Phenotyping the heterogeneity of chronic obstructive pulmonary disease

Bethan L. BARKER and Christopher E. BRIGHTLING

Institute for Lung Health, Department of Infection, Immunity & Inflammation, University of Leicester, Leicester LE3 9QF U.K.

Abstract
COPD (chronic obstructive pulmonary disease) is a heterogeneous disease associated with significant morbidity and mortality. Current diagnostic criteria based on the presence of fixed airflow obstruction and symptoms do not integrate the complex pathological changes occurring within lung, do not define different airway inflammatory patterns, nor do they define different physiological changes or differences in structure as can be defined by imaging. Over recent years, there has been interest in describing this heterogeneity and using this information to subgroup patients into COPD phenotypes. Most approaches to phenotyping have considered disease at a single scale and have not integrated information from different scales (e.g. organ–whole person, tissue–organ, cell–tissue and gene–cell) of disease to provide multi-dimensional phenotypes. Integration of disease biology with clinical expression is critical to improve understanding of this disease. When combined with biostatistical modelling, this information may lead to identification of new drug targets, new end points for clinical trials and targeted treatment for subgroups of COPD patients. It is hoped this will ultimately improve COPD outcomes and represent a move towards personalised medicine. In the present review, we will consider these aspects of multi-dimensional phenotyping in more detail.

Key words: chronic obstructive pulmonary disease (COPD), computed tomography imaging (CT imaging), genome-wide association, inflammation, remodelling

INTRODUCTION
COPD (chronic obstructive pulmonary disease) is an important cause of morbidity and mortality, and is predicted to become the third leading cause of mortality worldwide by 2030 [1]. In the U.K., it is responsible for approximately 30 000 deaths per year and for >£ 800 million per year in direct healthcare costs [2].

COPD is defined by airflow obstruction [post-bronchodilator FEV₁ (forced expiratory volume in 1 s)/FVC (forced vital capacity) ratio <0.7] that is not fully reversible, does not change markedly over time and that is usually progressive [3]. Cigarette smoke is the main causative agent, although other exposures (e.g. occupational and biomass fuel) are increasingly being recognized as important. Exacerbations are episodes involving an acute onset of sustained worsening of the patient’s symptoms from his or her usual stable state that is beyond normal day-to-day variations, and which often necessitate a change in medication [3]. Exacerbations are particularly important as they are associated with high economic costs and accelerated lung function decline, as well as having a negative impact on quality of life and mortality [4–6].

The current diagnostic criteria are based on symptoms, exposure to a potential aetiopathological agent and the presence of fixed airflow obstruction. Shortcomings of these criteria include the fact that they do not reflect the heterogeneous histopathological conditions observed in COPD. They do not integrate the complex pathological changes occurring within the lung, do not define different airway inflammatory patterns, nor do they define different physiological changes or differences in structure as can be defined by imaging. This use of a fixed FEV₁/FVC ratio to define airflow obstruction may result in an overestimation of disease in normal older individuals and an underestimation in young patients with true disease but normal FEV₁/FVC ratio [7,8]. An alternative is to use the lower limit of normal value for FEV₁/FVC ratio. The lower limit of normal value is based on a normal distribution and classifies the bottom 5% of a healthy population as abnormal. Use of the lower limit of normal value rather than a fixed
PHENOTYPING COPD

A phenotype is any observable characteristic that results from gene–environment interactions. To fulfill clinical and research goals, it has been suggested that ‘COPD phenotypes’ should be able to classify patients into distinct subgroups, provide prognostic information, be prospectively validated for each outcome and ultimately alter clinically meaningful outcomes [10].

Most approaches to phenotyping have considered disease at a single scale and have not integrated information from different scales to provide multi-dimensional phenotypes. Examples of information gathered relevant to these scales include the phenotyping or subgrouping of patients at the whole-person scale on the basis of clinical symptoms, such as breathlessness or chronic cough with sputum production (chronic bronchitis). At an organ scale, patients can be phenotyped on the basis of their lung function or radiology, where low-attenuation areas on CT (computed tomography) images are consistent with emphysema and bronchial wall thickening is suggestive of airways disease. At the cell–tissue scale, measures of airway inflammation, for example by sputum analysis, can determine phenotypes as defined by cell type or from inflammatory mediators. At the gene–cell scale, genetic variation can be identified, with the best example in COPD being α₁-antitrypsin deficiency.

This integration of disease biology with clinical expression is critical to improve the understanding of disease. In the present review, we will consider these aspects of multi-dimensional phenotyping in more detail (Figure 1).

MULTI-DIMENSIONAL COPD PHENOTYPING

Tissue to organ: image functional modelling

Radiological imaging techniques, such as CT and MRI (magnetic resonance imaging), are playing an increasing role in defining disease heterogeneity in COPD. With CT imaging, emphysema and airways disease can be demonstrated to exist separately or co-exist, and other common pathologies such as bronchiectasis and interstitial lung abnormalities can be identified [17,18]. This use
of imaging has added value, as the pathologies present cannot be predicted from lung function alone, and even those having characteristic lung function abnormalities may have evidence of a combination of lung pathologies [17,18].

