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

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Chapter 8
General Discussion
General Discussion

Inflammation and chronic obstructive pulmonary disease (COPD) are still not well-understood and there is no real cure of COPD to date. The studies, described in this thesis have added further knowledge on the best use of induced sputum to assess airway inflammation in COPD non-invasively, on the way CD8+ T lymphocytes contribute to the pathophysiology of COPD, on the anti-inflammatory and clinical effects of inhaled corticosteroids and on the relation between the latter two.

Similar to previous observations, in the present studies the magnitude and nature of the inflammatory reactions in the airways of smokers and of patients with COPD were found to be related to the degree of airflow limitation. It must, however, be kept in mind that this way of classification of severity, solely on the FEV1 as % of predicted may underestimate the rate of decline in airflow limitation in relatively young subjects, since a FEV1 of 60% at an age of 45 indicates a more rapid decline compared to the same 60% in subjects aged 70. Within this limitation, in the studies in this thesis, FEV1 as % of predicted was found to be correlated with the % of neutrophils in induced sputum (Chapter 2), with the number of CD8+ T-lymphocytes in airway mucosa and with the level of granzyme B in sputum (Chapter 7 for the latter two). These associations do not provide proof for a cause (inflammation) - effect (low FEV1) relationship. Direct evidence of such a kind can only be obtained in prospective, longitudinal studies in subjects at risk (i.e. smokers) or patients with established COPD, looking for (changes in) inflammatory parameters that precede or predict the (further) development of airflow limitation. Within such a design also the rate of decline in FEV1 may be estimated, accommodating the other limitation mentioned above. More indirect evidence should also come from intervention studies focusing on simultaneous changes in clinical and inflammatory parameters, for example as was recently shown for surfactant protein D. In the small-sized budesonide intervention study, described in Chapter 2, no relation between baseline inflammatory characteristics and clinical effects of 6 months’ treatment with inhaled budesonide was observed. Nevertheless, despite the small size of that study, improvements in lung function and in some inflammatory parameters went in parallel. Statistically significant relationships were found for sputum % eosinophils, IL-8 and ECP (for % eosinophils shown in Figure 1 below).

![Figure 1](image.png)

**Figure 1.** Relationship between change in % eosinophils in sputum and change in postbronchodilator FEV1 in % predicted after 6 months budesonide treatment compared to placebo (rho = -0.57, p=0.026).
The exact mechanisms behind these clinical effects of inhaled budesonide (as improvement in FEV₁) are not completely known, but possibly include a reduction of airway edema due to vasoconstriction, an improvement in the barrier function of the airway epithelium leading to reduced permeability, and especially a reduced influx of inflammatory cells (such as eosinophils) towards the airway mucosa.

While designing and performing the budesonide treatment study, presented in Chapter 2, several other clinical studies were published, investigating the effect of inhaled corticosteroids on inflammatory parameters in COPD. The results in these studies were partly confirmed by the findings in the study in Chapter 2 and partly showed conflicting data. A later meta-analysis of several short-term studies concluded some effect on sputum eosinophil numbers and sputum neutrophil numbers. In the two largest studies, analyzing both induced sputum and bronchial biopsies, reduced inflammatory cell numbers (especially lymphocytes) in the airway mucosa were observed, and some effects were observed on inflammatory parameters in sputum (reduced numbers of neutrophils and eosinophils). It is tempting to conclude that these effects of inhaled corticosteroids on inflammation and on clinical outcomes have a cause-effect relationship, but proof for this is lacking.

Clinical effects of corticosteroids in COPD

Simultaneous with the conduct of the studies in this thesis, other studies were published, investigating lung function in COPD patients during treatment with inhaled corticosteroids. These studies showed that such treatment is firstly capable of improving lung function during the first few months of treatment and secondly of partly affecting the long term decline in lung function. This latter effect was later estimated to be approximately one third of the additional COPD- or smoking-related decline on top of the physiological age-related decline. Inhaled corticosteroid treatment was also shown to affect the incidence of COPD exacerbations and was suggested to affect long-term mortality in COPD.

