COPD exacerbations, inflammation and treatment
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2007

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Discussion
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Steroid withdrawal in clinical trials

In chapter 3, we described that withdrawal of inhaled corticosteroids (ICS) in patients with stable COPD results in an increase in inflammation and airway obstruction. We furthermore explored the inflammatory mechanisms possibly responsible for these effects. These detrimental effects occurred even when the patients seemed clinically stable since patients experiencing an exacerbation were treated accordingly and left out of this analysis. These effects of steroid withdrawal have been described in several studies (1;2), but there is little insight on what causes this deterioration. We found that particularly sputum eosinophils increased after steroid withdrawal, which was accompanied by worsening of airway obstruction and quality of life.

Many studies have a run-in phase, in which ICS are withdrawn to avoid the bias that the baseline airway inflammation is variably modified by different ICS usage. New trials should reconsider this approach, for several reasons. Firstly, withdrawal by itself also induces bias: withdrawal of ICS induces differences in baseline inflammation, as not all patients respond the same to ICS withdrawal. Secondly, many patients exacerbate soon after withdrawal. Frequently, these patients are then excluded from the trial, resulting in a selection bias of patients towards a group with less tendency to deteriorate after ICS withdrawal. A third consideration is the dilemma whether it is ethical stop ICS with a subsequent increased risk of exacerbations for the sake of a study. These patients become more symptomatic, and may need additional (systemic) steroid treatment with associated potential side effects. For real life (not experimentally induced) exacerbations it has been demonstrated that patients sometimes do not fully recover form exacerbations to pre-exacerbation levels particularly for peak expiratory flow values (3).

ICS withdrawal is applied to obtain equal baseline values in COPD patients, and as mentioned above, this is a pitfall. Should we then investigate other ways that avoid steroid withdrawal? Reduction of steroid treatment in all patients to a similar low dose maintenance treatment may be a solution to this problem. But again a similar dose of ICS may have different effects in an individual patient with COPD. Another option is a gradual withdrawal of steroids, which might results in less deterioration of inflammation and respiratory symptoms. Other ways around this problem would be stratification for baseline steroid use, or even an increase in steroid use to a high dose for all study participants. Future studies should investigate if these options improve the methodology of trials and reduces symptoms for patients.

Non-invasive measurement of inflammation during COPD exacerbations

In chapter 4 we showed that it is safe and feasible to study the cellular patterns of airway inflammation by sputum induction using a hypertonic saline solution.
even during acute exacerbations of COPD. We assessed the safety in mild to moderate exacerbations that did not require hospitalisation. Sputum inductions have been assessed in several settings, but there is little data on safety (4-6). These studies did not all use the same induction protocol (i.e. duration, concentration and number of nebulised saline inhalations), which makes it difficult to compare both the safety and the results of the various studies on airway inflammation during COPD exacerbations. It would be worthwhile to study the various protocols systematically, in order to reach consensus on a universal protocol for sputum induction during COPD exacerbations with the best properties with respect to safety and a reproducible reflection of the cellular airway inflammation.

Not only inflammatory cells, but also non-cellular biomarkers provide information on airway inflammation. Thus far, cytokines have given us insight in the mechanisms that play a role in COPD in a stable phase and during exacerbation. However, the measurement of cytokines in sputum of COPD patients plays little to no role in current clinical practice.

We foresee a potential future role for measurement of cytokines during COPD exacerbations in the decision to prescribe antibiotics: in chapter 5, we have shown that sputum biomarkers might be useful to distinguish a bacterial from a non-bacterial origin of exacerbation. C-reactive protein (CRP) and procalcitonin are the most elaboratively investigated markers for this purpose. CRP has been evaluated as a marker to define an exacerbation, but was only useful in combination with a major exacerbation symptom. Procalcitonin-guided therapy has been shown to reduce antibiotics prescription, however the marker does not have ideal predictive characteristics of bacterial infections in COPD exacerbations, since both its specificity and sensitivity appear to be poor (7-9). From our data, sputum tumor necrosis factor-a seemed to be a potential marker to identify a bacterial cause of exacerbation. However, our study population was not large enough to provide firm data on its usefulness or on the best cut-off point. Nevertheless, if the favourable predictive value for a bacterial infection of sputum tumor necrosis factor-a levels could be confirmed in a large study, this might lead to a novel objective guide for administering antibiotics in COPD exacerbations, perhaps in conjunction with other parameters such as procalcitonin.

In chapter 5, we studied the relationship between the cause of an exacerbation and the inflammatory pattern. In 7 out of 28 exacerbations we identified a bacterial infection as the cause. Viral and bacterial infections and air pollution are well known causes of exacerbations (10;11). Nevertheless, in up to 30 % of exacerbations, the cause remains unknown (12). Perhaps these causes remain unknown due to limitations in our ability to detect viruses, or due to our lack of understanding of other causes. Identification of these causes would lead to a better understanding of the inflammation associated with COPD exacerbations, and explain why some patients do not respond to conventional steroid therapy. It might also open windows to new pathways of treatment.