CT scanning can provide both qualitative and quantitative measurements of lung structure. Large airways can be measured directly, but smaller airways (<2 mm) are beyond the conventional resolution of CT scanning. To address this, CT lung densitometry (from inspiratory and expiratory scans) can be used to quantitatively estimate gas trapping. The role for CT scanning in COPD patients continues to evolve and much information has been learned from studies of patients with α1-antitrypsin deficiency [19–25]. CT calibration techniques now allow quantitative analysis of emphysema in multi-centre and longitudinal studies [22], and CT densitometry studies have suggested that the optimal method for assessment of emphysema progression uses targeted sampling of the middle lung region [20]. Of note, CT lung densitometry has benefits over lung function in terms of its sensitivity for detecting emphysema progression [21], which has led to the suggestion that CT may be more useful than lung function to assess emphysema progression in clinical trials.

CT imaging findings also have clinical relevance. Emphysema distribution influences lung function impairment, with those having more basal emphysema having a larger reduction in FEV1 than lung function to assess emphysema progression in clinical trials.

CT imaging findings also have clinical relevance. Emphysema distribution influences lung function impairment, with those having more basal emphysema having a larger reduction in FEV1 and less impairment of gas exchange than those with apical emphysema [23]. Airway dimensions correlate with lung function [26] and also relate to symptoms [27], and proximal airway wall thickening is inversely correlated with lung function and also relates to exacerbation frequency [28].

CT-defined ‘airway predominant’ and ‘emphysema predominant’ phenotypes [26] are being explored in several large studies including ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints; http://www.eclipse-copd.com) [29], COPDGene® (http://www.copdgene.org) [30] and EvA (Emphysema versus Airway; http://www.eva-copd.eu) [31], which are seeking to identify biomarkers of COPD phenotypes. Within these and other studies, such as Spiromics (http://www.cecsc.unc.edu/spir/) and the Lung Genomics Research Consortium (http://www.lung-genomics.org), findings at the organ level (e.g. from imaging) are being matched with blood and lung samples with the aim of identifying biomarkers in lung at the DNA, RNA and protein level. Integration of CT information with that from other disease scales should ultimately help improve understanding of disease.

A disadvantage of CT scanning is the associated radiation risk, which has limited the use of multiple longitudinal scans in clinical trials. By contrast, hyperpolarized 3He MRI does not involve radiation and can be repeated in longitudinal and intervention studies [32–34]. Several functional measures can be derived using hyperpolarized MRI, including static airway functional measurement of 3He ventilation, structural measurement of airspaces using 3He apparent diffusion coefficient and dynamic measurement of 3He gas washin and washout characteristics. Several small studies have demonstrated that 3He MRI is a sensitive measure of emphysema and ventilation inhomogeneity [35–37]. Although MRI shows promise as a tool to assess airway function, a clear role for it in routine practice has yet to be defined. Its use is currently limited to research settings with the cost, availability of scanning equipment and the time-consuming nature of scans recognized as barriers to its use in clinical practice.

Physiological measurements can also be used to define phenotypes and heterogeneity within COPD. Current guidelines advocate using spirometry values to define COPD severity [3], even though COPD is predominantly a small airway disease, and spirometry values do not discriminate between large and small airway function. Impulse oscillometry and plethysmography are tools used to assess small airway function. Both impulse oscillometry and specific airway conductance using plethysmography have been shown to be more sensitive than FEV1 at detecting bronchodilation in COPD [38,39] and have been suggested as more appropriate end points than FEV1 for use in clinical trials. Multiple breath washout tests use inert marker gases such as sulfur hexafluoride or measure nitrogen washout after pre-filling the lungs with 100% oxygen [40]. These tests allow values including the lung clearance index, a measure of ventilation heterogeneity, to be obtained. In CF (cystic fibrosis), the multiple breath washout has been shown to be reproducible [41] and better at detecting abnormal lung function than plethysmography or spirometry [42], with the lung clearance index shown to be more sensitive than FEV1 for detecting structural lung disease [43]. The lung clearance index has been suggested to be a useful marker of small airways dysfunction, with abnormal lung clearance index values in CF patients with normal lung CT scans suggested to be due to detection of small airway abnormalities below the resolution of CT scans [43]. If these findings were replicated in COPD patients, the multiple breath washout could play a useful role, particularly in the detection of small airways disease.

Cell-to-tissue

A wide variety of changes are observed at the cell–tissue level in COPD (Figure 2). These changes are a consequence of lung damage and remodelling, due to underlying airways inflammation associated with impaired host defence and ongoing oxidative stress.

Tissue destruction: emphysema

Activated neutrophils release neutrophil elastase, a proteinase that can destroy elastin [44]. In health, this is counterbalanced by α1-antitrypsin, a proteinase inhibitor that protects lung tissue from neutrophil elastase damage. An imbalance between proteinases and antiproteinases leads to uncontrolled elastin destruction, resulting in parenchymal lung destruction. This is observed in the genetic condition α1-antitrypsin deficiency, which is characterized by early-onset emphysema [45].

Whether a ‘proteinase–antiproteinase imbalance’ is the main mechanism responsible for emphysema in those without α1-antitrypsin deficiency is less clear, although some degree of proteinase imbalance does exist in these patients. Macrophages and neutrophils are found in greater numbers in COPD airways [46] and, when activated, these can release elastolytic proteinases [e.g. MMPs (matrix metalloproteinases), cathepsins and collagenases] implicated in degradation of the extracellular matrix. Moreover,
alveolar macrophages from COPD patients exhibit increased elastase activity compared with controls, implicating them in the tissue destruction observed in emphysema [47].

