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# Biomarkers in obstructive respiratory diseases: an update

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**Chronic obstructive pulmonary disease (COPD) is characterized by chronic inflammation of the airways, with the involvement of many inflammatory cells and mediators. Traditionally, this inflammation is thought to spread to a systemic level, thus inducing damage of different organs. However, other pathogenetic mechanisms could take part to the above-described process, and some open questions need to be solved. Due to the burden and increasing prevalence of COPD, the opportunity to find biomarkers that can potentially be useful in identifying individuals with the disease, or better, prior to symptoms onset, to diagnose and properly manage the respiratory symptoms, as well as to evaluate the response to treatment and to select specific subtypes of patients for tailored treatments is strongly advocated. Several mediators, enzymes, hormones and cells have been claimed to adhere to this objective. Moreover, the presence of comorbid or concomitant diseases can variably influence the concentration of specific biomarkers in samples of individuals with COPD, and age-related functional and structural changes (inflammaging) can further confuse the biological pattern. Several observations have been performed in the last decades; nevertheless, no biomarker is currently considered as satisfying all the above-mentioned issues. The "Evaluation of COPD longitudinally to identify predictive surrogates and points (ECLIPSE)" study has specifically explored the possibility to identify novel biomarkers that correlate with clinically relevant COPD subtypes and with markers of disease progression. Among the thirty-four biomarkers considered, 15 resulted to be increased in COPD patients rather than in smoker and non-smoker controls. Specific lung proteins such as CC-16 and SPD are promising in detecting lung damage, exacerbation susceptibility or responsiveness to treatment. The ECLIPSE findings confirm that, to date, the use of a single biomarker is not sufficient, but the combination of novel biomarkers with the already existing tools could improve our skills in optimizing treatment of COPD patients.**

**KEYWORDS:** Biomarkers - Pulmonary disease, chronic obstructive - Outcome assessment.

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Chronic obstructive pulmonary disease (COPD) is projected to rank 5th worldwide in burden of disease according to a study published by World Bank/World Health Organization.[1] The need for biomarkers in COPD research and management to better diagnose and assess disease subtypes, as well as the effect of treatment is largely recognized. Biomarkers are objective indicators of normal biological processes, pathogenetical processes or pharmacological responses to therapeutic interventions.[2, 3] If the efforts of clinical research achieve the recognition of a biomarker, clinician may have the tools to identify individuals with the disease, or, even better, patients before the onset of symptoms, to accurately follow the progression of the disease, and to demonstrate the effect of drug intervention.

From the pharmacological research point of view, biomarkers could be useful as a proof of early stage trials to demonstrate that the intervention had the hypothesized pharmacological or biological effect, to

identify patients' sub-groups that benefit most and to monitor patients' safety. Currently, no biomarkers are widely accepted as reproducible discriminatory or amenable for the use in large clinical trials in obstructive respiratory disorders.

## Biomarkers in COPD

COPD is a syndrome characterized by inflammatory chronic bronchitis, poorly reversible airways disease with small-airway obstruction, and emphysema secondary to lung tissue destruction and loss of lung recoil.[4] COPD is a leading global cause of morbidity and mortality worldwide. The World Health Organisation (WHO) predicts that within the next 20 years, COPD will become the third-leading cause of death, after coronary artery disease and stroke. The growing impact of COPD is partly due to the continued use of tobacco and to the ageing of the world's population.[5] Despite the well documented role of cigarette smoking in the genesis of COPD, the pathogenetic mechanisms are not fully understood. COPD is characterized by chronic inflammation in small airways and lung parenchyma, with the activation of epithelial cells and resident macrophages and the recruitment and activation of neutrophils, eosinophils, monocytes and several cytokines and chemokines. Moreover, a meta-analysis of observational studies shows the association between COPD and increased serum concentrations of inflammatory molecules (C-reactive protein [CRP], fibrinogen, tumour necrosis factor α [TNF-α]) and cells.[6] Recently, the role of cysteinyl leukotriene antagonists in the treatment of COPD, suggests that the high levels of cysteinyl leukotriene could be a good marker of inflammation detectable in COPD patient's sputum.[7]

The activation of immune cells generates reactive oxidative species (ROS), which are involved in the pathogenesis of COPD. T lymphocytes, with a preponderance of the CD8+ subtype, and macrophages are found in the walls of central and peripheral airways and the parenchyma.[8] The number of CD8+ cytotoxic T lymphocytes is associated to the degree of airflow limitation, suggesting the role of this cell type in the COPD pathophysiology.[9] The abnormal inflammation in COPD was observed not only at pulmonary but also at systemic level.

