Review Article

Biomarkers of progression of chronic obstructive pulmonary disease (COPD)

Janet G. Shaw[1,2], Annalicia Vaughan[1,2], Annette G. Dent[1,2], Phoebe E. O'Hare[1,2], Felicia Goh[1,2], Rayleen V. Bowman[1,2], Kwun M. Fong[1,2], Ian A. Yang[1,2]

[1]Department of Thoracic Medicine, The Prince Charles Hospital, Brisbane, Australia; [2]UQ Thoracic Research Centre, School of Medicine, The University of Queensland, Brisbane, Australia

Correspondence to: Janet G. Shaw. Department of Thoracic Medicine, The Prince Charles Hospital, Rode Rd, Chermside, Brisbane QLD 4032, Australia. Email: Janet.Shaw2@health.qld.gov.au.

Abstract: Disease progression of chronic obstructive pulmonary disease (COPD) is variable, with some patients having a relatively stable course, while others suffer relentless progression leading to severe breathlessness, frequent acute exacerbations of COPD (AECOPD), respiratory failure and death. Radiological markers such as CT emphysema index, bronchiectasis and coronary artery calcification (CAC) have been linked with increased mortality in COPD patients. Molecular changes in lung tissue reflect alterations in lung pathology that occur with disease progression; however, lung tissue is not routinely accessible. Cell counts (including neutrophils) and mediators in induced sputum have been associated with lung function and risk of exacerbations. Examples of peripheral blood biological markers (biomarkers) include those associated with lung function (reduced CC-16), emphysema severity (increased adiponectin, reduced sRAGE), exacerbations and mortality [increased CRP, fibrinogen, leukocyte count, IL-6, IL-8, and tumor necrosis factor α (TNF-α)] including increased YKL-40 with mortality. Emerging approaches to discovering markers of gene-environment interaction include exhaled breath analysis [volatile organic compounds (VOCs), exhaled breath condensate], cellular and systemic responses to exposure to air pollution, alterations in the lung microbiome, and biomarkers of lung ageing such as telomere length shortening and reduced levels of sirtuins. Overcoming methodological challenges in sampling and quality control will enable more robust yet easily accessible biomarkers to be developed and qualified, in order to optimise personalised medicine in patients with COPD.

Keywords: Pulmonary disease; chronic obstructive; disease progression; biological markers (biomarkers); lung; sputum; blood

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Chronic obstructive pulmonary disease (COPD) is a chronic, inflammatory lung disease that arises from exposure to cigarette smoke and other inhaled toxins, and results from a gene-environment interaction (1). Disease progression of COPD is variable, with some patients having a relatively stable course, while others suffer relentless progression leading to severe breathlessness, frequent acute exacerbations of COPD (AECOPD), respiratory failure and death. This review will initially focus on radiological markers, and biological markers (biomarkers) in lung tissue, sputum and blood, which may be useful in predicting disease progression in COPD. Emerging approaches to discovering markers of gene-environment interaction will then be discussed, including exhaled breath analysis, exposure to air pollution, the lung microbiome, and lung ageing.

Measurements of disease progression in patients with COPD

Decline in lung function has been the classical objective

measure of progression of COPD over time. However, other clinically important measures have been used in epidemiological studies and clinical trials, including symptoms and health status, exacerbations and health care utilisation, and mortality.

Lung function

Lung function, particularly the forced expiratory volume in 1 second (FEV_1), provides an objective, physiological measure of worsening airflow obstruction in COPD. The classic Fletcher and Peto study (2) described variable decline in lung function in a cohort of male workers, with some smokers being more susceptible to accelerated decline. A range of clinical and demographic factors has been shown to influence decline in lung function in COPD, such as environmental and occupational pollutants, cigarette smoking, respiratory infections, exacerbations and comorbidities (3). The Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) cohort study of 2,163 patients observed a mean rate of decline in FEV_1 of 33 mL/year, with higher rates of FEV_1 decline in the presence of current smoking, emphysema and bronchodilator reversibility (4).

Symptoms and exacerbations

The Global Initiative for chronic obstructive lung disease (GOLD) guidelines recommend the inclusion of symptom assessment and exacerbation history, together with measurement of the severity of airflow limitation measured by FEV_1 (5). The ECLIPSE study confirmed that patients with 2 or more exacerbations per year (frequent exacerbators) were at higher risk of future exacerbations, and this risk was further increased with more severe airflow limitation (6). Change in health status is also an important measure of disease progression (1).

Mortality

COPD is the third leading cause of death world-wide, after ischaemic heart disease and stroke (7). This high rate of mortality is driven by increased smoking worldwide, reduced mortality from other treatable diseases and an ageing world population (5). Groups at high risk of mortality, as described in the Copenhagen City Heart Study of 10,457 participants, included those with lower baseline FEV_1 and excessive longitudinal decline in FEV_1, even before the point where their lung function becomes abnormal (8).

It is evident that a multitude of relevant clinical phenotypes portrays the disease progression of COPD, which reflects the heterogeneous and complex nature of this chronic disease.

Biomarkers for disease progression of COPD

Biomarkers are any clinical features, imaging quantification or laboratory-based test markers that characterise disease activity, which are useful for diagnosing and monitoring disease processes and response to therapy. Recent excellent reviews have summarised putative biomarkers for detecting the presence of COPD, characterising COPD phenotypes and monitoring response to treatment (9-11). Biomarkers of acute exacerbations have also been reviewed (12).