Increased apoptosis has also been observed in emphysematous lung [48,49] and is implicated in emphysematous tissue destruction. VEGF (vascular endothelial growth factor) is an endothelial cell survival factor found in large quantities in lung. Signalling pathways involving VEGF may be important in these processes as reduced expression of VEGF and its receptor VEGFR-2 is observed in emphysema [50]. Moreover, in animal models VEGF receptor blockers lead to lung cell apoptosis, and air space enlargement resembling emphysema [51].

Pulmonary surfactant is formed by type II alveolar cells and reduces surface tension in the lung. Abnormalities in surfactant resulting in the failure of alveolar wall maintenance have also been implicated in emphysema. In animal models, deficiency of SP (surfactant protein)-A and -D results in air space enlargement and reduced alveolar tissue volume consistent with emphysema [52]. By contrast, the role of serum SP-D in human disease is less clear. In a study of 1888 COPD subjects, SP-D levels were higher in subjects than in healthy smoking controls. No correlation was however found between SP-D levels and number of exacerbations at 12-month follow-up [53].

Small airway obliteration: airway inflammation and remodelling

Airway remodelling refers to structural and cellular changes that occur in airway walls. Remodelling changes observed in COPD include disruption and loss of epithelial cilia, squamous metaplasia of the respiratory epithelium, goblet cell hyperplasia and mucous gland enlargement, bronchiolar smooth muscle hypertrophy, airway wall fibrosis and inflammatory cell infiltration [54–56]. Small airways are the major site of airway obstruction in COPD. This airways obstruction is due to a combination of remodelling and accumulation of inflammatory exudates within the airway lumen, both of which increase with disease severity [57,58]. More recently, using data from multi-detector CT and microCT (Figure 3), a reduction in the number and luminal area of terminal bronchioles has been observed in COPD of varying severities [59]. This has led to the suggestion that narrowing and disappearance of small conducting airways can explain the increased peripheral airway resistance reported in COPD [59].

Cigarette smoke is a key trigger for tissue damage and is also implicated in the abnormal lung repair processes seen in COPD. Among the multiple effects of cigarette smoking, smoking causes increased epithelial permeability [60] and can activate EGFRs [EGF (epidermal growth factor) receptors]. Increased EGF and EGFR expression is observed in bronchial epithelium in COPD [61–64] and has been implicated in the remodelling
Phenotyping the heterogeneity of COPD

Figure 3 COPD can be examined at the tissue–organ level using radiological imaging (multi-detector and microCT)

These findings are comparable with those obtained through examination of histological samples. (A) Frozen lung slice from a patient with severe emphysema. (B) The same lung slice after samples were removed for analysis. (C) Matching slice from multi-detector CT of intact lung specimen. (D) Single control lung sample after processing for microCT. (E) MicroCT image of a control lung at a resolution of 16.24 μm, with a terminal bronchiole (indicated by the white line) at the point at which it branches into respiratory bronchioles. (F) The same terminal bronchiole reoriented to show the cross-section of the airway. This Figure is from N. Engl. J. Med., McDonough, J.E., Yuan, R., Suzuki, M., Seyedinjadeh, N., Elliott, W.M., Sanchez, P.G., Wright, A.C., Gefter, W.B., Litzy, L., Coxon, H.D., Pare, P.D., Sin, D.D., Pierce, R.A., Woods, J.C., McWilliams, A.M., Mayo, J.R., Lam, S.C., Cooper, J.D. and Hogg, J.C., Small-Airway Obstruction and Emphysema in Chronic Obstructive Pulmonary Disease, volume 365, pp. 1567–1575. Copyright © (2011) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

processes. Activation of EGFR can lead to mucin synthesis and goblet cell hyperplasia, and EGF is also a stimulator of airway smooth muscle proliferation [65]. This increase in airway smooth muscle in the small airways of COPD is negatively correlated with FEV1 [66,67].

Epithelial changes observed in COPD include small airway squamous metaplasia, which increases with increasing COPD severity [68]. These squamous cells express increased IL (interleukin)-1β, which induces a fibrotic response in adjacent airway fibroblasts and is implicated in activation of TGF (transforming growth factor)-β. TGF-β is a potent fibrogenic factor that is increased in the small airway epithelial cells in COPD. This activation of TGF-β correlates with disease severity and small airway thickening in COPD, and TGF-β mRNA levels correlate with smoking history and airflow obstruction [69,70]. The EGF and TGF-β released from small airways may be involved in fibroblast activation and proliferation, which results in peribronchiolar fibrosis. In keeping with this, genes involved in extracellular matrix synthesis and degradation are up-regulated in small airways in patients with COPD [71]. Increased bronchial deposition of extracellular matrix proteins including collagens, fibronectin and laminin is observed with deposition increased on the epithelial basement membrane at sites of damage.

The negative correlation between bronchial extracellular matrix deposition and FEV1 supports the view that bronchial deposition of extracellular matrix is related to airway remodelling [72].

