Airway pathology in COPD : smoking cessation and pharmacological treatment intervention. Results from the GLUCOLD study

Lapperre, T.S.

Chapter 1

General introduction and aims of the study

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide. Patients suffer from progressive dyspnea at rest or on exertion, chronic cough and sputum expectoration. The disease is characterized by progressive and largely irreversible airways obstruction, which is demonstrated by an accelerated decline of lung function (FEV_1: forced expiratory volume in 1 second) with age. In addition, a subgroup of patients experience frequent exacerbations, which may require hospital admission. The disease leads to impaired quality of life, disablement and eventually death. The main risk factor for development of COPD is cigarette smoking, and smoking-induced inflammation in the lung is thought to play a key role in the pathogenesis of COPD. In the studies described in this thesis we therefore focused on the role of airway inflammation in relation to lung function, smoking and therapeutic intervention.

Definition of COPD

COPD is defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) as *"a preventable and treatable disease with some significant extrapulmonary 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"* (1;2). Consequently, spirometry is essential for the diagnosis and is used for classification of the severity of COPD (table 1). The characteristic symptoms of COPD are chronic and progressive dyspnea, cough and sputum production.

Table 1. GOLD classification of COPD severity based on post-bronchodilator FEV_1 (1;2).

Stage I: Mild COPD	$FEV_1/FVC < 0.70$ $FEV_1 \geq 80\%$ predicted
Stage II: Moderate COPD	$FEV_1/FVC < 0.70$ $50\% \leq FEV_1 < 80\%$ predicted
Stage III: Severe COPD	$FEV_1/FVC < 0.70$ $30\% \leq FEV_1 < 50\%$ predicted
Stage IV: Very Severe COPD	$FEV_1/FVC < 0.70$ $FEV_1 < 30\%$ predicted or FEV_1 < 50% predicted plus chronic respiratory failure

Abbreviations: FEV_1: Forced Expiratory Volume in one second; FVC: Forced Vital Capacity; respiratory failure: arterial pressure of oxygen (PaO_2) less than 8.0 kPa (60 mmHg) with or without arterial pressure of CO_2 ($PaCO_2$) greater than 6.7 kPa (50 mmHg) while breathing air at sea level.

Epidemiology of COPD

COPD is one of the leading causes of mortality and morbidity worldwide (3). The prevalence of COPD defined by lung function criteria in adults aged ≥40 years is ~9-10% (4). According to the latest WHO estimates (2007), currently 210 million people have COPD and 3 million people died of COPD in 2005 (5). In the Netherlands, the prevalence of COPD was 316,400 individuals in 2003, there were 18,500 hospital admissions due to COPD in 2004, and 5,662 people died of COPD in 2004 (6). However, underrecognition and underdiagnosis of COPD leads to significant underreporting, and therefore underestimation of prevalence data. The prevalence, morbidity, and mortality of COPD vary across countries and different groups within countries, but are directly related to tobacco smoking. In addition, these figures are projected to increase in the coming decades as smoking frequencies rise and the population ages (7;8). The WHO predicts that COPD will become the fourth leading cause of death worldwide by 2030 (5).

Risk factors for COPD

Cigarette smoking is the most common risk factor for COPD. Indeed, Fletcher *et al*. have shown that the decline in lung function is faster in smokers compared to non-smokers (9). However, only 15-20% of all smokers, and up to 50% of elderly (>75 years) smokers (10), developed COPD suggesting a role for age and individual susceptibility. Alpha-1 antitrypsin deficiency (SERPINE1 gene; a serine protease inhibitor protein) is the most important known genetic risk factor for COPD (11). Polymorphisms in another related gene, SERPINE2, has also been proposed as a potential risk factor (12;13). Other candidate genes have not been reliably replicated (14), such as tumor necrosis factor (TNF)-α gene (15), transforming growth factor (TGF)-β1 gene (16), microsomal epoxide hydrolase (mEPHX)1 gene (17), and A Disintegrin and Metalloprotease (ADAM) 33 gene (18). Further risk factors include: exposure to occupational dust and chemicals, air pollution, reduced lung growth and development, oxidative stress, female gender, infections, low socioeconomic status, inadequate nutrition, cooking and heating in poorly ventilated spaces, and asthma (1;2). Furthermore, it has been reported that increased airway hyperresponsiveness (19), elevated serum IgE and peripheral blood eosinophilia are associated with a more rapid decline of FEV_1 in smokers (20).

