Inflammation in chronic obstructive pulmonary disease: its assessment and the effects of corticosteroids
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Chapter 1

Introduction
Chronic Obstructive Pulmonary Disease (COPD) is a major cause of morbidity and mortality, affecting approximately 10% of the adult world population. In the most recent version of the Global Initiative for COPD (GOLD) working group, COPD has been defined as:

“Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease with some significant extra-pulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases.”

Due to unlimited cigarette smoking (the major cause of COPD) in the past decades and the long-lasting effects of cigarette smoking, COPD is expected to become the third cause of death world-wide in the next decades and thus a major and increasing economic burden for society, irrespective of the success of attempts to limit exposure to cigarette smoke. Understanding the pathogenesis of COPD in terms of the (causative) links between cigarette smoke exposure, airway inflammation and the development of chronic airflow obstruction should eventually lead to new strategies for the treatment and prevention of COPD.

COPD comprises a heterogeneous group of respiratory conditions related to abnormalities in the large and small airways and to alveolar destruction. These abnormalities can be defined in structural terms (e.g. emphysema), pathological terms (bronchiolitis), clinical terms (chronic bronchitis) or functional terms (irreversible airflow limitation, gas exchange abnormalities and rapid decline in lung function). Clinically, COPD is characterized by slowly progressive airflow limitation, resulting in symptoms of dyspnea and limited exercise tolerance, with coughing and production of sputum and by the incidence of disease exacerbations, partly due to infections. A decreased value of one lung function parameter: the ratio of Forced Expiratory Volume in the first second (FEV₁) over Forced Vital Capacity (FVC) below the value of 0.70, has been used to define COPD in functional terms, while the FEV₁ itself (as % of the predicted value) is used to characterize severity. With progression of the disease, there is a gradual loss of quality of life, which is related to the severity of the disease, and is associated with the incidence of exacerbations. These exacerbations itself have a negative influence on the patient’s quality of life, are a likely contributor to the enhanced decline in lung function, and carry a major risk for hospitalizations and mortality, both on the short term and the long term.

The major cause of COPD is the smoking of cigarettes, be it that approximately in only 20% of all adult smokers a clinically significant COPD develops. With increasing age and with increasing smoke exposure, the incidence of COPD in smokers, based on symptoms but predominantly on lung function criteria, can increase up to 50%. Mild COPD does not always inevitably evolve into moderate or severe disease in the long
Smoking cessation benefits patients with existing COPD for multiple reasons, though reducing the excess mortality, hospital admissions and the excessive decline in lung function to some extent only. Factors have been identified that determine the risk of development of the disease and progression of the disease. Besides cigarette smoking, indoor and outdoor air pollution and occupational exposure can contribute to the induction of COPD and to maintenance of the disease.

Since most smokers do not develop COPD, attempts have been made to identify risk factors for the development and progression of COPD and hereby the susceptible minority. This has been approached by searching for specific clinical, dietary, genetic or inflammatory risk factors for the development of, or progression of COPD. To date this search has not been successful. The extensive search for genetic susceptibility has resulted in only one firmly established genetic risk factor: α-1 anti-trypsin deficiency. However, this deficiency, which leads to a disturbed protease-antiprotease balance and likely hereby to tissue destruction and emphysema, is present only in a very small proportion (approximately 2%) of patients with COPD. Polymorphisms in other genes, like those encoding for other potentially relevant enzymes, such as glutathion S-transferase P1 (an enzyme involved in detoxifying cigarette smoke constituents), superoxide dismutase (involved in the oxidant / antioxidant balance), microsomal epoxide hydrolase (involved in detoxifying cigarette smoke epoxides), metalloproteinases (involved in enhanced lung tissue breakdown), glutamate-cysteine ligase (involved in the oxidant / antioxidant balance) and for inflammatory and immune parameters such as the vitamin D-binding protein, tumor necrosis factor (TNF)-α, interleukins (IL) such as IL–1, IL-4 and IL-13 have all been suggested to be linked to the occurrence of COPD, to its severity, or to a poorer prognosis. So far, however, none of these genes have been established firmly as predisposing factors. The limited prognostic value of these separate genetic factors indicate that most likely multiple genes, in conjunction with environmental factors ultimately determine the clinical expression of the disease.

In an attempt to explain the differences in response to cigarette smoke exposure and the ongoing inflammation after quitting smoking, an autoimmune component within COPD has been suggested. One supportive finding hereof is the recent observation of circulating antibodies against (fractions of) bronchial epithelial cells, which were found more often in plasma of patients with COPD than in control subjects.

Besides the slowly progressive airflow limitation, accompanied by an increase in symptoms, patients with COPD experience exacerbations of the disease with temporarily increased dyspnea and sputum production. There is evidence to support a precipitating role of infections with both viral and bacterial pathogens in many exacerbations of
COPD, but whether and how these infections also have a disease modifying effect in COPD on the long run is far from clear. Besides a potential role for micro-organisms in exacerbations, the presence of respiratory syncytial virus and of hepatitis C has been proposed to be linked to faster lung function decline.\textsuperscript{37,38} A latent adenovirus infection of airway epithelial cells has been suggested to play a role in maintaining a status of enhanced inflammation,\textsuperscript{39,40} and (in an animal model) caused corticosteroids to lose their anti-inflammatory effect.\textsuperscript{41} Additionally, in sputum of patients with COPD, compared to healthy subjects, more often Epstein Barr virus DNA was detectable.\textsuperscript{42} Furthermore, pathogens like \textit{Chlamydia} and \textit{Pneumocystis} species have been reported to be associated with the chronicity of COPD.\textsuperscript{43,44}

Patients with COPD may become persistently colonized with bacteria like \textit{Haemophilus influenzae} \textsuperscript{45-47} and persisting strains may induce a different inflammatory response than non-persisting strains.\textsuperscript{48,49} The presence of lower airway bacterial colonization does not prove their direct role in the pathogenesis of COPD and its exacerbations; patients with COPD may be simply more prone to experience exacerbations due to the facilitating role of inefficient mucociliary clearance, caused by cigarette smoking. On the other hand, chronic infections may also be representative of an impaired defense mechanism which is not able to eradicate the microbes,\textsuperscript{39,46} and a vicious circle is present of infections, leading to epithelial damage, which itself facilitates subsequent infections. Taken together, these data suggest that concomitant infections with viruses and/or bacteria may play an important role in the development of COPD.