Airway inflammation

The presence of airway inflammation in both stable COPD and exacerbation episodes is well recognized, with severity of airflow obstruction correlating with airway inflammation, and with severe airflow obstruction associated with increased neutrophils, macrophages and CD8+ lymphocytes in the bronchial mucosa [73]. Cigarette smoking is important in the aetiology of this airway inflammation. It can induce epithelial cells to produce cytokines, which stimulate neutrophils and macrophages, as well as having a direct action leading to the activation of neutrophils and macrophages [74]. Other factors which have not yet been definitively described may, however, play an important role as inflammation is shown to persist despite smoking cessation [75,76]. These factors implicated in persistent airway inflammation include bacterial colonization, latent viral infections, genetic predisposition, autoimmunity and persistent apoptosis [75,77–79].

Neutrophils respond to infection and inflammation in all tissues and are important in innate immunity. Neutrophilic airway inflammation is considered the hallmark of COPD; elevated levels
of neutrophils are found in the sputum and bronchoalveolar lavage fluid during stable state [46], and these neutrophil levels relate to severity of airflow limitation [80]. An increased neutrophilic response is demonstrated by increased levels of neutrophil chemoattractants, such as TNF (tumour necrosis factor)-α, GM-CSF (granulocyte/macrophage colony-stimulating factor) and IL-8 [81–84], and this neutrophilic response is related to microbial load [81]. Eosinophilic airway inflammation is traditionally associated with asthma, but has also been found in 15–40% of stable COPD patients [85]. Eosinophils have been found in sputum, bronchoalveolar fluid and tissue [46,76,86], with activation correlating with disease severity [86–88]. An increase in the number of eosinophils has been found during exacerbations [89–91], along with secretory eosinophil products ECP (eosinophil cationic protein) and IL-5Rα (IL-5 receptor-α) [92,93]. CD8+ T-lymphocytes have a key role in inducing apoptosis of infected and damaged cells. An increased number of CD8+ cells is found in sputum, bronchoalveolar fluid, bronchial biopsies and lung parenchyma in COPD [94–98]. CD8+ T-lymphocyte number correlates negatively with lung function [97,99–101] and is associated with increased peripheral airway smooth muscle area [67], which suggests CD8+ may have a role in remodelling. These CD8+ cells are also highly activated, implicating them in the inflammatory processes of COPD [94].

Host–environment interactions
The airway is continuously exposed to a variety of insults which include pollutants such as cigarette smoke, allergens and pathogens. In health, dynamic interactions between the environment and the host’s innate and adaptive immune responses co-ordinate a cascade of inflammation and repair in response to these insults. These interactions seek to prevent aberrant or overexuberant inflammation and remodelling, and can be examined at a cell–tissue level.

Infection caused by pathogens, such as bacteria, viruses or a combination of these, is thought to have an important role in COPD pathogenesis. Traditional culture techniques report high rates of positive sputum cultures in stable patients, so-called ‘colonization’ [102–104]. This colonization is associated with increased airways inflammation, poorer health status and more rapid lung function decline [105–107]. Molecular techniques which allow identification of bacterial sequences are providing new insights into the types and quantities of bacteria in normal and diseased lung [108,109] and may help improve our understanding of host–pathogen interactions in COPD.

Both pathogenic and host factors influence whether or not a patient with COPD will experience an exacerbation following exposure to a pathogen.

Host factors
The host’s innate and adaptive immune system influence the clinical features observed following the acquisition of a bacterial pathogen. These factors also influence whether the pathogen is cleared by the host [110]. There is evidence that cigarette smoke has a detrimental effect on the host’s innate immunity. These effects include causing a defect in mucociliary clearance [111], the promotion of bacterial adhesion to airway epithelia [112], abnormalities of the lung epithelial host defence system [113,114] and abnormalities of the function of pulmonary host defence cells including macrophages [115].

Abnormalities of innate immunity, such as mucociliary dysfunction and alveolar macrophage dysfunction, also persist despite smoking cessation [116]. In COPD, the number of macrophages is increased in the airways, bronchoalveolar lavage fluid and tissue [117,118], and there is evidence that their function is abnormal; in COPD, alveolar macrophage phagocytosis of Haemophilus influenzae is reduced [119], and monocyte-derived macrophages show reduced phagocytic responses to Streptococcus pneumoniae and H. influenzae when compared with nonsmokers and smokers [120]. This macrophage dysfunction may be a major factor leading to bacterial colonization and increased exacerbation frequency, and novel therapies targeting macrophage function may prove beneficial.

In asthma, an abnormal innate immune response to rhinovirus infection has been observed [121,122], and a similar abnormal response may occur in COPD. In asthma, rhinovirus-infected bronchial epithelial cells produce less virus-induced IFN (interferon)-β [121] and IFN-λ [122], and alveolar macrophages induce less IFN-λ in response to rhinovirus infection [122] than controls. Whether impaired IFN production is also a mechanism leading to viral infection susceptibility in COPD is unclear [123,124]. Viruses have also been demonstrated to alter host defence [114,125,126]. Viruses can promote bacterial adhesion to respiratory epithelial cells which might increase colonization [127] and can up-regulate cell-surface receptors for respiratory bacteria [128]. The finding of CD8+ T-lymphocytes in small airways of COPD may be due to recurrent or persistent viral infections in this patient group, and might implicate latent viral infection in COPD pathophysiology. Host adaptive immune responses also influence whether or not exposure to bacterial pathogens leads to exacerbations. Adaptive immune responses to bacteria that may lead to protection from exacerbations include a mucosal IgA response after acquisition of Moraxella catarrhalis [129], and the development of T-lymphocyte responses to the P6 protein of H. influenzae [130].

