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

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Chapter 7

Conclusions and General discussion

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow limitation and airway inflammation mainly caused by tobacco smoking. COPD is a heterogeneous disease in terms of clinical, physiological, and pathological presentation. Hence, COPD has multiple disease domains that each can be highly relevant for disease monitoring, progression and treatment. This thesis has focused on: the potential independent roles of airway inflammation and functional impairment in COPD, the monitoring of airway function and inflammation in patients with COPD, the effects of smoking cessation on inflammation and epithelial remodeling in COPD, and the integrative effects of anti-inflammatory treatment on each of the disease domains in COPD.
This thesis comprises cross-sectional and follow-up analyses of data from the GLUCOLD study (Groningen Leiden Universities and Corticosteroids in Obstructive Lung Disease). The GLUCOLD Study is a prospective, four-arm, placebo-controlled, and double-blind study, comparing the effects of 30 months treatment with inhaled steroids, inhaled steroids and a long-acting β_2-agonist combination therapy, placebo, and 6 months inhaled steroids followed by 24 months placebo on clinical and pathological outcome parameters. First, the conclusions of the studies in this thesis will be summed up, followed by a general discussion and directions for future research.

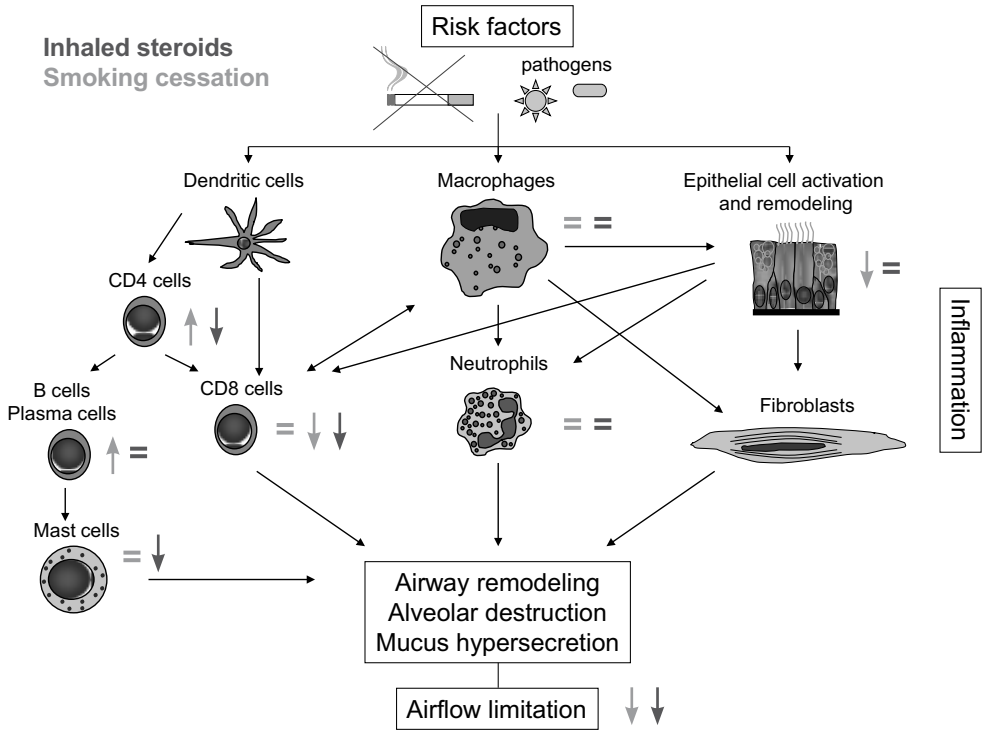
Conclusions of the thesis

Relation between airway inflammation and pathophysiology in COPD

- Airflow limitation, asthma-like components (i.e. airway responsiveness and IgE), exhaled nitric oxide and sputum inflammatory cell counts (neutrophils and eosinophils) offer separate and additive information about the pathophysiological condition of COPD patients (*Chapter 2*).
- Uneven distribution of ventilation and airway closure in patients with stable COPD are associated with neutrophil numbers in bronchial biopsies and bronchoalveolar lavage (BAL) fluid, and with neutrophil elastase and its local inhibitor secretory leukocyte proteinase inhibitor (SLPI) in BAL (*Chapter 3*).