This inflammation is associated with fibrosis and narrowing of small airways and lung parenchyma destruction, resulting from the action of different proteinases. The predominance of expiratory airflow limitation (bronchitis) or parenchymal destruction (emphysema) leads to the principal clinical phenotypes.

Although COPD is a pulmonary disease, it is associated with systemic manifestations and comorbid conditions. The frequent association of cardiovascular, neurologic and skeletal comorbidities suggests common risk factors and pathogenetic pathways. Firstly, COPD is a disease whose prevalence and severity increase with age. Because most chronic disorders of adults also increase with ageing, comorbidities will be relatively common among COPD patients. In addition, cigarette smoking represents a risk factor for the development of COPD and cardiovascular diseases. As a result of sharing common risk factors, patients with COPD are at further increased risk to suffer these comorbidities. Moreover, the theory that systemic inflammation is the common driver of chronic diseases could explain the high prevalence of multiple chronic diseases. It is a matter of fact that the inflammatory cascade present in COPD lung parenchyma could spread at systemic level and induce further organ damages. This systemic inflammation persists after smoking cessation. Nevertheless, it must be considered that concomitant diseases can be the cause of the increased biomarkers in elderly subjects who present comorbidities and COPD. Whether these systemic markers spill over from the lungs into the systemic circulation or merely reflect the proinflammatory state remains unclear.[10]

COPD and heart failure (HF) are the two most frequent and severe chronic diseases. It has been recently suggested that up to 32% of all HF patients have comorbid COPD. In addition, COPD patients have been found to be 4.5 times more likely to develop HF than disease-free control subjects [11] with these values suggested to be an underestimation due to the misdiagnosis of COPD.[12] Both COPD and HF find in aging and smoking the main risk factors, and in chronic inflammation the potential common pathogenic mechanism. In other words, the presence of concomitant pathologies such as HF or sleep apnea syndrome (OSAS) could represent a factor that makes difficult to ascribe the presence of a specific biomarkers to COPD. Therefore, the increase of mediators involved in the induction of an organ damage can be found in all these pathologies. Cytokines In-



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terleukin (IL-6) and Interleukin-1β (IL-1β) constitute a trigger for systemic inflammatory response with an increase in the circulating levels of platelets, leukocytes, proinflammatory and prothrombotic proteins. This complex response produces C reactive protein, fibrinogen and other coagulation factors which are associated not only to fatal cardiovascular events, but also to the remodelling of tracheobronchial tree present in COPD. Otherwise, despite this evidence, different studies suggest the hypothesis of a systemic inflammatory state called "inflammaging", that characterizes the aging process in an independent way from individual pathologies.[13]

The term "inflammaging", coined by Franceschi *et al*., refers to a progressive increase in proinflammatory status, a major characteristic of the aging process.[13-15] The inflammatory scenario characterizing inflammaging represents a highly complex response to various internal and environmental inflammatory stimuli, mainly mediated by the increased circulating levels of pro-inflammatory cytokines. Inflammaging also generates reactive oxygen species (ROS) causing both oxidative damage and eliciting an amplification of the release of cytokines, thus perpetuating a vicious cycle resulting in a chronic systemic pro-inflammatory state where tissue injury and healing mechanism proceed simultaneously and damage slowly accumulates without any symptoms. This condition is a major determinant of both the ageing process and of the development of age-associated diseases such as osteoporosis, cardiovascular diseases, sarcopenia, insulin resistance and diabetes, that can be noticed also in patients affected by COPD.[11, 14, 16] This could imply that systemic inflammation is present in the ageing process, even in healthy patients and it is not directly correlated to COPD.