Remodelling of bronchial structures can also influence host response to pathogens [114]. SlgA (secretory IgA) prevents adhesion to or invasion of bronchial epithelium by pathogens and is only expressed on normal bronchial epithelium. The abnormal remodelled bronchial epithelium in COPD is therefore associated with slgA deficiency. Strong correlations between the reduction in small airway slgA levels, latent viral infection, increased CD8 T-lymphocytes, airway remodelling and severity of airflow obstruction have been demonstrated. Together, these findings may suggest mucosal slgA may be important in COPD pathogenesis [114].

There has also been an increasing interest and understanding of the role of PAMP (pathogen-associated molecular pattern) and DAMP (danger-associated molecular pattern) receptors in host defence in airways disease such a COPD. RAGE [receptor for AGEs (advanced glycation end products)] is a pattern recognition receptor that interacts with several ligands, including AGEs and HMGB1 (high-mobility group box 1). In healthy tissues, RAGE is low or undetectable, but it is highly expressed in the
lungs [131,132]. RAGE binds to many DAMP receptors released from cells following tissue damage, and is secreted by activated immune cells and stressed structural cells. It has been suggested to play a role in lung homeostasis and may have a role in initiation and promotion of continuing inflammation. RAGE enhances adherence of type I epithelial cells to the extracellular matrix [133] and is implicated in the differentiation of type II to type I epithelial cells, which is thought to be crucial in alveolar repair [134]. Soluble RAGE acts as a decoy receptor for RAGE ligands and may offer protection against inflammation. Soluble RAGE levels are lower in COPD than controls and correlate with the amount of emphysema on CT, even after adjusting for smoking history [135,136]. HMGB1 levels and RAGE expression have been shown to be elevated both in smokers and smokers with COPD [137,138]. In COPD, HMGB1 levels are increased in bronchoalveolar lavage fluid, and correlate positively with markers of inflammation, such as IL-1β, and negatively with lung function. Lung and systemic levels of HMGB1 or other DAMPs may prove to be clinically relevant as biomarkers of neutrophilic inflammation and respiratory function in COPD.

Pathogenic factors
New strain acquisition is associated with an increased risk of exacerbations [139]. Pathogenic factors influencing development of COPD exacerbations include the finding that genome differences between infecting strains influence the outcome of encounters with *H. influenzae* [140]. Strains of *H. influenzae* causing exacerbations also show increased adherence to epithelial cells, increased induction of IL-8 and increased neutrophil recruitment compared with strains resulting in colonization [141]. New molecular techniques including quantitative PCR [104] and sequencing [142] may reveal further understanding of the relationship between the ecology of the airway and disease expression in COPD.

Oxidative stress
At the cell–tissue level, oxidative stress has also been implicated in many of the abnormal processes observed in COPD [143]. This increased oxidative stress found in COPD is due to a combination of inhaled ROS (reactive oxygen species) from cigarette smoke and ROS released from activated inflammatory cells in COPD airways [143]. The increased oxidative burden in COPD can be demonstrated by observations of increased H₂O₂ and lipid peroxidation products, such as 8-isoprostan and thiobarbituric acid-reacting substances, in exhaled breath condensate [144,145]. Increased blood levels of lipid peroxidation products including malondialdehyde have also been observed, with levels correlating negatively with FEV₁ [146].

The lung has protective antioxidant systems, but the increased oxidative burden in COPD leads to an oxidant–antioxidant imbalance which has multiple pathological effects. As well as causing direct lipid and protein damage, oxidative stress can also damage the respiratory epithelium, resulting in increased epithelial permeability [147,148]. This increased epithelial permeability is associated with a reduction in epithelial intracellular glutathione [149], which is important as glutathione has a protective antioxidant role in lung. A reduction in lung glutathione on its own can lead to increased epithelial permeability [149]. Other effects of oxidative stress on the respiratory epithelium include stimulation of increased mucus secretion [150] via signalling pathways for EGF, inhibition of ciliary function [151] and increased release of inflammatory mediators such as IL-8 [152]. This increased release of chemoattractants such as IL-8 leads to inflammatory cell influx into the lung and contributes to the increased airway inflammation observed in COPD [143,152]. Oxidative stress also contributes to airways inflammation through stimulation of pathways and signalling mechanisms, leading to up-regulation and activation of MAPKs (mitogen-activated protein kinases) and redox-sensitive transcription factors including NF-κB (nuclear factor κB) [153] and AP-1 (activator protein-1) [154]. Tissue destruction in COPD is also influenced by oxidative stress. The anti-proteinases that protect the lung from proteinase-induced damage may be inactivated by oxidants [155] released from inflammatory leukocytes in the lung. Apoptosis of alveolar cells is also thought to be influenced by oxidative stress as shown in an animal model where apoptosis caused by VEGFR (VEGF receptor) inhibition was associated with evidence of increased oxidative stress and could be prevented by antioxidants [156]. A potential adaptive consequence of oxidative stress is the up-regulation of epithelial antioxidant genes (e.g. glutathione peroxidase 2) through pathways involving Nrf2 (nuclear factor-erythroid 2-related factor 2) [157]. Nrf2 is a transcription factor that helps offer protection from ROS and is increased in the epithelium of cells exposed to cigarette smoke [158]. In animal models, absence of Nrf2 increases oxidative stress, apoptosis, airways inflammation and susceptibility to emphysema [159]. This may have clinical relevance and strategies to increase Nrf2 activation may help improve host responses to the increased oxidative burden.

