

## Special Article - Novel Markers in Chronic Obstructive Pulmonary Disease

# Novel and Emerging Blood Biomarkers in Chronic Obstructive Pulmonary Disease

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Recent advancement in the field of pulmonary biomarker research has produced a large number of potential biomarkers that are clinically relevant in assessment of chronic obstructive pulmonary disease (COPD). There is an ongoing research interest for new biomarkers and initially, compounds involved in the inflammatory cascade are potential candidates. Biomarkers are characteristics that are objectively measured and evaluated as indicators of biological or pathogenic processes, or responses to therapeutic interventions, and may provide information on the prognosis or progression of the disease and response to treatment. The developments of new technologies have generated a huge information data base and recent advances in biomarker research suggests that quantification of serum cytokines could play an important role in the diagnosis, classification, prognosis, and treatment response of COPD. They are more likely to be helpful in the management of airway diseases because of the heterogeneity of their pathobiology.

However, there is a paucity of information regarding their reproducibility and correlation with outcome measurements in COPD. The emerging knowledge in the field of Blood Biomarkers provides an enormous potential for understanding the disease pathophysiology, for developing markers specific for long-term outcomes, and for developing new therapeutic strategies. This review is based upon the consideration of the properties of ideal biomarkers for different clinical and research purposes. The current review explores some of these issues together and also explores those promising biomarkers that have already been proposed and investigated or being studied.

**Keywords:** Biomarkers; Chronic obstructive pulmonary Disease; Forced Expiratory Volume 1; Disease; Progression; Biomarkers; Lung; Sputum; Blood

## Introduction

Chronic obstructive pulmonary disease (COPD) is a significant health problem throughout the world and highly prevalent disease associated with long-term exposure to toxic gases and particles, mostly related to cigarette smoking and Global burden of Disease Study has projected COPD to be the third leading cause of death worldwide by 2020 [1]. COPD is a multi component disease characterized by progressive airflow limitation caused by chronic inflammation of the airways and lung parenchyma [2] and involves a range of pathological changes, which include mucus hyper secretion, airway narrowing and loss of alveoli within the lungs, and loss of lean body mass and cardiovascular effects outside the lungs. Extensive heterogeneity is observed among patients with COPD interms of their clinical presentation. Even though there have been significant advances in the understanding and management of COPD suggesting that the disease may largely be preventable, it remains marginally treatable.

Recent advances in understanding the pathogenetic mechanisms that underlie COPD have lead to the identification of “many novel therapeutic targets” as a result, a large number of agents have been explored as potential treatments. Newer technologies for investigating human diseases now offer significant potential to address the need for better diagnosis and improved understanding of COPD. Over the

past decades, there has been significant interest in biomarkers and lot of research has gone into identifying diagnostic biomarkers of disease activity [3]. In medicine, “biomarker” is a term often used to refer to a measurable characteristic that reflects the severity or presence of a disease state. According to *National Institute of Health (NIS)* a biomarker may be defined as a “characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [4]. Biomarkers are an integral part of the drug development and approval process. For COPD the only biomarker currently widely used in clinical trials is lung function testing, typically forced expiratory volume in 1 s (FEV1). Although FEV1 is easy to obtain and reproducible it does not provide information about underlying disease activity, does not separate out phenotypes of COPD, is not specific to COPD and is unresponsive to some therapies that clearly improve survival. The course of natural history of COPD is characterized by progressive deterioration of lung function and functional status, worsening quality of life and in many cases leads to mortality. Furthermore, COPD is extremely heterogeneous in nature and efficient strategies to develop treatments targeting specific groups of COPD patients have yet to emerge. There are evidences which suggest that extrapulmonary manifestations of COPD are becoming substantial causes of morbidity and mortality that need

timely characterization and treatment. Biomarkers have the potential to reflect reliably the disease process and activity, and provide a better understanding of the COPD subtypes.

Hence, there is a compelling need for biomarkers that can aid with the diagnosis, disease progression, risk stratification and the assessment of therapeutic interventions in COPD. Most of the search for biomarkers has revolved around proteins and other molecules in exhaled breath condensate, sputum, urine, bronchoalveolar lavage and blood that have been implicated in the pathogenesis of COPD [5]. Recently, profiling of blood biomarkers has identified a number of biomarkers that may distinguish individuals with COPD from control subjects [6]. Biomarkers have the immense therapeutic potential to play an important role in the assessment of COPD. To gain insight into the potential future uses and existing limitations of biomarkers in COPD, it is imperative to carefully evaluate the successful implementation of biomarkers in other fields of medicine. In near future we anticipate biomarkers may help us identify individuals at risk of developing COPD.