There is a strong heterogeneity in the effects, observed in the series of published clinical studies with inhaled corticosteroids in COPD. A few of the possible explanations for this heterogeneity (discussed in more detail below) are: the dose of the inhaled corticosteroids may have been too low; the inhaled drug did not reach the lower airways; there was an imbalance in the ratio of the active and inactive isoforms of the glucocorticosteroid receptor in the airways; the patient group was too small, too heterogeneous or not well-characterized; the study design led to selective withdrawal of treatment-responding patients; certain selection criteria (such as absence of reversibility) restricted the inclusion of responding patients; or the treatment duration was too short to show an effect on long-term decline in lung function. Obviously, one extreme ultimate explanation could also be that the complex inflammatory processes in COPD which lead to tissue destruction,
may actually be (almost) completely unresponsive to corticosteroid treatment, and the occasional positive findings occurred by chance alone.

Clinically, COPD is a heterogeneous syndrome with multiple phenotypes. During the past few years it has become evident that these different phenotypes may respond differently to treatments. By studying unselected or poorly characterized patients the risk of inconclusive results is increased. Thus, the beneficial effects in a certain “responding” subgroup of COPD patients can be obscured by lack of effect in a larger subgroup of non-responders. For example, in clinical studies with corticosteroids the concomitant treatment is restricted, and the use of other corticosteroids is usually temporarily stopped. When this corticosteroid treatment had an effect in some patients, withdrawal of previous medication can lead to disease deterioration, followed by withdrawal from the study and consequently to a biased patient population of predominantly non-responders in the remaining participants in the intervention study. This may have affected the results of the study in Chapter 2 as explained in Chapter 5. Also, strict inclusion criteria concerning reversibility in airflow obstruction will have excluded those patients with some concomitant asthma, but will also have excluded those patients who clinically improved after inhaled corticosteroid treatment, as can be concluded from the study in Chapter 2. Obviously, lack of knowledge on variability of all clinical outcome parameters in the study population and especially an underestimate of this variability will have led to underpowered studies. The study in Chapter 2 may have been underpowered for the detection of effects on sputum eosinophilia, since the study size was determined for the detection of an effect on sputum neutrophils.

In an early meta-analysis of the corticosteroid studies in COPD, available at the time of starting the studies in this thesis, it was suggested that the dose of the inhaled corticosteroids in COPD could have been too low in a number of studies. The dose which was considered to be clinically effective was assumed to be at least 800 μg budesonide per day or an equivalent dose of other corticosteroids. A higher dose was expected to give adverse effects on the long term, and therefore the dose of budesonide applied in later long-term studies as well as in the study in this thesis (Chapter 2) was set at 800 μg budesonide per day.

Inhaled corticosteroids, when given through dry powder inhalers may not reach the lower airways due to insufficient inspiratory flow, generated by COPD patients. In the study presented in this thesis in Chapter 6, we found that the lung tissue levels of two inhaled corticosteroids are higher than those required for adequate glucocorticosteroid receptor binding. The levels remained high for a long time after inhalation, enabling once daily or twice daily treatment for budesonide. It must be noted that besides tissue levels of the parent drug also formation of local metabolites is relevant, since, while budesonide tissue levels decreased, budesonide-esters (which are formed within the epithelium and
gradually release budesonide$^{18}$) remained high for a long time. In contrast, for fluticasone propionate, the suggestion has been made that “tissue levels” do not reflect the amount of drug available for glucocorticosteroid receptor binding, because of the presence of undissolved drug powder particles on top of the airway mucosa, especially in cases of excessive mucus.$^{19}$