Identifying individuals with COPD who are at higher risk of progression would enable more personalised management, in order to slow disease progression. Use of biomarkers would potentially add to existing strategies for smoking avoidance, pharmacotherapy, pulmonary rehabilitation and chronic disease management in COPD. Benefits from measuring biomarkers for COPD progression (and not only susceptibility to COPD) include identifying patients who are rapid decliners in the early stages of the disease, predicting disease progression in all severity groups of COPD, and quantifying response to treatment.

The search for reliable biomarkers in COPD, other than FEV_1, is ongoing [e.g., the international efforts by the COPD Biomarker Qualification Consortium (9)]. Providing reliable evidence to validate biomarkers before clinical implementation remains an important challenge. Important issues to be addressed include the accuracy and reliability of biomarkers for the clinical state of interest, evaluation of clinical utility and cost-effectiveness, and real world effectiveness compared to other biomarkers (13). The validation of biomarkers (biomarker qualification) for COPD would be clinically applicable to risk stratification of patients and outcome markers of efficacy and safety in drug development and other clinical trials (9).

Radiological markers for emphysema, airway thickness, bronchiectasis and multi-morbidities

Image biomarkers, especially radiological features of COPD morphology visualised on high resolution computed tomography (CT) chest scans, have been found to be useful

predictors of disease progression.

Emphysema and airway wall thickness

High resolution CT is able to assess emphysema and airway disease using quantitative indices (14). Inspiratory *vs.* expiratory analysis of distribution of parenchymal (emphysema) and functional small airways disease provides information about COPD phenotype (15), and change in lung density over time can itself be measured as an endpoint of COPD progression (16).

Quantitative CT measurements have been associated with outcomes of COPD progression in large cohort studies. Accelerated decline in lung function has been associated with more severe emphysema measured quantitatively by CT (17). The MESA (Multi-Ethnic Study of Atherosclerosis) study found that the presence of centrilobular and panlobular emphysema correlated with increased dyspnoea and reduced exercise capacity (18). Airway wall thickness correlated with reduced lung function and increased symptoms in smokers in a cross-sectional study (19). In the COPDGene study of 1,002 subjects, exacerbations were more frequent in those subjects who had a more severe CT emphysema index, and who displayed increased airway wall thickness (20). A higher CT emphysema index was associated with increased risk of respiratory (21,22) and COPD-specific mortality (23). Airway wall thickness was not independently associated with mortality (22).

Bronchiectasis

Bronchiectasis frequently coexists with COPD. Bronchiectasis is a persistent or progressive condition that is characterised by dilated, thick-walled bronchi that fail to clear airway secretions normally. This leads to bacterial infection and a chronic cough productive of sputum, recurrent infective exacerbations and ultimately, lung destruction and respiratory failure (24). In some COPD patients, bronchiectasis is an incidental finding on CT and may be subclinical, as observed in the ECLIPSE study where the overall prevalence of bronchiectasis was 4% in a highly selected population of milder COPD patients (25). In contrast, studies of moderate to severe COPD have demonstrated a higher prevalence of bronchiectasis of from 30% to 60%, with more extensive bronchiectasis in severe COPD (26-28).

The presence of bronchiectasis influences respiratory infections and other complications of COPD. In a study of patients with moderate to severe COPD, patients with COPD and co-existing bronchiectasis, compared to COPD alone, had more severe airflow obstruction (OR 3.9) and an increased yield of potentially pathogenic microorganisms on sputum culture (OR 3.6) (29). Furthermore, bronchiectasis increased the rate of at least one hospital admission for an AECOPD in the previous year (OR 3.0). In a subsequent study of 201 patients with moderate to severe COPD, the same investigators showed that bronchiectasis was independently associated with increased all-cause mortality (HR 2.5) (30). Conversely, a study of 245 patients with non-cystic fibrosis bronchiectasis in Belgium found that 17% of patients had co-existing COPD (31). Over 5 years of follow-up, patients with both bronchiectasis and COPD had a mortality rate of 55%, which was considerably higher than 13% in patients with bronchiectasis alone (31).

These studies emphasise the clinical impact of coexisting bronchiectasis in patients with COPD, especially in terms of excessive rates of AECOPD and mortality. Detecting bronchiectasis in patients with COPD from their routine HRCT chest scans may therefore be potentially clinically useful, identifying those patients who are predisposed to higher rates of exacerbations and increased mortality.

Coronary artery calcification (CAC)

Cardiovascular multi-morbidity is highly prevalent in patients with COPD, and adversely affects mortality. A high prevalence of coronary artery disease has been associated with emphysema severity (32). CAC is a marker of coronary artery disease (*Figure 1*), and its extent is directly associated with the total burden of coronary atherosclerosis (33). Whilst CAC can be measured using calcium scores on gated, non-contrast CT scans, the use of simple visual scores of CAC has also found utility in lung cancer screening studies (34). In a cross-sectional study of 200 patients with moderate to severe COPD, we observed a high prevalence of CAC (87%) on routine CT chest scans (35). Of prognostic importance, a moderate to high ordinal visual score for CAC (>4 out of a possible 12) was predictive of increased all-cause mortality (HR 2.0) in these patients with COPD (35) (*Figure 2*). This association was independent of duration of cigarette smoking. Similarly in the ECLIPSE study, a higher coronary artery calcium score percentile was associated with increased mortality (HR 1.77) in COPD patients (36). The results of these radiological studies suggest the scoring of CAC severity on CT chest