Effect of smoking cessation on airway pathology in COPD

- Ex-smokers with COPD have higher numbers of bronchial $CD4^+$ and plasma cells than current smokers, whereas numbers of neutrophils, macrophages, and $CD8^+$ cells are not different between both groups. T-lymphocytes are higher in short-



term quitters, whereas longer duration of smoking cessation is associated with lower CD8/CD3 ratios, and higher numbers of plasma cells (*Chapter 4*).

- Ex-smokers with COPD have less bronchial epithelial mucin stores, proliferating cells, and squamous cell metaplasia than current smokers with COPD, whereas epithelial epidermal growth factor receptor (EGFR) expression is not different between both groups. These epithelial changes in ex-smokers were more pronounced with longer duration of smoking cessation, and only significant after 3.5 years smoking cessation (*Chapter 5*).

Treatment effects on airway pathology in COPD

Long-term treatment with fluticasone propionate in COPD patients reduces the number of bronchial T-lymphocytes and mast cells, whilst increasing the integrity of bronchial epithelium and number of bronchial eosinophils, which was accompanied by a reduction in sputum cell counts. This is accompanied by a reduced rate of decline in FEV_1 and improvements in airway hyperresponsiveness, dyspnea and quality of life (*Chapter 6*).

- Fluticasone propionate-induced improvement in FEV_1 after long-term treatment in patients with COPD is associated with a reduction in bronchial $CD4^+$ cells, whilst the improvement in airway hyperresponsiveness is associated with a reduction in $CD3^+$, $CD4^+$, and mast cells and with an increase in intact epithelium (*Chapter 6*).
- Discontinuation of fluticasone at 6 months leads to a relapse of bronchial inflammation and airway hyperresponsiveness, dyspnea and quality of life, with acceleration of FEV_1-decline (*Chapter 6*).
- Adding salmeterol to fluticasone propionate does not provide further anti-inflammatory effects compared to fluticasone alone, but improves FEV_1-level without further influencing FEV_1-decline (*Chapter 6*).

General discussion

Airway inflammation and airflow limitation in COPD are not simply related

A crucial pathologic feature of COPD is airway inflammation and remodeling. The chronic inflammation in COPD is characterized by an accumulation of neutrophils, macrophages, B-cells, lymphoid aggregates and $CD8^+$ T-cells, particularly in and near the small airways (1). Multiple cross-sectional studies have demonstrated relationships between this inflammatory profile and airflow limitation in COPD, suggesting a role for inflammation in the pathogenesis of the disease. Increased airflow obstruction is associated with a higher degree of airway inflammation (2), and more specifically with increased numbers of $CD8^+$ cells (3-5), B-lymphocytes (2), neutrophils (6-10), macrophages (10), dendritic cells (11), and in some COPD patients even with eosinophils (12). In addition, one prospective study has shown that patients with an accelerated decline in lung function have an increased baseline sputum neutrophil differential count (13). However, prospective data examining the natural course of airway inflammation in relation to FEV_1 decline within COPD patients are still lacking. Also, cross-sectional correlations do not differentiate properly between cause and effect. So is chronic airway inflammation involved in COPD progression?

To clarify relationships between various parameters, without reference to a specific criterion, we performed a factor analysis using sputum inflammatory and functional parameters in patients with COPD. Interestingly, our data suggest that airflow limitation is greatly independent of neutrophilic and eosinophilic inflammation in the larger airways (chapter 2). Neutrophils are involved in the induction of mucous metaplasia and tissue damage through the release of serine proteases and oxidants (14). However, emphysema is not a prominent feature of other

pulmonary diseases where chronic airway neutrophilia is even more prominent, such as cystic fibrosis and bronchiectasis (15). In addition, increased neutrophil numbers are found in the airway lumen, but they are not a prominent feature of the inflammation in the airway wall or parenchyma in patients with COPD (16). This suggests that other factors are involved in the generation of emphysema. Obviously, sputum does not cover all the inflammatory and structural changes of the airways in COPD. It may well be that selection of other inflammatory cell types characteristic for COPD, such as $CD8^+$ T-lymphocytes and macrophages, or selection of cells in other compartments of the lung, would have given different results of the factor analysis. This is also supported by our observation that small airways dysfunction (uneven distribution of ventilation and early airway closure), as measured with the sbN_2-test, is associated with neutrophil numbers in bronchoalveolar lavage fluid and bronchial biopsies, and with NE and NE/neutrophil ratio, but not with sputum neutrophils or other inflammatory cell types (chapter 3). In patients with established airflow limitation the sbN_2-test contributes to prediction of the decline in FEV_1 (17). Therefore, we may speculate that neutrophilic inflammation is indirectly involved in the progression of COPD by contributing to small airways and/or alveolar pathology.

