Search for biomarkers in chronic obstructive pulmonary disease: current status

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Purpose of review
Chronic obstructive pulmonary disease (COPD) is a condition principally defined by airflow limitation that is not fully reversible. The main trigger, inhalation of noxious gases or particles (usually smoke) leads to complex pathology, including inflammation of the large and small airways, and destruction of the lung parenchyma. Overlap in pathophysiology with other chronic airways diseases leads to challenges in differential diagnosis, and furthermore, periodic exacerbations of disease symptoms also increase the complexity of the disease diagnosis and prediction of outcome. There is recognized need for biomarkers to aid in the determination of disease diagnosis, progression and response to intervention. This review describes the current status of biomarker identification in COPD.

Recent findings
Biomarkers of disease can take many forms other than the classical protein in serum, and their utility is dependent upon the clinical question to be addressed. No single protein marker has been adopted for routine clinical use to date. This review addresses the key issues around biomarker identification and utility in both stable and exacerbating COPD.

Summary
Biomarker identification in COPD is still a developing field, with increasing interest in patient phenotyping probably reflecting the challenges of biomarker development in a complex disease.

Keywords
biomarkers, chronic obstructive pulmonary disease, respiratory

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a condition primarily caused by the inhalation of noxious particles or gases (usually cigarette smoke) leading to inflammation and remodelling in the large and small airways, and destruction of the lung parenchyma in the form of emphysema. Multiple genetic and environmental factors affect the course of the disease, and because of a systemic inflammation component, the disease is often accompanied by significant comorbidities. Periodic exacerbations of disease symptoms (ECOPD), strongly associated with microbial infections, hasten disease progression and worsen prognosis.

Global prevalence of the disease is 9–10% [1], identified mainly according to the Global initiative for chronic Obstructive Lung Disease (GOLD) guidelines [postbronchodilator forced expiratory volume in 1 s (FEV_1)/forced vital capacity ratio less than 0.7; severity determined by FEV_1 alone] combined with a history of exposure to risk factors. There is considerable evidence of underdiagnosis, especially in the mild and moderate groups.

Whilst there is no cure, management of stable disease is principally directed by symptoms and spirometry. FEV_1 is the only validated clinical marker of COPD, but because of its use in the diagnosis of COPD, and its definition as incompletely reversible in the disease, it is not an ideal candidate for determining therapeutic effectiveness. FEV_1 correlates only poorly with clinical features and is not sensitive enough to indicate very early onset of disease.

There is a clear need for biomarkers not only to diagnose stable disease, especially at subclinical levels, but also to predict it, stage it, monitor

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KEY POINTS

- Many potential biomarkers require further validation; none are in clinical use except FEV_1.
- Longitudinal and prospective studies are enhancing the prospects of finding useful biomarkers of COPD.
- Subphenotyping of patients is likely to yield the best biomarkers in this heterogeneous disease.

progression and also to quantify therapeutic efficacy. This review describes the current knowledge of biomarkers in COPD.

THE ONGOING SEARCH FOR NOVEL BIOMARKERS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

The majority of current scientific research on COPD biomarkers is concerned with proof-of-concept or validation studies of single markers which have been identified either by deduction from related biological mechanisms in other diseases, or by previous proteomic or genomic studies; however, the attrition rate following the validation studies means that novel biomarker identification is a vital process.

Contributing to the biomarker canon, transcriptomic analysis of macrophages from bronchoalveolar lavage (BAL) fluid has identified differential mRNA profiles between healthy and COPD individuals. These genes were associated with apoptosis, metabolism and inflammation [2]. mRNA transcripts have also been compared in small airway epithelial cells from nonsmokers, smokers and COPD smokers [3▪▪]. A subset of healthy individuals were found to have altered gene expression in response to smoking (responders), and these were more similar in profile to COPD than to nonsmokers, suggesting that mRNA might be used to identify smokers at-risk of developing COPD.

Similarly, unsupervised clustering analysis of blood plasma from smokers with and without emphysema identified metabolites that could distinguish between emphysema and controls [4], and also found a group of healthy smokers with profiles similar to the emphysema group who may be at risk of developing the disease. Although the identity of these metabolites remains unknown, their potential use in disease prediction holds great promise.

Using exciting novel methodology, Terraciano *et al.* [5] examined the peptidome of induced sputum and discovered abnormal expression of α-defensins. This was, however, a proof-of-concept study and their validation is pending.