Heterogeneity of COPD

COPD has been recognized as a heterogeneous disorder in terms of clinical presentation, physiological and pathological characteristics (21;22). Clinical phenotypic distinctions in COPD include: symptoms of chronic bronchitis, frequent exacerbations, weight loss, rapid lung function decline, airways hyperresponsiveness,

impaired exercise tolerance, and in some patients perhaps features of asthma (23). This is accompanied by pathophysiological characteristics such as partial reversibility to bronchodilators, air trapping, impaired diffusing capacity, and airway hyperresponsiveness (24). Pathological findings include: features of emphysema, large and small airways disease, epithelial goblet cell hyperplasia, squamous cell metaplasia, and presence of eosinophilic airway inflammation in some patients with COPD (25). The presence and contribution of these features to the severity of COPD varies between patients and may be related to distinct pathophysiological mechanisms involved in development, clinical presentation, and course of the disease. It is increasingly recognized that disease heterogeneity provides opportunities for targeted interventions (23;26;27).

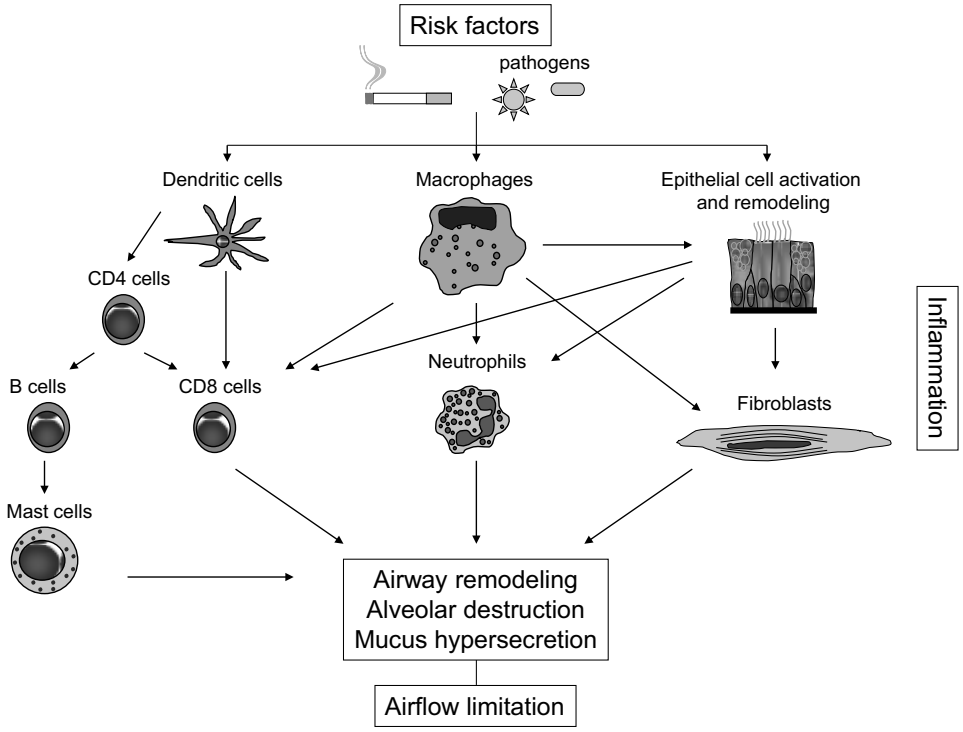
Pathology of COPD

Pathological changes are present at different levels and compartments of the lungs of patients with COPD: proximal airways, peripheral airways, lung parenchyma, and pulmonary vasculature. Induced sputum and endobronchial biopsies mainly represent the central airways. Bronchoalveolar lavage fluid (BAL) and surgical resection samples represent peripheral airways and lung parenchyma. Changes in the central airway wall include increased numbers of macrophages, $CD8^+$ T-lymphocytes and B-lymphocytes, increased epithelial goblet cell numbers and squamous cell metaplasia, and enlarged submucosal glands (28). The peripheral airways show increased numbers of macrophages, T-lymphocytes (predominantly $CD8^+$), B-lymphocytes, and mast cells (29). In addition, localization of neutrophils and $CD8^+$ cells has been observed in the airway smooth muscle layer in smokers with COPD (30). Structural changes of peripheral airways consist of airway wall thickening, with increased extracellular matrix components (31) and smooth muscle mass (32;33), peribronchial fibrosis, luminal inflammatory exudates, and airway narrowing (34). The lung parenchyma also demonstrates increased numbers of macrophages and $CD8^+$ T-lymphocytes, next to alveolar wall destruction, and apoptosis of epithelial and endothelial cells (35). The pulmonary vasculature shows increased numbers of macrophages and $CD8^+$ T-lymphocytes, thickening of the intima, endothelial cell dysfunction, and increased smooth muscle mass. Finally, the airway lumen of patients with COPD contains predominantly neutrophils (36) and increased numbers of $CD8^+$ T-lymphocytes (37) in larger airways, and increased numbers of macrophages and neutrophils in the periphery of the lung (38). In some studies increased numbers of eosinophils have been described in the airway lumen of (a subgroup of) COPD patients (39).