**Assessment of inflammation in COPD**

In the early 1970’s it was shown that peripheral airways in young smokers showed signs of inflammation, mainly of neutrophilic origin.\textsuperscript{50} As a result of increased research activities, the knowledge of the inflammatory processes in the airways of patients with COPD has increased considerably over the past decades. However, knowledge of the variability in inflammation during clinically stable conditions is still scarce, which is probably related to the lack of standardization of the different methods to investigate airway inflammation. No single inflammatory parameter has been identified to date which strongly relates to the severity of COPD or predicts the success of (anti-inflammatory) treatments.

To assess airway inflammation, several sampling techniques have been developed, providing information on different areas or compartments within the lungs. Flexible bronchoscopy enables direct access to the larger airways, and this method can be used to perform bronchial lavage and broncho-alveolar lavage allowing harvesting of cells and soluble mediators, present in or on the surface of the airway mucosa and epithelial lining fluid.\textsuperscript{51,52} Epithelial lining fluid can also be obtained directly via fluid absorbing probes. In addition, brushed material and biopsies can be taken from the mucosa and epithelium in (mainly) the larger airways.\textsuperscript{53} Samples from lung parenchyma and of
peripheral airways can be obtained during surgical procedures in patients (irrespective of concomitant COPD), who developed localized malignancies and in patients with severe COPD undergoing lung volume reduction surgery.\textsuperscript{54,55} The surgical method can provide larger sized biopsies allowing in depth immunohistochemical staining, but select patients with predominantly either mild to moderate or very severe disease. These methods are all invasive techniques potentially causing considerable distress of patients, and therefore there has been an urge to develop less invasive methods.

Many patients with COPD spontaneously produce sputum, especially during exacerbations, analysis hereof yields information on the presence of cells and mediators from the small and large airways.\textsuperscript{56} Inducing sputum by exposure to nebulized (hypertonic) saline provides material also from patients who do not produce sputum spontaneously. Standardization of sampling and processing of spontaneous and particularly induced sputum has been a further and major step forward in studying inflammatory processes in the airways in COPD.\textsuperscript{57,58} Repeatability of assessing inflammatory parameters in induced sputum (mainly while analyzing selected sputum plugs) has been documented to some extent in patients with asthma, COPD and in healthy subjects,\textsuperscript{57,59-63} but information on the intra-patient and inter-patient variability and the (short-term and long-term) repeatability of inflammatory parameters in whole sputum samples in patients with COPD is lacking. Some methodological aspects of analyzing induced sputum samples have not been fully elucidated either, such as the (potential) differences in applying the “whole sputum sample” methodology versus the “selected sputum plug” methodology, validation of the criteria of declaring a sputum sample as “valid” or “invalid” based on a certain % of squamous cell contamination and the magnitude of the potential contamination of whole sputum samples with non-sputum fluids. The two different approaches used to analyze induced sputum samples: the whole sputum sample or selecting plugs from the expectorate, can have effects on the analysis of sputum constituents.\textsuperscript{64} The selected plugs method discards the soluble fraction of sputum while the whole sample methodology carries the risk of admixture and thus contamination with other fluids such as upper airway secretions or saliva. This potential contamination is usually investigated via squamous epithelial cell counts in the sputum sample; a fixed cut-off value of either 20\%, 50\% and most often 80\% is used to designate a sputum sample as being “valid” or not.\textsuperscript{65-68} It can be envisaged that such contamination with other fluids will lead to dilution of the sputum sample and thus to lower levels of soluble inflammatory parameters and the number of inflammatory cells, but the extent of this dilution has not been quantified in detail.

A relatively new, and therefore not yet explored extensively, non-invasive approach is the analysis of exhaled air.\textsuperscript{69} In exhaled air and in exhaled breath condensate many compounds can be detected which may be produced in the airways and which may reflect ongoing inflammation, including volatile compounds like nitric oxide (NO), reactive oxygen species such as hydrogen peroxide, and larger molecules like hydrocarbons and proteins.\textsuperscript{69,70}
Using these different techniques samples from different parts of the lung parenchyma and airways can be obtained, providing supplementary, though potentially different information on the inflammatory processes. For example, biopsies of the airway mucosa taken during bronchoscopy are taken from the large airways, sputum is originating from both large and smaller airways, bronchoalveolar lavage will provide information on ongoing inflammatory processes in the alveoli and the smallest airways and exhaled breath condensate reflects inflammation from alveoli to larger airways.

Despite the obvious limitations of sampling only a single or a limited number of compartments, it may provide specific information about the different clinical manifestations of COPD. So, increased secretory activity of the bronchial glands present in the larger airways is likely associated with symptoms of chronic bronchitis, abnormalities in the peripheral airways underlie the chronic obstructive bronchitis associated with expiratory flow limitation, and alveolar abnormalities and destruction of the extracellular matrix represent the emphysematous component of COPD. It is of utmost importance to realize that data on inflammatory parameters in one compartment at one moment in time, provides only limited and temporal information on what is most likely a dynamic system of trafficking of inflammatory cells between the different compartments, driven by various mediators. For instance, the striking differences in neutrophil counts between samples from closely related pulmonary compartments (sputum versus bronchoalveolar lavage and biopsies) suggests differential trafficking of these cells from the vasculature through the connective tissue and the mucosa towards the airway lumen. Also, lymphocytes obtained from lung tissue have a more differentiated phenotype than those isolated from peripheral blood.

Although COPD is primarily a disease of the airways, other organs can be affected as well. Systemic manifestations can be present, in its most prominent form present as progressive weight loss and muscle wasting, having an independent association with increased mortality. Such observations can form the basis for a concept in which the airways and the systemic circulation represent different but connected “compartments“ of the inflammatory processes underlying the clinical signs and symptoms of COPD. Support for this view can be found in direct and indirect signs of inflammation in the peripheral circulation, such as high levels of acute phase proteins, cytokines and lipid peroxides, but also in an enhanced ex vivo inflammatory activity of leukocytes from patients with COPD.