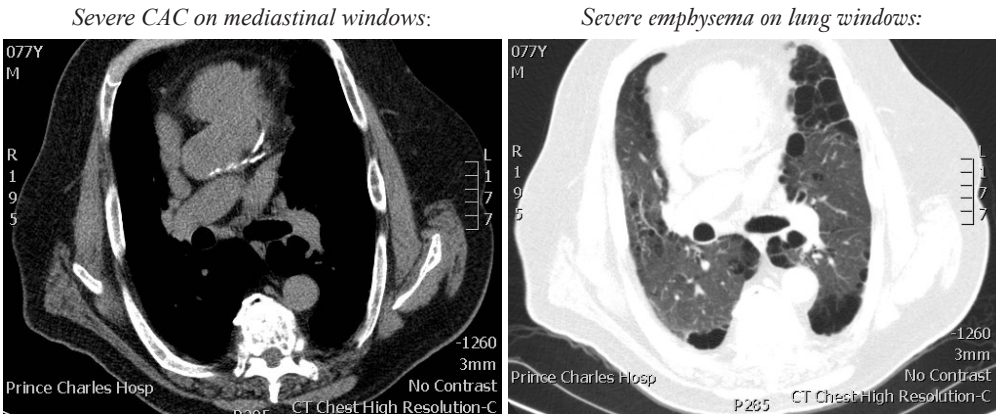

Figure 1 CAC on CT chest scan in a 77-year-old male patient with COPD and emphysema. CAC, coronary artery calcification; CT, computed tomography. Reproduced with permission from (3).

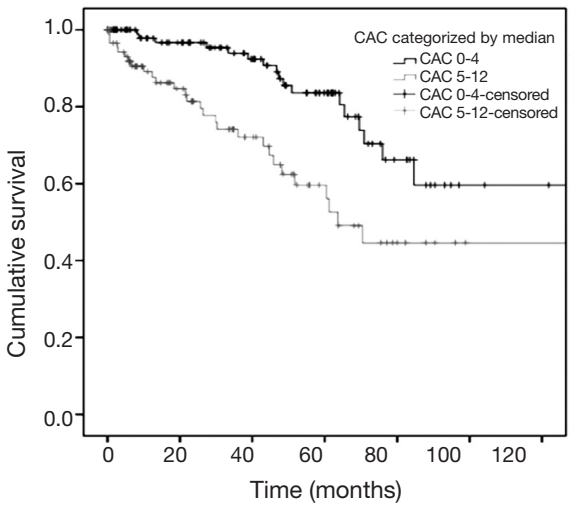

Figure 2 Kaplan-Meier survival curves for all-cause mortality for COPD patients, according to CAC score >4 (CAC 0-4 n=109, CAC 5-12 n=91). The Kaplan-Meier survival curve (censored at 120 months) shows that COPD patients with CAC scores of 5 to 12 (dichotomised by the median of 4) had higher mortality than patients with CAC scores of 0 to 4 (log rank test, P=0.001). CAC, coronary artery calcification. Reproduced with permission from (35).

scans can non-invasively screen for coronary artery disease in patients with COPD with important prognostic implications.

Lung tissue: gene expression markers

Molecular changes in lung parenchyma are a direct reflection of alterations in lung pathology that occur with disease progression in COPD. Routine collection of lung samples is only feasible in patients undergoing lung surgery. Nevertheless, molecular changes in lung tissue provide valuable insight into biomarkers that may be expressed and therefore usefully measured in accessible samples (e.g., sputum, exhaled breath condensate and blood).

A number of studies have used microarrays to examine differences in global mRNA expression between chronic lung disease and normal lung samples (37,38). Other studies have extended this approach by profiling gene expression across different severity stages of COPD. A study of lung tissue from COPD patients (n=21 GOLD stage 0; n=9 stage I; n=10 stage II; n=3 stage III) showed that gene expression correlated with forced expiratory flow between 25% and 75% of forced expiratory volume ($FEF_{25-75\%}$), a measure of small airways function (39). Upregulated genes included those involved in pathways of apoptosis and extracellular matrix synthesis and degradation; down-regulated genes included anti-inflammatory genes. A study of 56 lung tissues (no COPD, to COPD patients from mild to severe) found correlation of FEV_1 % predicted and FEV_1/FVC with functional classes of genes involved in DNA binding and transcription (40).

Studies from our group have also provided evidence for differences in gene expression signatures in the earlier stages of COPD disease progression. We have undertaken a study of lung tissue of 30 smokers with emphysema undergoing lung resection for lung cancer, with biological validation in an independent set of 62 patients (41). All patients had airflow limitation with FEV_1/VC ratio <0.70 and were arbitrarily classed, based on gas transfer, as mild (KCO ≥75% of predicted) or moderate (KCO <75% of predicted) emphysema. Gene expression profiling