Cell to genome
Information obtained at the cell–genome level may also increase our understanding of COPD pathogenesis. This is best demonstrated by our knowledge of the inherited condition α₁-antitrypsin deficiency, whereby a proteinase–antiproteinase imbalance favours proteinase-induced lung destruction and predisposes to early onset emphysema [58,160].

GWAS (genome-wide association studies) have identified several SNPs (single nucleotide polymorphisms) that are associated with FEV₁ and airflow obstruction. In a meta-analysis of GWAS for lung function, eight loci associated with FEV₁/FVC were identified [161], including GPR126 (gene encoding a G-protein-coupled receptor involved in cell adhesion and signalling), AGER-PPT2 (gene encoding RAGE) and HTR4 (gene encoding the 5-hydroxytryptamine receptor 4). One locus at INTS12-GSTCD-NPNT (encoding at least three genes, including glutathione transferase, C-terminal domain containing) was associated with FEV₁. A further study including 20 000 Europeans sought associations between lung function and genotypes or imputed SNPs [162]. Follow-up SNPs from the most significant loci were genotyped, leading to the identification of five additional loci including the GSTCD gene, with mRNA analysis revealing expression of all five loci in the lung.

Several SNPs have also been associated with COPD, including two SNPs at the CHRNA3/CHRNA5 (genes encoding α-nicotinic
acetylcholine receptor 3 and 5 respectively) [163]; this may, however, be an epiphenomenon representing an association between COPD and nicotine addiction, rather than providing additional mechanistic information about COPD pathogenesis. Other SNPs associated with COPD include loci at 19q13 [164], at HHIP (gene encoding Hedgehog interacting protein) and at FAM13A (gene encoding family with sequence similarity 13, member A), the products of which is suggested to have a role in signal transduction [165]. Of note the FAM13A locus was also implicated in the GWAS of lung function already described [161], and the FAM13A SNPs were not associated with smoking history.

These associations offer insight into the mechanisms regulating pulmonary function and examination of the products of these genes may identify potential pathways involved in COPD pathogenesis. Associations between airflow obstruction and genes coding for GSTCD and RAGE products implicate further oxidative stress and damage recognition receptors in the pathogenesis of airflow obstruction and thus the development of COPD.

**CLINICAL APPLICATIONS OF MULTI-SCALE COPD PHENOTYPING**

In the previous sections of the present review, we have attempted to describe COPD pathophysiology using information obtained at individual scales of disease (e.g. organ, tissue, cell and gene levels). The next stage is to integrate this knowledge to further improve our understanding of COPD. Further identification of inter-individual differences at multiple scales of disease should increase our ability to identify multi-dimensional phenotypes which can subsequently be targeted with phenotype-specific therapies. In the following sections, current examples of phenotype-targeted treatments available in clinical practice will be described.

**Phenotype-targeted treatment of stable COPD**

Personalized treatment for some groups of stable COPD patients already exists in routine practice. An example of how information gained at the tissue–organ level influences treatment arises from the results of the NETT (National Emphysema Treatment Trial) [166]. Within this study, patients with severe emphysema were randomized to LVRS (lung volume reduction surgery) or continued medical treatment. Although LVRS did not reduce overall mortality, subgroup analysis identified a group with upper lobe emphysema and low baseline exercise capacity who did obtain a survival benefit, and LVRS is now directed at this COPD phenotype. Likewise, personalized non-surgical lung volume reduction can be achieved by endobronchial valve insertion into lobes selected without collateral ventilation [167]. Personalization of long-acting bronchodilators in COPD has been challenging. In contrast with asthma, the response to β-agonists is not related strongly to genotype [168–173] and, although long-acting antimuscarinics have some additional benefit in reduction of dynamic hyperinflation in COPD patients with emphysema predominance [174], whether this distinction is clinically important is uncertain.

At the cellular scale, strategies can be adopted to target airways inflammation. Roflumilast is a selective PDF-4 (phosphodiesterase-4) inhibitor with anti-inflammatory effects. It has been shown to reduce sputum neutrophils, eosinophils, soluble IL-8 and neutrophil elastase levels [175], but, in spite of this, initial results from large COPD clinical trials were disappointing [176]. Post-hoc analyses did, however, identify a reduction in exacerbations in a subgroup with severe airflow obstruction (FEV<sub>1</sub> <50 % predicted), chronic bronchitis symptoms and frequent exacerbations [176,177]. These post-hoc analyses were used to design a prospective clinical trial which demonstrated reduced exacerbations in this specific subset of patients towards whom roflumilast therapy is now targeted [178]. Furthermore, targeting corticosteroid therapy to minimize eosinophilic airway inflammation in moderate-to-severe asthma reduces sputum eosinophil counts, severe exacerbations and hospital admissions [179]. Similarly, targeting corticosteroids in stable COPD according to sputum eosinophil levels reduces severe exacerbations, has no significant effect on mild-to-moderate exacerbation frequency and has no effect on average corticosteroid doses [180]. Concerns about corticosteroid side effects do, however, exist and novel drugs such as anti-IL-5 monoclonal antibodies that have reduced exacerbations and improved quality of life in refractory eosinophilic asthma [181,182] are being investigated in COPD.

