matrix metalloproteinases;  $\alpha_1$ -AT:  $\alpha_1$ -antitrypsin; SLPI: secretory leukocyte protease inhibitor; TIMPs: tissue inhibitors of matrix metalloproteinases; EGF: epidermal growth factor; D<sub>L,CO</sub>: diffusing capacity of the lung for carbon monoxide; CT: computed tomography; PET: positron emission tomography; MR: magnetic resonance; MRC: Medical Research Council; BDI/TDI: baseline and transition dyspnoea index; UCSD: University of California, San Diego; CRDQ: Chronic Respiratory Disease Questionnaire; SGRQ: St. George's Respiratory Questionnaire; BPQ: Breathing Problems Questionnaire; PFSDQ: Pulmonary Function Status & Dyspnoea Questionnaire; PFSS: Pulmonary Function Status Scale; CCQ: Clinical COPD Questionnaire; SF-36: Short Form-36; NHP: Nottingham Health Profile; EQ-5D: EuroQol 5D.

of inflammatory cells in the peripheral airways of smokers with and without COPD, higher levels of CD8+T-lymphocytes were found in biopsies from those patients with COPD. However, the levels of neutrophils, macrophages and CD4+ T-lymphocytes were similar in patients with or without COPD (37).

Whilst much attention has been paid to biomarkers from the lungs, biomarkers need not necessarily be present in the airways or sampled from the lungs. There is recent evidence that blood levels of the acute phase protein C-reactive protein (CRP) may be a marker of inflammation in the airways, and respond to inhaled therapy (38, 39).

The identification of biomarkers for exacerbations provides a good example of one of the key problems in developing a biomarker: the need for a precise definition of the disease phenotype. Currently there is no agreed method of identifying an acute exacerbation in clinical terms. This makes it very difficult to identify a precise, sensitive and specific biomarker.

#### **Physiological markers**

Identification and validation of physiological markers is not straightforward. Unlike biomarkers, which generally represent a well-defined chemical entity, physiological markers often require agreement between investigators over definitions and methods of measurement. Bronchial hyperreactivity is one example for which there is still no fully standardised approach after nearly three decades (40, 41). Physiological markers may change over time, and this has led to the development of derived parameters such as decline in FEV1 over 1 yr. This has become a well-accepted marker of deterioration in COPD that can change with therapeutic intervention, such as smoking cessation (42).

#### **Symptomatic markers**

Many aspects of COPD can still only be accessed through patients reporting symptoms. Some of these might just involve the straightforward recording of symptoms, such as cough, wheeze, breathlessness and sputum colour. The latter provides an example of a surrogate marker, since there is evidence that green-coloured sputum is associated with a higher likelihood of the presence of a bacterial infection in COPD than mucoid sputum (43). Issues concerning marker development become more complex when different

components are combined to produce a single overall measurement.

#### **Composite markers**

COPD is a complex, multifaceted disease, and reference has already been made to summary measures such as exercise capacity and health status measurements. These markers produce a score that reflects a range of effects of the disease, but another recent approach has been to create a composite made up of markers known to be predictors of mortality: BMI, FEV1, dyspnoea and exercise capacity (the BODE index) (44). This has proved to be a better predictor of mortality than the FEV1 alone.

#### **Role of Cytokines in Chronic Obstructive Pulmonary Disease:**

Cigarette smoking is a major risk factor for chronic obstructive pulmonary disease (COPD). Long-term smoking causes airway inflammation characterized by neutrophil, macrophage, and activated T lymphocyte infiltration and by increased cytokine concentrations such as tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukins (IL)-6 and IL-8 (56-59).

Several studies have shown systemic inflammation in COPD patients with increased neutrophil, macrophage, and T-lymphocyte numbers and high concentrations of inflammatory mediators in peripheral blood (C-reactive protein (CRP), IL-6, IL-8 and TNF- $\alpha$ ) (60-65). TNF- $\alpha$ , a powerful pro-inflammatory cytokine primarily produced by activated macrophages, is thought to play a critical role in the pathogenesis of COPD by promoting and maintaining the expression and release of various proinflammatory mediators which lead to tissue damage and remodelling (66,67).

An indirect marker of proinflammatory state related to systemic TNF- $\alpha$ , showed no influence of smoking on systemic inflammation in small sample of COPD patients (68). However, in a study with the aim to examine levels of inflammatory markers in COPD and asthma patients, the influence of smoking on TNF- $\alpha$  serum concentration was identified only in the subgroup of COPD patients (65). We hypothesised that active smoking may be associated with more severe systemic inflammation in COPD patients. In order to test our hypothesis, we analyzed concentrations of TNF- $\alpha$ , IL-6, IL-8 and CRP in the peripheral blood of current

smoker and exsmoker COPD patients, with a wide range of airway, current smoker and never-smoker controls (69).

Some patients of COPD are prone to frequent exacerbations, which are important determinants of health status (47). Cytokines are extracellular signal proteins (less than 80 kDa) formed by various types of cells in the body. IL-6 is secreted by monocytes, macrophages, T cells, B cells, fibroblasts, epithelial cells of the airway and endothelial cells. IL-8, also known as CXCL8, is a CXC chemokine that is a potent chemoattractant for neutrophils. In general, monocytes, tissue and alveolar macrophages, pulmonary epithelium, cells of the smooth muscles of the airway, eosinophils, fibroblasts, and endothelial cells are its important sources (48), (49).

The levels of many cytokines are known to be raised in serum in COPD (50), but their contribution to disease severity is still unknown. In this study, the relationship between the levels of IL-6 and IL-8 in the serum of patients with exacerbation of COPD, and PFT; FEV1, FEV1/FVC values CRP, and ESR were studied.

Presently, the medical literature contains numerous cross-sectional studies that have tried to correlate clinical variables, counts of cell types and concentrations of inflammatory mediators to FEV1 (51, 52). Examples include body mass index (BMI) (53), neutrophils cell counts, and tumour necrosis factor (TNF- ) (54). The elucidation of a marker's potential to discriminate between different disease stages would be useful in establishing appropriate clinical endpoints, particularly for the assessment of treatment effect (54).

#### CONCLUSION:

Substantial unmet needs remain in COPD and improved insight is required into the pathophysiology and effective treatments. From a diagnostic point of view, specific disease biomarkers, improved methods for early detection and diagnosis of exacerbations, and enhanced understanding of the relations between COPD and co morbidities would be helpful. Additionally, an important question that so far remains unanswered is whether different phenotypes of the disease exist and, if so, whether they respond differently to treatment. Research is being done into new compounds to treat COPD (71).

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