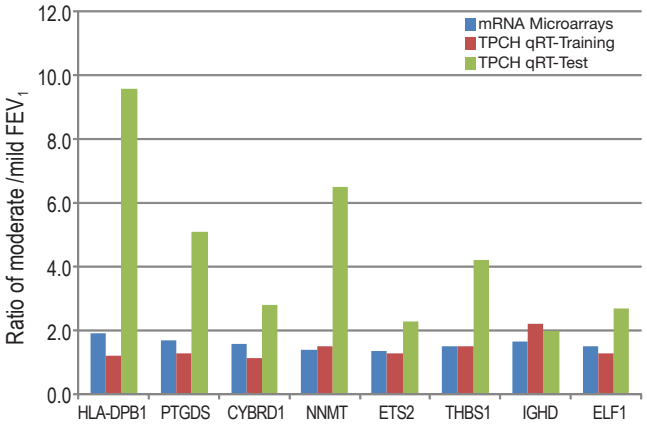

Figure 3 Comparison of mRNA expression differences in non-tumour lung tissue between mild and moderate COPD patients, based on FEV_1 % predicted. Fold-changes are shown for eight candidate genes measured by gene expression microarrays (TPCH training set, n=30), and qRT-PCR (technical replication: TPCH training set, n=30; biological validation: TPCH test set, n=58). NNMT, nicotinamide N-methyltransferase; THBS1, thrombospondin 1; IGHD, immunoglobulin heavy delta chain; HLA-DPB1, major histocompatability complex, class II, DP Beta 1; PTGDS, prostaglandin D2 synthase; CYBRD1, cytochrome B-Reductase 1; ETS2, erythroblastosis virus E26 oncogene homologue 2; ELF1, E74 like factor 1. Reproduced with permission from (42).

and confirmatory PCR identified seven genes that were differentially expressed in moderate emphysema, compared to mild emphysema by more than 1.3-fold: *COL6A3*, *SERPINF1*, *ZNHIT6*, *NEDD4*, *CDKN2A*, *NRN1* and *GSTM3* (41). Our additional study of lung tissue from patients with mild (n=9) or moderate (n=9) COPD, based on FEV_1 % predicted, with validation in an independent set of 58 lung samples, confirmed differential expression of eight genes (*NNMT*, *THBS1*, *HLA-DPB1*, *IGHD*, *ETS2*, *ELF1*, *PTGDS* and *CYRBD1*) by more than 1.8-fold between mild and moderate COPD severity (42) (*Figure 3*). Ontologies represented by these genes were predominantly cell migration, proliferation, angiogenesis and apoptosis (42). Using the same lung tissue, we have also shown that expression of microRNA-34c is associated with emphysema severity, and modulates SERPINE1 expression in COPD lung (43). Genes and pathways associated with severity of COPD, including the early stages of progression, could therefore be tested as lung biomarkers for progression of emphysema and airflow obstruction in COPD.

Gene expression profiling of specific cells or regions of COPD lung provide additional information about distinct gene signatures for disease progression. Expression of repair genes was examined in 136 paired small airways and emphysema lung tissue obtained by laser capture microdissection from 63 patients (44). Genes involved in tissue destruction were more commonly increased in expression in emphysematous lung tissue and correlated with impaired FEV_1, whereas these genes were not as highly expressed in the small airways, thereby promoting bronchiolar remodelling rather than destruction (44). In a study of 238 smokers with or without COPD, gene expression in bronchial brushings was similar to the expression in lung tissue, and this gene expression was regulated in part by activating transcription factor 4 (ATF4) (45). Finally, distinct gene signatures were observed in fibrotic and emphysematous areas of lung, in patients with combined pulmonary fibrosis and emphysema (46). Fibrotic regions expressed genes associated with immune function, and emphysematous areas expressed genes related to cellular fraction, membrane biology, and vascular biology (46), demonstrating that functional differences in gene expression occur with different lung pathologies. Overall, these studies show that specific cells and pathologies in the lung are likely to yield characteristic biomarkers that reflect individual COPD phenotypes of progression.

Sputum: inflammatory cells and mediators

Sputum has been studied as a non-invasive method of sampling biomarkers to assess disease severity and progression in COPD, including exacerbations. Many COPD patients can produce spontaneous sputum samples. However, these often contain a high proportion of non-viable cells which may influence the cell count and mediator profile. To overcome this, sputum can be induced with hypertonic saline in stable patients with COPD, with good safety and reproducibility for cell counts and inflammatory markers (47). Induced sputum also has an adequate safety profile during acute exacerbations, as demonstrated in studies of patients with mild to moderate (48) and moderate to severe COPD (49). Because of many technical and clinical confounding factors (such as interference with assays, smoking status of patients, bacterial infection and concomitant treatment), induced sputum is still undergoing investigation as a source of clinically useful biomarkers (9).

Sputum biomarkers during stability have been associated with severity of COPD. Sputum neutrophil count increased

with GOLD stage but was only weakly associated with lung function in the ECLIPSE study (50). Higher levels of human neutrophil peptides (HNP), neutrophil elastase (NE), interleukin (IL)-8 and matrix metalloproteinase (MMP)-9 in spontaneous sputum of COPD patients were associated with greater decline in lung function (FEV_1) over 2 years (51). In the ECLIPSE study, microarray profiling of gene expression in induced sputum from 148 COPD patients (and validated in 176 patients) found 277 genes differentially expressed between moderate, severe and very severe GOLD classes, and 198 genes that were differentially expressed between severities of emphysema (52). Further validation is required to test the clinical utility of these genes as biomarkers for COPD progression.