Dissociation of inflammation and lung function is partly supported by the results of intervention studies in COPD. For instance, although smoking cessation reduces lung function decline in COPD (18), inflammation persists (chapter 4) (19-21). On the other hand, intervention with inhaled steroids is able to slow down FEV_1 decline in a subgroup of COPD patients which is correlated with suppressive effects on the number of bronchial T-lymphocytes, but not with other inflammatory cells (chapter 6). This latter result suggests a role for at least T-lymphocytes in COPD progression.

Apparently airflow limitation requires more than the presence of inflammatory cells per se. Activity of these cells will obviously be of importance, and would therefore be of interest to investigate in more detail in future studies. Additionally, the degree of airflow limitation is also correlated with airway wall thickness (2), and in a recent study the annual changes in airway thickening assessed by CT measurements correlated with annual decline in air flow limitation (22). These data provide indirect evidence for a role for airway wall remodeling in airflow obstruction in COPD. This remodeling includes epithelial remodeling, including squamous metaplasia and mucous metaplasia, increased smooth muscle mass, and peribronchial fibrosis. In addition, breakdown of extracellular matrix occurs in parenchymal tissues, also resulting in airflow limitation.

COPD has been recognized as a heterogeneous disorder in terms of clinical presentation, physiological and pathological characteristics (23;24). This suggests

that distinct pathophysiological pathways contribute to COPD development. In agreement with this, we observed that multiple functional and inflammatory characteristics were categorized into 4 independent dimensions using factor analysis. Factor 1 included airflow limitation and hyperinflation; factor 2 features commonly associated with asthma (β_2-response, total serum IgE, airway hyperresponsiveness); factor 3 exhaled nitric oxide; and factor 4 sputum % neutrophils and eosinophils (chapter 2). Our data suggest that these four dimensions offer separate and additive information about the pathophysiological condition of COPD patients. Therefore, prospective long-term studies monitoring different aspects of pathological changes in the airways and different functional parameters may further explore the mechanisms leading to progressive airflow limitation in various phenotypic subgroups of COPD patients.

Ongoing airway inflammation and reversal of epithelial remodeling after smoking cessation in COPD

Smoking cessation is able to reduce COPD progression (18), respiratory symptoms and hyperresponsiveness as compared to continued smoking (25;26), and improves survival (27). What are the exact changes that occur in the airways and lung parenchyma after smoking cessation? Since airway inflammation is a key characteristic of COPD, it was hypothesized that this inflammation might be reduced after cessation. However, we have shown that ex-smokers with COPD have higher numbers of bronchial $CD4^+$ and plasma cells than current smokers, whereas numbers of neutrophils, macrophages, $CD8^+$ and mast cells are not different between both groups (chapter 4). The persistence of airway inflammation in ex-smokers is in line with other recent studies (19-21), although decreased numbers of macrophages in ex-smokers with COPD have been reported (19). In contrast, in subjects without COPD inflammatory changes are at least partially reversible with cessation (28). These results suggest that inflammation becomes or remains self-perpetuating after stopping smoking in COPD patients, which could be related to latent adenovirus or bacterial colonization, an auto-immune response (29), persistent apoptosis (30), or persistent and/or aberrant repair processes. The discrepancy between the improvement in FEV_1 decline and the ongoing airway inflammation suggests that inflammation does not necessarily contribute substantially to disease progression.