Furthermore, biomarkers could improve our ability to monitor disease progression and exacerbations, predict mortality and in some cases provide insight into disease mechanisms. However, additional research and rigorous evaluation is needed to determine the clinical utility of biomarkers aimed at treating COPD patients and the ability to serve as surrogate endpoints in clinical studies.

The objective of current review is to explore the examples of existing novel biomarkers that have been valuable in the clinical setting and to briefly describe these biomarkers that are used clinically to manage patients with a focus to highlight their associations with clinical variables in an attempt to illustrate the potential clinical implications based on current available evidences.

## Blood Biomarkers: Established and New Markers for the Assessment of Copd

Over the past few years, several biomarkers have been intensively studied in COPD and are relatively close to use in clinical practice. For COPD the only biomarker currently widely used in drug trials is lung function testing, typically forced expiratory volume in 1s (FEV1). Although FEV1 is easy to obtain and reproducible it does not inform about underlying disease activity, does not separate out phenotypes of COPD, is not specific to COPD and is unresponsive to some therapies that clearly improve survival (such as Long term oxygen therapy). Hence, novel markers are needed to allow a more complete and clinically relevant assessment of COPD, reflecting the substantial variation in the way in which the disorder presents in different patients. They may enable better phenotyping of different types or patterns of COPD and help improve assessment of disease severity, response to therapy and monitoring of disease progression. The process of identifying appropriate markers and outcomes is not straight forward and needs to reflect the needs of various clinical entities with differing priorities. Validation of these measures is time consuming and involves a substantial commitment of resources. However, this is critical to gaining a better understanding of the pathophysiology of COPD and the development of reliable, comprehensive and evidence-based assessments of the effectiveness of therapeutic interventions. Whilst there are currently very few well

validated markers, there is large number of candidate markers that could be potentially useful for the assessment of COPD. The better-validated ones are discussed below. But the list of these biomarkers is likely to be increased and should not be considered exhaustive.

Biomarkers derived from blood are appealing and are gaining more attention given the ease and uniformity of sample collection when compared with the more technically demanding invasive techniques. Although many proposed blood biomarkers remain relevant only for research purposes and have yet to be applied in the clinical setting, we review here several promising potential candidate that may soon play a role in the management of COPD patients. The most widely studied biomarkers in this population capitalize on the inflammatory nature of COPD, operating under the principle that there is a presence of persistent low grade systemic inflammation in patients with COPD and this lung inflammation spreads to the systemic circulation where it can be measured in the blood.

### Pro inflammatory markers of copd

It is widely accepted and well recognized that the majority of diseases in the COPD syndrome have inflammation as the key underlying mechanism, but also one marked by low-grade, chronic, systemic inflammation with extrapulmonary manifestations, including reduced BMI, skeletal muscle dysfunction, cardiovascular disease, and osteoporosis [7] and studies of a vast array of inflammatory mediators have been undertaken during last decade. As such, biomarker discovery has centered on both extrapulmonary biomarkers of systemic inflammation and pulmonary candidate molecules that are derived from respiratory tract inflammation and lung destruction/repair [8]. The concept that COPD is a systemic disease has led to the postulation that biomarkers of common inflammatory pathways may be useful in the assessment of COPD.

### C – Reactive protein

C-reactive protein (CRP), an acute-phase protein linked to the total systemic burden of inflammation, and is probably the most nonspecific marker of inflammation and yet, has been widely studied in COPD. It was initially thought to be promising because it was shown to be increased in COPD, to correlate with variables predictive of outcomes, and to be reduced in patients with COPD using inhaled corticosteroids. A literature search on pubmed gives more than 300 articles, on CRP and COPD. CRP levels, even those within the accepted normal range, predict future cardiac events in a general population [9] and they are thought to play an important role in the pathophysiology of vascular disease [10]. There is an inverse relationship between plasma CRP levels and lung function, even in subjects otherwise healthy subjects [11].

Evidences are not lacking which clearly established the mechanism to implicate CRP in the pathophysiology of COPD, nor does it reflect known genetic polymorphisms, indicating that it is merely a general marker of the underlying inflammatory process associated with COPD [12]. However, the available data did suggest that high CRP was associated with an increased risk for hospitalization, although probably reflecting disease severity. The conclusions were based on a large population cohort and as such, only indicate trends and will have little impact on managing individual patients [13].

