

# Blood and sputum biomarkers in COPD and asthma: a review

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**Abstract.** – Chronic obstructive pulmonary disease (COPD) and asthma are lung inflammatory diseases that represent major public health problems. The primary, and often unique, method to evaluate lung function is spirometry, which reflects disease severity rather than disease activity. Moreover, its measurements strictly depend on patient's compliance, physician's expertise and data interpretation. The limitations of clinical history and pulmonary function tests have encouraged focusing on new possible tracers of diseases.

The increase of the inflammatory response in the lungs represents an early pathological event, so biological markers related to inflammation may play key roles in earlier diagnosis, evaluation of functional impairment and prognosis.

Biomarkers are measurable indicators associated with the presence and/or severity of a biological or pathogenic process, which may predict functional impairment, prognosis and response to therapy.

The traditional approach based on invasive techniques (bronchoalveolar lavage and biopsies) may be replaced, at least in part, by using less invasive methods to collect specimens (sputum and blood), in which biomarkers could be measured. Proteomics, by the association between different protein profiles and pathogenic processes, is gaining an important role in pulmonary medicine allowing a more precise discrimination between patients with different outcomes and response to therapy. The aim of this review was to evaluate the use of biomarkers of airway inflammation in the context of both research and clinical practice.

*Key words:*

Asthma, COPD, Biomarkers, Early diagnosis, Prognosis.

## Abbreviation list

BAL	Bronchoalveolar lavage
BMI	Body Mass Index
CC-10	Clara cell protein 10
CC-16	Clara cell protein-16
CCL-2	Chemokine (C-C motif) ligand 2
CCL-5	Chemokine (C-C motif) ligand 5
CCL-18	Chemokine (C-C motif) ligand 18
CD4+	Cluster of differentiation 4
CD-8+	Cluster of differentiation 8
COPD	Chronic obstructive pulmonary disease
CRP	C reactive protein
CT	Computer Tomography
CXCL1	Chemokine (C-X-C motif) ligand 1
CXCL8	Chemokine (C-X-C motif) ligand 8
CXCL9	Chemokine (C-X-C motif) ligand 9
CXCL10	Chemokine (C-X-C motif) ligand 10
CXCL11	Chemokine (C-X-C motif) ligand 11
DEFA1	Defensin, alpha 1
DEFA2	Defensin, alpha 2
ECP	Eosinophil cationic protein
EGFR	Epidermal growth factor receptor
FE (NO)	Fractional exhaled nitric oxide
FEV1	Forced Expiratory Volume in the 1st second
FVC	Forced Vital Capacity
GM-CSF	Granulocyte-macrophage colony stimulating factor
GOLD	Global Initiative for chronic Obstructive Lung Disease
GRO $\alpha$	Growth-related-oncogene-alpha
HNP	Human Neutrophil Peptides
IFN	Interferon
IgE	Immunoglobulin E
IL-1 $\alpha$	Interleukin 1alpha
IL-1 $\beta$	Interleukin 1beta
IL-1RA	Interleukin 1 receptor antagonist
IL-2	Interleukin 2
IL-6	Interleukin 6
IL-8	Interleukin 8
IL-13	Interleukin 13

IL-17	Interleukin 17 activated
IL-18	Interleukin 18
IL- $\beta$	Interleukin beta
LTB4	Leukotriene B4
MCP-1	Monocyte chemoattractant protein-1
MMP-12	Matrix metalloproteinase-12
MMP-1	Matrix metalloproteinase-1
MMP-7	Matrix metalloproteinase-7
MMP-8	Matrix metalloproteinase-8
MMP-9	Matrix metalloproteinase-9
MPO	Myeloperoxidase
NE	Neutrophil elastase
NIH	National Institutes of Health
NO	Nitric Oxide
PEF	Peak expiratory flow
PRB4	Proline-rich protein 4
PRH1	Proline-rich protein HaeIII subfamily 1
PRH2	Proline-rich protein HaeIII subfamily 2
RNS	Reactive nitrogen species
SOD	Superoxide dismutase
SP-D	Surfactant protein-D
TGF- $\beta$	Transforming growth factor beta
Th17	T helper 17
Th2	T-helper 2
TNF- $\alpha$	Tumor Necrosis Factor alpha
VEGF	Vascular endothelial growth factor
WA%	Airway wall area percent
WHO	World Health Organization
$\alpha$ 1-AT	Alpha 1 antitrypsin