Verrills *et al.* [6▪] combined standard proteomic methodology with sophisticated unbiased statistical analysis to identify four blood-based biomarkers of COPD. All four were acute-phase proteins involved in inflammatory regulation.

Recent studies examining the smoking-associated proteome in humans [7] and animal models [8] may help to inform on the mechanisms leading to COPD, particularly at the subclinical stages.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE BIOMARKERS ARE RELATED TO DISEASE PATHOPHYSIOLOGY

COPD biomarkers appear to fall into several main categories, presumably reflecting their role in the disease process, including the acute-phase proteins, innate immune proteins/cells, inflammation, adaptive/autoimmunity, oxidative stress responses and tissue remodelling (Fig. 1). The latest findings in each of these categories are summarized here.

Acute phase and related proteins

Acute-phase proteins have been implicated in both stable and exacerbating COPD. For example, elevated plasma fibrinogen has been associated with poor prognosis, especially in severe disease [9]. Similarly, plasma C-reactive protein (CRP) has generally been found to be elevated in stable COPD, although not in every study; elevated CRP was recently shown to increase the risk of death only in severe COPD [10]. The lack of a link between genetic elevation of CRP levels and COPD risk suggests no causal relationship [11].

Innate immunity

Neutrophils may be mechanistically involved in COPD pathology [12], are elevated in the disease and therefore make attractive biomarkers for therapeutic efficacy; however, their levels appear to be refractory to some treatments [13]. Protein markers reflective of innate defence may be more responsive. Innate defence molecules, which are generally secreted by the epithelium or innate immune cells such as neutrophils, and have antimicrobial properties *in vitro*, were amongst the first biomarkers of COPD identified; examples of these include the α-defensins and β-defensins, and surfactant proteins A and D [14]. This has led to suggestions that a deficiency in innate defence proteins might contribute to disease by allowing increased microbial

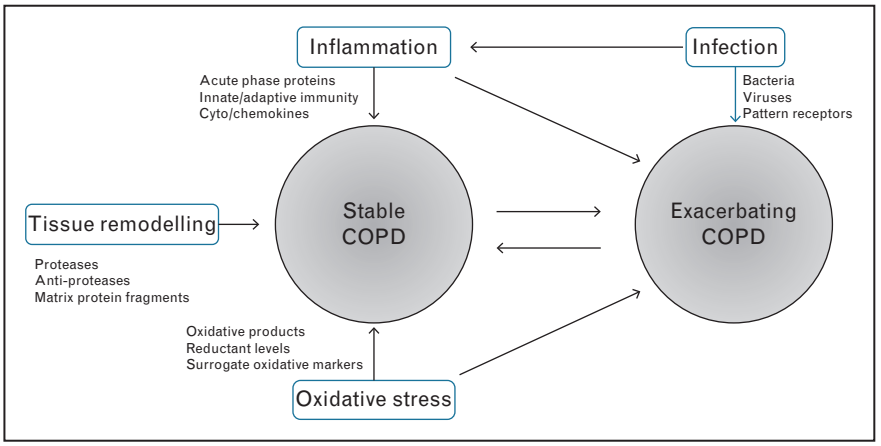

FIGURE 1. Key components of chronic obstructive pulmonary disease (COPD) pathophysiology and how they relate to the known functions of recently identified COPD biomarkers.

colonization. Recent analysis showed evidence of increased circulatory surfactant protein D and reduced pulmonary levels [15].

Pulmonary and activation regulated chemokine (PARC/CCL18) is a protein secreted by innate cells such as monocytes, macrophages and dendritic cells resident within the lung. Levels in plasma were elevated in COPD and associated with total mortality in COPD, although not correlated with disease severity [16]. This chemokine appears to be modulated in serum by steroid use, showing its potential as a therapeutic marker.

Inflammation

The receptor for advanced glycation end-products (RAGE) and one of its ligands, HMGB1, are recently discovered biomarkers elevated in COPD [17]. Binding of the receptor to its ligands (others include serum amyloid A and S100 proteins) appears to trigger a proinflammatory response. A soluble truncated form of the receptor (sRAGE) acts as a decoy for the receptor ligands and is thus thought to protect against excessive inflammation. sRAGE is reduced in COPD and is correlated to both airflow obstruction and emphysema [18]. Reduced sRAGE is indicative of inadequate inflammatory regulation, particularly within the alveolar compartment. Levels seem to be reduced further during exacerbation, and rise gradually during convalescence [19].