CRP levels rise during exacerbations particularly when there is an

increased influx of neutrophils due to a bacterial cause [14]. In addition, a raised CRP in the stable state predicts recurrent exacerbations due either to a failure to completely resolve the first episode or a persistent underlying airway colonization that predisposes to further episodes [15]. However, there is lack of evidence of a causal association or direct correlation with survival [12, 13, 16-19]. As such, it has been suggested that elevated levels of CRP may not reflect mortality from COPD, but rather vascular events, [20] or it may be secondary to increases in other proinflammatory cytokines, such as tumor necrosis factor-  $\alpha$  (TNF-  $\alpha$ ), IL-6, IL-8, or fibrinogen [21,22,23].

Two major studies explored the relationship between CRP level and mortality in COPD. The first evaluated CRP in 4803 subjects at the fifth annual visit of in the Lung Health Study (LHS) and a mortality and morbidity review board classified mortality end points [24]. The median duration of follow-up was 7.5 years, during which 329 (6.8%) participants died. Serum CRP levels were found to be a significant contributor of all-cause mortality. Both cancer and cardiovascular causes of mortality increased with increasing CRP. The risk of respiratory deaths did not increase with CRP increase also; the patients included were only those with mild-to-moderate COPD, which cannot be applied to all patients with COPD.

The second study investigated CRP levels in 1302 subjects with airway obstruction in the Copenhagen City Heart Study [19]. In the 08 follow-up period, 185 individuals (14%) were hospitalized due to COPD and 83 (6%) died from COPD. Baseline CRP levels were recorded higher in those who had COPD outcomes and the difference was greater in those who died from the disease, 4.3 versus 2.3 mg/l. The predictive impact of CRP for mortality was independent of smoking or lung function in COPD Patients. The fact that an elevated level of CRP in COPD may be predictive of mortality adds evidence to the hypothesis that a persistent low-grade systemic inflammation drives the disorder. However this hypothesis was contradicted by other studies failing to find an association between CRP levels and mortality in COPD. De torres et al; studied 218 stable, well-characterized patients with COPD who had moderate to very severe COPD followed over 4 years, and found that the baseline serum CRP level was not significantly associated with survival status [18]. Also it was noted that the CRP levels of patients who died was higher than that of the patients who survived over time, but the difference did not reach statistical significance.

## Fibrinogen

Of late, Fibrinogen has emerged as the most promising biomarker in COPD and is currently being considered for qualification as a drug development tool by the US Food and Drug Administration [25]. It is an acute phase soluble plasma glycoprotein, (as part of the systemic effects of the disease) synthesized primarily in the liver and converted by thrombin into fibrin during blood coagulation. Normal fibrinogen can increase during acute phase stimulation [26] in response to increased IL-6 production [27]. Several studies have been done to investigate the potential of fibrinogen as a blood biomarker of COPD and assess the evidence for an association between fibrinogen and risk of developing COPD, disease severity, progression and mortality. Many cross-sectional studies have found and reported that the blood fibrinogen levels are higher in patients with COPD compared with healthy controls [28-30]. Plasma fibrinogen has been variably associated with the risk of COPD, disease progression, and mortality

independent of other well-established risk factors, such as age, cigarette smoking, and lung function [31].

The relationship of blood fibrinogen levels with mortality is particularly significant and strong in both COPD specifically as well as general population cohorts. A large meta-analysis of prospective studies of over 154 000 individuals demonstrated a clear correlation between plasma fibrinogen and death from COPD (HR 3.7 (95% CI 2.75-4.97) per 1 g/litre increase in fibrinogen) [32]. In this meta-analysis, the relationship between plasma fibrinogen levels and COPD mortality was stronger among lifetime never smokers than in current or ex-smokers (hazard ratio of 5.5 vs 3.7 for all participants). Recent data from the ARIC/CHS and NHANES III general population cohorts also exhibits increased all-cause mortality in individuals with higher circulating fibrinogen [33].

However, Garcia-Rio and colleagues could not reproduce this finding in a smaller cohort. They found no association between fibrinogen and a diagnosis of COPD after adjusting for age, sex, body mass index (BMI) and smoking history [16]. Eickhoff and colleagues also failed to show any correlation between fibrinogen and disease severity (as defined by Global Initiative on Obstructive Lung Disease (GOLD) stage) in 60 patients with COPD recruited into a study of systemic vascular function in COPD [29] suggesting that a modest association between fibrinogen and disease severity that can only been seen in large sample population.