Characteristics of inflammation in COPD

Though smoking a single cigarette has only limited detrimental effects, chronic cigarette smoking leads to a non-specific inflammatory infiltrate in the airways and the lung parenchyma, consisting of neutrophils, macrophages and T-lymphocytes, which will be described below in more detail. In persons who smoke and who have developed
COPD these inflammatory changes are more prominent; moreover, inflammation does not subside rapidly after stopping smoking. The precise sequence of events in an individual smoker that offsets the enhanced inflammation and leads to the development of irreversible chronic airway obstruction is not clear.

The inflammation in the airways in COPD has not an uniform character. Some elements contributing to the inflammatory process are several cells (neutrophils, eosinophils, T-lymphocytes and structural cells such as epithelial cells), cytokines, chemotactic agents, proteolytic enzymes (neutrophil elastase, granzymes, matrix metalloproteinases) and reactive oxygen species. Support for the contribution of these elements can be found in the observed associations between disease severity or other disease characteristics and the characteristics of the airway inflammation, like the number or the activation state of inflammatory cells and the presence and concentration of soluble mediators. So the presence of neutrophils in higher quantities or in a more activated state, higher numbers of eosinophils, and lymphocytes in sputum, and the protein leak from blood into sputum were all found to correlate with the degree of airflow limitation. In addition, a high neutrophil content in sputum was associated with a more rapid decline in lung function in previous years. Also, a high eosinophil count in sputum was in some studies associated with a clinical response to corticosteroid treatment. One prospective study linking the airway inflammation with long-term changes in lung function showed that neutrophilic inflammation at baseline, assessed with levels of myeloperoxidase (MPO), leukotriene B4 (LTB4) and interleukin 8 (IL-8) in sputum were related to some extent with the subsequent long term decline in large airway function and (especially) in smaller airways function.

The inflammatory process is dynamic, both in time and with respect to an exchange between the different compartments. Inflammatory cells migrate from the peripheral circulation into the airway mucosa and from there into the airway lumen, attracted by the chemotactic factors, with their transport facilitated by endothelial adhesion molecules. The characteristics of the inflammation changes during exacerbations, both qualitatively and quantitatively from the state in the stable situation. The temporal, intermittent or permanent presence of infectious organisms, like Haemophilus influenzae modulates inflammatory reactions. Inflammatory cells that are in an active state in the lumen of the alveoli or on the surface of the smaller airways may achieve a change in their activation state when being transported to the larger airways by mucociliary clearance. Similarly, cells, present in blood or the airway mucosa, may loose or gain their activity after trafficking to the airway lumen.

The most extensively studied and probably most relevant inflammatory parameters in COPD are described in some more detail below.
**Neutrophils**

A contribution of neutrophilic granulocytes to the pathogenesis of COPD has been postulated decades ago because of their abundant presence in sputum and in airway biopsies, and their ability to release tissue destructive proteases. Neutrophils play an important role in the defense mechanism against microbes and are able to kill ingested organisms with a variety of enzymes that are released in the phagosome. Upon extracellular release these enzymes may harm the airway tissue and the extracellular matrix. Neutrophils may constitute up to 80% of all cells in sputum produced by COPD patients and this fraction is two-fold to four-fold higher than in sputum of asthmatic patients and of healthy smoking subjects. The proportion of neutrophils is however not that high in lavage fluid which is sampled from more peripheral airways and from the alveoli. Airway neutrophilia is likely caused and maintained by cytokines and chemokines like TNF-α and interleukin-8 (IL-8) and neutrophil chemotactic factors like leukotriene B_4 (LTB_4) produced by the epithelial cells and inflammatory cells. In bronchial biopsies of patients with COPD epithelial cells express increased amounts of the adhesion molecule ICAM-1 which is involved in neutrophil influx. The presence in induced sputum of markers of neutrophil activation such as myeloperoxidase (MPO), human neutrophil lipocalin and neutrophil elastase indicates an increased activation state of the neutrophils and extracellular release. Higher levels of MPO in sputum are associated with a more severely disturbed lung function. During exacerbations, TNFα, IL-8, and MPO are present in increased amounts in sputum and higher levels of IL-8 in sputum in stable disease were associated with an increased exacerbation frequency. Remarkably, neutrophils obtained from the systemic circulation of patients with stable COPD are also in an activated state.

**Eosinophils**

Historically, eosinophils have not been linked to COPD; in airway secretions of patients with COPD, eosinophils are usually present in low to very low numbers. Additionally, it has been suggested that, when present, eosinophils of COPD patients may be less activated than those of asthmatic patients. Still, compared to healthy subjects, an increased number of eosinophils was observed in the sub-epithelial airway tissue of patients with COPD. Moreover, elevated levels of eosinophil cationic protein (ECP) in sputum in stable COPD indicate that eosinophils are actually activated. In COPD patients, increased serum-IgE levels and/or blood eosinophil counts have been found to be associated with a favorable response to inhaled β_2-agonists and systemic steroids, but also with an unfavorable enhanced decline in lung function. These observations suggest that COPD patients with airway eosinophilia may represent a different phenotype of COPD. This airway eosinophilia in some patients may be of a temporal nature since airway eosinophilia increases during COPD exacerbations, both in airway secretions and in tissue biopsies. This points to a different, especially
eosinophilic, inflammation during exacerbations. Whether eosinophils are also present in higher numbers in the weeks prior to an exacerbation is not known yet. Likely, this supplementary eosinophilic inflammation during an exacerbation responds differently to (anti-inflammatory) treatments than the inflammation in stable disease. High eosinophil numbers in sputum in stable COPD may appear to be predictive for a beneficial clinical effect of corticosteroids. Collectively, these findings point to a subtype or specific phenotype of COPD, characterized by either a continuous or a temporal airway eosinophilia, which may be more responsive to corticosteroid treatment.