## Introduction

Chronic obstructive pulmonary disease (COPD) and asthma are chronic inflammatory airways' diseases both arising as major public health concerns associated with a complex gene-environment interaction<sup>1</sup>.

COPD is a multicomponent disease characterized by a not fully reversible airflow limitation, due to an abnormal inflammatory response to noxious stimuli and linked to a range of pathological changes such as mucus hypersecretion and airway obstruction. In established disease the inflammatory cell infiltrate is primarily represented by neutrophils and cytotoxic T cells<sup>1</sup>.

COPD is currently a leading cause of morbidity and mortality worldwide and a recent projection suggested it will become the 4<sup>th</sup> cause of death in 2030<sup>2</sup>.

Asthma is a common chronic inflammatory disease characterized by bronchospasm and reversible airflow obstruction. The predominant mechanism involved in the pathogenesis of asthma is a Type 2 helper T cell cytokine-mediated eosinophilic airway inflammation associated with hyper-responsiveness of the lungs<sup>1,3</sup>.

The differential diagnosis between asthma and COPD has been traditionally based on age of onset, reversibility of airflow limitation, symptom variability and atopy. Nevertheless, misclassifications may occur because of the overlap of clinical features<sup>1</sup>.

Unfortunately early symptoms of COPD are subtle and tend to be ignored by individuals, so very often COPD is diagnosed at advanced stages of disease when patients experience a substantial impairment of their quality of life<sup>4</sup>.

Spirometry is by far the more reliable diagnostic approach used to evaluate disease status, and often the only one. Pulmonary function tests reflect disease severity rather than disease activity and its measurements strictly depend on proper execution and data interpretation. Moreover, clinical symptoms on which pulmonologists rely for a correct diagnostic approach are subjective and nonspecific. Spirometry and clinical history limitations have therefore encouraged focusing on new indicators of disease.

Increased airways inflammatory response may represent an early pathological event, and markers of inflammation may play key roles both in earlier diagnosis and evaluation of prognosis<sup>5,6</sup>. Efforts made in this field may also allow substantial improvement in predicting responsiveness to therapy and/or evaluating efficacy of therapy and disease activity<sup>7</sup>.

With this as a background, it is of primary importance to focus on improving early diagnostic methods, identifying patients with early COPD who could benefit from non-pharmacologic treatments<sup>8</sup>.

In this review, we have analyzed markers of early disease which may help to predict lung function impairment and prognosis of COPD and asthma.

## COPD and blood biomarkers

Although the observation that blood biomarkers could be measured by cost-effective and routinely used techniques makes this approach very appealing, it is important to underline that morbidities other than lung diseases may alter biomarker concentrations, making difficult to give a clearcut meaning to their measurements<sup>5</sup>.

The biomarkers of systemic inflammation that have been most widely studied are fibrinogen, interleukin (IL)-6, IL-8, and C-reactive protein (CRP)<sup>9</sup>. These markers can distinguish patients with COPD from controls with acceptable sensitivity; however, unfortunately, they lack specificity<sup>6</sup>.

Therefore, other molecules, such as extracellular matrix markers such as metalloproteinases (MMPs) 8 and 9 and lung-derived markers, including surfactant protein-D (SP-D), clara cell protein-16 (CC-16) and CCL-18 have also been studied in order to identify proteins that could better mirror the airway environment<sup>9</sup>.