In the ECLIPSE study (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints) one of the largest prospective COPD-specific cohort studies to date, plasma fibrinogen was found to be weakly associated with total mortality over 3 years and was outperformed by serum interleukin (IL)-6 [34].

Plasma fibrinogen may act as a surrogate marker of disease activity in individuals with COPD if it can predict decline in FEV1 over time. This would benefit us in current clinical setting in to predict those who will remain stable and who are likely to deteriorate rapidly. The ECLISPE study showed that fibrinogen was associated with baseline FEV1 but not longitudinal decline in FEV1, in a larger cohort of 1793 individuals [35]. These data suggest that fibrinogen is not associated with longitudinal lung function decline despite the presence of an association with baseline lung function.

The role of fibrinogen as a diagnostic biomarker for exacerbation has also been found to be variable. Elevated fibrinogen has been reported to be associated with an increased rate of exacerbations in individuals with COPD. In the ECLIPSE study, elevated plasma fibrinogen levels were associated with an increased risk of exacerbations in patients with moderate to severe COPD. A 1 SD increase in plasma fibrinogen level was associated with a 35% increase in the risk of exacerbations [36]. Some studies have reported that plasma fibrinogen levels increase during acute exacerbations, whereas others [37] have shown no significant difference in plasma levels between exacerbations and post stabilization period [38]. Most of these studies involved relatively small sample sizes. Additional large cohort studies will be needed in the future to ascertain the role of fibrinogen as a diagnostic biomarker for exacerbation and to clarify the role of fibrinogen in predicting recovery from exacerbations of COPD.

## Surfactant protein-D

Surfactant protein (SP)-D is a large hydrophilic protein that is a member of the collagen-containing C-type lectins or collectins [39]. This protein is found in the endoplasmic reticulum of type II pneumocytes and the secretory granules of Clara cells [40], and is important in surfactant homeostasis and pulmonary immunity. In COPD surfactant expression decreases in lungs whereas; there is paradoxical increase in protein expression in plasma. The latter has been associated with poor health outcomes in COPD. SP-D is thought to play an important role in the pathogenesis of COPD, including oxidant production, inflammatory responses in alveolar macrophages, and apoptotic cell clearance. It has been found that serum SP-D levels were higher in individuals with COPD and correlated significantly with changes in health status [41, 42]. In the ECLIPSE cohort, serum SP-D levels were recorded to be higher in patients with COPD compared with smokers with healthy controls and were also predictive of increased frequency of exacerbations [42]. The ECLISPE study failed to exhibit correlation between serum level of SP-D and mortality, and therefore its value as a predictor of outcome in COPD remains to be determined. Foreman et al; [43] have shown that certain genetic variants of SP-D are associated with changes in serum concentrations of SP-D and lung function, indicating that SP-D is involved in the pathogenesis of COPD. Sin and colleagues showed that inhaled corticosteroids with long-acting  $\beta$  agonists reduced lung-specific SP-D levels, but not the more generalized biomarkers of systemic inflammation (CRP and IL-6) in ECLISPE Study [44].

## Pro surfactant protein b (pro-SFTPb)

In chronic obstructive pulmonary disease (COPD), surfactant expression decreases in lungs whereas; there is a paradoxical increase in protein expression in plasma. The latter has been associated with poor health outcomes in COPD. Surfactants are particularly interesting in that they are important for reducing surface tension at the air-liquid interface of lungs and thus are essential for life [45,46]. SFTPb is synthesized as a hydrophilic 42-kD protein by type 2 alveolar pneumocytes and nonciliated bronchiolar cells as pro-SFTPb. On synthesis, pro-SFTPb quickly undergoes proteolytic cleavage by cysteine proteases in the endoplasmic reticulum resulting in the synthesis and secretion of a 9-kD noncollagenous hydrophobic SFTPb, which is the functional mature form of SFTPb [47]. SFTPb also has anti-inflammatory properties and may be involved in protecting the lung against oxidative stress [48, 49]. Most importantly, to our knowledge, no extra-pulmonary organs produce any appreciable amount of SFTPb, making SFTPb a highly specific lung biomarker. Plasma pro-surfactant protein B (pro-SFTPb) levels have recently been shown to predict the development of lung cancer in current and ex-smokers, but the ability of pro-SFTPb to predict measures of chronic obstructive pulmonary disease (COPD) severity is unknown. It is important to note that while SFTPb protein expression can be found in a variety of cells in lungs, SFTPb mRNA expression is localized exclusively to type II alveolar cells and nonciliated epithelial cells. Thus, SFTPb is a highly specific pneumoprotein unlike other surfactants such as SP-D and SP-A, which can be genetically expressed by other cells and organs [50]. This unique property of SFTPb makes it a very promising biomarker for evaluating disease severity and perhaps even disease activity in COPD and other inflammatory lung diseases.