One alternative explanation for the limited clinical effects of corticosteroids in COPD may be that in the airways of patients with COPD the glucocorticosteroid receptor exist predominantly in the ineffective $\beta$ isoform, rather than in the active $\alpha$ isoform.$^{20}$ In a small-sized pilot study, performed alongside the efficacy study of inhaled and oral corticosteroids (Chapter 2), the quantity of mRNA of these two isoforms of the glucocorticosteroid receptor was investigated in airway cells (Addendum to Chapter 2): epithelium (obtained by brushing) and alveolar macrophages (obtained by bronchoalveolar lavage), before and after three weeks oral prednisolone treatment. No mRNA of the inactive $\beta$ form of the glucocorticosteroid receptor could be detected. However, based on the lower level of quantification, if present, this $\beta$ isoform would be present in at least 18-fold lower quantities than the active $\alpha$ isoform, indicating that absence of significant clinical effects of corticosteroid treatment in COPD can not be explained by a dominant presence of inactive glucocorticosteroid receptors. These results are in contrast to an earlier report where mRNA of the two isoforms was found in similar quantities.$^{21}$ Likely, the discrepancies are due to large differences in present methodology compared to the previous study.

When planning a clinical study the sample size estimation is always performed with a delicate balance in mind. A too large sample size is unethical and unnecessary expensive, but a too small sample size is likely to provide inconclusive data and on these grounds unethical as well. At first, the primary parameters have to be defined, realizing that for secondary parameters the study may be either over-sized or (more likely) undersized. Lack of knowledge of the primary parameters (e.g. the expected value in the control group, the effects of the intervention and the variability herein) within the study population being investigated complicates the planning of clinical studies and may necessitate trial size adaptation during the conduct of the study.$^{14}$ For example, the long-term decline in FEV$_1$ under placebo treatment in the first long-term study in mild to moderate COPD was 69 ml/year,$^9$ whereas in the most recent study in COPD patients with a much more severe disease (lower FEV$_1$ as % predicted at enrolment), the decline under placebo was much less: 55 ml/year.$^{12}$ Also, in clinical studies investigating the effects of interventions on the incidence of COPD exacerbations usually patients are selected, who already previously experienced exacerbations, or who have a low lung function, in order to increase the exacerbation frequency and to increase the possibility to detect an effect of the intervention with a study population as small as possible. Similar, in studies where an effect is investigated on the long-term decline in lung function, preferentially those
patients are to be selected who have a low lung function as % of predicted, and thus already have experienced an enhanced decline in lung function. These selection criteria of low lung function and past exacerbation frequency in these landmark studies may complicate extrapolation to other patient groups.

Insufficient characterization of the patient population in terms of inclusion criteria will lead to a heterogeneous population and will therefore complicate the interpretation of the results of clinical studies in COPD. In most published studies requirements were COPD-related symptoms, a minimum cigarette smoke exposure and a certain degree of airflow limitation, as well as absence of signs of a concomitant or previous asthma. Concerning reversibility in airflow limitation, different definitions and different levels of reversibility are used, either expressed in ml, in % of the initial value or in % of predicted. The strict selection criteria in most clinical studies have led to exclusion of a large proportion of patients with obvious clinical COPD but with some reversibility. This restriction leads to limitations in generalizing the clinical study findings and application in clinical practice. The study in this thesis focused on a well-defined phenotype of COPD and strict criteria were defined to exclude patients with concomitant allergy, asthma and reversibility; at the same time, patients with a wide range in disease severity were selected (Chapter 2). Overall, the clinical effect of 6 months’ treatment with inhaled budesonide on lung function parameters and symptoms was small and reached statistical significance only for some parameters, most likely because the power calculation was performed for a different parameter (% neutrophils in sputum). The level of improvement in lung function after 6 months was related to the magnitude of reversibility and, unexpectedly, also to the level of airflow limitation. Patients with the best FEV1 at enrolment improved most. These observations indicate, that the large proportion of patients with COPD who do not fulfill the more strict inclusion criteria of the long term intervention studies (usually a FEV1 below 50% or 60% of predicted or limited reversibility) might benefit of inhaled corticosteroids as well or even more.

