# NOVEL INSIGHTS INTO CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD): AN OVERVIEW

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## ABSTRACT

Chronic obstructive pulmonary disease (COPD) is mainly caused by smoking and presents with shortness of breath that is progressive and irreversible. It is a worldwide health problem and the fourth most common cause of chronic disability and mortality, even in developed countries. It is a complex disease in which both the airway and lung parenchyma are involved. In this review we will be mainly focusing on the airway component of the disease. We have reviewed the current literature on airway inflammation and remodelling in smoking-related COPD. It is not only the tobacco smoking which can lead to chronic inflammation, but also the persistent presence of pathogenic microorganisms in the airways. Detailed data on these in COPD are sparse. One potential mechanism contributing to small airway fibrosis/obliteration and change in extracellular matrix is epithelial mesenchymal transition (EMT). When associated with angiogenesis (so called EMT-Type-3) it may well also be the link with the development of cancer, which is closely associated with COPD, predominantly in large airways. In this paper we focused on: 1) the role of inflammation in developing COPD; 2) recent observations on structural and cellular changes which might have relevance to a major feature of COPD that is poorly understood, namely, the striking vulnerability of patients with COPD to develop lung cancer; 3) the potential role of respiratory infections in COPD.

Keywords: Chronic obstructive pulmonary disease (COPD), lung cancer, epithelial mesenchymal transition (EMT), inflammation, infections, fibrosis.

## INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a disease state which is characterised by a (not fully reversible) airflow limitation. This is commonly progressive and is associated with noxious particles/gases causing an abnormal inflammatory response of the airways.[1] The term COPD, now widely used, was introduced into the literature in 1964.[2] Later, in the 1970s and 1980s, subphenotypes such as emphysema, chronic bronchitis, chronic obstructive bronchitis, and chronic bronchitis with emphysema were used, and recommendations and international guidelines became available on how to use these specific terms to define the disease, all of which are today lumped together as COPD.[3]

Tobacco smoking is considered as the main aetiological factor in this condition, at least in Western countries. It is a complex disease, and can have both airway and lung parenchymal components involved. The airway component may lead to chronic cough and sputum production (classically termed chronic bronchitis) but also airway narrowing with airflow obstruction, most importantly with destruction/obliteration of small airways ('destructive bronchitis', which is more of a pathological entity with physiological consequences).[4-6] Small airway narrowing is the core COPD pathology with alveolar destruction occurring subsequently in about 50%, which is the process of emphysema. Emphysema is an anatomical definition, but it is associated

with enhanced breathlessness in COPD, and physiologically gives rise to a reduced diffusing capacity (a lower oxygen absorbing capacity of the lung). It also adds to airflow obstruction by reducing the elastic recoil properties of the lung tissue, so that the driving pressure for the air to leave the alveoli is reduced.[5-7]

### Inflammation

Over the past two decades the dogma that inflammation is the primary airway pathology in COPD has gained broad acceptance. Chronic inflammation in COPD has been mainly characterised as accumulation of neutrophils, macrophages, and CD8+ T cells. Inflammation is said to become worse with disease severity,[8] and in very advanced disease, lymphoid aggregates of B cells become evident in the small airway wall. COPD is a neutrophilic disease in the airway and lumen, with also a large increase in luminal macrophages. Neutrophils are also found in mucus glands in abundance but in airway epithelium and smooth muscle bundles.[9] To our surprise, we found a decrease in the total number of cells in the lamina propria in current smokers, with and without COPD, compared to normal controls.[10] There has been no previous differentiation between the hyper-cellularity around the reticular basement membrane (RBM)[11,12] and the hypo-cellular in the underlying layer.[10] We are currently doing a comprehensive cellular analysis of lamina propria in COPD with recent data indicating low numbers of neutrophils in smokers with COPD.[10] Blood vessel density is also decreased in the lamina propria,[13] but we do not know if these two phenomena are related, i.e. fewer vessels meaning less cellular trafficking to lamina propria tissue.

Although there have been a number of studies reporting inflammation in COPD, it is still hard to draw any firm conclusions based on the current literature. Markers used in these studies are often controversial.[14,15] The lamina propria in COPD is associated with decreased cellularity at least in mild-to-moderate disease, except in specific areas such as glands and airway lumen. We suggest that the role of inflammation in COPD needs more work, especially teasing out exactly which type of cells are going down, and if this is a feature of only mild-moderate disease or of severe disease as well.