Interestingly, we observed increased bronchial $CD4^+$ and plasma cells in ex-smokers compared to current smokers (chapter 4). In addition, a more recent study demonstrated that current smokers with COPD have lower numbers of bronchial dendritic cells which reversed upon smoking cessation (31). These results might be explained by reversal of immunosuppressive effects induced by tobacco smoke,

and thereby ameliorating lung defence mechanisms. Alternatively, smoking cessation may contribute to restoration of epithelial characteristics in the large airways of COPD patients, which are directly and continuously exposed to the noxious substances present in cigarette smoke, thereby contributing to the clinical benefits observed after smoking cessation. Consistent with this, we demonstrated that long-term ex-smokers with COPD had less bronchial epithelial mucin stores, proliferating cells, and squamous cell metaplasia than current smokers with COPD (chapter 5). These epithelial features might contribute to COPD by facilitating colonization of the airways by respiratory pathogens, secondary to loss of cilia, increased mucus secretion, and epithelial injury (32). The chronic colonization of the airways may enhance airway inflammation and further epithelial injury. In addition, mucus hypersecretion may cause airways obstruction in peripheral airways (2). Reversal of epithelial remodeling may therefore contribute to reduced progression of COPD attributable to restored mucociliary clearance, resulting in reduced respiratory tract colonization (33) and exacerbations, and less small airways obstruction.

Finally, our results demonstrate that longer duration of smoking cessation (>3.5 years) in COPD patients is associated with higher plasma cell numbers, lower CD8/CD3 ratios, and more pronounced reductions in epithelial mucin stores, proliferating cells, and squamous cell metaplasia (chapter 3 and 4). This suggests a long-term effect of smoking on bronchial regulatory networks, which is not restored immediately after removing the initial stimulus, i.e. cigarette smoke. In contrast, the greatest improvements in respiratory symptoms and lung function decline occur within the first year after cessation (18;25). Therefore, other pathological mechanisms that reverse more rapidly after cessation should be involved in the mechanism leading to the clinical beneficial effects of smoking cessation. It could therefore be interesting to study effects of smoking cessation on for instance cell activity, oedema in de airways, vascular changes and airway smooth muscle. In addition, these results indicate that when studying the effects of smoking cessation the duration of cessation should be taken into account when interpreting the results.

Anti-inflammatory properties of inhaled steroids in COPD

Systemic and local inflammation has been implicated in the pathogenesis of COPD, and consequently the use of systemic and inhaled corticosteroids (ICS) has been considered critical to COPD treatment. As a result, ICS treatment has been clinical practice for decades in stable COPD patients; however, their precise role remains controversial. Previous studies have shown clinical benefits of ICS in patients in terms of symptoms, exacerbation rate, and initial improvement in

FEV_1 (34;35). The attenuated decline in FEV_1 in COPD patients in the present thesis (chapter 6) contrasts with large COPD trials from the 90's showing initial improvement in FEV_1 without benefits on the subsequent progressive FEV_1-decline (34-36). Interestingly, the more recent TORCH study did show slight reductions of FEV_1-decline in COPD patients by inhaled fluticasone, but also by LABA (37). Discrepancies between the negative trials and the present study may be due to differences in study populations, which may provide a clinical message. We studied steroid-naïve patients, with predominantly moderate degrees of airway obstruction and the majority demonstrated airway hyperresponsiveness and/or modest reversibility of FEV_1, although patients with a previous or concurrent diagnosis of asthma were excluded. Recent studies showed that these characteristics, previously attributed to asthma, can also be components of COPD (38;39). It may well be that this COPD phenotype is particularly sensitive to inhaled corticosteroids, similar to beneficial effects of smoking cessation (40). Therefore, our findings raise the option that inhaled corticosteroids, when given for the first time and for longer duration to steroid-naïve patients as recruited from general practices having relatively moderate disease, have a realistic potential to change the clinical course of COPD. Moreover, there seems to be a great deal of patient-to-patient variability regarding efficacy of inhaled steroids. As a result, it would be highly valuable to identify phenotypic disease markers (clinical or pathophysiological) that can distinguish which patients with COPD experience the greatest benefit by inhaled corticosteroids. Previous studies have suggested that smoking cessation (34;41;42), larger bronchodilator response (34;35;43), airway hyperresponsiveness (43-45), and eosinophilic airway inflammation (46;47) may be able to predict a beneficial response of steroid treatment in patients with established COPD. However, larger long-term prospective studies should confirm whether these markers can predict the effects of inhaled steroids on FEV_1 decline.