Immunity

Although previously considered as an inflammatory-driven disease, evidence for an involvement of the adaptive immune system in COPD is emerging. For example, elevated plasma immunoglobulin light-chain levels have been found in COPD patients compared with controls [20]. There have been mixed reports of the involvement of antielastin antibodies and cell-mediated immunity in COPD and emphysema, which might have provided a mechanism of antibody-mediated matrix degradation. Bonarius *et al.* [21] recently found elevated antinuclear autoantibodies (ANAs) in COPD patients compared with controls. The levels of ANAs were not related to disease severity, suggesting that the autoimmune response in COPD may be a result of current disease rather than reflecting activity.

$CD8^+$ T cells are known to be elevated in the lungs in COPD, and their accumulation has been associated with airflow limitation. These cells are believed to contribute to chronic inflammation through the release of inflammatory mediators and perforin, a protein stored in their secretory granules. A recent study [22] found elevated perforin levels in the epithelial-lining fluid of ex-smoker COPD patients compared with controls, and also found greater levels in the peripheral compared with the central airways. Perforin may therefore act as a useful marker of $CD8^+$ T-cell accumulation in the lung.

Oxidative stress

Oxidative imbalance has been considered a major feature of COPD for some time. Cigarette smoke is one potential source of oxidants, with another being the products of neutrophil degranulation. The oxidant and antioxidant status of the COPD airways is thus of major concern.

A study of the potential relationship between the polymorphisms in genes encoding antioxidant response and the measured antioxidant capacity in erythrocytes and serum from control and COPD patients [23] found that COPD patients showed reduced antioxidant levels. Analysis of polymorphisms in some targeted antioxidant genes showed a significant correlation with total antioxidant

status. The levels of oxidative markers, on the other hand, appear to be elevated during exacerbation [24].

Heat shock proteins (HSPs) are known to be elevated following oxidative stress. Rather than having classical cytoprotective functions, the extracellular HSPs are thought to be immune signalling molecules which can affect cytokine secretion. Recent evidence shows that plasma HSP60 is elevated in COPD [25].

Tissue turnover

Tissue destruction is a cardinal feature of emphysema. The lung matrix components affected appear to be the collagens and elastins. Therefore, there has been interest in measuring these components, their breakdown products or the agents responsible for matrix destruction in relation to COPD. Desmosine and isodesmosine are cross-links of mature elastin, that when found in plasma are a measure of its degradation. Encouraging new evidence finds a link between urinary and plasma desmosines and lung function in COPD patients [26], although levels were also influenced by factors such as age and smoking.

Another exciting finding has been the measurement of neutrophil elastase-mediated fibrinogen cleavage by quantifying plasma concentrations of $A\alpha\text{-}Val_{360}$ [27]. Levels were elevated in stable disease and even further during exacerbation. Notably, baseline levels were related to subsequent severity and disease progression, particularly in emphysema [27]. This biomarker is likely to be measuring neutrophil-mediated lung tissue destruction, so its specificity for COPD is open to question.

Matrix metalloprotease-9 (MMP-9) is a putative biomarker for emphysema. EMMPRIN, a molecule that increases MMP-9 release from epithelial cells, was recently found to be elevated in COPD compared with controls in BAL fluid [28], providing further evidence for protease imbalance in COPD within a compartment relevant to emphysema.

Microbial infection

The majority of exacerbations are known to have a microbiological trigger. Elevation of symptoms and sputum purulence are standard tests for ECOPD. Clinical indicators such as serum albumin, urea and arterial pCO_2 can also be useful in predicting mortality in acute exacerbations [29] but do not predict microbial involvement, and the limitation of sputum purulence is that it can exist in stable disease because of persistent airways colonization.

In the clinical setting, the cause of the majority of exacerbations is not determined but would be desirable if it could guide treatment. Direct measurement of bacteria in the airways by culturing from sputum samples has proven insensitive, because of limitations in the range of bacteria that can be cultured. Molecular typing of bacteria in such biofluids is beginning to overcome these problems. For example, measurement of bacteria in exhaled breath condensate (EBC) by molecular typing [30] showed poor correlation between sputum and EBC bacterial readings, but in the absence of a gold standard of bacterial detection, the implications of this are unclear.