During exacerbations, sputum cell and mediator profiles are heterogeneous and can predict response to therapy of the exacerbation (53). The presence of a mixed inflammatory cell profile in the sputum, together with increased concentrations of sputum and serum biomarkers, were found in patients with exacerbations who had lower FEV_1 and increased hospital length of stay (53). Inflammatory mediators in induced sputum during stability may predict future risk of exacerbations. A review by Koutsokera and co-workers found that levels of some mediators in sputum [including in sputum IL-6, IL-8 and myeloperoxidase (MPO)] may be associated with frequency of exacerbations, although more confirmatory studies are needed (12). In a longitudinal study with monthly visits, sputum levels of leukotriene B_4 were found to be elevated prior to an exacerbation, and were suggested as possible biomarkers for exacerbation risk (54).

Blood biomarkers: monitoring the systemic compartment

Blood samples provide a convenient source of biomarkers of lung disease. The relevance of blood biomarkers depends on release of markers from the lung into the bloodstream, or systemic markers present in the blood that reflect active disease processes in the lung.

A range of blood biomarkers has been associated with severity of airflow limitation and emphysema. Reduced levels of serum club (Clara) cell protein 16 (CC-16), a protein produced in the lungs and released to the serum, were weakly associated with accelerated decline in lung function (FEV_1) in both the Lung Health Study (55) and ECLIPSE study (4). In the TESRA (Treatment of Emphysema with a Selective Retinoid Agonist) and ECLIPSE studies, reduced serum levels of soluble receptor for advanced glycation endproducts (sRAGE) were associated with more severe GOLD stage and more extensive emphysema (56). Lower levels of sRAGE were similarly associated with more advanced emphysema or lower FEV_1 in two other studies (57,58). In the ECLIPSE cohort, low levels of vitamin D were correlated with FEV_1 and severity of emphysema and associated with 6-minute walk distance, bronchodilator response and CC-16 levels (59). In a subset of the COPDGene cohort, emphysema quantified on CT was associated with higher plasma levels of the adipokine, adiponectin (60) and lower levels of plasma IL-16 (61). Plasma YKL-40 has been associated with higher all-cause mortality (HR 1.4) in a cohort of 493 COPD patients in Denmark (62). Thus a range of biomarkers detectable in peripheral blood show potentially promising relationships with COPD phenotypes.

Panels of blood biomarkers may provide more accurate modelling of future risk. In the Grosshansdorf COPD cohort of 140 COPD patients, clusters of plasma proteins involved in neutrophil function were associated with parameters related to FEV_1 (63). Furthermore, proteins related to the epidermal growth factor receptor (EGFR) pathway were associated with gas transfer (DLCO) and FEV_1 (63). A panel of three systemic inflammatory markers in peripheral blood (CRP, fibrinogen and leukocyte count) was tested in 6,574 individuals with COPD (defined as FEV_1/VC ratio <0.7) in the Copenhagen City Heart Study and the Copenhagen General Population Study (64). Elevation of all three biomarkers simultaneously was associated with an increased risk (OR 3.7) of having frequent exacerbations. This association was observed even in subjects with milder COPD and those with no history of frequent exacerbations (64). In the ECLIPSE study, adding the full range of studied blood biomarkers [white blood cell counts, fibrinogen, chemokine ligand 18, surfactant protein D, CRP, Clara cell secretory protein-16, IL-6, IL-8, tumor necrosis factor α (TNF-α)] to the model of age, BODE index and previous COPD hospitalisations improved prediction of mortality (65). A refined panel of six systemic inflammatory markers in peripheral blood (white cell count, fibrinogen, CRP, IL-6, IL-8, TNF-α) in the ECLIPSE study was able to predict increased mortality and exacerbation rates in COPD patients with inflammation, compared to patients without inflammation (66). Of these, currently plasma fibrinogen is being considered for regulatory qualification as a prognostic marker by the US Food and Drug Administration and the European Medicines Agency (9,67).

Because of the large number of putative biomarkers, heterogeneity in study design and evaluation, a formal systematic review of this emerging field is beyond the scope of this review.

Emerging gene-environment approaches to biomarkers of disease progression in COPD

In addition to the more traditional sampling of biomarkers described above, emerging approaches to capturing the effects of gene-environment interaction on COPD disease progression are receiving more focus in research studies. Of these, analysing biomarkers in exhaled breath is a potentially useful, non-invasive method of sampling the airways and epithelial lining fluid that is exposed to the environment. In addition to cigarette smoking, exposure to air pollution and infection are important environmental drivers of COPD progression and phenotypes. Finally, lung ageing, whilst an endogenous chronological factor, also brings with it exposure to internal and external factors over many years, and should be integrated into the complex profiling of COPD.

Exhaled breath analysis

Volatile organic compounds (VOCs)

Advances in technology have produced small, portable array type devices (electronic noses) that are highly applicable to the clinical setting. Electronic noses use a variety of technologies to emulate the human nose, with VOCs adsorbing onto sensors to produce a change in conductivity, colour or oscillation of a crystal, leading to readouts that are analysed. These devices approach the problem of detection from an entirely different viewpoint from that of the gas chromatograph: in the same way a human nose can tell the difference between the bouquet of chocolate and a rose without needing to know the chemical constituents of the vapour, so the electronic nose is able to discriminate between two vapour mixtures without needing to characterise the exact molecules responsible.

Exhaled breath analysis using differing technologies, including gas chromatography-mass spectrometry and the electronic nose, can discriminate between a range of pulmonary diseases (68), including COPD and asthma (69,70). Relatively few studies to date have linked VOCs profiling of the exhaled breath with COPD progression. A recent study showed that the VOCs pattern is reasonably reproducible in healthy subjects and patients with severe COPD and has some correlation with tests of small airways disease (71). VOCs pattern was shown to differentiate between some phenotypes of COPD, such as patients with higher sputum eosinophilia or frequent exacerbations (72).