Clinical benefits of ICS in COPD may be mediated, at least partially, by their anti-inflammatory efficacy. Short-term (2-3 months) ICS treatment in COPD was previously shown to reduce numbers of bronchial mast cells, but not $CD8^+$ cells, neutrophils or macrophages (48;49), cells that are characteristic for COPD. We observed differential effects of ICS on inflammatory cell numbers with long-term treatment. Bronchial T-cells, mast cells and sputum neutrophils, macrophages, and lymphocytes were reduced, whereas bronchial neutrophils and macrophages were not (chapter 6). Although smoking may reduce corticosteroid responsiveness (50), our data show that at least part of the inflammation in COPD is not insensitive to this treatment. The contribution of $CD8^+$ cells to inflammation and the relevant antigen-specific triggers (e.g. microbial or autoantigens) in COPD are still unknown. $CD4^+$ cells may contribute to activation and memory formation of $CD8^+$ cells as well as

providing help for B cells (51). Mast cells and their secreted enzymes can drive a variety of processes relevant to inflammation and remodeling, including fibrosis, extracellular matrix turn-over, angiogenesis, smooth muscle and epithelial hyperplasia, and hypersecretion (52). This aspect requires follow-up studies including inflammatory cell activity in addition to cell numbers as outcome parameter. Notably, the observed increase in intact epithelium by long-term corticosteroid treatment has also been found in asthmatic patients (53). This might be due to alterations in inflammation and/or in fragility of the epithelium secondary to changes in epithelial integrity, apoptosis or proliferation status. We can exclude the latter since we did not find such treatment effects within the intact epithelium, or effects on mucin stores, squamous cell metaplasia or EGFR expression. Interestingly, corticosteroid-induced changes in epithelial integrity (and inflammation) correlated with improvements in PC_{20}, supporting the notion that airway hyperresponsiveness in COPD can be a marker of disease activity (40;54).

The reduction in $CD3^+$ cells and mast cells and the functional benefits are reversed after stopping inhaled steroids (chapter 6), indicating that the anti-inflammatory effects are not persistent and that those processes inducing ongoing inflammation in COPD are not affected permanently by steroids. This suggests a role for continuous and long-term treatment with inhaled steroids in COPD as opposed to intermittent treatment. We cannot infer from our study how fast inflammation increases after stopping steroids or whether duration of treatment influences this process. Previous biopsy studies included patients with COPD who withdrew for at least 4-8 weeks from inhaled steroid treatment before entry, and therefore may have interfered with the effects of stopping steroids on inflammation (44;48;55). The next step required when examining effects of corticosteroid intervention in the airways of patients with COPD, is to focus on inflammatory and epithelial cell activity and on airway wall remodeling and fibrosis.

Although both smoking cessation and treatment with inhaled steroids are able to reduce FEV_1 decline in COPD, airway inflammation is partly reduced with steroid treatment whereas it persists after smoking cessation. In addition, whereas smoking cessation reverses epithelial remodeling, treatment with inhaled steroids does not. These apparent discrepancies indicate that other factors, next to inflammatory cell numbers and epithelial remodeling, play a role in lung function decline in COPD. These may include for instance activity of inflammatory cells, remodeling of the airway wall (matrix remodelling and airway smooth muscle hyperplasia/hypertrophy), or alveolar destruction. It should be noted however that the observed discrepancies may also be a consequence of methodological differences between the studies on smoking cessation and inhaled steroid treatment in the present thesis (i.e. retrospective versus prospective, time effect).

Limitations of the studies and methodological considerations

The patients recruited for the GLUCOLD study had to meet the in- and exclusion criteria as discussed in the introduction. These comprised: GOLD stage II and III, no maintenance treatment with inhaled steroids in the past 6 months, no asthma, willing to undergo three bronchoscopies, and participate in a long-term follow-up study with 3-monthly outpatient clinic visits. It turned out to be difficult to find these patients, because the majority of these patients diagnosed with COPD already were treated with inhaled steroids. Therefore, we had to change our recruitment strategy to find those patients who were not yet diagnosed with the disease. It took 2.5 years of intensive recruitment in outpatient clinics and general practices, including approximately 40,000 letters send to potential candidates and 3600 lung function screenings in Leiden only. Finally, we included 114 patients in the study, which was less than the aimed number, but still one of the largest studies including bronchial biopsies world-wide. Steroid withdrawal has shown to result in detoriation of clinical as well as inflammatory parameters in COPD (55-57) (chapter 6). Therefore, we believe that the fact that we included mainly steroid-naïve patients is one of the strengths of the study. It should be taken into account that COPD is a heterogeneous disease in terms of clinical, physiological and pathological presentation. As a result of our inclusion criteria we selected a specific subgroup of the total COPD population, and therefore our results may not be representative for COPD in general.