According to its definition, an ideal biomarker must be reproducible in stable disease. Among blood markers, this has been the case of SP-D, fibrinogen and CC-16, while other candidate molecules, including IL-6 and IL-8, CCL-18 and CRP, need further evaluation<sup>10</sup>.

SP-D is emerging as one of the promising markers, being an important molecule involved in pulmonary system immunity and surfactant homeostasis: blood median levels of SP-D are higher in COPD patients and in smokers. While SP-D levels do not correlate with GOLD-defined disease severity status, peak blood levels seem to be linked to exacerbations risk and are associated with extension of CT-documented emphysema and its progression<sup>11,12</sup>.

Currently, another emerging biomarker for COPD is fibrinogen, an acute phase plasma protein, which is synthesized primarily in the liver and converted by thrombin into fibrin during blood coagulation. According to Duvoix et al, while there is a significant association between fibrinogen levels and number of COPD exacerbations<sup>10,12</sup>, and rate of hospital admissions<sup>13,14</sup>, it appears unable to predict lung function decline<sup>10</sup>.

Another predominant plasma biomarker is CRP, a routinely measured acute-phase protein, which is involved in COPD pathogenesis together with other inflammatory molecules such as matrix metalloproteinases. Although data need further studies, it has been shown that CRP levels at baseline are associated with lung function decline and increases of this molecule are inversely associated with forced expiratory volume in the first second (FEV<sub>1</sub>). Similar findings were obtained with other markers such as MMP-1, 7 and 9, not only in COPD associated to tobacco smoking, but even in COPD due to biomass smoke exposure<sup>15,16</sup>.

Fibronectin, a high molecular weight glycoprotein whose primary role is promoting wound repair after injury, has been investigated as biomarkers in COPD, with controversial results. According to some authors, fibronectin seems independently associated with mortality<sup>17</sup>, but these findings are not confirmed by more recent studies. In fact Kelly et al<sup>18</sup> have studied the role of systemic

inflammatory biomarkers such as fibronectin, C-reactive protein, and IL-6 in predicting COPD mortality. Only C-reactive protein was proven to be independently associated with increased risk of death; while IL-6 has been shown to contribute to mortality prediction only when added to known clinical variables such as dyspnea, obstruction, BMI, and exercise capacity index.

Another recent study<sup>13</sup> showed that the incidence of hospital admissions for COPD was significantly associated with increased levels of fibrinogen,  $\alpha$ 1-antitrypsin, haptoglobin, ceruloplasmin, and orosomucoid.

Studies on the relationship between markers of eosinophilic and T cell activation and the development of progressive COPD are promising. In fact, IL-2 levels were higher in patients with stable disease as compared to patients with progressive COPD. Moreover, Eotaxin-1 appeared to be lower in patients with stable disease, suggesting that both molecules may become important markers of stability in patients with COPD<sup>19</sup>.

Airway remodeling in asthma and chronic obstructive pulmonary disease results in thickening of bronchial walls and may affect lung function. A significant correlation between CRP, IFN-gamma, IL-6, and IL-13 and parameters measured by high resolution computerized tomography, such as wall area as a percentage of total airway area (WA%), was recently demonstrated by Bon et al<sup>20</sup>. On the contrary EGFR was inversely related to this parameter but was able to reflect airway functional impairment<sup>21</sup>.

## Asthma and blood biomarkers

The management of asthma, as that of COPD, has always been focused on the monitoring of lung function using spirometric parameters such as FEV<sub>1</sub> and peak expiratory flow (PEF), possibly adding the evaluation of airway hyper-responsiveness. Only recently, the identification of molecules of airway inflammation as possible biomarkers of clinical utility has been approached. Due to asthma pathogenesis, the most widely investigated markers are B-cell and T-helper 2 (Th2) derived molecules<sup>7</sup>.

As recently discussed, high levels of blood eosinophils indicate a switch to Th2 cell phenotype and may help to predict responsiveness to corticosteroid therapy<sup>22</sup>.