Identifying the neutrophilic and eosinophilic inflammatory phenotypes of COPD would further aid in tailoring effective treatment. A strong association between sputum cell count and exhaled breath compounds has been demonstrated in subjects with mild to moderate COPD (GOLD stages I and II) (73). Moreover, VOCs profiling was able to discriminate between subjects with COPD and α_1-antitrypsin (AAT) deficiency, with very high accuracy, and the VOCs profile of AAT deficiency patients changed with human recombinant AAT therapy, indicating a possible marker of response to treatment (74). However, before widespread application in the clinical setting, methodological issues of VOCs testing need to be overcome, and more extensive validation is required (75).

Exhaled breath condensate (EBC)

Collection of cooled exhaled breath as condensate is a non-invasive method of sampling the airway lining fluid (76). To date, a small number of studies have examined EBC biomarkers and COPD progression. EBC pH was found to be lower in former smokers with GOLD stage III to IV COPD, compared to stage I (77), suggesting that airway acidification could be a marker of airway inflammation and disease severity in COPD, although not all studies have shown a relationship with FEV_1 (78). EBC pH is also reduced during acute exacerbations (79). EBC hydrogen peroxide (H_2O_2), a marker of oxidative stress, has been shown to correlate with COPD health status as measured by the COPD assessment test (CAT) (80). Methodological issues such as dilution and sensitivity of assays, as well as interpretation of clinical factors that impact on EBC analysis, still require to be solved in larger studies (9).

Exposure to air pollution

The predominant sources of particulate matter in the lungs of COPD patients are cigarette smoke and ambient air pollution (81). With up to 25-45% of patients with chronic airflow limitation being never smokers (82), it is evident that non-smoking-related factors (e.g., air pollution) play a role in the progression of COPD (82,83). Exposure to air pollution should therefore be characterised as a factor that influences disease outcomes in COPD.

Vehicle emissions are a major contributor to air pollution

in the urban environment. The main components of vehicle emissions are particulate matter less than 10 μm in diameter (PM_{10}), nitrogen dioxide (NO_2) and sulfur dioxide (SO_2) (84). Recent epidemiological studies have observed strong associations between air pollution exposure and COPD outcomes, including exacerbations, hospital admissions and mortality (*Table 1*). The repetitive nature of the inhalation injury caused by air pollution is considered a major mediator in the COPD progression (81). Chronic exposure to air pollution, specifically vehicle emissions, has been linked to increased hospital admissions of COPD patients, including those who are never smokers (89). Analysis of early evidence showed that long-term exposure to particulate matter can lead to a reduction in lung function and increased COPD incidence and progression (81). These studies and others (86,87) support the notion that exposure to air pollution is a driver of COPD progression in susceptible individuals.

Monitoring of air quality occurs for legislative and public health requirements, as well as epidemiological research. However, real-time monitoring of personal air pollution exposure and biomarkers of the adverse effects of ambient air pollution are still in development (93). *In vitro* studies have elucidated gene and protein expression profiles of human bronchial epithelial cells, in response to air pollutant exposure (3), which could be brought to clinical testing with further validation. EBC levels of nitrite and nitrate (markers of oxidative stress) were associated with concentrations of ambient coarse particles, but not indoor air pollutant levels, in four cities in Europe (94). Systemic responses to air pollutants were studied in 242 stable COPD patients in Spain (95). In this time series analysis, blood levels of CRP, fibrinogen, HGF and IL-8 were associated with increased ambient NO_2 levels, mainly detected in former smokers.

At present, little is known about the molecular mechanisms by which air pollution can promote progression of COPD, and further studies are needed in this field.

Lung microbiome

Bacteria are strongly associated with AECOPD, with bacteria cultured in ~50% of patients with an AECOPD (96). Chronic airway infection with bacteria (colonisation of the airways by bacteria) is more common in patients with severe COPD (97). Whether chronic infection contributes to the pathogenesis of airway inflammation and increasing frequency and severity of AECOPD is not known. The 'vicious circle' hypothesis outlines the principles that chronic microbial colonisation, alters innate immunity and airway epithelial injury contributes to the progression of both COPD and other chronic lung diseases such as bronchiectasis (28,98). According to this paradigm, the presence of chronic bacterial infection in the airways, (including during stable disease), may drive inflammation and disease outcomes.

The microbiome describes the microbial community that share an environment in a particular body site. Next-generation sequencing is used to identify these microbial populations which include microbes that are unculturable (99). Characterising the microbiome is rapidly emerging as an important approach to unravelling the complex microbiology of chronic lung diseases (100) [outlined in detail in this issue of the Journal by Daniel Chambers and colleagues (101)]. The community composition of microbial communities can be determined by sequencing the variable regions of the 16S gene, which encodes bacterial ribosomal RNA (rRNA) (98). Published studies of the lung microbiome in COPD have recruited relatively small numbers of patients, with a range of methods of sampling the microbiome (*Table 2*). Furthermore, few studies to date have applied study of the lung microbiome to outcomes of COPD progression. In general, tobacco smoking in the absence of COPD does not appear to alter the lung microbiome, but severe COPD is associated with less population diversity of resident bacterial communities, although even this result seems dependent on whether BAL or airway tissue is being sampled (96,103,106).

