Summary
Summary

This thesis describes the results of two clinical trials: the Symbexco trial, in which the anti-inflammatory effects of inhaled budesonide/formoterol in treatment of COPD exacerbations were investigated, and the “CO in COPD” trial, in which the anti-inflammatory capacity of inhaled carbon monoxide in stable COPD was explored.

In Chapter 2, we reviewed the literature on airways inflammation and treatment of exacerbations of COPD. We found that the sputum counts of eosinophils, neutrophils, and lymphocytes are increased during COPD exacerbations, and that there are associations between the cell type that is increased and the cause of the exacerbation. Eosinophils are more increased in exacerbations caused by a virus, whereas neutrophils are more elevated in exacerbations of bacterial aetiology. Lymphocytes seem to play an important regulatory role in exacerbations; however, literature so far has provided little insight in the precise inflammatory mechanisms.

We reviewed the anti-inflammatory drugs that were studied for their efficacy in treating COPD exacerbations. We concluded that only oral steroids have been proven successful until now. However, the effects of oral steroids are modest, and oral steroids do have important systemic side effects. The use of inhaled steroids (in combination with long acting bronchodilators) has been reported as an alternative to systemic steroids in the treatment of exacerbations with less potential for systemic side effects. For the future treatment of COPD, we found that several more drugs aiming at novel targets are in development for stable COPD. A further step will be to test some of these drugs also in COPD exacerbations.

We concluded in our review that further research is required to more fully understand the inflammatory mechanisms in the onset and development of COPD exacerbations. This may make inflammatory pathway-specific interventions possible, resulting in a more effective treatment of COPD exacerbations with fewer side effects.

In Chapter 3, we described the results of our evaluation of whether the induction of sputum is safe during COPD exacerbations. Sputum induction is a validated method to acquire information on airway inflammation. During sputum induction, patients inhale nebulised saline of various concentrations, by which the coughing-up of sputum is facilitated. Furthermore, the samples gained by sputum induction are of a better quality compared to spontaneously produced sputum.

Unfortunately, some patients develop a bronchoconstrictive reaction to inhaled saline. Therefore the forced respiratory volume in 1 second (FEV₁) of each patient was monitored after each step of saline inhalation during the sputum induction. With this close monitoring, sputum induction is generally considered safe, even in patients with COPD with a severe airway obstruction. To the best of our knowledge, the safety of sputum induction during exacerbations of COPD...
had however not been assessed. To do so, we analysed our data on safety and bronchoconstriction during exacerbations, and compared this to the data of the same patients during sputum induction in a stable phase.

We found that considerable decreases in FEV$_1$ can occur, but they are sustained well. The decreases in FEV$_1$ by sputum induction during an exacerbation are of similar absolute magnitude or even smaller than in the stable phase. Monovariate analysis showed that a larger decrease in FEV$_1$ due to sputum inductions during exacerbations can be predicted by a larger decrease in FEV$_1$, a lower sputum total cell count, and a higher eosinophil percentage in the induced sputum differential count during the stable phase of COPD. The sole independent predictor of the fall in FEV$_1$ during sputum induction in COPD patients with an exacerbation was the decrease in FEV$_1$ during sputum induction during a stable phase. We concluded that induction of sputum in mild to moderate exacerbations of COPD can be performed as safely as during stable phase, using a cautious protocol in patients with severe COPD.

In Chapter 4, we studied the effects of steroid withdrawal in patients with COPD in a stable phase. In the Symbexco trial, inhaled steroids were withdrawn for methodological reasons: we wanted to avoid that the anti-inflammatory effects of inhaled steroids influenced the baseline measurements. Two months after the steroid withdrawal, we performed baseline measurements. We noticed that many patients did not sustain this withdrawal well: their FEV$_1$ decreased, and quality of life worsened. This has been reported in other trials. We investigated which inflammatory mechanisms were associated with the worsening of FEV$_1$ and quality of life. We found that sputum eosinophils were increased after steroid withdrawal, and that this increase was associated with the worsening of FEV$_1$ and quality of life.

In Chapter 5, we studied the change in inflammation from a stable phase of COPD to an exacerbation. We found a general increase in airway inflammation during COPD exacerbations: sputum eosinophil, lymphocyte, and neutrophil numbers all rose compared to the stable phase of the disease.