**Lymphocytes**

Lymphocytes may also be involved in COPD, especially CD8+ T-lymphocytes, as an increased presence of CD8+ T lymphocytes has been demonstrated in lung tissue of COPD patients and in smokers who have not (yet) developed COPD. In some studies, the number of CD8+ T-lymphocytes was found to be related to the degree of airflow limitation, but in one study the number of lymphocytes in the airway epithelium was shown to be more strongly related to the extent of cigarette smoke exposure. Cytotoxic CD8+ T lymphocytes play an important role in normal immunological defense, in particular with respect to the killing of virus-infected or otherwise altered or injured cells. It may be hypothesized that these cytotoxic effector cells are directed against the airway epithelium which is altered by noxious injuries such as cigarette smoke and/or active or latent viral infections. In addition, CD8+ T-lymphocytes may contribute to the initiation and perpetuation of neutrophilic airway inflammation because of their ability to secrete the neutrophil chemotactic cytokine IL-8. Proteolytic granzymes from cytotoxic T-lymphocytes may be introduced intracellularly, inducing apoptosis of airway epithelial cells. Granzymes, when released extracellularly, may act as chemotactic mediators, affecting the extracellular matrix in the lungs, and may induce neurotoxicity. However, only scarce information is available on the presence of granzyme containing inflammatory cells such as CD8+ T-lymphocytes in airway tissue, or of granzymes in airway fluids, such as in bronchoalveolar lavage fluid or sputum, and its relation to severity of COPD.

**Epithelial cells**

The airway epithelium is a physical barrier against foreign particles and organisms, but has also a role in directing and modulating inflammatory responses. Bronchial epithelial cells can react with the production of pro-inflammatory cytokines (a.o. leading to a neutrophilic influx) such as IL-6 or IL-8 in response to a number of stimuli, such as exposure to cigarette smoke, temperature gradients, osmolarity changes, TNF-α, granzyme A, Fas ligation and certain strains of bacterial airways pathogens like *Haemophilus influenzae*. Epithelial cells of patients with COPD, as compared to those of healthy controls, may be more sensitive in this respect. Corticosteroids may affect the production of some cytokines by epithelial cells. It has also been suggested that epithelial cells infected with...
adenovirus may induce or maintain the vicious circle of a high inflammatory state. In addition, damaged epithelial cells may also be a source for an adapted immune response, which could lead to continued inflammation, despite smoking cessation.

Proteases

The physical structure of the lung parenchyma is based upon both elastic and non-elastic fibers. The integrity of this structure is maintained in a delicate balance of degradation and synthesis. Disturbance of this balance may lead to a destruction of the alveolar walls and emphysema or to fibrosis. Important proteolytic enzymes are elastase, trypsin and a series of matrix metalloproteinases (MMP’s) which are present in higher quantities in lung tissue, sputum and lavage fluid of patients with COPD. The presence of MMP’s in the peripheral airways was correlated with the degree of airflow limitation (FEV₁) and with the radiologically assessed emphysema severity. Many proteases are counterbalanced by a number of specific antiproteases, like α₁-antitrypsin, secretory antileukoprotease and various tissue inhibitors of metalloproteases. Components of cigarette smoke can inactivate these antiproteases like α₁-antitrypsin, thereby disturbing this balance. Several MMP’s are present in high quantities in lung tissue of patients with COPD, and alveolar macrophages of COPD patients are more prone to release MMP’s. MMP-12 levels in sputum of patients with COPD are higher than those in sputum healthy smokers and healthy non-smokers, importantly, MMP-12 levels were also high in COPD patients who were ex-smokers. Macrophages immunostaining for MMP-12 were more abundant in induced sputum and in BAL of patients with COPD than in healthy smokers and a relationship between the exposure to cigarette smoke (as packyears) and the proportion of MMP+ macrophages has been reported. Degradation products of elastin were found in higher quantities in the urine of patients with COPD, especially during exacerbations. Potentially harmful enzymes released by neutrophils, like neutrophil elastase are present in sputum of COPD patients in larger quantities than in healthy subjects. The presence in the airway lumen of enzymes such as granzymes which are involved in the process of cell apoptosis may also be linked to enhanced tissue breakdown and loss of lung function. Interestingly, an increased secretion of anti-proteases has been reported during systemic corticosteroid therapy. Inhibition of neutrophil elastase by a specific inhibitor was found to decrease smoke-induced emphysema in a guinea pig model and to decrease inhaled elastase-induced damage in a rat model of COPD. The release of MMP’s from alveolar macrophages in vitro was found to be reduced by the corticosteroid dexamethasone.

Oxidative stress

Inflammatory cells such as neutrophils produce reactive oxygen species to kill bacteria. In addition, cigarette smoke contains high quantities of oxidants, like oxygen radicals. In plasma, lung lavage fluid, exhaled air and urine of patients with COPD, reactive oxygen species and other products of oxidative stress have been identified, including isoprostane
F$_2$O–III and lipid peroxides. It has been suggested that reduced glutathione, an endogeneous antioxidant, important as protective agent against oxygen radicals and other oxidants, is reduced or even becomes depleted in patients with several lung diseases. Upon stimulation, peripheral blood neutrophils and alveolar macrophages of patients with COPD were shown to be able to produce more superoxide anions than cells from healthy (smoking) subjects. During infections, alveolar macrophages may additionally produce such reactive oxygen species locally, leading to further tissue damage. An intriguing finding in this respect is the detection of increased amounts of hydrogen peroxide in the condensate of exhaled air of COPD patients, especially during exacerbations in patients with unstable COPD. On this basis, detection of oxidant markers in exhaled air has been advocated as a non-invasive method to monitor inflammatory reactions in the airways.

**Effects of treatment on inflammation in COPD**

Although clinical effects of the different treatments in COPD have been studied in great detail and on a large scale during long-term treatment, only a limited series and mainly small scale studies have been performed, investigating the effects of treatments on airway inflammation. Most of the latter studies were performed with systemic and inhaled corticosteroids.