Another difficult issue in the design of long term intervention studies is the withdrawal of medication in the run-in period. Usually, a run-in period with a minimum of allowed medical treatments is designed to assess correct and “clean” baseline values of several parameters. The data, observed under the subsequent treatment, is compared to this baseline data. However, during withdrawal of corticosteroid treatment, COPD patients may deteriorate clinically and such a deterioration may lead to a COPD exacerbation, requiring medical intervention, leading to withdrawal from the study. In part, this is a statistical dilemma, since with a frequency of one exacerbation per year for COPD patients in general, some exacerbations may occur in the run-in period by chance. The incidence of exacerbations and withdrawals in most studies is however larger than this chance incidence and in the study in this thesis such a high withdrawal was also observed (Chapter 5). The occurrence of exacerbations was found to be related to a prominent eosinophilic inflammation. These
patients, who deteriorate upon withdrawal of corticosteroid treatment in the run-in period, can be assumed to be responsive to corticosteroid treatment. Therefore, in many studies, including the study in this thesis, the withdrawal of these patients will have resulted in a limited potential for observing beneficial effects of the inhaled corticosteroid treatment in the subsequent intervention study.15

The duration of treatment is of great importance in COPD studies since COPD is a slowly progressive disease. The efficacy of treatments reversing bronchoconstriction and symptoms can be analyzed in a matter of weeks, but the effects on the long term obviously need to be studied under long-term treatments. For a correct estimate of the individual decline in lung function over time, many observations over multiple years are required. In the first large-scale long-term study on the decline in lung function it was observed that initially, corticosteroid treatment induced an increase in FEV1 (later referred to as a “shoulder”), peaking at 6 months. A correct estimate of the effect of interventions on the long-term decline has therefore to take place after this shoulder.9;12 In the study in Chapter 2 the effects of inhaled budesonide were studied for six months and thus until the peak of this shoulder. Therefore, no conclusions can be drawn for longer-term treatment, such as whether the present observation that patients with the best lung function at baseline responded most to treatment at 6 months, will also hold for longer term treatment.

Assessing inflammation and anti-inflammatory effects of corticosteroids

The studies presented in this thesis have provided supplementary information on methodological aspects of assessing airway inflammation via collection of induced sputum. Repeatability of inflammatory parameters in whole sputum samples, both on the short-term and on the long-term, has been documented for patients with COPD (Chapter 3). Variability appeared to be large, both between subjects and within subjects. Based on the present data on repeatability and variability of induced sputum parameters, projections could be made for the statistical power of intervention studies. Generally speaking, 20 to 30 patients need to be enrolled in a clinical study in order to document a two-fold increase or decrease in a single inflammatory parameter in induced sputum and much more patients are needed, when the effect size is smaller. Repeatability of inflammatory parameters in whole sputum samples was similar to repeatability, previously documented in sputum samples, analyzed via the selected plugs methodology.23;24 A second important observation on methodological aspects of analyzing induced sputum samples is that the content of squamous epithelial cells within a whole sputum sample is a quantitative marker for contamination of the sputum sample with other fluids from the throat, mouth or nose (Chapter 4). In most studies, when the % of squamous cells within the total cell count in a sputum sample was below a certain cut-off value (usually 80%), the sample was used in the analysis, when % squamous cells was higher, the sample was discarded. Knowing that this % of squamous epithelial cells is a quantitative marker of contamination, this % of squamous epithelial cells could be used to correct
the observed values of inflammatory parameters for this contamination. This correction was applied, consequently, repeatability was enhanced and variability reduced in two sets of data: the data on repeatability in Chapter 3 and the data from a separate study in patients with asthma. At low % of squamous cells, the magnitude of contamination is limited and correction not necessary, but at higher % squamous cells, the magnitude of contamination can be very high. The differential count within the non-squamous Total Cell Count was not affected by contamination and the study in Chapter 4 showed that a differential count can be made, up to a value of 90% squamous epithelial cells, instead of up to other, arbitrary, cut-off values such as 20%, 50% or 80%. Herewith a smaller number of samples is rejected and discarded as being invalid for statistical analysis of differential cell counts.

At present, based on the studies presented in this thesis, no specific marker within the multitude of cellular and soluble markers has been identified which can be related with certainty to the clinical effect of inhaled corticosteroids. However, two cell types in the lungs and airways appear linked in some way to the clinical effects of inhaled corticosteroids: eosinophils and CD8+ T-lymphocytes.