A study by Wagener et al<sup>23</sup> evaluated the role of blood eosinophils, FE (NO) and serum periostin as

surrogates for sputum eosinophils in asthma, showing that blood eosinophils had the highest accuracy value and suggesting that blood eosinophils evaluation may facilitate asthma management and may help the search for individualised treatment.

In contrast, a previous research<sup>24</sup> showed that blood eosinophils, exhaled nitric oxide and total IgE levels, despite being associated with sputum eosinophils, did not accurately predict their percentages.

Periostin is a very promising molecule that recently emerged as a potential biomarker of Th-2 dependent eosinophilic activation. Though not yet applied in clinical practice nor accurately evaluated in COPD or other airway diseases, the levels of periostin in blood seem to be strictly linked to airway eosinophilia, even more than eosinophil count and IgE levels<sup>25</sup>. Lately, Corren et al<sup>26</sup> conducted a trial on asthmatic adults in treatment with Lebrikizumab, an anti-IL-13 monoclonal antibody, demonstrating that higher levels of periostin are associated with a better response to therapy.

In this field special attention has been given to Immunoglobulin E (IgE), as it is a marker for B-cell activation<sup>26</sup>.

Liang et al<sup>27</sup> recently conducted an epigenome-wide association study, surveying association of total serum IgE concentration with methylation at 36 loci, which encode inflammatory mediators, eosinophil products, and specific transcription factors. This study confirmed that methylation at these loci was significantly different in eosinophils from subjects with and without asthma and high IgE levels, suggesting new biomarkers and therapeutic targets for atopic patients.

### **COPD and sputum biomarkers**

Sputum represents an important diagnostic tool for assessing airway inflammation in patients with COPD. It can be spontaneous or induced by inhalation of hypertonic saline solution<sup>28</sup>.

In contrast to other sampling procedures, such as bronchoalveolar lavage, in which the main cell type are the alveolar macrophage<sup>21</sup>, the typical COPD feature in sputum is an increased number of neutrophils although with not univocal findings. While Shaw et al suggest<sup>29</sup> a significant association between neutrophil count and GOLD stage<sup>30</sup>, the ECLIPSE study<sup>30</sup> did not reveal a straightforward correlation with pulmonary function.

While at all COPD stages we can find an activation of innate immunity, characterized by high-

er levels of neutrophils and macrophages, in later stages, also lymphocytes seem to play a key role<sup>31,32</sup>, with increased sputum concentrations of type 1 CD-8<sup>+</sup> T-cells<sup>21</sup>.

Interestingly some COPD patients have an increase in eosinophil counts. Higher levels of eosinophils seem to be associated with a better responsiveness to corticosteroids and bronchodilators<sup>33</sup>.

In the last years, the levels of an enormous amount of molecules, released by activated respiratory tract cells, such as cytokines, chemokines, lymphokines, growth factors, molecules related to oxidative stress, proteases and antiproteases, have been found altered in the lungs of patients with COPD.

More than fifty cytokines have been identified for being responsible of the inflammatory process in COPD, and it appears to be a certain overlap in their functions. More studies are needed to clarify the role of each molecule in the pathogenesis of COPD<sup>34,35</sup>.

Pro-inflammatory cytokines amplify and perpetuate the inflammatory response, in part through the activation of transcription factors that lead to the increased expression of certain genes. Among proinflammatory cytokines, several were found to be increased in the sputum of patients with COPD, (e.g. TNF- $\alpha$ , IL- $\beta$ , and IL-6).

Tumor necrosis factor (TNF)- $\alpha$  is a pleiotropic cytokine that plays a significant role in chronic bronchitis, COPD and asthma, but the analysis of its levels yielded contrasting results. Some studies have shown that COPD cultured sputum cells synthesize significantly less TNF- $\alpha$  than healthy nonsmoking subjects, while no significant differences were found between sputum cells from COPD patients and healthy smokers<sup>36</sup>. On the contrary, Hacievliyagil et al<sup>37</sup> found higher levels of TNF- $\alpha$  in the sputum of COPD patients compared to the smoking and non-smoking control subjects, as also reported by Barnes<sup>34</sup>.

