Inflammation in chronic obstructive pulmonary disease: its assessment and the effects of corticosteroids
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Summary
The studies in the present thesis were performed to address some questions concerning the development and treatment of Chronic Obstructive Pulmonary Disease (abbreviated as COPD).

In Chapter 1 a general introduction is given on the inflammatory reactions in COPD, their relationship with long-term cigarette smoke exposure and its probable contribution to the development of COPD. As a likely consequence of these inflammatory reactions, irreversible damage is inflicted to the airways, which becomes apparent as a decline in lung function. This decline is faster than the ageing-related decline in subjects who do not smoke. Additionally, current treatments of COPD are described, with a focus on influencing inflammation and with a focus on the clinical effects of corticosteroids. Also, the aims of the studies, presented in this thesis, are described.

In Chapter 2 the results of a randomized, controlled, cross-over corticosteroid-treatment study are described. Patients with a significant smoking history, having mild to severe COPD, limited reversibility in airflow limitation, and absent history of asthma or allergy, were studied. This study showed that treatment for 6 months with the inhaled corticosteroid budesonide had a small effect on lung function and modestly affected eosinophil count in induced sputum. The study also showed that improvements in lung function went in parallel with a decrease in inflammation. Of particular interest was the observation that in the presently studied group of patients, those with the best lung function (and those with the largest reversibility) improved most on inhaled corticosteroid therapy. This contrasted with international guidelines, such as GOLD, which state that mainly patients with a lung function (FEV\textsubscript{1}) below 50% of predicted will benefit from long-term inhaled corticosteroid treatment and thus should receive such treatment. A lower incidence of exacerbations was observed during active inhaled corticosteroid treatment compared to placebo, though the difference did not reach statistical significance. Of all exacerbations, the proportion requiring medical intervention was significantly lower during budesonide treatment. The effect on COPD exacerbations may be a consequence of reducing airway eosinophilia.

A short course of treatment with a systemic glucocorticosteroid was not able to identify patients who benefited later from inhaled corticosteroid use. The effects of the prednisolone treatment on inflammation in individual patients did not predict the effects of long term inhaled corticosteroid treatment on inflammation or on clinical outcome parameters.

In an addendum a small sub-study is described. One of the explanations for a limited effect of corticosteroids in COPD may be the relatively high presence of the non-functional β-isoform of the glucocorticosteroid receptor, compared to the functional α-isoform. Before and after the systemic corticosteroid treatment the quantity of mRNA of the two isoforms was assessed in cells obtained by bronchoalveolar lavage (mainly alveolar macrophages) and by brushing the airways (mainly bronchial epithelial cells). It
appeared very difficult to obtain enough material to make a reliable estimate of the two isoforms of the glucocorticosteroid receptor. In a minority of the samples the α-isoform was quantified. The mRNA of the β-isoform could not be quantified in any of the samples and was therefore assumed to be present in at least a 18-fold lower quantity than the α-isoform. This observation implies that the presence of the non-functional β-isoform could not form an explanation for the relatively corticosteroid insensitivity of COPD.

In **Chapter 3** the results are shown of an in depth analysis of repeatability and variability of assessing inflammatory markers in induced sputum. Data were used from the prospective study on the effects of inhaled and systemic corticosteroids, which is described in Chapter 2. The analysis showed, that repeatability of assessing inflammatory parameters in induced sputum (obtained twice within one week and processed as the whole sputum sample) was good, and comparable to the previously reported repeatability of sputum, processed by the sputum plug method. Within-subject variability was approximately two-fold to three-fold for most parameters, and between-subjects variability was approximately two-fold higher than within-subjects variability. Long-term repeatability, using samples, obtained at 3 and 6 months placebo treatment, was only slightly less than the short term repeatability. From the present data extrapolations were made for the power calculation of intervention studies in COPD and it was concluded that minimally 20 patients are required for detecting a two-fold decrease or increase in an inflammatory marker in induced sputum.

In **Chapter 4** the assumption was challenged, that those induced sputum samples, containing more than 80% squamous epithelial cells, should be considered invalid for analysis. Such samples are generally considered contaminated with saliva or other fluids, while only samples with a lower % squamous cells are considered valid. Data from the prospective corticosteroid study in COPD was used (Chapter 2), together with data from patients with asthma, where sputum was collected alongside a study, investigating effects of treatments and allergen provocations. That level of 80% epithelial cells turned out to be too arbitrary, the present analysis showed that differential cell counts in samples with up to 90% squamous cells can be used. This adaptation will decrease the number of discarded sputum samples.