COPD is an heterogeneous syndrome, and expiratory forced volume in 1 second (FEV1) alone does not capture the disease complexity (air-flow limitation, parenchymal destruction, mucus hyper-secretion, extra pulmonary effects).[17]

Very few studies have related proteases, neutrophil-elastases and matrix metalloproteinase-9 to the degree of emphysema documented with high resolution computed tomography. Interleukin-8 (IL-8) is reported to be elevated in bronchoalveolar lavage (BAL) of smokers with emphysema compared with those without it.[18] Other authors found that vascular endothelial growth factor concentration in induced sputum increased proportionally with airflow limitation in chronic bronchitis patients whereas decreased proportionally with airflow limitation in subjects with emphysema.[19] The number of neutrophils in BAL and bronchial biopsies correlates with the nitrogen wash-out testing, suggesting that neutrophils count could be useful in identifying COPD patients with small airways involvement.[20]

Sputum eosinophils seem to be linked with COPD airway responsiveness and reversibility and with the response to inhaled corticosteroids.[21-23] The presence of systemic inflammation in COPD has been assessed, and the role of specific biomarkers such as TNF-α, IL-6, and nitric oxide (NO), has been evaluated.[24] The results of a study considering three different groups of subjects (stable COPD, COPD during exacerbation and healthy subjects) showed that TNF-α and IL-6 levels in both stable and exacerbation groups were higher than in controls. In addition, no significant differences in NO between stable COPD group and controls were detected, even if NO levels were higher in the exacerbation group. When comparing cytokine and NO levels at admission and after exacerbation, no significant difference was found. However, the studied circulating biomarkers cannot be considered an useful indicator of COPD exacerbation, neither a parameter for monitoring recovery after exacerbation. Since these biomarkers were high both in stable and in exacerbation groups, and no differences in NO concentration between COPD and controls could be detected, it is plausible to hypothesize that inflammation can be a possible, independent result of the inflammaging process, as already discussed.

This hypothesis is supported by a study [25] in which aging resulted to be associated with low-grade increase in circulating levels of TNF-α and IL-6. Several concomitant diseases (cardiovascular diseases, insulin resistance, type 2 diabetes mellitus and endothelial dysfunction) are indeed controlled by these biomarkers. Moreover, TNF-α and IL-6 are also differently and independently associated with mortality in elderly patients. In conclusion, the association between cytokines and mortality seems to be independent by comorbidities.

Among blood-based biomarkers CRP has proven to be promising. CRP is an acute-phase protein synthetised predominantly by the hepatocytes in response to tissue damage or inflammation. It reflects the total systemic impact of inflammation [26] and has been found to be increased both in stable COPD [6]



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and during exacerbation.[27] It can also be used as a predictor of hospitalization and mortality in patients with chronic respiratory failure.[28] There is now a large consensus about the association between the levels of CRP and the presence of airflow obstruction.[29] However, only few studies that provide an assessment of the relationship between CRP levels and other clinical variables known to predict outcome in COPD patients are available.[30] De Torres JP *et al.* conducted one of the largest clinical studies aimed at investigating the influence of predictive outcomes on CRP levels in a well-defined population of stable COPD patients.[30] The most interesting and novel result of this study is that in stable COPD patients, CRP levels are strongly associated with $PaO_2$ and 6MWD. The study also found that CRP levels represent an independent factor related to important prognostic clinical variables such as FEV1, FVC, IC/TLC, GOLD stage and BODE index. These findings lend support to the concept that the presence of "extra-pulmonary or systemic" consequences of COPD can be clinically detected.[31] Moreover, by determining the level of increased systemic inflammatory markers it is possible to assess the systemic burden of COPD.[6] This study also confirmed that the worsening of lung function is associated to the increase in CRP levels.[32] This correlation could also demonstrate the direct relationship between CRP levels and GOLD stages or the BODE index, which includes some of the previously mentioned outcome parameters (FEV1, 6MWD and BMI).[30] One of the novel findings of this study was the negative correlation between CRP levels and $PaO_2$, which requires further investigations. Indeed, it is now accepted that COPD patients with hypoxaemia present higher mortality, which is improved with oxygen therapy. Hypoxaemia is a trigger for both oxidative stress and inflammation in COPD but, until now, no data has shown that oxygen therapy decreases systemic inflammation in stable COPD patients.[33] The results of the present study supports the need to further explore the relationship between oxygenation, inflammatory markers and outcomes.

In a more recent study [34] the same authors seemed to change opinion with regards to the meaning and the role of CRP levels in COPD. The aim of this study was to determine if CRP levels were associated with survival in patients with moderate to very severe COPD in comparison with other well-known prognostic parameters of the disease (serum CRP level, BODE index, $PaO_2$, inspiratory capacity, TLC and Charlson comorbidity score) in 218 stable COPD patients. The results showed that CRP levels were similar between survivors and deceased and they were not significantly associated with survival.