## Clara cell secretory protein-16 (CC-16)

Various proteins have been assessed as potentially useful tools in monitoring airway inflammation and epithelial damage [51, 52]. Clara cells, namely located in the terminal bronchioles, are one of the most multifunctional epithelial cell types in mammalian lungs. They appear to be devoted to the protection of the respiratory tract from inhaled toxic agents. Indeed, Clara cells repair damaged epithelium, detoxify xenobiotics, and secrete proteins with important biological activities such as leukocyte-protease inhibitors and the 16 kDa Clara cell protein (CC-16) [53]. (CC-16) a protein secreted by non-ciliated cells of the bronchioles, has also been studied as an indicator of epithelial barrier disruption in the lower airways. Significant efforts have been undertaken to explain its role in airway inflammation and, although its function has not been fully elucidated, CC16 is known to have anti-inflammatory and anti-oxidative properties [54]. However, CC16 levels in serum depend not only on its production by Clara cells and possible leakage through the disrupted epithelial barrier, but also on renal clearance. It can be detectable in bronchoalveolar lavage fluid and also in the serum, where its presence has been proposed to demonstrate a noninvasive marker of lung epithelial injury, based on a passive leakage of CC-16 into the blood circulation [54]. Several lines of evidence suggest that CC16 has an important role in lung defence in experimental animals.

Lower levels of CC-16 had been reported in patients with more severe disease compared with those with moderate airflow obstruction in a small study of 20 patients with COPD [55]. Reduced levels of serum CC-16 have been reported in individuals with COPD and weak but significant correlations with disease severity have been found in former smokers [42]. In a large cohort of patients with COPD, the ECLIPSE investigators explored the relationship between the change of FEV1 over time and the level many proinflammatory biomarkers at baseline [29]. Only the CC-16 levels were significantly associated with the rate of change in FEV1 even when adjusted by age, gender, GOLD COPD severity stage, current smoking status or smoking history, or patient subgroup. Although the relationship to mortality was not significant, the association with rate of decline in FEV1 as a surrogate marker of disease activity suggests a potential role of this biomarker in the comprehensive assessment of patients with COPD.

## Interleukin- 6 (IL-6)

Recently a lot of attention has been given to the contribution of systemic inflammation, as reflected by increased plasma levels of protein interleukin-6 (IL-6) and C-reactive protein (CRP), to reduced muscle strength, decreased exercise endurance, shorter 6-minute walk distance (6MWD), and poor health status [56-59]. IL-6 is one of the most significant mediators of fever and of the acute phase response in the liver [56]. Protein IL-6, besides its central role in initiating and modulating acute-phase inflammatory responses to injuries and infections, increases in plasma during stress unrelated to inflammation. In particular, previous *in vivo* [59] and recent *in vitro* [60] studies have suggested that oxidative stress in hard contractive respiratory muscles elicits expression of IL-6, interleukin-1 $\beta$ , and tumor necrosis factor- $\alpha$ , and this cytokine interplay accounts for most of the hypothalamic-pituitary-adrenal axis stimulating activity in plasma.