## ROLE OF EPITHELIAL MESENCHYMAL TRANSITION (EMT) IN COPD

One of the features of chronic inflammatory airway diseases, including COPD, is airway remodelling.[16] In COPD, remodelling may occur as a response to smoking-induced damage to the airways, but the details of structural changes and underlying mechanisms are poorly described and understood.[17] One potential mechanism contributing to small airway fibrosis and epithelial malignancy (predominantly in large airways) is the transition of airway epithelial cells to a mesenchymal phenotype with myofibroblast characteristics, which then migrate into the lamina propria; a process termed EMT.[18] Milara et al.[19] recently reported that EMT is an active process in small airways of COPD patients and potentially contributes to small airway fibrosis. Wang and colleagues[20] further elaborated this concept by demonstrating increased urokinase-type plasminogen activator receptor expression in the small airway epithelium of patients with COPD, participating in an active EMT process. In our group, description of central airway remodelling, we observed hyper vascularity in relation to the RBM, i.e. EMT-Type 3. It is of note that large airways are classically the site of most lung cancers, especially squamous cell type.[21] Approximately 70% of patients with lung cancer have pre-existing mild-to-moderate COPD,[22,23] with COPD increasing cancer risk 6-fold, even allowing for the underlying smoking habit. We believe this link is under-appreciated and warrants further investigation.

Several pathways are proposed as a link between COPD and lung cancer.[22,24] Yang et al.[25,26] outlined pathways such as inflammation, tissue damage due to oxidative stress, altered DNA repair mechanism, and angiogenesis, as well as EMT being implicated in both COPD and lung cancer. EMT demonstrates many of these pathogenic processes suggested leading to lung cancer. Further, EMT caused both inhibition of growth arrest and apoptosis to increasing transitional cell survival. Such increased cell survival ultimately leads to tissue damaging pro-inflammatory cell necrosis, which itself can lead to an increased chance of malignant change.[27-31] Another important cancer modulator is the cancer-associated fibroblast (CAF), which plays a vital role in the development of solid cancer. Myofibroblasts or stromal cells derived from EMT, bone marrow derived blood fibrocytes, vessel endothelium derived stromal cells (Endo-EMT), or resident fibroblasts have all been proposed to be the

source of aberrant CAFs. These activated cells in 'pro-tumour stroma' facilitate angiogenesis, tumour induction, growth, and progression.[29,32] As already inferred; angiogenesis can be an important aspect of both premalignant and malignant phases of cancer development and, as described above, in large airways in COPD, EMT is associated with local angiogenesis, RBM, and epithelial hyper vascularity (EMT-Type 3). Hiroshima et al.[33] demonstrated angiogenesis and penetration of capillary loops into bronchial epithelium of smokers, who proved to be at high risk of developing lung cancer.

Proangiogenic vascular endothelial growth factor has been shown to be hyper expressed in bronchial and alveolar epithelial cell, airway smooth muscle cells of COPD.[34] RBM associated vessels were shown to be hyper permeable (positive for albumin), while those in the lamina propria were not.[35] This protein-rich tissue micro with fibrinogen extravasation may be stimulatory to angiogenesis.[36] A subsequent study found enhanced staining for transforming growth factor-beta-1 in RBM vessels in smoker COPD subjects, implicating it in this angiogenesis.[35] It is thought that endocan upregulation in blood vessel endothelium provides an immune defense for developing epithelial tumours by inhibiting accumulation and local activity of natural killer cells, which would otherwise keep check on tumour cell proliferation.[32,37]

## THE EXTRACELLULAR MATRIX (ECM) IN COPD

Ultimately, the airflow limitation in COPD is the result of airway wall tissue remodelling and scarring, i.e. reorganisation of the ECM. These changes in the ECM have profound effects, the most important being gradual obliteration of the small airway. The cell type that is involved in ECM production is the myofibroblast. Studies in COPD based on this protein marker for myofibroblasts (alpha-smooth muscle actin [αSMA]) using human bronchi and bronchiolar tissue have been variable. Lofdahl et al.,[38] in their histological staining of large and small airway tissue from operative resection, showed an increased expression in αSMA positive cells in the lamina propria of the large airway in COPD patients when compared to non-smoker controls, although similar differences in the expression level were not observed in the small airway. In contrast, findings from *in vitro* studies with fibroblasts isolated from the distil end of the airway from COPD patients showed increased contractile properties associated with increased myofibroblasts.[39] These findings suggest myofibroblasts may be important in both the small and the large airways but the situation needs to be classified.

Myofibroblasts are known to secrete a large array of ECM proteins including fibrous proteins (collagens and elastin) and glycoproteins (fibronectin, tenascin C [TN-C], and proteoglycans). Fibrillar collagens Type 1, 2, 3, 5, and 11 are the most abundant matrix proteins and constitute approximately 15-20% of the dry weight of the tissue.[40] In patients with COPD, variability in collagen subtype deposition in both the large and small airways has been related to disease stage. In large airway biopsies, Harju et al.[41] observed an increase in expression of both collagens 1 and 3 in Stage 1 and 2 COPD in the lamina propria region, while Stage 4 COPD patients showed a decrease in expression of collagen 1 and an increase only in expression of collagen 3 when compared to normal smokers and non-smoker controls. Small airway tissues showed an overall increase in both collagen sub-types in the early stages (1 and 2), which subsequently decline in Stage 4. In contrast again, Annoni et al.[42] showed a decrease in collagen Type 1 and no change in Types 3 and 4 in mild-to-moderate COPD patients over that of non-smokers in resected tissue, in both large and small airways.