Studies with inhaled glucocorticosteroids have shown inconsistent results, and when some markers were influenced in one study, other studies were not able to reproduce these findings. This may be the consequence of studying small patient numbers, heterogeneity of patient material and lack of knowledge on variability and repeatability of the assessment of inflammatory parameters, leading to underpowered studies. Short term treatment with systemic glucocorticosteroids reduced sputum eosinophilia and ECP levels in patients with COPD. In some studies, also a reduction in sputum neutrophilia, as well as a decrease in sputum eosinophilia was observed. In a meta-analysis of a series of small-scale studies an overall effect (reduction) on both sputum neutrophilia and eosinophilia was concluded. Inhaled corticosteroid treatment was also shown to modestly reduce the content of hydrogen peroxide in exhaled breath condensate. In bronchial biopsy studies a slight reduction in the CD8+/CD4+ ratio as well as a decrease in mucosal mast cells have been observed. Decreases have been found in circulating and sputum IL-8 levels, MPO levels in sputum and plasma protein leakage. Also, modest beneficial effects were shown on the protease – antiprotease imbalance. Interestingly, corticosteroids may also be capable of reducing angiogenesis. Corticosteroids were shown to reduce neutrophil chemotactic activity when cells were incubated in vitro and to affect inhaled ozone-induced neutrophilic inflammation in patients with asthma. Despite an inhibition of neutrophil apoptosis, glucocorticosteroids were shown not to influence IL-8 production by neutrophils in vitro. 
In studies, investigating bronchial biopsies, inhaled corticosteroid treatment (alone or in combination with $\beta_2$-agonists) has been reported to affect the number of lymphocytes in the airway epithelium.\textsuperscript{147;152;165;166}

Systemic inflammation, visualized by raised levels of C-reactive protein (CRP) was suppressed by inhaled corticosteroid treatment in one study,\textsuperscript{167} but was not affected in another study, where a closer link was observed between clinical improvement after inhaled corticosteroids (be it after short-term treatment) and changes in surfactant protein D.\textsuperscript{168}

During in vitro experiments, epithelial cells were shown to respond to glucocorticosteroids with a decreased production of IL-8 and GM-CSF.\textsuperscript{169} Additionally, glucocorticosteroids have been suggested to protect cultured epithelial cells against elastase-induced damage and against viral infections.\textsuperscript{170;171} Apoptosis of epithelial cells was reduced by glucocorticosteroids at low concentrations in one study, but was induced at high concentrations in another study.\textsuperscript{172;173}

Only a very limited number of studies has been devoted to the anti-inflammatory effects of non-steroid drugs in COPD. N-Acetyl-Cysteine (NAC, also used as a mucolytic agent in COPD) has some antioxidant properties,\textsuperscript{140} and one study indicated a decrease in exhaled hydrogen peroxide after long-term NAC treatment.\textsuperscript{174} Inhaled tiotropium was shown to have no significant effect on inflammatory parameters, assessed in induced sputum, despite significantly reducing exacerbation frequency.\textsuperscript{175} Theophylline induced a small reduction in sputum neutrophil count, in IL-8, in MPO levels and in the amount of reactive nitrogen species in sputum of patients with COPD.\textsuperscript{153;176} The specific phosphodiesterase inhibitor cilomilast reduced the presence of CD8+ T-lymphocytes in the airway epithelium.\textsuperscript{177} A second phosphodiesterase inhibitor, roflumilast, reduced absolute cell numbers and levels of inflammatory mediators, however without affecting relative cell counts.\textsuperscript{178} Supplementation of $\alpha_1$-antitrypsin in a small scale study has lead to a reduction in elastase activity and also to a reduction in LTB$_4$ levels in sputum and a trend towards a reduction in MPO and IL-8 levels.\textsuperscript{179} Treatment with macrolide antibiotics was suggested to have an additional, non-antibacterial effect, supplementary to reducing the bacterial load. One study showed oral clarithromycin to improve quality of life in patients with severe COPD,\textsuperscript{180} and one study indicated azithromycin to improve macrophage phagocytic function.\textsuperscript{181} These antibiotics were reported to reduce the activity of MMP’s.\textsuperscript{182;183}

Effects of treatment on COPD, clinical effects

Long term management of COPD is aimed at multiple targets.\textsuperscript{184} Besides treating the disease manifestations in the airways, smoking cessation should be assisted by extensive non-pharmacological and pharmacological treatments.\textsuperscript{185;186} The reduction in exposure to cigarette smoke has been shown to limit the excess decline in lung function and
the inflammatory processes to some extent. Additionally, a physical rehabilitation
program using anabolic steroids may induce an improvement in the physical condition
and a reduction of skeletal muscle wasting, one of the non-pulmonary features of severe
COPD.

Symptomatic treatment of dyspnea in COPD is achieved by maximal bronchodilator
treatment such as with short-acting and long-acting $\beta_2$-adrenergic and anticholinergic
agents. Especially long-acting bronchodilators have proven to be very effective, and
concomitantly improve quality of life. These effects were observed in patients with
and without short-term reversibility in airflow obstruction.

Additional aims in the treatment of COPD include the suppression of inflammation and
the prevention and treatment of exacerbations (see below). Treatment of COPD via
suppression of the underlying inflammation with corticosteroids was attempted first with
systemic corticosteroids, aimed to influence lung function or symptoms on the short
term. The response rate, however, was small and quite heterogeneous and a positive
response to treatment, defined as a certain improvement in lung function, was observed
in 10% to 50% of COPD patients. The large differences between the different studies on
clinical parameters may be related to differences in patient selection such as the cigarette
smoke exposure, age, baseline lung function and reversibility in airflow obstruction. The
choice of FEV$_1$ as primary outcome parameter in the long term follow-up studies may
have restricted the potential for demonstrating beneficial effects, since in COPD lung
function parameters, specific for small airways, are generally relatively more disturbed
than parameters for large airways function, and thus may also be more susceptible to
detect changes. However, FEV$_1$ is regarded as the golden standard in assessing
severity of disease and the annual decline in FEV$_1$ is regarded as the optimal parameter to
describe the course of disease.