At exacerbation, serum IL-6 levels are correlated with selected

**Table 1:** Summary of Pro Inflammatory Biomarkers for predicting risk mortality and progression of disease in COPD.

BIOMARKERS	AUTHOR/YEAR/REFERENCE	MAIN FINDINGS
<b>C-Reactive Protein(CRP)</b>	Dhal et al;/2007 [19]	8-yr follow-up of 1,302 individuals with airway obstruction (Copenhagen City Heart Study) to predict whether increased serum CRP predicts future hospitalization and death from COPD. CRP was found to be a strong and independent predictor of future COPD outcomes.
	Man SF et al;/2006 [24]	4803 subjects in the Lung Health Study with mild to moderate COPD; 05 yrs follow up. Risk of all-cause and disease specific causes of mortality was determined as well as cardiovascular event rates. CRP levels were associated with all-cause, cardiovascular causes of mortality and were associated with an accelerated decline in FEV1. CRP measurements provide incremental prognostic information beyond that achieved by traditional markers of prognosis.
<b>Fibrinogen</b>	Mannino DM; et al /2012 [33]	Follow up study (NHANES III population) (8570 subjects). Mortality data to determine the relation between fibrinogen levels and how fibrinogen levels at baseline affected long-term outcomes in COPD Patients. An elevated fibrinogen level increased the risk of mortality in subjects with Stage III or IV and impaired lung function correlated with higher fibrinogen levels. Also elevated fibrinogen level increased the risk of mortality.
	Celli et al;/2012 [34]	1,843 patients in ECLIPSE cohort to Identify Predictive Surrogate Endpoints. An elevated plasma fibrinogen level was associated with an increased risk of exacerbations in patients with moderate to severe COPD and was also associated with baseline FEV1 but not longitudinal decline in FEV1. Currently undergoing a regulatory qualification process.
<b>Surfactant Protein-D</b>	Lomas DA; et al/2009 [42]	Serum SP-D was evaluated as a biomarker for components of COPD in the ECLIPSE cohort and its response assessed to the administration of the anti-inflammatory agent prednisolone. Serum SP-D levels were recorded to be higher in patients with COPD (1,888 individuals) compared with smokers with healthy controls and were also predictive of increased frequency of exacerbations. The ECLIPSE study failed to exhibit correlation between serum level of SP-D and mortality.
	Sin DD; et al/2008 [44]	The effect of Oral or inhaled corticosteroids on systemic biomarkers of inflammation was evaluated in a small sample population. Inhaled corticosteroids with long-acting $\beta$ agonists reduced lung-specific SP-D levels, but not the more generalized biomarkers of systemic inflammation (CRP and IL-6) in ECLIPSE Study.
<b>Clara Cell Secretory Protein-16</b>	Lomas DA; et al/2008 [42]	Serum CC-16 levels were measured in 2083 individuals with COPD and a smoking history of $\geq 10$ pack-years, 332 controls with a smoking history of $> 10$ pack-years and normal lung function and 237 non-smoking controls. Serum CC-16 level was significantly reduced in a replication group of 1888 current and former smokers with COPD compared with 296 current and former smokers without airflow obstruction.
	Vestbo J; et al/2011 [92]	Weakly associated with lung function decline, emphysema
<b>Interleukin -6 (IL-6)</b>	Bucchioni E; et al/2003 [63]	The presence of interleukin-6 in the exhaled breath condensate of 16 ex-smokers with moderate COPD, 12 healthy non-smokers. IL-6 was measured. IL-6 levels were detectable in all of the subjects, but were higher in the COPD patients suggesting that increased IL-6 levels in exhaled breath condensate may reflect airway inflammation.
	Celli BR; et al/2012 [21]	The ECLIPSE investigators investigated whether an addition of a panel of biomarkers to clinical variables, already proven to predict mortality in COPD, improved the accuracy for predicting mortality in COPD patients. PARC, SP-D, IL-6, IL-8, CC-16, TNF- $\alpha$ , fibrinogen and CRP were investigated. They found only IL-6 independently added predictive power to the basic clinical model, whereas the other biomarkers individually improved the model only marginally.
<b>Pulmonary And Activation-Regulated Chemokine (PARC)</b>	Pinto plata V; et al/2007 [70]	PARC levels were found to be increased in association with reduced FEV1 and higher BODE index scores.
	Sin DD ;et al/2011 [71]	Whether PARC/CCL-18 levels are elevated and modifiable in COPD and to determine their relationship to clinical end points of hospitalization and mortality in the ECLIPSE cohort and Lung Health Cohort. Serum PARC/CCL-18 levels were higher in subjects with COPD than in smokers or lifetime nonsmokers without COPD. Elevated PARC/CCL-18 levels were associated with increased risk of cardiovascular hospitalization or mortality in the LHS cohort and with total mortality in the ECLIPSE cohort.

markers of airway inflammation, and are higher in the presence of a bacterial pathogenesis and tissue repair elements [61]. Thus, the analysis of systemic inflammatory markers may reflect inflammatory load in the airway [62].

Bucchioni et al. [63] investigated the levels of IL-6 in the EBC of ex-smokers with moderate COPD and compared them with the value recorded in healthy non-smokers. IL-6 was detectable in the breath condensate of all the healthy non-smokers ( $4.9 \pm 0.1$  pg/ml), but was significantly higher in the COPD patients ( $8.0 \pm 0.2$  pg/ml;  $P < 0.001$ ). Hence, more studies are needed in order to determine the importance of IL-6 and its effect in FEV1 and mortality. Pro inflammatory cytokine IL-6 plays different biological roles in the context of the acute phase response, as well as in the progression from acute to chronic inflammation. In acute airway and alveolar inflammation,

the role of protein IL-6 has been documented by studies showing its association with faster decline in lung function [64] and it is related to exacerbations in patients with COPD [65].