Eosinophils, since increased numbers of these cells in airway specimen are related to the incidence of exacerbation of COPD and higher numbers of eosinophils seem to be related with an imminent exacerbation (as shown in Chapter 5). Also, since the incidence of exacerbations is linked with the prognosis of the disease and since in the study in Chapter 2 a decrease in sputum eosinophil numbers was paralleled with an improvement in FEV1. Importantly, inhaled corticosteroids are able to reduce airway eosinophilia as well as exacerbation frequency, this may be linked.8,26-28 Additionally, a treatment algorithm directed at controlling sputum eosinophilia is able to decrease the frequency of exacerbations in COPD.29

CD8+ T-lymphocytes, since in a large number of studies (including the study in Chapter 7) an increased presence of CD8+ T-lymphocytes in the airway mucosa was linked to the severity of airflow limitation and since in some prospective studies with inhaled corticosteroids (be it combined with a long-acting bronchodilator) a reduction of the number of CD8+ T-lymphocytes in the airways was observed.4-6 In the corticosteroid intervention study in Chapter 2 of this thesis, no effect on lymphocyte numbers in sputum was detected (data not shown), probably due to the very low numbers of lymphocytes in sputum, additionally, subsets of lymphocytes were not quantified. The importance of CD8+ T-lymphocytes is underlined by the new and interesting observation, reported in this thesis, that the level of the proteolytic granzyme B in the airway lumen, sampled via induced sputum, and likely derived from the CD8+ T-lymphocytes in the airway mucosa, was also related to the severity of airflow limitation (Chapter 7). This granzyme is normally present intracellularly in, among other cells, CD8+ T-lymphocytes. One of the functions of granzyme is lysis of virus-infected cells. Previous studies have found associations between loss of lung function and the number of CD8+ T-lymphocytes in the airways. Our data
confirmed this association and suggest that extracellular release of this proteolytic enzyme could have contributed to remodeling of the airway mucosa and loss of lung function since the number of CD8+ T-lymphocytes containing granules with granzyme B in the walls of small airways as well as the concentration of granzyme B in sputum were related to lung function.

The observations with respect to the importance of CD8+ T lymphocytes and eosinophils contrast to that of neutrophils. Unlike induced sputum, where neutrophils are the most prominently present cell type in patients with COPD, in the airway mucosa of smokers who participated in the study in Chapter 7, similar numbers of neutrophils and lymphocytes were present. In patients with a more severe degree of airflow limitation and a clinical diagnosis of COPD, such as in Chapter 2 and in multiple previous studies, the magnitude of airflow limitation was correlated to the number of neutrophils in sputum. In contrast, in Chapter 7, in (ex-)smokers of whom a minority has COPD, this relationship between sputum % neutrophils and airflow limitation was not observed. Similarly, in the study in Chapter 7 no such relation with airflow limitation was observed for the level in sputum of the proteolytic neutrophil elastase, which is considered to contribute to the pathophysiology in COPD. In most studies, no consistent decrease of airway neutrophilia by inhaled corticosteroids has been found, though a meta-analysis concluded some effect on sputum neutrophil numbers.8 The observation of a significant and large reduction in neutrophil numbers in sputum after inhaled corticosteroid therapy was found in only one earlier study,30 these results may thus also consist of a chance finding. Of importance, this study formed the basis of the power calculation for the corticosteroid intervention study in Chapter 2, in which no reduction in sputum neutrophil numbers was observed.

A “test treatment” for COPD?

In the study in Chapter 2 the effects of a systemic corticosteroid and of an inhaled corticosteroid were studied and compared. Simultaneously, the effects on inflammatory parameters and on clinical parameters were investigated, be it in a relatively small group of patients - though with a specific and well-defined phenotype, selected on the absence of “allergic”, “reversible” and “asthmatic” characteristics. A first observation was that in the studied population, the systemic corticosteroid hardly influenced parameters of inflammation (except for % eosinophils in blood and in induced sputum). Moreover, it did not have a significant clinical effect. The second observation was that inhaled corticosteroid therapy influenced inflammatory parameters to a lesser degree (only a small effect on sputum eosinophilia) than did systemic corticosteroid treatment but did have some clinical effect after six months of treatment. The effect of systemic corticosteroid treatment on inflammation was not predictive for the effects of inhaled corticosteroid treatment, precluding its use as “test treatment” to identify those COPD patients who are more likely to respond to long-term treatment. Neither could patients be identified for whom such treatment had no benefit. Previous studies have documented
that the effects (or lack of effects) of a systemic corticosteroid on clinical parameters appear to be unsuitable to predict the clinical effects of inhaled corticosteroids.\textsuperscript{10,31,32}