Pathogenesis of COPD

The pathogenesis of COPD is strongly linked to the effects of cigarette smoke on the lungs. Different important processes have been suggested to play a role in the development and progression of COPD. The key processes include pulmonary inflammation, oxidants-antioxidants imbalance, and protease-antiprotease imbalance (40-42). In addition, it is thought that the observed airway remodeling, including epithelial alterations and changes in the composition of the extracellular matrix, may be caused by an aberrant repair process following initial injury. The enhanced or abnormal inflammatory response to cigarette smoke is a characteristic feature of COPD and has the potential to produce lung injury (Figure 1). Both innate and adaptive inflammatory and immune responses are involved in pulmonary inflammation in COPD. Since inflammation and epithelial remodeling are the main topics of this thesis, the role of inflammatory cells and epithelial cells in COPD will be discussed more extensively below.

Figure 1. Inflammatory cascade in COPD.



Innate immune responses and COPD

Neutrophils

The role of neutrophils in the pathogenesis of COPD is not entirely clear. Multiple studies have shown increased neutrophil counts in sputum (36;43;44) and airway lavage fluid (38;45-47) from patients with COPD, whereas studies about their presence in the airway wall are inconsistent (30;48;49). Relationships have been shown between airflow limitation and sputum (36;43), BAL (46;47), and bronchial neutrophils (49), and between rate of FEV_1 decline and sputum neutrophils (50) in patients with COPD. Neutrophils can migrate to the respiratory tract under control of chemotactic factors, such as LTB4, IL-8, and other CXC chemokines, which are increased in COPD airways (51). Neutrophils are a source of reactive oxygen metabolites, inflammatory cytokines, lipid mediators, antimicrobial peptides, and tissue damaging proteinases, such as neutrophil elastase, cathepsin G, and proteinase 3, as well as matrix metalloproteinase (MMP)-8 and MMP-9. These compounds are implicated in the generation of mucous metaplasia in chronic bronchitis and the destruction of lung tissue in emphysema (52), and thereby may play a role in progression of airflow limitation in COPD.

Macrophages

Increased macrophage numbers have been observed in lavage fluid (38), the airway wall (48;53;54), the lung parenchyma (55), and bronchial glands (56) of patients with COPD. Macrophage numbers in the airways correlate with the severity of COPD (49). Macrophages play a central role in inflammation and host defense against microorganisms, but they also participate actively in the resolution of inflammation after alternative activation. It is unclear which of these macrophage sub-phenotypes predominates in the airways of COPD patients. Macrophages may release reactive oxygen species, chemotactic factors, inflammatory cytokines, smooth muscle constrictors, mucus gland activators, extracellular matrix proteins, and matrix metalloproteinase enzymes (MMPs). Particularly, the latter are thought to be involved in emphysema. *In vivo* studies have indeed observed increased expression of MMP-2, MMP-9, and MMP-12 in patients with COPD compared with healthy controls (57). In addition, alveolar macrophage number in lung parenchyma has been correlated to the severity of lung destruction (55), suggesting a role for macrophages in the development of emphysema.

Eosinophils

Airway eosinophilia can be observed in patients with stable COPD in sputum (39;58-60), BAL fluid (59), and the airway wall (45;59;61). In addition, sputum eosinophilia has been found to be associated with airways obstruction (39) and

with hyperresponsiveness to adenosine 5'-monophosphate (AMP) in COPD (62). During exacerbations of chronic bronchitis eosinophil counts can even be as high as in asthma (63-65). These findings suggest that airway eosinophilia is functionally important in patients with COPD. It has been suggested that eosinophilia in COPD is related to the intensity of the inflammatory process in the airways, leading to a nonspecific recruitment and activation of eosinophils (59). However, the eosinophilic inflammation seen in patients with COPD might also identify a subgroup of COPD patients that shares some characteristics with patients with asthma, including not only eosinophilia, but also a partial bronchodilator response to inhaled salbutamol (60).