Recent evidence has shown that ECM glycoproteins, such as TN-C and fibronectin, have an essential role in tissue remodelling in COPD. Karvonen et al.[43] showed an increased expression of TN-C in mild-to-moderate COPD patients and increased correlation with myofibroblasts in the lamina propria area of the large airway biopsies. Similar changes were reported by Annoni et al.[42] For fibronectin, however, neither groups found any change in expression in COPD patients. Similarly, *in vitro* studies for evaluation of secretory fibronectin from fibroblasts isolated from non-smokers and COPD patients also showed no differences.[44] The findings are surprising as both glycoproteins are known to be secreted by myofibroblast, and the apparent differential expression level could be due to spatial and temporal changes that occur in the ECM under disease conditions.

Proteoglycans consist of a protein core covalently attached to one or more glycosaminoglycan (GAG) chain(s) and have an essential role in maintaining tissue homeostasis. Proteoglycans are further subdivided into three subtypes: basement

membrane proteoglycans (e.g. perlecan), small leucine-rich proteoglycans (e.g. decorin, biglycan, lumican), and hyalectans (versican, aggrecan).[45] Annoni et al.[42] recently observed no changes in versican, decorin, biglycan, or lumican expression in resected large or small airways or in lung parenchyma among COPD patients in comparison to non-obstructive smoker and non-smoker controls. In contrast, van Straaten et al.[46] had earlier observed a decreased expression of decorin and biglycan in the peribronchiolar area of the emphysematous lung tissues of COPD patients, and associated it to decreased elastic recoil and increase in bronchiolar obstruction. Further, Hallgren et al.[47] described that distal airway fibroblasts from COPD patients showed enhanced production of versican, which correlated with decreased elastic recoil emphysema. Lower perlecan production was observed from centrally derived cells in COPD.

Although there are substantial reports on ECM changes in other lung diseases such as interstitial lung disease (including idiopathic pulmonary fibrosis) and asthma, investigations into changes in the ECM in COPD patients are limited. The lack of differential markers to distinguish myofibroblasts from other fibroblasts and mesenchymal stromal cells has been an impediment to this research. New markers such as CD44 and CD90 (Thy1) have emerged as plausible specific tools that could improve sensitivity.[43,48] There is also great interest over recent results in the roles played by other mesenchymal cells, such as pericytes and endothelial cells, and their potential transition to myofibroblasts, and also the role of macrophage subtypes in maintaining and/or disrupting the ECM homeostasis in airway and lung tissue of COPD patients.

## CHRONIC AIRWAY INFECTIONS IN COPD

Airway infection is not a primary driver of COPD, as tobacco smoking is, but it seems undeniable that chronic airway infection with either bacteria or viruses, or both, is important in the progression of disease. Lungs are 'sterile' based on traditional microbiological techniques, while potentially pathogenic microorganisms are present in lower airway secretions in 29-45% of COPD patients (sputum and bronchoalveolar lavage). The most common 'colonisers' in the stable state of COPD are *Haemophilus influenzae, Streptococcus pneumoniae,* and *Moraxella catarrhalis.*[49-53] Chronic airway 'colonisation' by respiratory viruses in COPD is controversial.[54] However, low-grade respiratory syncytial virus (RSV) infection in 30% of sputum samples in stable patients has been detected. Moreover, latent adenoviral infection has been proposed, and detection rates (approximately 6%) were similar in both stable COPD and exacerbations. Adding to this, lung tissue from COPD patients was found to carry more group C adenoviral DNA compared to non-obstructed smokers.[55] Recent applications of highly sensitive molecular techniques (e.g. reverse transcription polymerase chain reactions) have perspective on whether normal respiratory tracts are really sterile, with traditionally 'non-culturable' bacterial communities found in the human lung. The microbiome may alter in COPD but data are highly inconsistent across the various studies published so far.[56-59] It seems likely that in COPD there is decreased bacterial diversity as disease severity increases.[58,60] The main change seems to be an increase in *Haemophilus*, which is much the same conclusion for traditional culture methods.