Interleukine (IL)-1 $\alpha$ , IL-1  $\beta$ , and IL-1 receptor antagonist (IL-1RA) constitute the IL-1 superfamily, involved in cell proliferation, differentiation and apoptosis, and in the induction of macrophage related cytokines and metalloproteinase MMP-9 release. Data on these molecules are conflictual. According to recent studies, there is an increase in the levels of this pro-inflammatory cytokine in COPD patients sputum, which is associated with disease severity. Moreover, a decrease in the levels of both IL1 receptor antagonist and soluble IL1 receptor was demonstrated<sup>38</sup>.

On the other hand, Comandini et al<sup>36</sup> report that the expression of IL-1 in sputum bronchial cells is present also in normal individuals, but data are not sufficient to draw a clear role in tobacco smoke exposed subjects.

Also IL-6 was recently studied, with the observation that its levels appear to be increased in the sputum of COPD patients, primarily during exacerbations<sup>39</sup>.

Chemokines have been observed in sputum samples, as they play a key role in the recruitment of inflammatory cells, especially neutrophils, to the lungs of COPD patients: CCL2, CXCL8, CXCL1 and CCL5 levels appeared markedly increased in induced sputum of patients with COPD<sup>40,41</sup>.

CXCL9, CXCL10 and CXCL11 correlate with disease severity and are increased in sputum of COPD patients. These findings were confirmed by Costa et al<sup>42</sup> that analyzed their levels in COPD patients and in normal volunteers.

In their review Comandini et al<sup>36</sup> suggest that the increased levels of chemotactic protein-1 (MCP-1) and growth-related-oncogene-alpha (GRO- $\alpha$ ) may be related to an inflammatory state due to disease process and not just to smoke exposure.

Some T-cell cytokines, also known as lymphokines, seem to be increased in the sputum of individuals with advanced COPD<sup>31</sup>. IL-17A, produced by Th17 cells, a subset of CD4<sup>+</sup> T cells, was found to be increased in the sputum of individuals with COPD<sup>43</sup>. Similar findings were observed for IL-18, with data also related to disease severity<sup>44</sup>.

Several growth factors have been studied in sputum samples of patients with COPD. Profita et al<sup>36</sup> demonstrated higher levels of granulocyte-macrophage colony stimulating factor (GM-CSF) in induced sputum cells of COPD patients, while data on sputum induced transforming growth factor (TGF)- $\beta$  are still scarce<sup>28</sup>.

Vascular endothelial growth factor (VEGF) was found to be increased in induced sputum of patients with COPD and its levels were negatively correlated with lung function<sup>45</sup>.

Among oxidants and oxidative stress related factors, several molecules have been studied in their association with COPD in sputum samples.

Myeloperoxidase (MPO) is contained in neutrophils granules and in monocytes. According to a recent metanalysis, its sputum levels were higher during exacerbations and in stable disease in comparison to normal controls<sup>46</sup>. Superoxide dismutase (SOD) has been more intensively stud-

ied using other sampling procedures, while there are not enough data on sputum<sup>28</sup>.

8-isoprostane is an important prostaglandin-isomer, which is emerging as a relevant marker associated to the physiopathology of oxidative damage. According to Kinnula et al<sup>46</sup>, 8-isoprostane increases in the sputum of COPD patients and is correlated with smoking attitude and with the reduction of FEV<sub>1</sub> and FEV<sub>1</sub>/FVC.

Levels of nitrotyrosine are considered to be reliable indicators of the production of Reactive nitrogen species (RNS), due to higher levels of NO during airway inflammation. Nitrotyrosine levels are increased in the induced sputum of smokers, as documented by Rityla et al<sup>46</sup>.