Mast cells

A role for mast cells in the pathogenesis of COPD is yet unclear. Whereas increased numbers of mast cells have been found in the airways of COPD patients by our group (29), and in patients with chronic bronchitis by other investigators (66;67), most previous studies show no evidence of mast cell abundance in the airways or parenchyma of COPD patients (49;56;68;69). Mast cells and their secreted cytokines (IL-8, TNF-α) and enzymes (tryptase, chymase), have been shown to initiate and drive a variety of processes relevant to airway inflammation and remodeling. These include airway fibrosis and extracellular matrix turnover (70;71), angiogenesis, airway smooth muscle and epithelial cell hyperplasia, inflammation, alterations in bronchial tone, and mucus hypersecretion (72;73). It is yet unclear whether these mast cell induced mechanisms play a role in the pathogenesis of COPD. Interestingly, a more recent study demonstrated similar distributions of tryptase and chymase positive mast cells in the airways of COPD patients compared to controls (74). Moreover, higher numbers of these cells in peripheral airways were associated with less severe airflow limitation in COPD. It is unclear whether these results reflect a protective role of mast cells in the pathogenesis of COPD or increased degranulation of mast cells.

Dendritic cells

Dendritic cells initiate and regulate both innate and adaptive immune responses to inhaled antigens, viruses and bacteria. Recently, increased numbers of dendritic cells have been observed in small airways and induced sputum from patients with COPD compared to smokers without COPD, increasing with disease severity (75). CCL20, the most potent chemokine in attracting dendritic cell precursors to sites of inflammation, is also increased in the airways of COPD patients (75). The role of dendritic cells in de pathophysiology of COPD remains to be clarified.

Epithelial cells

The airway epithelium of patients with COPD undergoes alterations, including squamous cell metaplasia, and goblet and basal cell hyperplasia (76). Bronchial epithelial cells contribute to an adequate maintenance of lung homeostasis by mucus production, ciliary beating, secretion of antimicrobial products and adequate immunological drive in response to noxious stimuli. Therefore, epithelial remodelling in COPD may be involved in the pathogenesis of the disease. Goblet cell hyperplasia is more pronounced in smokers with COPD compared to those without airflow limitation (77). In addition, it contributes to mucus hypersecretion, which is associated with morbidity and mortality in COPD (78;79). Squamous cell metaplasia impairs mucociliary clearance and contributes to the increased risk of squamous cell carcinoma as observed in COPD (80). The mechanisms underlying epithelial alterations in COPD are incompletely understood. The epidermal growth factor receptor (EGFR) cascade has been shown to be involved in mucin production and goblet cell hyperplasia (81;82), repair of damaged epithelium (81;82), as well as development of squamous cell carcinoma (83). In addition to EGFR ligands, a wide variety of stimuli can induce EGFR activation *in vitro* and in animals, including cigarette smoke (81;82). Additionally, epithelial EGFR expression is increased in bronchial biopsies from smokers with (84;85) and without (85;86) COPD compared to non-smokers. Previously, we have observed higher epithelial EGFR expression in ex-smokers with COPD compared to non-COPD, but not in current smokers, suggesting that current smoking may obscure differences in EGFR expression (87). Therefore, EGFR activation may play a role in epithelial phenotypic alterations observed in COPD through active smoking.

Adaptive immune responses and COPD

T-Lymphocytes

In COPD the $CD8^+$ T-cell is the most prominent lymphocyte subtype. Increased $CD8^+$ T-lymphocyte numbers have been found in sputum (37), the airway wall (32;53;88), and lung parenchyma (69;89) of COPD patients. Moreover, a strong correlation has been found between severity of airflow limitation and the number of $CD8^+$ cells (53;88). Mucosal $CD8^+$ cells have also been associated with airway hyperresponsiveness to AMP (62). Additionally, sputum $CD8^+$ cells of COPD patients have elevated cytotoxic activity (90). A key function of $CD8^+$ cells is to combat viruses, which may consequently lead to collateral tissue damage via release of lytic substances such as perforins and granzymes (25). $CD8^+$ cells can induce structural cell apoptosis (25), as suggested for alveolar endothelial and epithelial cells (89). A more recent study demonstrated that patients with COPD had a blunted regulatory T-cell response to tobacco smoke, and higher $CD8^+CD45RA^+$ and lower