The oxidant–antioxidant imbalance observed in COPD is another potential therapeutic target. Therapeutic options include thiol antioxidants, such as N-acetylcysteine, which neutralize oxidant species and increase intracellular glutathione in lungs, but have limited bioavailability and so far have had mixed clinical results [183,184]. Nrf2 is also of interest. Nrf2 is protective in animal emphysema models [159] and its activation by sulforaphane can influence some of the biochemical changes found in smokers and COPD [185]. These observations suggest that further investigation as to whether this translates into clinical benefit is warranted.

**Phenotype-targeted treatment of COPD exacerbations**

The diagnosis of an acute exacerbation of COPD is currently based on symptoms. Treatment with systemic corticosteroids is recommended for patients hospitalized with exacerbations or if there is an increase in breathlessness which interferes with daily activities. Antibiotics are recommended if there is an increase in three symptoms (dyspnoea, sputum volume and sputum purulence) or if there is an increase in two of these symptoms and one of these is an increase in sputum purulence [3,186].

Although there is evidence supporting the use of corticosteroids and antibiotics for COPD exacerbations, both agents have significant potential to cause harm and there is evidence that not all patients benefit equally. A systematic review of treatment for acute exacerbations of COPD found that systemic corticosteroids reduce treatment failure and length of hospital stay, along with increasing the rate of improvement in lung function when compared with placebo [187]. Corticosteroids have not, however, been demonstrated to reduce mortality and are associated with significantly more adverse events such as hyperglycaemia [187]. Meta-analyses and systematic reviews of randomized
controlled trials comparing antibiotics to placebo for acute COPD exacerbations [188–191] conclude that antibiotics reduce treatment failures and mortality in severe exacerbations and hospitalized patients [188] and lead to a small, but statistically significant, benefit in terms of peak flow measurements [190]. Antibiotics do not, however, appear to reduce treatment failure in mild-to-moderate exacerbations [188] and in out-patient exacerbations [189]. Conclusions from these pooled group analyses do, however, need to be interpreted with caution as there is significant heterogeneity both within trials and between trials in terms of COPD severity and exacerbation severity.

The ultimate aim is to be able to target COPD exacerbation treatment to subgroups most likely to respond to particular therapies, as well as identifying those at most risk of harm. Targeted antibiotic therapy can reduce costs, reduce subsequent colonization and infection with antibiotic-resistant organisms [192] and can help reduce rates of antibiotic-related diarrhoea, including Clostridium difficile infection [188,191]. Targeted corticosteroid therapy may similarly reduce adverse events such as corticosteroid-induced hyperglycaemia.

Recent research efforts have focused on improving objectivity of COPD exacerbation diagnoses and on accurately identifying the underlying aetiology of exacerbations. Biomarkers have been used to try to objectively diagnose exacerbations [193] and have also been used to phenotype exacerbations according to aetiology [104]. At the patient level, classifying exacerbations according to the presence or absence of three symptoms (sputum purulence, sputum quantity and increased breathlessness) as described by Anthonisen et al. [194] is one simple way to try to target treatments. Those with Type 1 exacerbations (increase in all three symptoms) are more likely to have positive bacterial sputum cultures and respond to antibiotic treatment, whereas antibodies have been shown to have no significant benefits in Type 2 and 3 exacerbations [188]. Purulent sputum has been associated with bacterial growth [195] and is associated with higher rates of positive bacterial culture in the presence of exacerbation symptoms [102]. In single centre studies, sputum colour assessed by a colour chart is a strong predictor of bacterial aetiology, although the diagnostic yield is less convincing in multi-centre trials [102,195,196]. PCT (pro-calcitonin), a peptide released in bacterial infections, has potential in identifying bacterial exacerbations of COPD and guiding treatment duration. A PCT algorithm (predefined criteria for starting/stoping antibiotics) has been shown to reduce the duration of antibiotic courses, without increasing adverse events [197]. Thus using PCT to guide treatment could reduce inappropriate antibiotics in acute exacerbations of COPD.

Following on from observations in stable COPD [180], a strategy to target eosinophilic inflammation in out-patient COPD exacerbations has also been investigated [198]. Peripheral blood eosinophil count has been identified as a biomarker of eosinophilic airways inflammation [104] and, in a single centre study, targeting oral corticosteroid treatment to those with evidence of eosinophilic airways inflammation has been shown to lead to a safe reduction in oral corticosteroid prescriptions [198]. Moreover, within this study, those receiving oral corticosteroids, but without an eosinophilia, experienced more adverse events and poorer recovery rates. These findings now require replication in a multi-centre study, but may represent a further step towards personalized treatment of COPD exacerbations.