Levels of CRP were associated with cardiovascular risk of death in the general population [35] as well as in patients with other chronic conditions, such as diabetes [36] and renal disease.[37] Although the important amount of data concerning CRP levels as a cardiovascular mortality marker suggests a role in daily clinical practice, its exact role has been postulated but not yet fully accepted.[34, 38] Two epidemiologic studies have shown that increased CRP levels are independently associated with global and cardiovascular mortality in COPD patients with mild-to-moderate degrees of airway obstruction. As already discussed, CRP is an acute-phase reactant that increases in a very sensitive but non-specific way during tissue damage, inflammation, and infection [26] all processes occurring in COPD patients.[39] Furthermore, CRP levels are influenced by many different effectors such as cardiovascular disease, degree of physical activity, diabetes, renal disease, hypertension, metabolic syndrome, obstructive sleep apnea, smoking status, diet and medications.[34] On this basis, other long-term studies in different settings are needed to evaluate the role of CRP and other biomarkers [40] in patients with clinical COPD. Notwithstanding these issue, the study performed by De Torres [34] is important because it suggests that although the CRP levels reported in large studies correlate loosely with morbidity and mortality, they are unlikely to be a good biomarker because of the lack of specificity for COPD outcomes. This may not be surprising, given that CRP is synthesized predominantly by hepatocytes (and not by the lungs) and for this reason it is a general (and not a lung-specific) biomarker of systemic inflammation.

The above-mentioned reported data show how a systematic research on well-selected patients is needed in order to clarify the role of single biomarkers or biomarkers patterns in disease pathogenesis. The ideal biomarker for COPD has to demonstrate a strong biological plausibility in terms of its role in the pathogenesis of the disease, it must have a strong consistent and independent association with COPD and hard clinical outcomes such as mortality and hospitalization, it has to be modifiable by an intervention, and its change after an intervention must



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be related to a change in an important and accepted clinical outcome (*e.g.*, mortality, exacerbation, rate of decline in FEV1, health status).[41] In the past, induced sputum, bronchoalveolar lavage BAL fluid, bronchial biopsy specimens, and exhaled breath condensates have been investigated as potential sources for biomarker discovery. However, major methodological shortcomings, including the invasiveness of the procedures, poor reproducibility, and/or lack of a standardization of measurements have limited their clinical application.[42] With the growing awareness of COPD as a systemic disease, there has been a shift in the focus of biomarkers discovery away from lung sources and toward blood specimens.[43] However, ongoing researches are evaluating biomarkers in organ-related samples (such as induced sputum or exhaled breath condensate, BAL and bronchial biopsy) or in blood/urine samples. Obviously, some proteins secreted in the lungs can be evaluated in the blood.

The criteria mentioned above have been the guide in developing the "Evaluation of COPD longitudinally to identify predictive surrogates and points (ECLIPSE)" study. The aims of the study were to define clinically relevant COPD subtypes in individuals with GOLD stage II-IV COPD, to establish the parameters that predict disease progression over three years in the clinically relevant COPD subtypes, to acquire data on known clinical biomarkers in order to identify those that correlate with clinically relevant COPD subtypes and to identify novel genetic factors and/or biomarkers that correlate with clinically relevant COPD subtypes and with markers of disease progression.[44]

The study was conducted in 46 centers in 12 countries, enrolling 2180 COPD subjects and 566 control subjects (343 smoking and 223 non-smoking controls) aged 40-75 years. All patients were evaluated periodically for three years. Several biomarkers were assessed both in the general population and in *ad hoc* subgroups of patients. In details, 34 blood biomarkers were assessed in 201 subjects with COPD, 37 in ex-smokers control with normal lung function and 37 in healthy non-smokers. Fifteen biomarkers were significantly different in individuals with COPD when compared to former or non-smokers controls.[45]

Fibrinogen was significantly higher in COPD patients than in ex-smoker and non-smoker controls. It was not associated with COPD severity nor with the severity of emphysema. There was a weak association with the number of reported exacerbations in the year prior to the study. Fibrinogen was also associated with decreased exercise tolerance as measured by 6-minute walk distance (r=-0.23, P=0.001), BODE index (r=0.20, P=0.007) and MRC score (r=0.17, P=0.021). Based on these data, fibrinogen seems to reflect a global assessment of disease severity and can be therefore considered a useful biomarker in COPD.

CRP was found significantly higher in COPD patients than in smokers (P=0.036) and non-smoker controls (P=0.002). As expected, COPD further raised during exacerbation. Unfortunately, this biomarker showed a high variability with only 21% of COPD subjects having a three-month value within 25% of the baseline value. According to these data, its utility as biomarker seems to be limited.