COPD patients are prone to exacerbations, which are characterised by worsening dyspnoea, cough, and sputum (increased purulence) production, requiring antibiotic treatment.[61] Traditional microbial culture techniques have demonstrated that approximately 50% of COPD exacerbations are associated with increased bacterial loads, mainly *S. pneumoniae, H. influenzae*, and *M. catarrhalis*.[62] *Pseudomonas aeruginosa* becomes prevalent only in advanced disease.[63] In addition, exacerbations may very well be elicited by acquisition of a new bacterial species or by the acquisition of a different bacterial strain of an established bacterial species.[64] The pathogen-directed host innate-immunity is really the effector of acute exacerbations of COPD, leading to further lung injury and progression of airflow obstruction.[65] Novel diagnostic techniques based on the viral genome have highlighted that viruses likely initiate 22-64% of COPD exacerbations.[66] Of particular interest is rhinovirus, which makes up to 50% of the total viral genome isolated, followed by influenza, para-influenza, RSV, and adenovirus.[67] In addition, up to 25% of exacerbations occur when patients encounter a co-infection involving both bacteria and viruses, and are thus associated with more severe disease.[68] It would seem that the initial respiratory viral infection alters the host immunity by significantly increasing the levels of inflammatory cytokines and also enhancing bacterial adherence/proliferation.[50,62] Thus, bacterial-viral interactions

are associated with increased airway inflammation, bacterial load and symptoms, and comparatively more reduced lung function.[69]

Apart from bacterial and viral infections in COPD, there has also been a focus on the possible presence of atypical bacteria including *Mycoplasma pneumoniae*, *Chlamydia pneumonia*, and *Legionella pneumophila*.[70-73] Although convincing evidence is sparse, chronic colonisation with *C. pneumoniae* may be associated with more frequent exacerbations as well as amplified inflammatory responses in the airways of COPD patients, especially ciliostasis that promotes infection with other respiratory infections.[71]

## ADHESION MECHANISM: A NOVEL RESEARCH AREA

An important question to address is why only a limited number of pathogens (mostly only three bacteria) actually gain access to the airways. Epithelial adhesion is a prerequisite for colonisation of mucous membranes, and one possible mechanism employed by 'the' major respiratory pathogens, *H. influenzae* and *S. pneumonia*, is adaptively enhanced airway epithelial adherence by physico-chemical interaction of phosphorylcholine on the bacterial cell wall surface with platelet activating factor receptors (PAFr) on the airway epithelial cells.[74]

Another major 'receptor' for pathogens is intercellular adhesion molecule-1, which is one of the important receptors for attachment and invasion of lung epithelia in >90% of Rhinovirus serotypes.[75,76] Some reports also suggest relevance for another receptor family, namely Toll-like receptors (TLRs), largely found on innate immune cells and structural cells. In this case, reduced TLR expression in COPD on airway inflammatory cells can lead to constrained and inadequate pathogen identification and clearance, facilitating bacterial colonisation, viral invasion, and an increased risk of exacerbations.[77] Several less investigated pathogen-sensing receptors include RIG-I-(retinoic acid-inducible gene 1)-like receptors (RLRs) and NOD (nucleotide-binding oligomerisation domain)-like receptors (NLRs).[78] RLRs are believed to be anti-viral, whereas NLRs are known to interact with bacteria. It may be that upregulation of such cell surface adhesion molecules and/or downregulation of innate immune pathogen-sensor receptors is key to pathogenesis of chronic colonisation and/or acute and chronic infection by 'pathogens' in COPD. This is a relatively new area of research in airway microbiology of COPD, but we have shown that PAFr is upregulated by cigarette smoke acting on epithelial cells, and expression of PAFr is especially marked in COPD.[74,79]

## CONCLUSIONS

COPD is a disease of enormous international importance. Unfortunately, the international research effort into COPD has been disproportionately weak compared to its social importance, and is the least researched of all common chronic conditions. Tobacco smoking is the major aetiological factor for COPD in developed countries, but the clinical outcomes are poorly understood. To some extent, inflammation has been broadly studied in COPD but there are studies reporting contradictory results, which warrant further studies. The prime pathology associated with COPD involves destructive airway remodelling including obliteration of small airways. These individuals are also especially at risk of lung cancer, with approximately 70% of lung cancer occurring in this group. We believe that recent work on EMT in COPD may lead to a radical rethink of the airway pathology of COPD and its linkage to physiological dysfunction, destructive airway remodelling including obliteration of small airways, and also to lung cancer development; teasing out these mechanisms may have therapeutic implications. This will also lead to better understanding of the ECM changes in the airway wall. Current evidence suggests the role of chronic infection in the pathogenesis of COPD (both stable and exacerbations) in a considerable subset of patients but the underlying mechanisms, which increase the susceptibility to infections, are far from clear. As this research field advances in the future, we anticipate a better understanding of respiratory host-pathogen relationship; understanding of its detailed pathogenesis is needed to design better translational treatments and management strategies.

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