It needs to be emphasised that the studies on smoking cessation (chapter 4 and 5) were cross-sectional studies, and it cannot be ruled out that our ex-smoking group is a selected group of patients who quit smoking because they suffered more from smoking related symptoms, and may already have had different cell numbers before quitting. Nevertheless, ex-smokers had significantly less respiratory symptoms than current smokers, whilst having similar pack-years and duration of smoking. In addition, in the analysis we did adjust for clinical differences between the groups.

Bronchoscopy with biopsy and induced sputum access the proximal bronchi only, and we have therefore not been able to assess the effects of therapy on small airways and lung parenchyma, the site considered to contribute most to reduced lung function in COPD (2). However, the predominance of $CD8^+$ cells is seen in both proximal and small airways and the correlation between this cell type and impaired lung function is similar in both large and small airways and lung parenchyma (2;3). This and other data (58) suggest that similar processes of inflammation and airway wall thickening appear to be taking place in both large and small airways. Thus, biopsy samples of large airways may be a reasonable surrogate for assessing the potential effects of treatment on small airway inflammation and

remodeling. Additionally, most studies examining peripheral airways pathology have used resection material from patients with lung cancer. It may well be that airway inflammation is influenced by these malignant processes in the airways. We attempted to investigate treatment effects on peripheral airways inflammation assessed in bronchoalveolar lavage fluid. Unfortunately, because of ethical considerations, the BAL procedure was discontinued (i.e. a few patients reported side effects in relation to the BAL) and therefore it was performed only in the first half of patients. An alternative approach of studying treatment effect on smaller airways pathology could be using ultra thin bronchoscopes.

A fully automated image analysis system was applied for cell counting in airway area sections (59). We are aware that counting cells in a 2 dimensional manner has limitations, since it does not take into account the volume of the cell in a given sample – the smallest cells have the least chance to be counted in a single biopsy. Nevertheless, we were able to demonstrate differences in the smallest cells (lymphocytes) in our studies. There is still debate in the literature whether the theoretic basis of stereology fits well with the limitations of endobronchial biopsies (60). Still, most of the present data in the literature is based on counting profiles/area, which allows, although somehow limited by other methodological factors, comparison among studies.

Directions for future research

The studies described in this thesis have provided more insight into the role of airway inflammation in the pathogenesis and treatment of patients with COPD. However, many issues remain to be explored. Interesting questions for future studies may include:

- Are airflow limitation, airway responsiveness, and airway inflammation in bronchial biopsies separate entities underlying the pathophysiology of COPD by using factor analysis?
- What is the natural course of airway pathology in relation to FEV_1 decline within COPD patients in a prospective long-term study? (this is currently ongoing)
- Can the long-term clinical and pathological course of COPD in patients with usual medical care, in a long-term follow-up study of patients from the GLUCOLD study, be predicted by phenotypic disease markers of cellular, physiological and clinical origin? (this is currently ongoing)
- What is the effect of long-term smoking cessation on inflammatory cell activity and airway remodeling in COPD?
- What is the effect of long-term treatment with inhaled corticosteroids on inflammatory cell activity and regulation of matrix remodelling and airway smooth muscle hyperplasia/hypertrophy in airways of COPD patients?
- Is it possible to predict which COPD patients benefit from treatment with inhaled corticosteroids? (this currently ongoing)
- Which non-invasive markers are useful for monitoring COPD patients and treatment effects?
- Can development of novel treatments for COPD result in reversal of disease progression and reduction in mortality?
- How can the apparent discrepancy between beneficial effects of smoking cessation and ICS on lung function decline in COPD, but differential effects on airway inflammation and epithelial features be explained?

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