$CD8^+CD45R0^+$ than smokers with normal lung function, indicating a shift to more final maturation-activation state of CD8+ T-lymphocytes (91). In addition, It has been hypothesized that smoking and inflammation induced injury to the lung may result in structural alterations of cell- and tissue proteins into "autoantigens" that are recognized by T-lymphocytes leading to further lung injury (89). Alternatively, a persistent intracellular pathogen, such as adenovirus (92), may provide a foreign antigenic stimulus that activates T-lymphocytes.
The role of $CD4^+$ cells in COPD is unknown. $CD4^+$ cells are a diverse group of lymphocytes in which various subtypes are recognized that may contribute to COPD pathogenesis. These include not only Th1 and Th2 cells, but also regulatory and Th17 cells. $CD4^+$ cells may contribute to the inflammatory process by production of pro-inflammatory cytokines, providing help for B cell responses, and may be important by their actions as T-helper cells, priming $CD8^+$ cytotoxic responses, maintaining their memory and ensuring survival (25).

B-lymphocytes and plasma cells
It has been demonstrated that B-lymphocyte numbers and lymphoid follicles in the small airways of COPD patients are increased (34;93), and associated with disease severity (34). While previous studies observed similar B cell counts in large airways of COPD patients compared to controls (53;54), we have shown increased numbers in bronchial biopsies associated with severity of airflow limitation (94). These B cells can result from a local inflammatory process, an altered T-helper (Th)1-Th2 balance and/or can reflect an antigen-specific reaction to pathogens, components of cigarette smoke, or auto-antigens. In line with this, B-cell follicles with an oligoclonal, antigen-specific reaction were found in men and mice with emphysema (95). Furthermore, the presence of anti-elastin antibodies (96) and IgG anti-epithelial cell antibodies, and the potential to mediate cytotoxicity (97), support a role for auto-reactive adaptive immune responses in patients with COPD. Plasma cells are terminally differentiated effector B cells, and are the cellular source of mucosal immunoglobulin production. There is limited data on their role in COPD. One single study by Zhu *et al.* examined their numbers in patients with chronic bronchitis with or without airflow limitation (67). It was concluded that patients with chronic bronchitis have increased plasma cell counts in submucosal glands and subepithelial compartments compared to controls, which was not significant for patients with airflow limitation.

Pathophysiology of COPD
Irreversible airflow limitation, the main characteristic of impaired lung function in COPD, results from the loss of elastic recoil of the parenchyma and from the

increase in airway resistance. The pathological substrate of airflow limitation is predominantly located in the periphery of the lung (98). In the emphysematous lungs, parenchyma destruction and loss of alveolar integrity is observed, which leads to reduced recoil and collapsed small airway lumens. Additionally, due to destruction of the parenchyma, gas exchange may be impaired, resulting in reduced diffusing capacity. The current working hypothesis is that inflammatory cell infiltration of small airways, together with fibrosis and smooth muscle cell proliferation results in reduced diameter and increased resistance. In addition, mucus hypersecretion may also contribute to airflow limitation. The altered compliance and resistance of small airways may result in uneven distribution of ventilation and early airway closure, as can be measured by "small airways tests", such as the single-breath nitrogen test (sbN_2-test) (99). The physiological abnormalities observed in COPD are progressive and finally cause hypoxia.

Management of stable COPD

Current guidelines recommend smoking cessation and on demand use of short-acting bronchodilators for all GOLD severity stages of COPD (1;2). Addition of long-acting bronchodilators is advised for patients with at least GOLD stage II COPD, and treatment with inhaled corticosteroids for patients with at least GOLD stage III COPD and frequent exacerbations (1;2). In the following paragraphs, the effects of smoking cessation, bronchodilators, inhaled steroids, and combination therapy of inhaled steroids with long-acting bronchodilators on clinical and, in particular, inflammatory parameters in COPD patients will be discussed more extensively.