The ECLIPSE investigators investigated whether an addition of a panel of biomarkers to clinical variables, already proven to predict mortality in COPD, improved the accuracy for predicting mortality in COPD patients [21]. They investigated PARC, SP-D, IL-6, IL-8, CC-16, TNF- $\alpha$ , fibrinogen and CRP. With use of C-statistics, they found only IL-6 independently added predictive power to the basic clinical model, whereas the other biomarkers individually improved the model only marginally.

#### **Pulmonary and activation-regulated chemokine (PARC)**

Pulmonary and activation-regulated chemokine (PARC) is

a protein secreted mainly by the lungs. It is a 7-kD protein that is constitutively expressed by monocytes/macrophages and dendritic cells and is secreted predominantly in the lungs [66]. Although the exact biological role of PARC/CCL-18 is not known it is also reported to be elevated in acute coronary syndrome [67]. PARC Levels are known to be elevated in idiopathic pulmonary fibrosis [68], and interestingly, in idiopathic pulmonary fibrosis, serum PARC/CCL-18 levels may reflect fibrotic activity and correlate with survival [69].

Pertinent to COPD, one previous study had reported PARC levels to be increased in association with reduced FEV1 and higher BODE index scores [70]. In another study, PARC was also found to be associated with acute exacerbations [71]. Following these reports, a large study using data from two major COPD cohorts, the LHS and the ECLIPSE, explored the association of PARC levels and the relationship with lung function and mortality [29]. PARC was found to be independently associated with lung function, morbidity and mortality.

Although these data are promising, several critical questions remain unanswered regarding the possible use of PARC/CCL-18 as a biomarker in COPD, including its relationship with clinical outcomes such as hospitalization and mortality and its responsiveness to pharmacologic therapy hence, more studies are needed to fully determine the pathobiological role of this marker and its value as a marker of disease activity or progression in COPD (Table 1).

## Other Emerging Non Inflammatory Blood Biomarkers

Other than inflammatory biomarkers described above, several novel systemic molecules that have been shown to have a promising role in predicting cardiovascular mortality have been investigated as potential predictors of Mortality in COPD.

### Adrenomedullin (ADM)

The first emerging biomarker of interest is Adrenomedullin (ADM), a pluripotent regulatory peptide that has range of biological function. It acts both as a hormone and a cytokine to exert intensive vascular, immunomodulatory and metabolic effects [72]. ADM is a 50/52-amino acid peptide that was first isolated from human pheochromocytoma [73]. It can be synthesized by many tissues and cells, such as the adrenal medulla, myocardium, central nervous system, and vascular smooth muscle cells [74]. ADM has a variety of biological actions, including vasodilatory, bactericidal, and anti-inflammatory activities [75]. Plasma ADM has been shown to be elevated in a number of diseases, such as arterial hypertension, myocardial infarction, heart failure, [76] and septic shock [77]. Furthermore, it is involved in the pathophysiology of these disorders. This amino acid peptide is expressed in bronchoalveolar epithelial cells, alveolar macrophages and pulmonary endothelium [78]. ADM is induced in response to bacterial exposure and hypoxia [79, 80]. In COPD airway inflammation, ADM may act primarily to promote tissue repair and maintain microvascular function [81, 82].

### Atrial natriuretic peptide (ANP)

Atrial natriuretic peptide (ANP), is a 28 amino acid peptide hormone synthesized by the cardiac atria, has a wide range of cardioprotective effects, including inhibition of sympathetic nervous

system activity and the renin-angiotensin-aldosterone system [83]. Since Nishikimi T, et al showed that human cardiocytes in both atria produce and store a potent natriuretic peptide-atrial natriuretic peptide (ANP)-this substance has been extensively studied in patients with congestive cardiac failure. ANP also attenuates activation of inflammatory signaling by lipopolysaccharide and tumour necrosis factor- $\alpha$  in human pulmonary endothelial cell, and protects against bacterium-induced lung injury and pulmonary endothelial barrier dysfunction [84]. These mechanisms support its protective role against cardiopulmonary complications in patients with COPD undergoing lung cancer surgery [85]. The role of ANP in the development of the clinical syndrome of cor pulmonale is unknown. Recent reports of measurement of ANP levels in patients with various chronic lung diseases including COPD have shown that these patients have higher levels of plasma ANP than normal subjects [86].