Initially, small-scale short-term studies investigated effects of inhaled corticosteroids
on symptoms and on lung function parameters, later studies assessed airway
hyperresponsiveness as well. These studies showed some positive effects on symptoms
and lung function, however, the effects were limited, and in retrospect these studies must
be regarded as being underpowered, since in the majority of studies the effects were
not statistically significant. A first retrospective study suggested that long-term
treatment with high doses of systemic corticosteroids attenuated the annual decline in
lung function. However, later studies indicated that continuous use of systemic steroids
may be associated with a worse survival, especially in severe COPD. Studies, in which
inhaled treatment was given for many months, showed some effect on lung function and
on the incidence of exacerbations. Later, positive effects on lung function or
lung function decline and on the incidence of exacerbations were documented in larger-
scaled studies lasting 2 to 3 years of treatment. At starting inhaled corticosteroid
treatment in patients with COPD, in general, a modest improvement in lung function was observed after the first 6 months, followed by a small reduction of the annual decline in lung function.\textsuperscript{202-205}

Several meta-analyses discussed, rejected, confirmed and substantiated these long-term effects of inhaled corticosteroids, with or without additional long-acting bronchodilator treatment.\textsuperscript{206-210} In these meta-analyses the extra decline in lung function in patients with COPD, compared to healthy subjects is reduced by approximately 10 ml/year, which is equivalent with 30\% of the excess decline, observed in patients with COPD. The most recent analysis of a large-scale study indicated a limitation of the decline in \textit{FEV}_1 with 13 and 16 ml/year respectively over 3 years of treatment with respectively fluticasone propionate and the combination of fluticasone propionate with salmeterol.\textsuperscript{205} These effects, observed in these large scale studies and in meta-analyses are valid for groups of patients fulfilling the usually strict inclusion criteria of these studies such as \textit{FEV}_1 values below 50\% or 60\% of predicted. Unfortunately, to date, the individuals or specific sub-populations having the worst prognosis have not been identified, other than those with a high exacerbation frequency over the last years, which could predict further exacerbations, and those with a low lung function, which was related to further decline in lung function. In particular, these studies have not identified individuals who are most likely to benefit clinically of the treatment. Partially, strict inclusion criteria, such as a lung function below a certain level and absent reversibility of airflow obstruction in these large scale studies, prevent extrapolation of study results to patients with e.g. better lung function or partial reversibility. This selection bias complicates interpretation of the data and the institution of treatment guidelines, since only a minority of patients in clinical practice fulfils the inclusion criteria applied in these landmark studies.\textsuperscript{211}

Expecting that inhaled corticosteroids could be effective in a subgroup of patients, though with unknown characteristics, effects of systemic corticosteroid treatment on lung function parameters have been evaluated prior to commencing long-term inhaled corticosteroid treatment. This “test treatment” was given in an attempt to select potential “responders” to long-term inhaled corticosteroid treatment. To date none of these studies had a positive outcome.\textsuperscript{194,212} Therefore, it might be fruitful to further analyze the above mentioned large scale studies with respect to clinical characteristics of those patient sub-populations, benefiting least or most of long-term treatment with inhaled corticosteroids. Also, other ways of identifying potential responders may be undertaken, such as characterizing patients, based on their individual inflammatory profile.

Whether prescribing inhaled corticosteroids to those COPD patients, who were hospitalized for an exacerbation of COPD, will reduce further hospitalizations and mortality requires further investigation. Though initial analyses pointed to both a reduction in mortality and morbidity by starting inhaled corticosteroid therapy after hospitalizations, later analyses
of the same data showed a smaller positive effect of such therapy.\textsuperscript{213,214} In a subsequent meta-analysis of all available prospective long-term study data, it was concluded that the reduction in mortality was statistically significant and amounted to 25\% over the observation period.\textsuperscript{208}

Reports in which the effects of inhaled corticosteroids were studied in combination with long-acting bronchodilators confirmed the beneficial effects on lung function decline, exacerbation frequency and potentially mortality of corticosteroid treatment alone.\textsuperscript{204,205,215-217}

To date, investigations have offered only some clues as to why glucocorticosteroids with their impressive short-term and long-term anti-inflammatory effects and disease modifying effect in asthma have shown only such a limited short-term success in COPD. This relative ineffectiveness of glucocorticosteroids in COPD holds true both for the limited ability to suppress the ongoing inflammation as well as for the limited ability to significantly influence or normalize the clinical course of the disease.\textsuperscript{202,203} The observation, that in asthmatics glucocorticosteroids were less effective when the patient was a smoker,\textsuperscript{218,219} could be understood as one explanation for their ineffectiveness in (cigarette smoke related-) COPD,\textsuperscript{220} but could not explain the observation that inflammation and relative corticosteroid unresponsiveness persist in COPD after smoking cessation. Additionally, in asthmatics, smoking cessation only partially reversed the relative insensitivity to corticosteroids.\textsuperscript{221} One hypothesis underlying the relative ineffectiveness of corticosteroids in smokers is based on irreversible inactivation of histone deacetylase in smokers (and COPD patients with increased oxidative stress), which leads to decreased transcription of specific corticosteroid-specific mRNA and thereby to reduced synthesis of anti-inflammatory proteins.\textsuperscript{220}

Treatment with inhaled corticosteroids may be relatively ineffective in COPD, partly because of the dose being sub-optimal. Early studies suggested some dose-response relationship for inhaled corticosteroids in COPD,\textsuperscript{206} and patients with moderate to severe airflow obstruction might be unable to inhale optimally through certain inhalers, resulting in low lung deposition, requiring an increase in nominal dose for an optimal effect.\textsuperscript{222-224} In contrast, other studies showed that irrespective of limitations in expiratory lung function, patients with severe COPD were not so much restricted in inspiratory lung function as they were in expiratory lung function, and were able to benefit from inhaled treatment.\textsuperscript{225,226} In patients with asthma, lung deposition of inhaled corticosteroids from certain inhalers was indeed affected when expiratory lung function was affected.\textsuperscript{223,227} Further studies are required to establish whether the doses of inhaled corticosteroids which have been used in most studies are high enough to reach adequate tissue levels of the corticosteroids.
An alternative explanation for the low efficacy of inhaled corticosteroids was sought in the suggested presence of the inactive β subtype of the glucocorticosteroid receptor.\textsuperscript{228,229} When present, this inactive β subtype will bind the corticosteroid and will form an inactive glucocorticosteroid-receptor complex. As a consequence, the active α subtype can not bind the corticosteroid and clinical effects may be reduced.