There are various causes which lead to exacerbations of COPD: viral, or bacterial airway infections, or exposure to air pollution have been identified as causes. We investigated whether these causes are associated with different inflammatory cellular patterns. We found that some inflammatory markers, specifically sputum LTB4, MPO, IL-6, TNF-$\alpha$, and serum CRP and IL-6, which are commonly associated with exacerbations, are increased during bacterial exacerbations, but little or not increased at all during non-bacterial exacerbations.

It is important to identify a bacterial cause of exacerbation, since these exacerbations should be treated with antibiotics. Sputum cultures are often used in the decision to prescribe antibiotics. The sputum gram staining, which can be determined immediately, is often not specific enough for this purpose, whereas
waiting for the results of sputum culture takes about 5 days, which is too long. Therefore inflammatory markers, if specific and sensitive for a bacterial airway infection, would be of clinical use. Using receiver operated curves, we determined the predictive values of those markers we that we had found to be increased in bacterial infections. We concluded that sputum TNF-a has the best predictive values to differentiate a bacterial infection from a non-bacterial cause of exacerbation.

In Chapter 6, we described the results of a study of the treatment of exacerbations: the Symbexco study. COPD exacerbations are generally treated with oral steroids and increased use of bronchodilators. As described earlier in the summary of Chapter 2, the effects of oral steroids in COPD exacerbations are modest, and come with considerable systemic adverse effects. It would be a step forward if we could treat patients with steroids avoiding these systemic adverse effects. Inhaled steroids have been reported to cause less systemic effects. Therefore, we explored the anti-inflammatory and therapeutic effects of inhaled budesonide/formoterol. We compared the effects of inhaled budesonide/formoterol to placebo, and to oral prednisolone, primarily on sputum eosinophilic inflammation during COPD exacerbations. Furthermore, we compared effects of these treatments on lung function parameters, respiratory symptoms, quality of life, treatment failure and adverse events.

We found that treatment of COPD exacerbations with high dose budesonide/formoterol reduced sputum eosinophils compared to placebo and resulted in an improvement in respiratory symptoms. Prednisolone treatment also reduced airway inflammation and respiratory symptoms. Treatment with prednisolone resulted in a suppression of plasma cortisol levels. Our study was not designed to determine whether budesonide/formoterol treatment is as effective as prednisolone treatment, which would require a larger study population. We concluded that future studies should prove this non-inferiority, before the standard prednisolone treatment can be replaced with inhaled budesonide/formoterol treatment.

In Chapter 7, we explored the anti-inflammatory effects of inhaled low dose carbon monoxide. Both in vivo and in vitro studies have shown strong anti-inflammatory effects of carbon monoxide. Next to this, carbon monoxide has relaxing effects on the airway smooth muscles, since it is a neurotransmitter via cyclic GMP causing bronchodilation in the airways. Additionally, carbon monoxide is a powerful anti-oxidant. Since inflammation, oxidative stress, and airway obstruction are important features in COPD, we decided to explore the therapeutic capacity of carbon monoxide in this disease.

The first objective of the study was to assess safety and feasibility of inhalation of low dose carbon monoxide by COPD patients. We titrated the dosage of carbon monoxide at carboxyhemoglobin (COHb) levels below that "achieved" by smoking 20 cigarettes a day where the 24 hour average COHb levels reach 5.3%, with peaks above 6%. A protocol of 100 ppm CO for two hours had been shown to lead to COHb levels of approximately 4%. Therefore we explored the therapeutic effects of CO. The inhalation of 100 ppm led to a maximal COHb
level of 3.1% in our patients with COPD and the highest COHb level reached with 125 ppm was 4.5%, which is in the range of the maximal COHb values we had expected.

We did not find a significant effect of the inhaled CO on vital signs. One patient reported haemoptesis. This patient had a long history of frequent haemoptesis of unknown origin before this trial. There were two exacerbations in the CO periods; one patient experienced a COPD exacerbation starting on day 4 of CO inhalation, 18 hours after the last inhalation. A severe exacerbation occurred in another patient, 2 months after the last inhalation. Both patients had experienced regular exacerbations in the past. We concluded that inhalation of low dose carbon monoxide is feasible. Future studies should assess whether the adverse events during treatment with carbon monoxide were unrelated to the CO inhalation, which we speculate, or caused by the CO inhalation itself.

The second objective of this pilot study was to explore effects of inhaled carbon monoxide on inflammation. We found that inhalation of low dose carbon monoxide results in a trend of reduction of airway inflammation, specifically eosinophils, and responsiveness to methacholine. We conclude that our data on these effects are very useful to design larger studies, which may confirm the trends we found.