IL-6 seemed to be significantly increased in COPD ex-smokers than in smokers without airways obstruction, whereas its concentration was different from COPD patients and non-smokers.

Several chemoattractans for monocytes/macrophages (IL-68 IL-12 p40, CCL2), lymphocytes (CCL18, CXCL10) and neutrophils have been explored. CCL12 e IL12p40 were not different in COPD patients compared to smoker controls, while both were significantly higher in COPD patients compared to non-smoker controls. CCL18, molecule of great interest due to its correlation with cardiovascular comorbidities, was significantly higher in COPD as regard smoker and non-smoker controls, while CxCL10 and IL-8 resulted significantly increased only in COPD *vs*. smokers. The above-mentioned biomarkers could be useful in identifying subgroups of COPD patients.

The analysis of this cohort of subjects involved in the ECLIPSE study did not show differences among COPD smoker and non-smoker controls, concerning TNF-α seric concentration. Therefore, this mediator does not seem to be significantly associated with COPD, and a detectable concentration of TNF-α was shown only in 10% of COPD patients evaluated. These results could explain the failure of a previous study,[46] in which a monoclonal antibody against TNF-α (infliximab) resulted clinically effective when administered in patients with moderate to severe COPD. Nevertheless, the above-mentioned results open the perspective to use this drug in well-tailored subpopulations of COPD patients characterized by high blood concentration of TNF-α.

Matrix metalloproteinases 8 and 9 (MMP8 and

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MMP9), potential biomarkers of lung parenchymal injury, were found significantly increased in COPD compared to non-smokers, while MMP8 was also higher in COPD than in smoker patients. MMP8 and 9 were shown to be related to GOLD spirometric classification of severity and with the amount of emphysema assessed by means of computed tomography. Until now, MMP8 repeatability needs to be tested while, unfortunately, MMP8 has shown a great variability among repeated dosages. Beyond this evidence, these biomarkers seem to be potentially useful in differentiating patients with emphysema from those with predominant airways obstruction. Mieloperoxidases, enzymes involved in several redox pathways, were significantly higher in COPD than in non-smoker patients but not different when compared to smoker controls. On the contrary, prolactin, peptide hormone involved in the regulation of the immune system, was significantly higher in COPD *vs*. smoker patients but not different when compared to non-smokers. Adiponectine, a protein involved in regulating glucose levels, as well as fatty acid breakdown, was higher in COPD patients than in smokers but resulted not different when compared to non-smokers. Finally, beta-defensin-2, a low molecular weight peptide with antimicrobial properties was found significantly higher in COPD *vs*. smoker and non-smokers controls.

These enzymes and hormones are supposed to be useful at a diagnostic level when combined in a panel of inflammatory markers to better describe the type of inflammatory process of specific subgroups of patients.

A protein of great interest is protein Clara cell 16 (CC-16), produced by the terminal bronchiolous of Clara cells. Recent evidence suggest that Clara cells play an important role in host defence, immunomodulatory response and airways remodelling through the production of specific factors such as CC-16. This protein has never been linked to patients' lung function tests, diseases severity and blood gases parameters. A study [47] performed in 2007 assessed a potential correlation between CC-16 expression in sputum, measured by a new methodological approach, and the degree of severity in patients suffering from moderate and severe COPD. A significantly different expression of CC-16 was detected in COPD patients, according to their stage of severity, as defined by the GOLD 2006 guidelines. Taking into consideration CC-16 properties in innate immunity, a possible link among innate immune system, protein expression and COPD infectious exacerbations may be hypothesized, but further investigation is necessary. Circulating levels of CC-16 have been related to Clara cell toxicity and, therefore, this protein has been suggested as being a COPD marker. The aim of the study performed by Lomas [48] was to verify this hypothesis in a real life study. Serum CC-16 was measured in three different groups of patients. The median level of CC-16 was significantly reduced in a replication group of 1888 current and former smokers with COPD when compared to 296 current and former smokers with COPD without airflow obstruction and 201 non-smokers. Serum levels of CC-16 were found lower in current smokers rather than former ones with GOLD stage II and III COPD, but were not different in individuals with stage IV of the disease. CC-16 does not differ across GOLD stages II-IV, while it was significantly lower in GOLD stages II-III current smokers compared to former ones. After adjusting for age, gender, smoking status and lung function, serum CC-16 was significantly lower in COPD subjects compared with smoker controls. On the above-mentioned data and on the evidence that CC-16 proved to be related to the annual rate of change in FEV1 (P=0.04),[44] it can be assumed that its dosage could be used in longitudinal studies aimed to assess the repairing process of bronchial epithelium, or combined with other markers in the diagnosis and monitoring of COPD progression.[48] Another possible biomarker, which can be found altered in COPD patients is the urinary concentration of the F2-isoprostane (iPF2-III), proposed as a reliable marker of oxidant stress in vivo. It needs determination of overnight urinary excretion of the F2-isoprostane iPF-III, using repeated collections over 3 consecutive days, and shows significantly increased concentrations in subjects with COPD.[49]