**THE FUTURE OF COPD PHENOTYPING: MOVING TOWARDS MULTI-SCALE MODELLING**

Until recently, COPD treatment has been stratified according to disease severity as described by FEV₁. Times are, however, changing; the latest GOLD (Global Initiative for Chronic Obstructive Lung Disease) strategy document moves away from recommending treatment stratified according to severity as defined by FEV₁, acknowledging the increasing evidence that FEV₁ alone is inadequate for describing COPD status. The new approach recommended represents a move towards personalized treatment for COPD patients, matching therapy more closely to individuals' needs and includes multi-dimensional assessment of symptoms, spirometric classification and evaluation of the risk of future adverse events, particularly exacerbations [3].

This movement towards multi-dimensional patient assessment reflects the progress made in COPD research strategies over recent years. Previous observational studies have identified clinical and pathological features associated with airflow obstruction and exacerbation frequency using multiple regression analysis [27,80,88,90,97]. Integration of data from non-invasive markers of airflow inflammation has provided new inflammatory phenotypes, e.g. eosinophilic and non-eosinophilic [46,85]. Genetic variation has been identified at the genome level in patients with COPD and is being used to suggest pathways implicated in COPD pathogenesis [161–163]. More recently, efforts to integrate data from individual disease scales with statistical modelling such as factor and cluster analysis have been made in order to provide more insight into the complexity of COPD phenotypes and to phenotype both stable COPD [199,200] and exacerbations [198].

Cluster analysis is a technique for data exploration that groups subjects without an a priori hypothesis. It seeks to organize information so that heterogeneous groups of variables can be classified into more homogeneous groups [201]. Cluster analyses have been performed on random community population samples [202] and on well-characterized COPD subjects [199,200,203]. These techniques have identified ‘clusters’ or ‘phenotype’ that cannot be predicted using spirometry alone and which exhibit marked differences in age, symptoms, co-morbidities and predicted mortality [199,200,204]. Prospective validation of some of these phenotypes has also demonstrated that they have relevance in terms of predicting long-term outcomes. Garcia-Aymerich et al. [200] identified three groups using cluster analysis; a ‘severe respiratory COPD’ group, a ‘mild respiratory COPD’ group and a ‘systemic COPD’ group. A 4-year longitudinal follow-up demonstrated that subjects in the ‘severe respiratory COPD’ group had increased hospitalizations due to COPD and increased all-cause mortality, whereas subjects in the ‘systemic COPD’ group had an increased incidence of cardiovascular disease [200].

There are currently several large COPD studies gathering data available from multiple scales in COPD patients,
including COPDMAP (http://www.copdmap.org), Spiromics (http://www.cscc.unc.edu/spir/), ECLIPSE [29], COPDGene® [30], EvA [31], Lung Genomics Research Consortium (http://www.lung-genomics.org) and AirPROM (Airway Disease Predicting Outcomes through Patient Specific Computational Modelling; http://www.airprom.eu). Several COPD phenotypes have been suggested using data gathered from ECLIPSE [29], including a ‘frequent exacerbator’ and ‘persistent systemic inflammation’ phenotype. The ‘frequent exacerbator’ phenotype [205] describes subjects who experience two or more exacerbations per year. This phenotype is relatively stable over time and can be identified by asking patients about their exacerbation frequency over the previous year. These ‘frequent exacerbators’ are an important subgroup to identify, as exacerbations are associated with decline in lung function, deterioration in health status and increased mortality [4,206,207]. The term ‘persistent systemic inflammation COPD phenotype’ [208] has been suggested to describe the subgroup of COPD subjects who demonstrate persistent systemic inflammation in spite of similar lung function impairment, and who have increased all-cause mortality and exacerbation frequency. Both of these phenotypes are at increased risk of adverse outcomes and may be a target for specific research and ultimately treatments. The term ‘chronic bronchitis phenotype’, describing the subgroup experiencing chronic cough with sputum production has also been suggested [205,209], although whether this subgroup meets the criteria for a COPD phenotype remains unclear. Although several authors have shown chronic bronchitis to be associated with increased FEV\(_1\) decline, exacerbations, dyspnoea and mortality [206,210–212], the ECLIPSE investigators did not find increased dyspnoea in those with chronic bronchitis, and the association between chronic cough and exacerbations was not significant in multivariate analysis [205]. It has been suggested that chronic bronchitis symptoms alone may be insufficient to define a phenotype [204] and, in keeping with the hypotheses of the large COPD study groups described, a combination of characteristics (e.g. symptoms, imaging, biomarkers and genetics) which may include ‘chronic bronchitis’ symptoms may prove to be a more successful way of defining COPD phenotypes.

Within these large COPD studies high-throughput technology is being used which will improve the ability to screen, identify and validate biomarkers, and will allow analysis of many different biomarkers simultaneously. Validation of these phenotypes will then require longitudinal data collection in carefully characterized patient populations, but ultimately these data may be used to develop statistical models of future risk of lung function decline, risk of future exacerbations or the likely treatment response.

CONCLUSIONS

COPD is a heterogeneous disease. Our understanding of this heterogeneity can be improved through integration of disease-specific information obtained at multiple disease scales. When combined with biostatistical modelling, integration of this information may help identification of new drug targets, new end points for clinical trials and targeted treatment for subgroups of COPD patients. This approach has already improved, and is likely to further improve, COPD outcomes and represents a move towards personalized medicine.

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