In COPD, bacterial community profiles in BAL samples from patients using inhaled steroids and long-acting bronchodilators clustered differently from the profiles observed in patients not using these medications (105). Infection with respiratory viruses increases the total bacterial load in patients with COPD, compared to similarly infect healthy controls, but with no obvious difference in bacterial diversity based on analysis of sputum samples (106).

These alterations to the lung microbiome have considerable potential implications for the pathogenesis and progression of COPD. Predominance of one bacterial species in an anatomical lung region (e.g., affected by bronchiectasis) could reduce bacterial diversity, leading to disruption of the balance between mucosal immunity and the bacterial communities present (airway dysbiosis). Alteration of the normal balance of bacterial flora may lead to an excessive inflammatory response, perpetuating the airway inflammation that is characteristic of COPD (98). The microbiome is an emerging source of biomarkers of respiratory infection and possibly COPD progression.

Table 1 Examples of recent studies of air pollution exposure and COPD outcomes

Study	Location	Study design	Main findings
Waked 2012 (85)	Lebanon	• Cross-sectional analysis of Lebanese residents over 40 years from October 2009 to September 2010 • Out of 2201 individuals, 732 were never smokers, and 3.4% had COPD • Lung function and exhaled carbon monoxide levels were measured	• Correlation between COPD incidence and indoor air pollution from house warming with diesel, as well as childhood respiratory disease and older age
Gan 2013 (86)	Canada	• Longitudinal study with a 5-year exposure period and 4 year follow up period • 467,994 participants, with no COPD baseline and an age range between 45-85 years old	• Black carbon was strongly associated with risk of COPD hospitalization and mortality (which was attenuated when demographic factors such as age and sex were accounted for) • 6% increased risk of mortality and 7% increased risk of hospitalization with higher levels of exposure
Hansel 2013 (87)	USA	• Longitudinal study to investigate the effects of indoor PM and NO_2 concentrations on COPD morbidity in a peri-urban community • Pollutant concentrations measured at baseline, 3 and 6 months • 84 participants with moderate to severe COPD	• Indoor pollutant exposure to $PM_{2.5}$ and NO_2 was associated with increased respiratory symptoms and risk of COPD exacerbations
Wang 2013 (88)	Taiwan	• Time series analysis of outpatient visits and air pollution in the context of a heavily polluted urban area	• NO and NO_2 were positively associated with respiratory disease, but also some association was seen for PM_{10}, $PM_{2.5}$, O_3, CO and SO_2 • COPD outpatients were most sensitive to air pollution and weather
Schikowski 2014 (89)	Europe	• Impact of chronic exposure to air pollution on COPD in four cohorts • The annual average of PM_{10}, NO_2 and road traffic exposure was assessed	• In meta analyses, NO_2, SO_2, PM_{10} and the traffic indicators were positively associated with COPD • Significant positive association with traffic intensity and COPD incidence in never smokers
Tao 2014 (90)	China	• Time series analysis of PM_{10}, SO_2 and NO_2 and respiratory hospitalizations • 28,057 recorded admissions for respiratory disease	• Significant positive association between air pollutants and respiratory hospitalizations, and stronger effects were observed for females and persons aged ≥65 years
Zhou 2014 (91)	China	• Cohort of 71,431 middle-aged Chinese men from 25 different cities • Annual average particulate matter exposure was estimated and compared to total, cardiovascular and respiratory disease mortality	• 1.7% increase in respiratory mortality per 10 $\mu g/m^3$ increase in PM_{10}
Vanos 2014 (92)	Canada	• Investigation into the relative risk of mortality from all non-accidental, respiratory, and cardiovascular related causes, associated with exposure to four air pollutants, by weather type and season, in ten major Canadian cities	• In total, 61% of the respiratory-related mortality relative risk estimates were significantly higher than for cardiovascular-related mortality. The combined effect of weather and air pollution is greatest when tropical-type weather is present in the spring or summer

COPD, chronic obstructive pulmonary disease; PM_{10}, particulate matter less than 10 μm in diameter; NO_2, nitrogen dioxide; SO_2, sulfur dioxide.

Table 2 Microbiome studies of COPD patients and smokers

Study	Population	Main findings
Hilty 2010 (102)	11 asthma, 5 COPD, 8 controls—bronchial brushings	Microbiota present in bronchial tree. Proteobacteria more frequent in COPD and asthma
Huang 2010 (96)	8 COPD, mechanically ventilated—endotracheal aspirates	Presence of diverse bacterial communities in airways of COPD patients with severe exacerbation
Erb-Downward 2011 (103)	4 COPD, 7 smokers, 3 never-smokers—BAL; 6 severe COPD—lung tissue explants	Lung microbiome distinct from oral microbiome. Lower diversity in more severe COPD; Pseudomonas predominance. Differences in bacterial communities within lung sites in severe COPD
Cabrera-Rubio 2012 (104)	6 moderate COPD—sputum, bronchial wash, bronchial biopsy, BAL	High bacterial diversity. Sputum and washings – different microbiota to BAL and biopsies
Pragman 2012 (105)	22 moderate to severe COPD, 22 controls—BAL	Increase in microbial diversity in COPD. No difference with increasing severity. Differences based on inhaler therapy
Sze 2012 (99)	8 severe COPD, 8 CF, 8 smokers, 8 never-smokers—lung tissue	Lower bacterial density and differences in bacterial populations in severe COPD lung tissue
Molyneaux 2013 (106)	14 COPD, 17 controls infected with rhinovirus—induced sputum	Increase in bacterial burden in COPD after RV infection, including Haemophilus influenzae
Morris 2013 (107)	19 smokers, 45 non-smokers—BAL, oral wash	Higher abundance of some bacteria in the lungs. No difference between smoker and non-smoker lung microbiota
Zakharkina 2013 (108)	9 COPD, 9 controls—BAL	Diverse bacteria present in healthy lungs and COPD, different bacterial taxa in COPD
Galiana 2014 (109)	9 mild/moderate COPD, 10 severe COPD—sputum	Increased total bacterial load in severe COPD
Huang 2014 (110)	60 samples from 12 mild/moderate/severe COPD patients in a longitudinal study—sputum	Altered microbial communities with acute exacerbation, and with type of treatment for exacerbation (antibiotics, steroids or both)
Millares 2014 (111)	11 severe COPD; 5 COPD colonised by PA—sputum	Increased biodiversity during exacerbation in PA-infected sputum, to equal non-PA sputum