Procalcitonin (PCT) is elevated in bacterial infections but remains low in viral infections and other inflammatory conditions, suggesting it may be more specific than CRP in determining exacerbations. Recent studies have highlighted the poor correlation between CRP, PCT and detection of bacteria in sputum samples of ECOPD patients, possibly reflecting the limitations of the bacteriology. Considerable overlap in CRP and PCT levels between stable and ECOPD is probably caused by colonization of stable patients with bacteria [31]. PCT appears to better predict microbial involvement in pneumonia than in ECOPD [32], consistent with the concept that exacerbations result from the acquisition of new strains of bacteria rather than novel colonization [33]. PCT guidance does however seem to result in reduced antibiotic use in ECOPD without increasing adverse patient outcomes [34].

Determination of viral cause is not a usual test in exacerbation. Several biomarkers have been proposed as indicators of viral infection, including IP-10, sIL5Rα and fibrinogen. Again, agreement between the studies is limited, possibly because of differences in viral type/strain, reflecting the wide range of pathogen–host interactions. Almansa *et al.* [35] performed a study of ECOPD patients which, despite relatively low numbers of participants, found a range of cytokines and chemokines that were altered between ECOPD with and without viral infection; these included innate immunity cytokines such as IP-10, IL-8 and eotaxin, the TH_1 cytokines IL12p70 and IL-15, and the immunomodulatory cytokine IL-10. This cytokine profile represents a typical antiviral response, and although not strain specific, could be used in conjunction with bacterial markers to lead to more directed therapies.

COMPLEX APPROACHES ARE NEEDED FOR BIOMARKERS OF A HETEROGENEOUS DISEASE

As previously mentioned, there is no shortage of single biomarkers implicated in COPD; however, it

is controversial whether they have advanced the diagnostic or disease-monitoring functions as no single marker has yet been widely accepted for the purpose. It is becoming increasingly clear that the heterogeneity of the disease is becoming a barrier to biomarker utility in COPD.

There are three current alternative approaches to the biomarker question. The first uses longitudinal or prospective studies which could better define the periods of stable and exacerbating disease. The second approach considers groups of biomarkers using multicomponent analysis to increase their predictive power, and the third considers improving the subphenotyping of patients, thus accepting biomarkers of, for example, emphysema, rather than COPD per se.

One significant recent development has been the establishment of several large cohorts for longitudinal study of multiple potential biomarkers of COPD. These large cohorts enable well controlled comparisons of multiple analytes. One analysis of 34 potential COPD biomarkers in plasma samples from the Evaluation of COPD longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) cohort [36▪] found 8 to be differentially expressed in COPD (CRP, β-defensin, adiponectin, IL-6, fibrinogen, CCL18, MMP-8 and prolactin), many of which have already been discussed here. One major finding was that many of these analytes were variable between measures, making them unlikely biomarker candidates.

A second study from the same cohort combined clinical and biomarker variables and risk of death in a longitudinal study of COPD patients [37]. Importantly, they found that the clinical indicators were the strongest at predicting death; however, some biomarkers were able to add strength to the predictive C-statistic and, of those, particularly IL-6. Individually, a number of the biomarkers were associated with increased risk of death when adjusted for clinical parameters such as age, BODE index and hospitalizations.

Recognizing the risk that clinical phenotyping of patients may pose limitations on biomarkers responsive to disease, Bafadhel *et al.* [38▪] used unbiased cluster analysis on biomarker data to identify four subphenotypes of exacerbating patients: bacteria predominant, viral predominant, eosinophil predominant and pauci-inflammatory. These clusters were not clinically different, but had inflammatory profiles which may indicate a likely differential response to treatment. Continuing this theme, inflammatory marker profiles were recently compared between asthmatic and COPD individuals [39], and demonstrated very clearly the overlap in cellular and mediator inflammatory indices between COPD and asthma, particularly severe asthma, whereas differentiation between eosinophilic and noneosinophilic subphenotypes showed greater inflammatory differences.

The eosinophilic subphenotype has a potential clinical application, in that blood eosinophil-guided treatment resulted in reduced prednisolone prescription during exacerbations without affecting the clinical outcome [40▪▪].

CONCLUSION

The potential for subphenotypes (endotypes) of diseases in leading to personalized treatment is clear, even if our understanding of the basis for such clusters is incomplete. It is likely that, if synchronization of clinical and 'omics' studies can occur, then large numbers of endotypes may be identified, even without known clinical differences, and that they may eventually be connected with specific treatment strategies to either improve outcome or reduce inappropriate therapeutic use. Further integration with high-resolution imaging to identify pathological endotypes and screening for genetic susceptibility should help to strengthen the personalized medicine agenda in COPD and its exacerbations. Time will show whether the systems biology approach needed for integration of these scientific and clinical elements is up to the task.

Acknowledgements

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

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