With regard to proteases and antiproteases, matrix metalloproteinases (MMPs) are involved in the destruction of extra cellular matrix components, and their levels have been accurately studied in the sputum of patients with COPD. Ilumets et al<sup>48</sup> suggest that MMP-8 can differentiate symptomatic smokers and individuals who risk to develop COPD among non-symptomatic chronic smokers. Moreover, MMP-8 also seems to be associated with lung function. Also MMP-9 and MMP-12 appear increased in symptomatic smokers but neither of them accurately differentiate healthy from symptomatic smokers.

Neutrophil elastase (NE) is a neutrophilic serine protease, and its enzymatic activity appears to be considerably increased in current smokers versus former smokers with COPD<sup>49</sup>. Paone et al<sup>50</sup> confirmed an increased concentration of NE in the sputum of patients with COPD, and demonstrated an increase of HNP (Human Neutrophil Peptide), IL-8 and MMP-9. Furthermore NE, IL-8, and HNP, seemed to have a significant negative correlation with FEV<sub>1</sub> and FEV<sub>1</sub>/FVC.

Concerning leukocyte pro-inflammatory and antibacterial products, one of the main molecules that have been studied in sputum is leukotriene B4 (LTB4), an arachidonic-derived molecule released by both neutrophils and macrophages. Tufvesson et al showed that sputum levels of LTB4 could be reliable predictors of COPD exacerbations<sup>51</sup>.

Another approach in biomarker classification has been proposed by Comandini et al<sup>36</sup>, who stratified biomarkers into five principal groups: 1) molecules that are associated both with tobacco smoke and COPD, such as MMP-9, MMP-8, LTB4, GM-CSF and 8-isoprostane; 2) COPD biomarkers that are not in association with tobacco smoke, such as IL-8,  $\alpha$ 1-AT, and GRO- $\alpha$ ; 3) biomarkers that are influenced by tobacco smoke exposition but are

not correlated with COPD activity (NE, CXCL9, CXCL10, CXCL11 and CCL5); 4) markers that are negatively associated with COPD and/or tobacco smoke, such as TGF- $\beta$  and SOD; 5) biomarkers that are variably associated with COPD and tobacco smoke (TNA- $\alpha$ , MPO, and VEGF).

### Asthma and sputum biomarkers

Sputum analysis is also a remarkable technique applied to investigate respiratory tract in patients with asthma. Since asthma is not associated with productive cough, it is often necessary to perform sputum induction.

Asthma is traditionally considered as a prevalent eosinophilic disease so that several Authors have suggested to apply eosinophil counts to support diagnosis<sup>52</sup>. Because cases of non-eosinophilic asthma may exist, as well as patients showing eosinophilic predominant COPD: studies on induced sputum have led to the definition of four inflammatory phenotypes of asthma: eosinophilic, neutrophilic, mixed and paucigranulocytic pattern<sup>53</sup>.

Recent observations<sup>54</sup> pointed out that patients with higher numbers of sputum eosinophils seem to have better responsiveness to steroid therapy. Moreover, Petsky et al<sup>55</sup> carried out a meta-analysis in which asthma exacerbations resulted prevented by treatment protocols based on sputum eosinophil counts rather than clinical symptoms. However, other trials did not confirm these results<sup>56</sup>.

Another potential sputum biomarker for asthma is eosinophil cationic protein (ECP). It is an eosinophil-derived degranulation product that is released during several inflammatory conditions, therefore does not represent a specific marker of asthmatic disease<sup>57</sup>. Nevertheless, in patients with asthma diagnosis, ECP levels could be used for the assessment of the extension and severity of inflammation<sup>58</sup>. In addition, VEGF appears increased in sputum during asthmatic exacerbations and high levels of VEGF in sputum seem to be related to airflow obstruction<sup>59</sup>.

### COPD and biomarkers in BAL

Besides sputum and blood samples, potential useful COPD biomarkers have been evaluated using bronchoalveolar lavage (BAL), such as several

pro and anti-inflammatory molecules sampled from the lower respiratory tract.