Smoking cessation

Smoking cessation is able to reduce COPD progression (100) and improve survival (101). Moreover, patients who quit smoking experience fewer respiratory symptoms and less hyperresponsiveness as compared to those who continue smoking (102;103). These beneficial clinical effects of smoking cessation do not appear to be accompanied by a simultaneous simple reduction of airway inflammation characteristic for COPD. Induced sputum from COPD smokers and ex-smokers reveal similar inflammatory cell counts in one study (44), and higher macrophage counts in smokers in another (104). There are only few cross-sectional studies comparing smokers and ex-smokers regarding bronchial inflammation in heterogeneous and relatively small groups of patients without an established diagnosis of COPD (105). Most of these previous studies were performed in patients with chronic bronchitis (66;106). In patients with symptoms of chronic cough and expectoration, ex-smokers tended to have lower mast cell numbers in the lamina propria

than current smokers (66), whereas the number of neutrophils, macrophages, eosinophils, and lymphocytes in bronchial biopsies have been reported to be similar (106). Recently, it was observed that in patients with established COPD there are also no differences in bronchial T-lymphocytes, neutrophils, macrophages and mast cells between current and ex-smokers (107), but that current smokers have lower numbers of dendritic cells (108). In contrast, another study observed a positive relation between macrophages in bronchial biopsies and current smoking in COPD (104). Finally, in a prospective study of COPD patients bronchial inflammation also persisted after smoking cessation, while the number of sputum neutrophils, lymphocytes, IL-8 and ECP levels significantly increased (109). It needs to be emphasized that the majority of studies did not take duration of smoking cessation into account when comparing current and ex-smokers. However, it has been shown that this may influence the inflammatory response in small airways (61). In summary, airway inflammation characteristic for COPD seems to persist after smoking cessation, in contrast to clinical beneficial effects on symptoms and decline of lung function. Possible explanations for this ongoing inflammatory response have been proposed, and include persistent presence of a microbial stimulus or the development of autoimmune disease or alternatively to represent the inflammatory component of tissue repair.

Pharmacological intervention

Bronchodilators

Inhaled bronchodilator medications (β_2-agonists and anticholinergics) are central to the symptomatic management of COPD (110;111). The principal action of β_2-agonists is to relax airway smooth muscle by β_2-adrenergic receptor stimulation, which increases cAMP and produces functional antagonism to bronchoconstriction. The most important effect of anticholinergics is to block the acetylcholine effect on muscarinic receptors. Long-acting β_2-agonists (salmeterol) and long-acting anticholinergics (tiotropium) are more effective than short-acting bronchodilators (112;113). Both salmeterol and tiotropium have beneficial effects on symptoms, exacerbation rates and lung function level in COPD patients (114-117). In addition, salmeterol has an effect on decline in lung function (118), which was not observed with tiotropium treatment (117). It has been suggested that bronchodilators also exhibit some anti-inflammatory effects. In fact, salmeterol inhibits inflammatory responses by neutrophils and mononuclear cells *in vitro* (119;120), and in mouse models of lung inflammation *in vivo* (121). In addition, salmeterol has anti-inflammatory effects in LPS exposed healthy volunteers (122) and in mild asthmatics (123;124). To our knowledge, the effects of salmeterol mono-therapy on airway inflammation in COPD have not been studied. Although tiotropium is

able to suppress acetylcholine-induced release of chemotactic mediators *in vitro* (125) and inhibit allergen-induced increase in MUC5AC-positive goblet cell numbers and eosinophil infiltration *in vivo* in guinea pigs (126), it does not seem to attenuate sputum and systemic inflammatory markers in COPD patients (127).

Inhaled steroids

Regular therapy with inhaled corticosteroids leads to clinical benefit in terms of symptoms, exacerbation rate, and initial improvement in FEV_1 (128;129). In addition, withdrawal of inhaled steroids in COPD results in deterioration of lung function, symptoms and exacerbation rate (130;131). So far, it has not been settled whether ICS alone or combined with LABA changes FEV_1-decline in COPD (132-137) and if so, in which COPD phenotypes. ICS may have the potential of 'disease modification' since long-term therapy improves airway hyperresponsiveness (138), a disease feature that is present in about 70% of the COPD population and by itself constitutes a risk factor for accelerated FEV_1 decline (139;140). Interestingly, a recent analysis of the TORCH study suggests that prolonged therapy with ICS and/or LABA attenuates FEV_1-decline in COPD (118). Several short-term studies have investigated the anti-inflammatory effects of ICS in patients with COPD. 2-3 Months treatment with inhaled steroids in patients with COPD reduces bronchial mast cells, but not $CD8^+$ cells, neutrophils or macrophages (141;142), and may reduce sputum total cell counts, neutrophils and lymphocytes (143). In addition, the presence of sputum eosinophilia predicts the response of airways obstruction to oral and inhaled corticosteroid treatment in COPD (58;144-146), and can even be used to guide it (27). Therefore, the clinical effects of long-term treatment with inhaled steroids in COPD may be mediated, at least partially, by anti-inflammatory effects. However, the longer-term anti-inflammatory effects of inhaled corticosteroids have not been studied before.