### Copeptin

Copeptin, is a 39-amino-acid-long peptide, is the C-terminal part of pro-arginine vasopressin and is released together with vasopressin during processing of the precursor peptide vasopressin, the antidiuretic hormone produced by the hypothalamus, is the key hormone involved in the hemodynamic and osmotic control and mirrors the individual stress level; but its instability precludes its routine use and makes reliable measurements difficult to achieve [87]. In contrast to vasopressin, copeptin is stable at room temperature in serum and plasma and can be measured as a 'shadow' fragment of vasopressin in the circulation [88].

In the last few years Copeptin has been studied as a diagnostic marker and as a prognostic marker in different diseases as sepsis, shock, pneumonia, acute exacerbation of COPD, heart failure, and myocardial infarction [89]. In patients with AECOPD, Copeptin level may reflect the inflammatory cytokine response correlated with the severity of lower respiratory tract infection [90].

### Serum adiponectin

Serum adiponectin has emerged as promising biomarker in COPD. Adiponectin is a hormone produced by adipose tissues that has anti-inflammatory, antidiabetic and antiatherogenic activities. In COPD, it has been reported to be associated with decreased risk of cardiovascular events but with an increased risk of respiratory mortality [91].

In humans, adiponectin is exclusively produced in adipose tissue and is considered as main regulator of proinflammatory adipokines, such as TNF- $\alpha$  and IL-6. Although it is mainly synthesized by adipose tissue, blood levels decrease with the increasing body mass and fat content of individuals. In COPD, as in the general population, high serum adiponectin levels are associated with a reduced risk of cardiovascular morbidity and mortality. However, high serum levels are associated with an increased risk of disease progression and COPD-related mortality. Thus, serum adiponectin levels have a neutral effect on total mortality in COPD [91].

## Future of Pulmonary Biomarkers in Chronic Obstructive Pulmonary Disease

To date, biomarkers are still can't be considered as the reliable element of clinical status or physiological impairment in COPD.

The identification of robust, reliable, and reproducible biomarkers in COPD would be a major boost to the development of new drugs and other interventions to improve the health outcomes of our patients with COPD. With the evidences available, there is currently no established and well validated blood biomarker with clinical applications for smoking-related lung diseases and it is evident from the review detailed here that research community can be expected to develop a panel of biomarkers that can be used clinically to correlate with disease progression, identify exacerbations, and predict mortality. In this context, it seems unlikely that biomarkers will be able to substitute for clinical parameters, but rather will improve diagnostic accuracy through their incorporation into integrated clinical, molecular, and genetic predictive models. We anticipate that a single biomarker a panel of biomarker could serve as a surrogate end point for functional outcome measures in clinical research. Using a biomarker or biomarker panel that more effectively measures disease outcomes when compared with a conventional clinical end point such as lung function will allow for the design of clinical trials of shorter duration with smaller study cohorts. Biomarkers may eventually help us identify high-risk individuals (ie, smokers) likely to develop COPD. Additionally, biomarkers are expected to play a prominent role in the diagnosis of COPD enabling us to identify clinical phenotypes of the patients such as rapid decliners and patient who experience frequent exacerbations. Finally, we anticipate that biomarkers will be a part of a more stratified and personalized approach to the classification, prognostication, and treatment of the chronic respiratory diseases like COPD [92].

## Conclusion

COPD is a complex and heterogeneous disease that affects more than 200 million patients worldwide. The development of novel therapeutics has been slow owing in part to a lack of simple, sensitive, specific, and repeatable biomarker that can predict disease progression and other clinical outcomes. The range of markers available for the assessment of chronic obstructive pulmonary disease and comparison of the effectiveness of different management strategies is currently rather limited. The forced expiratory volume in one second (FEV1) has come to be used almost as a global marker of chronic obstructive pulmonary disease but it does not reflect the multi component nature of the disease. While the development and validation of markers is a difficult task requiring considerable time and resources, progress in the area is critical to improved understanding and management of this chronic, debilitating and increasingly common disorder. Biomarker discovery has become rapidly expanding field of research in COPD and; several biomarkers are poised to be accepted clinical setting, with fibrinogen being the closest to FDA approval and qualification. Despite the rapid gains in knowledge in the pathogenesis of COPD, there remain substantial gaps. Consequently, the identification, validation and understanding of such markers, either in a hypothesis-testing or hypothesis-generating approach, may prove sufficiently informative for understanding the disease process and for developing new therapeutic interventions in COPD. Nevertheless, without accurate patient characterization, biomarker validation, and an understanding of the issues that likely influence the measurements this process may prove to be both fruitless and confusing.

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