An interesting protein assessed in the ECLIPSE study was also the surfactant protein D (SPD). This protein is produced by second type pneumocytes of the terminal bronchiolus. The method of analysis of this protein performed in the ECLIPSE study was the same as the one previously described for CC-16. The aim of the research was to assess the relationship between the level of SPD and the exacerbation risk, and the possibility to modulate the protein level with the treatment with systemic steroids. SPD appeared significantly increased in COPD *vs*. smoker and non-smokers control, while no difference was



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found among spirometric stages of severity according to GOLD. The reproducibility of SPD dosage was high. Of great interest seems to be relationship between protein concentration and exacerbation risk: for each increment of 100 ng/mL$^{-1}$ the odds ratio for exacerbation was 1.22. Moreover, SPD was shown to be modifiable with the treatment with systemic prednisolone; therefore it should be used as intermediate measure in developing new anti-inflammatory drugs for COPD treatment.[50]

Recent evidence suggested that SPD genetic mutations can influence its blood concentration, pulmonary function and susceptibility to COPD, but these data need to be further confirmed.[51] Besides the above-mentioned mediators, also the neutrophils count in sputum was considered. The results showed that the neutrophils count is notable to predict the FEV1 variability and the rate of exacerbations. Moreover, it is not associated with the degree of emphysema and is weakly related to other biomarkers and weakly associated with patient health status. In addition, a relevant variability among different disease GOLD stages has been assessed.[52] A promising field of research is represented by proteome analysis of samples such as induced sputum, exhaled breath condensate, BAL. It may provide presumptive evidence for biomarkers and may discern molecular patterns that are predictive of susceptibility, development, and progression of COPD.[53]

Unfortunately, also the evaluation of pH in the exhaled breath condensate seems not useful. As a matter of fact, pH was lower in COPD patients than in non-smokers, while it was not different in COPD *vs*. healthy smokers. It did not correlate with disease severity, airways inflammation and it was not modified by the treatment with steroids.[54] Data coming from the ECLIPSE study open new perspective in the identification and use of biomarkers in COPD management. However, beyond the relationship within COPD and exacerbation risk, the possibilities to use a single biomarker in predicting, assessing or following COPD patients seems limited. The combination of several biomarkers seem promising. For instance, a group of patients was followed for three months; the level of fibrinogen, IL-6, SPD and CRP were significantly higher in those patients who had a COPD exacerbation in the first month of exacerbation rather than in those who did not have it.[45]

Very recently, Celli *et al*. published the results of an evaluation aimed at analyzing if the biomarkers assessment is able to ameliorate the sensibility of the available tools in predicting patients' mortality. The predictive value of age, BODE score and rate of previous hospitalizations resulted to be improved by integrating it with IL-6 dosage.[55]

## Conclusions

All the data we have disposable in this moment cannot be sufficient answers to all our questions about COPD. ECLIPSE study and other research data confirm that, at the moment, the use of a single biomarker is not sufficient, but the use of a pattern of biomarkers or the combination of a biomarker with the already existing evaluating tools could improve our skills in managing COPD. In particular, as underlined by Cazzola *et al*.[42] there is little information regarding correlation of biomarkers not only with traditional outcome measures (*i.e*., dyspnea, exacerbation frequency and mortality) but also with patient reported outcomes.[56, 57] The final goal will be to match disease severity, biological mechanisms reflecting COPD pathogenesis and the impact of the disease from the patient's perspective. Clear is the example of asthma in which we have assisted to the integration of knowledge about pathogenesis,[58] with the selection of specific biomarkers for targeting the therapy [59] and disease control both in clinical trials [60] and in real life.[61]

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