Combination therapy

Addition of LABA to inhaled corticosteroids treatment in COPD is more effective in improving lung function level (147;148), health status (114;149) and reducing exacerbations (114;150), although it does not have supplementary effects on FEV_1 decline (118). Combination treatment with a LABA for 3 months may have additional anti-inflammatory effects on bronchial $CD8^+$ and $CD68^+$ cells compared to mono-therapy with inhaled steroids (151), and reduces sputum neutrophils and eosinophils compared to placebo (152). To our knowledge, the longer-term anti-inflammatory effects of combination therapy have not been studied previously. Finally, although triple therapy with salmeterol/fluticasone propionate and tiotropium seems to have additional bronchodilator effect after 2 weeks treatment (153), long-term effects of this combination have not been studied so far.

Aims of the present studies

In summary, this thesis addresses airway pathology in relation to three different aspects of COPD: pathophysiology, smoking cessation and pharmacological treatment. The questions mentioned in the introduction have been addressed in cross-sectional and longitudinal analyses from the Groningen Leiden Universities Corticosteroids in Obstructive Lung Disease (GLUCOLD) study.

The GLUCOLD study

The GLUCOLD study is a two-centre, randomized, double-blind, four armed, placebo-controlled, parallel-group trial. It was designed to investigate the effect of long-term versus short-term treatment with inhaled corticosteroids, either or not combined with a long-acting β_2-agonist, on bronchial inflammation in patients with stable COPD. The in- and exclusion criteria are presented in table 2.
114 COPD patients were randomized to 30 months treatment with fluticasone propionate, fluticasone propionate/salmeterol combination therapy, placebo, and 6 months fluticasone propionate followed by 24 months placebo (figure 2). Patients visited the out-patient clinic every three months to perform spirometry and obtain symptoms- and health status questionnaires. At baseline, after 6, and 30 months treatment more extensive measurements were performed including: peripheral blood analysis, diffusion capacity, bodyplethysmography, methacholine provocation, sputum induction, and bronchoscopy (figure 3).

Figure 2. Design of the GLUCOLD study.

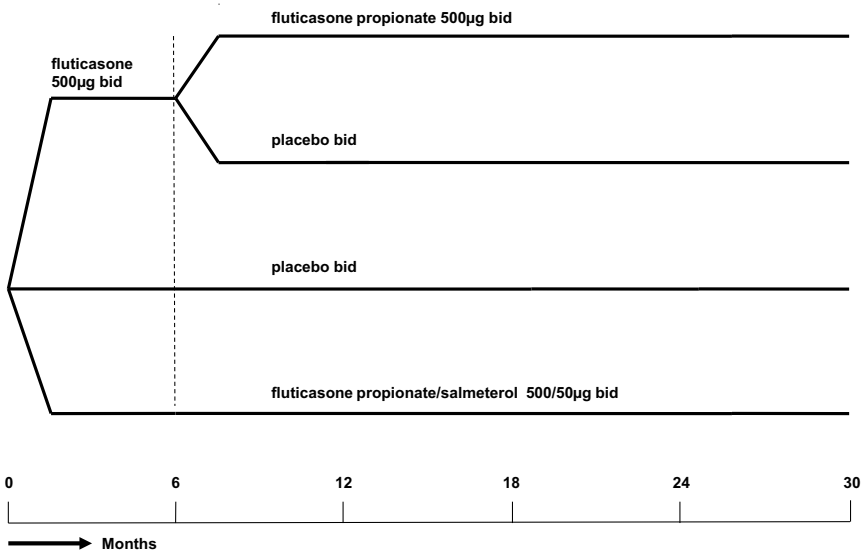
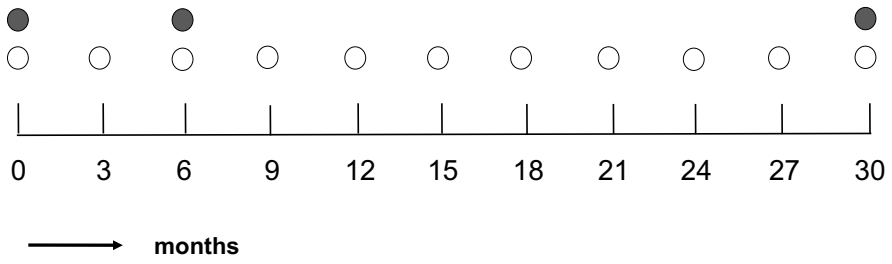


Table 2. In- and exclusion criteria of the GLUCOLD study.