Importantly, several shortcomings must be considered: COPD inflammation may alter the characteristics of alveolar fluid making it quantitatively inappropriate for analysis; the exact amount of biomarkers may be difficult to measure due to sample dilution because of saline solution lavage<sup>21</sup>. In addition, the effectiveness of the results could be limited by manipulation during BAL processing<sup>33,39</sup>. Thus, the validation of a diagnostic protocol based on BAL requires a significant number of patients.

As BAL analyzes distal airways, there is a high prevalence of alveolar macrophages in the cell count of COPD patients (>80%)<sup>60</sup>, but, interestingly, Drost et al<sup>61</sup> found that a severe stage of disease might be associated with mononuclear cell reduction. Of note, smokers show a decreased quote of lymphocytes as compared to ex-smokers<sup>60</sup>. In particular, while the amount of CD-8<sup>+</sup> T-cells appears higher, CD-4<sup>+</sup> T-cells number is lower in COPD smokers and healthy smokers compared to non-smoking subjects. It is difficult to gather data about eosinophils; however, some studies demonstrated a higher number of this cell type in COPD<sup>60</sup>.

COPD pathogenesis is linked to neutrophils and their amount in the BAL correlates with FEV<sub>1</sub>/FVC ratio<sup>61</sup>. In fact, there is a rise of both neutrophils and their derived molecules levels during stable status and also during exacerbations, compared to healthy nonsmokers. This is probably due to smoking exposition, as some studies document<sup>60</sup>. Notably, in mild emphysema, this cell type cannot be detected in BAL, even though in BAL fluid neutrophil derived enzymes and neutrophil chemokine IL-8 are higher than in healthy condition<sup>60</sup>.

Rohuani et al<sup>62</sup> found that NE baseline levels in BAL from individuals with alpha 1 antitrypsin deficiency correlates with FEV<sub>1</sub> rate of decline.

BAL fluid is a source of different molecules which may be detected in order to analyze respiratory tract inflammation. Paone et al<sup>63</sup> found that human neutrophil peptides (HNP), small antimicrobial molecules released by activated neutrophils, are increased in smoker BAL.

COPD causes a rise of surfactant protein D (SP-D) in serum but a decrease of this molecule in BAL whereas steroid treatment leads to an opposite condition. For this reason, SP-D may be considered a marker of both COPD and response to therapy<sup>33</sup>.

Miller et al<sup>64</sup> demonstrated that some eosinophil

markers in BAL such as eosinophil cationic protein (ECP) and eotaxin were associated with emphysema extension reported in CT scan and they were higher in COPD patients after bronchodilator therapy, in contrast to neutrophil marker levels. In addition, healthy smokers have higher BAL levels of ECP, myeloperoxidase and IL-8 in comparison with nonsmokers. However, it is useful to underline that increased ECP and MPO levels are found also in other chronic respiratory disorders<sup>65,66</sup>.

Another interesting finding is that prostaglandin D<sub>2</sub> and eicosapentaenoic acid levels in BAL lavage are positively correlated with lung function in COPD patients<sup>67</sup>. Antczak et al<sup>68</sup> also demonstrated positive correlations between 8-isoprostane and LTB<sub>4</sub> amount in BAL.

Among studies on COPD biomarkers, some authors investigated an innovative research field, the regulation of gene expression involved in lung inflammation. In BAL lavage, basic salivary proline-rich protein 4 (PRB4) and lysozyme C have been reported to be up-regulated in smokers, neutrophil defensins 1 and 2 (DEFA) were positively associated to COPD, and transcription of calgranulina A gene has been found after smoke exposition, both in patients with and without COPD. On the other hand, salivary acidic proline-rich phosphoprotein 1/2 (PRH1 and PRH2) and clara cell phospholipid binding protein (CC10) showed a reduced expression<sup>69</sup>.