To date, little effort has been made to select or adapt treatment in COPD based on the patient’s individual inflammatory profile. However, one study showed that adjusting (corticosteroid) treatment using sputum eosinophil counts was successful in lowering the incidence of exacerbations.\textsuperscript{230}

Anti-inflammatory effects of other COPD treatments have been studied to a limited extent. Long-acting bronchodilators, such as the long-acting β\textsubscript{2}-agonists salmeterol and formoterol (especially in the combination with inhaled corticosteroids) and the long-acting anticholinergic agent tiotropium are extremely beneficial in relieving airflow limitation and also have been reported to have some beneficial effect on the incidence of exacerbations.\textsuperscript{175,191,204,215-217,231} Only limited data on the anti-inflammatory effects of bronchodilators is available. Rather, the effects of β\textsubscript{2}-agonists seem to be indirect by enhancing the effects of corticosteroids intracellularly.\textsuperscript{232} Treatment with oral N-acetyl cysteine was shown to reduce exhaled hydrogen peroxide,\textsuperscript{174} possibly due to the antioxidant properties of N-acetyl cysteine.\textsuperscript{233}

Concerning other and newer pharmacological agents, one study has shown a bronchodilating effect of a single dose of the oral leukotriene receptor antagonist zafirlukast in patients with COPD,\textsuperscript{234} and short term studies showed an effect on lung function of the phosphodiesterase inhibitor cilomilast.\textsuperscript{177,235} A 6 months study with cilomilast showed a modest 40 ml difference in FEV\textsubscript{1} but more importantly a clinically relevant difference in quality of life and in exacerbation frequency.\textsuperscript{236} Other specific agents in development for the treatment of COPD are 5-lipoxygenase inhibitors, leukotriene (LTB\textsubscript{4}) antagonists,\textsuperscript{237} newer mucolytics,\textsuperscript{238} other phosphodiesterase inhibitors,\textsuperscript{239} neutrophil elastase inhibitors\textsuperscript{240} and MMP inhibitors.\textsuperscript{241} Extensive clinical studies with a combined dopamine-2 agonist/ β\textsubscript{2}-agonist aiming at additional suppression of dyspnea and of inflammation did not result in a supplementary clinically relevant improvement, beyond bronchodilation, in patients with COPD.\textsuperscript{242,243} New and specific agents directed towards TNF\textsubscript{α}, like infliximab and etanercept which are successfully used in patients with other inflammatory diseases, like rheumatoid arthritis and Crohn’s disease were also tested in patients with COPD, despite their potential of facilitating pulmonary infections.\textsuperscript{244,245} The first two studies did not show a relevant clinical effect of infliximab in patients with COPD.\textsuperscript{246,247} In addition, stimulating alveolar repair with retinoids did not result in a clinically meaningful improvement in lung function parameters in patients with emphysema.\textsuperscript{248}
Treatment with statins for other and usually concomitant smoking-related diseases such as hypertension and hypercholesterolemia (which probably resulted in a reduced systemic inflammation) and treatment with inhaled corticosteroids both reduced mortality of COPD patients following hospitalization for an exacerbation of COPD, these effects were additive. 

The limited clinical effect of pharmacological treatments of COPD on the long term has intensified attempts to elucidate the complex inflammatory reactions. This should ultimately lead to individually targeted treatments and novel therapies, aimed at influencing the most crucial steps in the inflammatory cascade. At present, the selection of patients who would benefit most of corticosteroid or any other treatment is at best described as “trial and error”, in the current guidelines on the treatment of COPD, corticosteroids are advised for patients with severe to very severe COPD based on the rather arbitrary value for FEV$_1$ of 50% of predicted or the frequency of prior exacerbations, but not on individual inflammatory characteristics. An in depth search for parameters, which can predict responders to (corticosteroid) treatment, is therefore needed. Knowing that the inflammation in COPD is very heterogeneous within and between patients, there would be many ways to influence inflammation and thus there is hope that in the future specific agents can be developed which significantly alter the long-term prognosis of COPD.

Exacerbations of COPD and their treatment

An exacerbation of COPD may be elicited by different causes: a bacterial or viral infection, air pollution or other and unknown causes. They are characterized by increased symptoms such as sputum production, sputum purulence and dyspnea, and usually they require intensification of medical treatment, such as with corticosteroids and antibiotics. In severe cases, exacerbations may require hospitalization. Of importance, hospitalizations for COPD exacerbations are associated with a high mortality in the year thereafter. Exacerbations may be paralleled with a change in the airway inflammation. During exacerbations, inflammatory reactions in the airways are more severe and have a different characteristic, predominantly eosinophilic, and there are signs of an increased systemic inflammation, which may take weeks or months to normalize. Differences between patients in systemic inflammation in the stable phase may predict the occurrence or frequency of exacerbations on the long-term, but studies in this field have yielded conflicting results, probably due to the multiplicity of parameters investigated and due to differences in selection of patients.

Treatment of COPD exacerbations itself consist of bronchodilators, corticosteroids and, when indicated, antibiotics. Corticosteroids are routinely given intravenously or orally and are very effective. This efficacy may be due to the different characteristics of the inflammation during exacerbations as compared to stable disease, as described above. A
recent study suggest that treatment with a high dosed inhaled budesonide/formoterol combination was equally effective in reducing airway eosinophilia and improving lung function as treatment with an oral corticosteroid.259

Prevention of exacerbations is of great importance since every exacerbation carries the risk of severe complications, hospitalizations and mortality, and attributes to the decreased quality of life in patients with COPD.7 With an incidence of severe exacerbations of approximately once per year and with large differences in exacerbation frequency among patients, identification a priori of patients most at risk is difficult. Therefore, usually large-scale and long-term clinical studies are required when investigating (effects of interventions in) exacerbations of COPD. Long-term treatment with inhaled corticosteroids, with or without additional long-acting bronchodilator treatment, has shown in most studies to reduce the incidence of exacerbations by up to 30% and to decrease the severity of exacerbations.204;215;217 Additionally, withdrawal from inhaled corticosteroid treatment may trigger an exacerbation or may reveal patients who were benefiting of inhaled corticosteroid treatment, this complicates the interpretation of studies with corticosteroid-free periods.260;261 Treatment with the long-acting anticholinergic agent tiotropium has also been shown to reduce the incidence of exacerbations,175;231 an effect suggested to be obtained by improved airway mechanics such as decreased hyperinflation,262 since it was also observed after lung volume reduction surgery in patients with severe COPD.263

Adjusting corticosteroid treatment towards normalizing sputum eosinophilia was recently shown to significantly reduce the incidence of severe COPD exacerbations.230

In contrast to earlier and small-scale studies, the most recent long-term and large-scale study with oral N-acetyl-cysteine did not show a beneficial effect of this treatment on exacerbation frequency.264 A similar negative finding was made with an analogue of N-acetyl-cysteine, having a higher bioavailability.265

Aims and scope of the studies in this thesis

The studies presented in this thesis were directed to address some issues concerning the development and treatment of COPD. Three independent studies formed the basis of the results, described in the chapters in this thesis.