Inclusion criteria
Age: 45-75 years
≥ 10 Packyears of smoking
≥ 1 of the following symptoms: chronic cough, chronic sputum production, frequent exacerbations, or dyspnoea on exertion
No course of oral corticosteroids during last 3 months, no maintenance treatment with inhaled or oral steroids during last 6 months
Postbronchodilator FEV_1 (after 400 μg of inhaled salbutamol) < 90% confidence interval (90% CI) of the predicted FEV_1, and postbronchodilator FEV_1/IVC ratio below the 90% CI of the predicted FEV_1/IVC ratio (154)
Postbronchodilator FEV_1 > 1.3 litre and > 20% of predicted value.
Exclusion criteria
Prior or concomitant history of asthma
Alpha-1 antitrypsin deficiency (SZ, ZZ, zero phenotype)
Other active lung disease, except for mild bronchiectasis
Contra-indications for elective bronchoscopy, such as: O_2 saturation <90%, abnormal coagulability, anti-coagulant therapy which cannot be temporarily withheld for performance of bronchoscopy, history of pneumothorax, uncontrolled angina pectoris
Other diseases likely to interfere with the purpose of the study.
Inability to keep diary and to understand written and oral instructions in Dutch

Figure 3. Measurements during the GLUCOLD study.



○ Symptoms, questionnaires, lung function

● CO diffusion, bodybox, peripheral blood, PC_{20} methacholine, sputum induction, bronchoscopy: bronchial biopsies

Research questions

Relation between airway inflammation and pathophysiology in COPD

1. *Are the various functional and inflammatory features of COPD separate, complementary domains of this heterogeneous disease?*
   In chapter 2 we applied a factor analysis including lung function indices and markers of inflammation in induced sputum and exhaled air from patients with clinically stable COPD.
2. *Is small airways dysfunction in patients with COPD associated with the inflammatory profile characteristic for COPD?*
   In chapter 3 we examined, in a cross-sectional study in 51 patients with COPD, the relationship of uneven ventilation and airway closure, measured by the single breath nitrogen (sbN_2)-test, with the number of inflammatory cells in bronchial biopsies, bronchoalveolar lavage (BAL) fluid, and induced sputum, together with the levels of neutrophil elastase (NE), IL-8, and secretory leukocyte proteinase inhibitor (SLPI) in BAL fluid.

Effect of smoking cessation on airway pathology in COPD

3. *Does bronchial inflammation in patients with established COPD differ between active smokers and patients who stopped smoking? Is airway inflammation associated with duration of smoking cessation?*
   In chapter 4 the number of inflammatory cells in bronchial biopsies of current and ex-smokers with COPD was investigated, and related to duration of smoking cessation.
4. *Is bronchial epithelial cell proliferation and differentiation in patients with COPD different between active smokers and those who stopped smoking, and is this difference influenced by the duration of smoking cessation? Are the epithelial changes associated with epithelial growth factor receptor (EGFR) expression?* In chapter 5 the extent of epithelial goblet cell hyperplasia, proliferation, squamous cell metaplasia, and EGFR expression in bronchial biopsies of current and ex-smokers with established COPD was measured and related to smoking cessation duration.

Treatment effects on airway pathology in COPD

5. *Are there additional anti-inflammatory effects of long-term treatment compared to short-term treatment with inhaled steroids in patients with COPD? Does discontinuation of inhaled steroids lead to a flare up of inflammation? Does addition of long-acting β_2-agonists to inhaled steroids lead to additional anti-inflammatory effects after long-term therapy?*
   In chapter 6 a longitudinal randomized trial was performed examining the effects of 30 months treatment with fluticasone propionate, fluticasone propionate/salmeterol combination therapy, placebo, or 6 months fluticasone propionate followed by 24 months placebo on sputum and bronchial inflammatory cells and bronchial epithelial remodelling.

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