Tobacco smoke is by far the most commonly encountered risk factor for COPD; however, not all smokers develop clinically significant COPD. This suggests that cigarette smoking cannot be the only cause of COPD and that additional factors are involved in determining each individual susceptibility. According to Dahl et al<sup>5</sup> the earliest potential risk factors of developing obstructive lung diseases are due to genetic predisposition, and as suggested by a meta-analysis by Joanna Smolonska<sup>70</sup> on suspected obstructive airway diseases genes, a polygenic inheritance seems involved, with each gene having a small effect on the development of the disease.

In addition, COPD is a progressive disease and it is demonstrated to begin many years before a diagnosis is made, so focusing on indicators of earlier diagnosis and treatment is of primary importance<sup>71</sup>.

At present, spirometry is the most reliable detection method for obstructive pulmonary diseases; however, airway obstruction does not seem to correspond to the very beginning of the disease<sup>71</sup>.

When spirometry is used as the only method of analysis, all the other pathological components of such multicomponent diseases remain ignored<sup>5</sup>. New proven diagnostic tests are therefore needed in order to allow an early and complete disease assessment.

An earlier diagnosis would bring several advantages, including the use of therapy when it would have a good chance to modify the course of the disease. Therefore, the identification of biological markers of susceptibility or progression of disease would be crucial.

In this background, and considering the key role of aberrant inflammatory response in the lung as a primary pathogenic process of obstructive airway diseases, inflammatory markers have a promising predictive value in COPD and asthma<sup>5</sup>.

Although an overwhelming number of biomarkers has been proposed in airways diseases, there are still many unanswered questions about their utility in "real life".

One of the most widespread definition of a biomarker is that of a molecule of clinical utility by being associated with an alteration in physiology or progression of disease.

Several characteristics distinguish an ideal biomarker: its association with disease, the demonstration that therapy has a consistent effect on the marker concentrations and the observation that these changes are associated with positive effects in clinical outcomes. Other necessary feature is that of being easily measured using standard procedures<sup>72</sup>.

The identification of potential markers of airway diseases is, therefore, one of the most challenging aims of research<sup>5</sup> and markers of pulmonary diseases will be very useful in the upcoming future especially in predicting prognosis and responsiveness to treatment<sup>34</sup>.

However, as Dahl et al<sup>5</sup> notice, biomarkers may be contemporarily associated to various pathogenic processes, possibly representing diseases other than COPD, making more difficult the interpretation of the results.

An interesting suggestion offered by Tzortzaki et al<sup>73</sup> is to combine panels of markers together to achieve higher specificity. This alternative approach would confer clinical utility to markers that currently are not sufficiently specific to be used alone.

Paone et al<sup>74</sup> evaluated a panel of sputum markers such as HNP, MMP-9, and IL-8 analyzing its diagnostic role in discriminating COPD patients from smokers.

As underlined by Ptolemi et al<sup>75</sup>, special attention must be given to cost benefit ratio, as the

number of NIH grants from 1986 to 2009 for biomarker research has enormously increased without comparable findings of clinical utility, especially in the latest years.

## Conclusions

Although at the present the knowledge about reproducibility is too immature and very few markers have been validated, there is urgency to approve obstructive pulmonary disease biomarkers to give consistent help to clinicians in achieving a complete and prompt assessment of patients, including diagnosis, follow-up and response to therapy<sup>76</sup>.

Recently proteomics approach, by the association between different protein profiles and pathogenic processes, is gaining an important role in pulmonary medicine allowing a more precise discrimination between patients with different outcomes and response to therapy. Lee et al<sup>77</sup> undertake proteomic analysis in lung tissues of smokers, nonsmokers, and COPD patients demonstrating a significant difference in the three groups.

It is likely that an integrative approach that combines biomarkers with clinical parameters, associated with new information from the fields of genomics, transcriptomics, and proteomics, will improve the ability of clinicians in monitoring obstructive lung diseases progression and predicting their response to therapy<sup>78</sup>.

## Conflict of Interests

The Authors declare that they have no conflict of interests.

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