Additionally, the % of non-squamous epithelial cells in an induced sputum sample was shown to be a quantitative measure of contamination (and thus dilution) of the sputum sample, leading to a proportional decrease in the level of the soluble inflammatory markers and in absolute cell counts in the sputum samples. From the linear relationship between the levels of the inflammatory markers and the proportion of squamous cells within the sample a formula was developed allowing correcting for this dilution. While applying this correction, variability of inflammatory data (as described in Chapter 3) decreased and repeatability improved. When applied, these adaptations may improve the power of future studies, investigating induced sputum.
In Chapter 5 a retrospective statistical analysis was done on the characteristics of the patients who developed an exacerbation, early in the prospective study, described in Chapter 2. This analysis was done after observing that an unexpected high proportion of patients experienced a deterioration of their disease in the run-in phase of the prospective study. This deterioration ended in many patients in an overt COPD exacerbation. Of importance, in several patients this resulted in withdrawal from the prospective study. In that run-in phase of the study, previously clinically stable patients were followed for two months, while their inhaled corticosteroid treatment (which was given to the majority of patients before the study) was stopped. This occurrence of exacerbations could have been a chance finding, since patients had to be stable at enrolment in the study for at least a month prior to enrolment and the run-in period lasted 2 months. With a frequency of 1 exacerbation per year for an average COPD patient, there is a fair chance that some patients will have an exacerbation by chance alone during the two-months run-in period. But since the incidence of exacerbations was much higher than expected and since in all patients it occurred within the first 4 of the 8 weeks of that run-in period this explanation was rejected. Patients with most severe disease (the lowest lung function) had the highest chance of such an early exacerbations and the eosinophilic inflammation (in sputum and in peripheral blood) was most pronounced in the patients who developed an exacerbation in the 4 weeks thereafter. Seven of the 20 patients who received inhaled corticosteroid treatment before the run-in period experienced an exacerbation, compared to only one of the nine patients who received no inhaled corticosteroid treatment. This difference was however not statistically significant.

The selective withdrawal of a specific type of COPD patients in the run-in period may have had an important influence on the effects observed under the subsequent study treatments, since it is likely that those patients, being most corticosteroid-sensitive, were excluded from participation, leaving the least responsive patients in the long-term study. This selective withdrawal may therefore have reduced the potential for detecting beneficial effects of the corticosteroid treatments.

In Chapter 6 the results of a study are described, in which the lung tissue concentrations of two inhaled corticosteroids were determined: budesonide and fluticasone propionate. Patients, subjected to lung surgery, inhaled a single dose of the inhaled corticosteroids prior to surgery and the corticosteroids were assessed in the parts of the lungs which remained after the operation. In addition, budesonide-esters were determined, compounds which were believed to be formed within the airway epithelium and which prolong the presence of budesonide in airway tissue. The study showed that both corticosteroids are detectable in lung tissue for many hours after inhalation and in concentrations high enough to occupy glucocorticosteroid receptors. Budesonide and budesonide-esters were detectable up to 10 hours and 43 hours, respectively, and fluticasone propionate up to 22 hours after inhalation. This enables a twice daily dosing of the inhaled corticosteroids and potentially a once daily dosing for budesonide, while
maintaining tissue levels high enough to influence inflammation. Though the single dose of the corticosteroids was rather high (1000 μg), during the steady state situation in the study in Chapter 2 a maintenance dose of 400 μg budesonide twice daily was given, and tissue concentrations of budesonide and budesonide-esters can be assumed to have been in the same order of magnitude and thus of clinical relevance.

In Chapter 7 the results of a study are described, in which the relation was investigated between the presence of cytotoxic T-lymphocytes in lung tissue and the development of COPD. Lung tissue of patients (ex-smokers and current smokers) with an isolated malignancy in the lung was investigated postoperatively. Though only a minority of the selected (ex-)smokers was already known with a clinical diagnosis of COPD, 41% had COPD based on lung function criteria, which is much higher than the generally quoted 15% to 20%. A higher number of cytotoxic CD8+ T-lymphocytes in the airway mucosa was related to lower lung function values, confirming earlier studies. Moreover, both the presence in the airway mucosa of CD8+ T-lymphocytes expressing granzyme B and the level of granzyme B in induced sputum were also related to loss in lung function. This may point to granzymes, released into the airway lumen, having an important role in tissue remodeling and hereby to the development of COPD.

In Chapter 8, the general discussion, these observations are discussed in more detail and are brought into a broader perspective.

In conclusion, the present studies confirm other studies in which inhaled corticosteroids were shown to have some, be it modest, effect in COPD, both concerning clinical effects as well as concerning an effect on airway inflammation. Small study sizes limits the potential for obtaining a statistically significant treatment effect in many studies, including the present study, and larger scale studies are required to make a more correct estimate of the effects of maintenance treatment. These studies must also be used as a tool in better characterizing patients who will benefit most from the therapeutic interventions. By applying strict inclusion criteria in clinical studies, such as those on (reversibility in) lung function, and by excluding the potentially most corticosteroid sensitive patients already at the start of the intervention studies (during corticosteroid withdrawal), these studies have limited possibilities to obtain significant treatment effects. Additionally, by these strict inclusion criteria the subsequent extrapolation of the research findings to the general population of COPD patients is limited. Inflammation in COPD is very heterogeneous and many cells and mediators are playing an important role in COPD, most prominently neutrophils, eosinophils, lymphocytes and proteolytic enzymes such as neutrophil elastase and the granzymes. Long term prospective studies rather than cross-sectional studies are required to fully elucidate the role of these cells and mediators either being secondary or the real cause in smokers of developing the devastating disease COPD.