In the present study, this paradigm is extended by documenting the effects of the test treatment on inflammatory parameters. It cannot be excluded that with the relatively low number of patients studied, the actual effects of the test treatment were too small to reach statistically significance. However, the actual effects observed with the number of patients studied were that small, so that the effects may be clinically irrelevant. It could also be imagined that in our study either the duration of treatment with the systemic corticosteroid was too short to influence the key steps in the inflammatory cascade to obtain clinical improvement, or that the effects of inhaled and systemic corticosteroid treatments on inflammation in the airways are to a certain level different, due to the potentially very high local concentrations in the target organ after inhalation. The study in Chapter 6 in which lung tissue levels of inhaled corticosteroids were assessed provide some support for the latter explanation. The observed effect of the inhaled corticosteroid on sputum eosinophilia may have a relation with the long-term clinical effect, since the inhaled corticosteroid treatment also influenced (severity of) exacerbations, and these exacerbations went in parallel with increased eosinophilia in sputum.

Future research and treatment

Observing the multitude of inflammatory reactions, the largely irreversible remodeling of the airways, the limited effect of current anti-inflammatory treatments, used so far, the complexity of interactions between the different cells and between the different compartments in which inflammation is being investigated, it will be obvious that there are potentially many ways to influence the inflammatory processes in COPD. However, at the same time it is very unlikely that inhibiting or blocking a single cell or mediator will lead to a disease remission or restoration of remodeling. In such heterogeneous inflammatory reactions, many pathways can be taken as target for intervention in order to potentially influence the disease. Of importance, with existing or newly developed agents that are specific and powerful in vitro antagonists or inhibitors of single mediators, the relative importance of these separate mediators can be elucidated.\textsuperscript{33-35}

Not all tools to assess airway inflammation have been validated in full, such as the analysis of exhaled breath, being non-invasive but requiring further standardization and the assessment of repeatability and variability in COPD. Furthermore, existing tools should be used to investigate differences in inflammatory profile in patients with different phenotypes of COPD, such as recently done for chronic bronchitis.\textsuperscript{36} The next step would be to extend current knowledge on the differences in response concerning clinical and inflammatory parameters based on patient characteristics. Meta analyses have shown that inhaled corticosteroids are able to reduce the long-term decline and to improve the long-term survival of patients with COPD.\textsuperscript{13} These effects were confirmed later in specifically designed studies.\textsuperscript{12,14} Similar meta-analyses should also provide more
insight in the effects of corticosteroids on inflammatory parameters in different patient populations with COPD.

For short-term treatment of symptoms and bronchoconstriction, existing and novel bronchodilators will be used, such as $\beta_2$-agonists and anticholinergics, or a combination of these, with a prolonged duration of action and newer agents with other modes of action. Probably, these compounds will continue to positively modify the quality of life of patients with COPD. Long-term management of COPD will have to focus also on other goals, such as reducing the decline in lung function, prevention of further remodeling of the small airways, prevention of exacerbations, reducing co-morbidity and reducing the mortality, such as associated with hospitalizations for exacerbations. Though activities, directed at a reduction of cigarette smoking, will take a long time to result in a decreased prevalence of COPD, intensive stop-smoking programs should be performed and extended, using both pharmacological and psychological tools, and commencing cigarette smoking in young people should be prevented by all means.