The first study (Chapters 2, 3, 4 and 5) was aimed to answer several questions around the efficacy of inhaled corticosteroids in the treatment of COPD. The first question herein was whether there is an effect of the inhaled corticosteroid budesonide on inflammatory parameters in COPD, and whether such an effect is associated with effects on lung function and symptoms. The second question was whether these effects of inhaled corticosteroid treatment could be predicted by a prior assessment of the effects of a test treatment with a systemic glucocorticosteroid. From previous studies it was known that
assessing the effects of such a test treatment on lung function parameters did not help in predicting the response to inhaled corticosteroids. Therefore, in the present study the effects of systemic corticosteroids on inflammatory parameters were investigated with respect to their predictive value for future inhaled corticosteroid treatment. The third question was whether the effects of inhaled corticosteroid treatment could be predicted from patient characteristics such as lung function data or from the characteristics of the airway inflammation.

The second study (Chapter 6) addressed another issue concerning the efficacy of inhaled corticosteroids and aimed to investigate whether the lack of effects of inhaled corticosteroids in COPD could be explained by the pharmacokinetic profile of inhaled corticosteroids. Inhaled corticosteroids could reach predominantly the larger airways in patients with COPD and could hereby have reached too low levels in the smaller airways and the airway epithelium to induce an anti-inflammatory effect. The participants in this study were patients, subjected to surgical removal of a local pulmonary malignancy. The tissue levels of two inhaled corticosteroids were to be determined at certain time points following inhalation. For one of these inhaled corticosteroids (budesonide) the presence of corticosteroid esters was also investigated, since esterification of budesonide was documented previously in nasal tissue and in animal lung tissue and esterification in the airways was assumed to attribute to prolonging the presence of inhaled budesonide in airway tissue.

The third study (Chapter 7) was directed to investigate why only a minority of smoking individuals developed COPD, more specifically: to relate airway inflammation, assessed via immunostaining of biopsies, to the development of airflow obstruction. A group of subjects was selected, who had been exposed to cigarette smoke to a variable extent. These subjects, with a planned surgical removal of a localized pulmonary malignancy, were considered to form a group of subjects who all had the potency to develop COPD. Only a minority of these subjects was expected to have a current clinical diagnosis of COPD. Differences in the inflammatory profile in the airways and in the resected lung tissue of subjects with and without COPD (based on the GOLD lung function criteria) were to be investigated. Differences could point to the mechanism by which the chronic cigarette smoke exposure induced COPD in some subjects or could point to aspects of inflammation which were not affected in those subjects who did not (yet) developed COPD. The presence of CD8+ T-lymphocytes was to be investigated in the airway mucosa and was to be related to airflow limitation and cigarette smoke exposure. Additionally, the presence of CD8+ T-lymphocytes and of cytotoxic granzymes in airway mucosa and in the airway lumen (assessed via induced sputum) was to be investigated and related to airflow obstruction.
In order to make valid conclusions on the data obtained on inflammatory markers in induced sputum, which is an attractive and rather non-invasive method of obtaining information on the inflammatory status of the airways, an analysis was made on the repeatability of assessing inflammatory markers in induced sputum in patients with COPD (Chapter 3). Some data was already known on repeatability, but mainly assessed in healthy volunteers and in patients with asthma and predominantly using the selected plugs method. The analysis of the large set of data, obtained in the above mentioned first study could provide additional information on the repeatability of obtaining information on the inflammatory processes in patients with COPD via collecting induced sputum and analyzed as whole sputum sample.

Whole sputum samples may be contaminated in a variable way with saliva, with upper airway secretions and other sources of squamous epithelial cells. Therefore, only sputum samples with squamous epithelial cell content in the sputum sample below a certain cut-off value (usually 80%) have generally been considered to represent valid samples originating mainly from the lower airways. This contamination and cut-off value were challenged in a statistical analysis of the sputum data obtained in the first study and of sputum data obtained in a previously conducted study in asthma patients, executed in the same laboratory, in order to provide a rationale for declaring sputum samples valid and to assess the level of contamination in a quantitative way (Chapter 4).

In several published clinical studies in COPD, an unexpected and disproportionally high withdrawal rate at the start of the study was seen, when treatment with inhaled corticosteroids was stopped. An analysis was therefore performed on the withdrawal of patients in the first study (Chapter 5). It was speculated, that those patients who are most likely to respond to inhaled corticosteroid treatment are also the patients who experience an exacerbation of COPD and were subsequently withdrawn. Selective withdrawal of those “relatively corticosteroid-sensitive” patients would affect the interpretation of subsequent intervention studies, since performed on the remaining patients which could be considered to be less corticosteroid sensitive. Using the data from the first study, the (inflammatory) characteristics of the two groups of patients, those who were withdrawn because of an exacerbation and those who did not, were to be compared in an attempt to explain the limited effects of inhaled corticosteroids in studies in COPD by selective withdrawal. An explorative analysis was to be made attempting to predict the occurrence of the exacerbation in the run-in period of the first study based on the (inflammatory) characteristics.
References


42. McManus TE, Marley AM, Baxter N, Christie SN, Elborn JS, O’Neill HJ, Coyle PV, Kidney JC. High levels of Epstein-Barr virus in COPD. Eur Respir J. 2008;31:1221-1226.


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98. Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in Interleukin-8 and Tumor Necrosis Factor-α in induced sputum from patients with chronic obstructive pulmonary disease or asthma. Am J Respir Crit Care Med. 1996;153:530-534.


Introduction


231. Dusser D, Bravo ML, Iacono P, on behalf the MISTRAL study group. The effect of tiotropium on exacerbations and airflow in patients with COPD. Eur Respir J. 2006;27:547-555.


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