COPD, chronic obstructive pulmonary disease; PA, Pseudomonas aeruginosa.

Lung ageing

Ageing is an endogenous rather than exogenous factor, representing cumulative exposures to environmental factors over time. A wide range of phenotypes and biomarkers of ageing are currently being investigated in chronic diseases, including COPD (112,113). Examples of potential relevance to COPD progression include telomere shortening and sirtuins.

Telomeres are protective structures of repetitive sequence that stabilise the ends of chromosome by preserving genetic information and preventing DNA degradation (114,115). Telomere length varies between different cell types, tissues and individuals. Shortening of telomere repeats occurs naturally with cell division, with the shortened telomere ends eventually acting as a signal for apoptosis (116,117). For example, the reduction rate of telomere repeats in peripheral blood mononuclear cells is measured at approximately 84 bp per year, with an accompanying progressive decrease in telomerase activity, in healthy individuals under 40 years of age (118). Telomere length is also a predictor of years of healthy life in older persons (119). Because of this relationship with biological age, telomere length has been associated with ageing and age-related diseases such as COPD. Telomeres are shorter in peripheral blood leukocytes of COPD patients (120,121), particularly cigarette smokers (122,123), providing a common risk factor for accelerated ageing and replicative senescence in COPD.

Telomere length has been linked with lung function

in large population studies. A population study of 46,396 subjects (120) found an association between reduced leukocyte telomere length and COPD, and a weak correlation with lung function (FEV_1, FVC, FEV_1/FVC). A second study found circulating leukocyte telomere length was reduced in patients with COPD (n=934) compared to controls (n=15,846), and more strongly correlated with lung function in never smokers than in smokers (122). Telomeres were found to be relatively preserved in patients with AAT deficiency, compared to non-AAT-related, aged-matched COPD subjects, and there was good correlation between blood and lung telomere lengths (with blood being shorter on average) (124). Of prognostic importance, short leukocyte telomere length was associated with increased risk of all-cause mortality (HR 1.29), compared to longer telomeres, in 4,271 subjects with mild to moderate COPD in the Lung Health Study (125).

Sirtuins (SIRTs) are NAD^+-dependent deacetylases and are members of the silent information regulator 2 (Sir2) family (126), with seven homologues in man, SIRT1-7 (127). This family of enzymes is involved in gene silencing and several studies have demonstrated that SIRT1, an anti-inflammatory and anti-ageing protein, is decreased in the lungs of patients with COPD and peripheral blood mononuclear cells in COPD (128-130). Sirtuins also control resistance to oxidative stress and DNA repair (130) and SIRT1 activation reduces cigarette smoke-induced oxidative stress (131). MMP-9 is down regulated by SIRT1 and reduced levels of SIRT1 may cause structural changes in the lung tissue (126,132,133). Sirtuins were shown to be suppressed by cigarette smoking in the large airways of asymptomatic smokers and not in the small airways, whereas in COPD a greater suppression of sirtuin expression was seen in both the large and the small airways (127). Thus there is emerging evidence to suggest that a reduction in sirtuin expression is involved in accelerated lung ageing and pathogenesis of COPD (112).

Conclusions

COPD is a heterogeneous and complex chronic lung disease with extrapulmonary manifestations. Identification of clinically applicable biomarkers would help to screen for and diagnose COPD, monitor disease activity and progression, and guide response to therapy. Similar to other chronic diseases, the search for relevant biomarkers is certainly expanding rapidly in COPD. However, access to samples remains a major issue. Gene expression profiling of lung tissue has identified genes whose expression differs in COPD according to severity, but markers derived from lung tissue are not routinely available for clinical disease monitoring, whereas sputum and blood are readily accessible. Biomarkers in blood, especially inflammatory markers such as fibrinogen, are associated with exacerbations and mortality in larger COPD cohort studies. Much more work is needed to assess blood and sputum biomarkers against disease progression outcomes in COPD. Emerging approaches to studying gene-environment interaction, which impacts on disease pathogenesis and progression in COPD, are providing promising leads for novel biomarkers. These include (I) sampling exhaled breath for VOCs and exhaled breath condensate for protein markers; (II) characterising responses of the lung to inhaled air pollutants; (III) applying knowledge of the lung microbiome to COPD phenotypes; and (IV) determining the significance of biomarkers of ageing such as telomere attrition. Overcoming methodological challenges in sampling and quality control will enable more robust yet easily accessible biomarkers to be developed and applied to optimise personalised medicine in patients with COPD.

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