Treatment with corticosteroids likely affects several aspects of COPD. On the short term, reducing edema in the airway mucosa may have a small effect on lung function parameters, this may reflect the modest effect on lung function parameters in the first months of treatment, also referred to as the “shoulder”. On the longer term, treatment with inhaled corticosteroids likely reduces airway eosinophilia. This may both be the constant eosinophilia in some patients and may be the temporary eosinophilia which is observed during COPD exacerbations and perhaps also prior to exacerbations. By reducing the eosinophilia the exacerbation may be reduced in severity and may be handled with fewer therapeutic interventions. And since every severe exacerbation may induce some irreversible damage to the airways and since every severe exacerbation carries the risk for mortality on the short and intermediate term, reducing the occurrence of exacerbation may have induced the observed effects on mortality and the long term decline in lung function.

In the inflammatory cascade multiple targets for intervention can be imagined: prevention of the activation and influx of inflammatory cells, prevention of their degranulation, antagonizing the effects of the granulocyte products, preventing or counteracting the local production of chemotactic factors like TNF$\alpha$, IL-8 and LTB$\alpha$, and of reactive oxygen and nitrogen species and influencing the disturbed proteases/antiproteases balance. Pro-inflammatory cytokines may be blocked using specific monoclonal antibodies. Phosphodiesterases play an important role in several aspects of inflammation. Inhibition of one or more of the various phosphodiesterase subtypes may have a role in stabilizing inflammatory cells and prevent the release of mediators. Theophylline is the classical example of a phosphodiesterase inhibitor, but subtype selective inhibitors have been developed, showing some anti-inflammatory effect. It has also been suggested that
theophylline can be used to reverse that part of the relative refractoriness to inhaled corticosteroids, which is attributed to decreased histone deacetylase activity, caused by smoking or oxidative stress.\textsuperscript{43}

The neutrophilic granulocyte forms a target since its proteolytic enzymes such as elastase are involved in the destruction of the lung parenchyma. β-Adrenergic bronchodilators have been shown in vitro to have some anti-inflammatory effect by stabilizing neutrophils, an effect of uncertain clinical relevance however.\textsuperscript{44} Interestingly, corticosteroids are reported to be relatively ineffective towards neutrophils, at least in vitro. Therefore, influx of neutrophils into the airways may be inhibited by antagonizing one or more of the chemotactic mediators involved.

The imbalance in proteases / antiproteases presents an additional target for intervention.\textsuperscript{45} By preventing further degradation of the extracellular matrix, progression of the disease may be diminished.\textsuperscript{46} In animal studies an inhibitor of metalloproteinases was capable of preventing smoke-induced emphysema and airway remodelling.\textsuperscript{33}

Antioxidants, agents that are able to neutralize reactive oxygen and nitrogen species, either by itself or via an enhanced synthesis of glutathion (like N-acetyl-cysteine or future more powerful agents) have a potential for influencing that oxidative stress.

An effect of these potential agents \textit{in vitro} is no guarantee for an effect \textit{in vivo}, because of the complexity of the inflammatory cascade. Investigating the effects of such specific agents as well as of nonspecific anti-inflammatory agents on a multitude of inflammatory markers may shed light on the cause and effect relationship of the different inflammatory cells and mediators and differentiating the most promising targets from “innocent bystanders”.

Of potentially highest importance for the treatment of individual patients with COPD is the identification of markers, which can be used to predict the patient’s individual prognosis of COPD and/or the effects of therapeutic interventions. This may differ between the different phenotypes of COPD, as characterized on the basis of their inflammatory profile.\textsuperscript{47} Directing treatment of COPD at lowering sputum eosinophilia proved successful in one study.\textsuperscript{29} Monitoring the effects of specific treatments on inflammation in individual patients may provide insight into the differences in therapeutic response between patients and may even be used to optimize treatment in individual patients.\textsuperscript{48} Linking effects on inflammation to clinical outcomes may identify the key steps in the inflammatory cascade as potential targets for intervention.\textsuperscript{49}

With sputum induction being standardized and available for routine use and being a tool to collect information on airway inflammation, it has become feasible to perform large
scale studies, which require multiple centers. Analysis of exhaled breath may provide the same information. Hereby, routine assessment of the inflammatory profile of individual patients may open the gate to individualized treatments, especially with future drugs, able to modulate specific inflammatory pathways. However, until that date treatment should be directed at multiple targets to provide maximal symptomatic relief and an optimal prevention of further deterioration of patients with COPD.
References