Gastro oesophageal reflux (GER) might be such an unidentified cause. GER is well known to cause respiratory symptoms. In asthma, GER is a cause of
increased respiratory symptoms, which responds well to anti-reflux treatment (13). These increased symptoms are thought to be attributable to aspiration of gastric acid, causing a neutrophilic inflammation in the airways (14). There is little research on the role of GER in COPD. Nevertheless, a few studies have shown that GER is more prevalent in COPD patients than in controls and furthermore a trend has been observed towards more severe COPD in individuals in whom GER exists (15-17). An increased prevalence of GER in COPD patients has been shown by reflux questionnaires and by oesophageal pH measurement (15;18).

At present, it is not known if an episode of increased GER can cause an exacerbation in COPD patients. However, patients with GER do have a much higher exacerbation rate compared to patients without GER, suggesting a causative role for GER in COPD exacerbations (19). In vitro research shows that exposure of bronchial epithelial cells with reflux, and particularly the stomach fluid component pepsin, an enzyme involved in protein break-down, induces release of interleukin-6 and interleukin-8, pro-inflammatory cytokines reported to be increased during COPD-exacerbations (figure 1). In summary, it seems important to ask patients with COPD about GER symptoms in clinical practice.

Treatment of COPD exacerbations with inhaled budesonide and formoterol.

In chapter 6, we analysed the effectiveness of budesonide/formoterol (B/F) in the treatment of COPD exacerbations. The treatment with B/F compared to oral prednisolone seemed to have similar effects on airway eosinophils and symptoms, and even better effects on both airflow limitation and health status. These encouraging results are to be confirmed in future large trials. Furthermore, we studied the outpatient treatment of mild to moderate COPD exacerbations. Whether the effects are the same in hospitalised patients with more severe exacerbations is an interesting and important research and clinical question to be studied in future trials. To answer, at least in part, these questions raised from the Symbexco trial, a large multicenter trial has already started (ClinicalTrials.gov Identifier: NCT00259779). We look forward to the results of these trials, since they might confirm our results of positive effects with B/F instead of prednisolone in the treatment of COPD exacerbations.
Figure 1: Induction of IL-8 release by pepsin: effect of pepsin dose and pH. Interleukin-8 was corrected for cell proliferation and expressed as a % of exposure with the corresponding pH without pepsin (control). There is a difference in interleukin-8 release induced by pepsin between the pH levels ($F=5.1; p<0.01$). Interleukin-8 release induced by pepsin is higher at pH 1.5 compared to pH 2.5 (mean difference 221%; $p<0.01$). Data expressed as mean (histograms) and SEM (bars) for 3 replicates.

It would be an interesting option to treat exacerbations at home at the onset of an evolving exacerbation by B/F. We hypothesize that treatment with an increased dose B/F, which the patients already might use as maintenance therapy, would reduce the delay of steroid treatment which frequently occurs with oral steroids. The early onset of steroid treatment, which is facilitated by this treatment strategy, might reduce the exacerbation severity, or even prevent an full-blown exacerbation In asthma, this treatment strategy improves the time to the first exacerbation, respiratory symptoms, and lung function, and reduced the rate of severe exacerbations (20). Figure 2 shows a suggestion for a simple double-blind randomised controlled trial design to assess whether the early increase of B/F treatment could prevent the development of full-blown exacerbations leading to hospitalisations. In this trial patients would be included in a stable phase, and all patients would receive B/F maintenance treatment in their stable phase. Patients would be randomised to use increased dose B/F or
placebo for two weeks at the onset of increasing symptoms, with additional short acting bronchodilators as needed in both groups. The patients should be instructed to contact the physician when a full blown exacerbation develops, requiring open label prednisolone treatment. The rescue use of prednisolone would be the (primary) endpoint of the study. If health improves, the patients continue their maintenance treatment after the 2 weeks of add-on trial treatment. During each subsequent period of increased symptoms the trial medication would again be used, until there is a need for open label prednisolone treatment, or 1000 days have passed, whichever comes first. During baseline and the trial treatment periods, symptoms and quality of life should be recorded, and at the end of baseline and treatment periods lung function, adverse symptoms related to steroid treatment, and responsiveness to methacholine would be measured. These would be analysed as secondary endpoints (need for open label prednisolone is the primary endpoint, as mentioned above).

**Figure 2:** Study design to investigate effectiveness of increasing B/F maintenance dose in the prevention of full blown COPD exacerbation*All patients receive maintenance therapy B/F in a dose of 200µg /6µg, twice daily, and shorting acting bronchodilators as needed. ‡ All patients continue to use the maintenance therapy. The patients randomised for B/F treatment use the inhaler containing the additional B/F in a dose of 200µg/6µg four times daily; the patients randomised for placebo use the placebo-inhaler four times daily.
Inflammatory cells to measure treatment outcome.

In the SYMBEXCO-study, described in chapter 6, we used sputum eosinophils as the primary endpoint to measure the outcome of treatment effect by steroids. We hypothesized that this inflammatory cell would be a valid endpoint for several reasons.

The first reason for choosing eosinophils as primary endpoint, is that various studies have found eosinophils to be increased during COPD exacerbations, as summarised in table 2 of the review of chapter 2 (4;5;21;22). We confirmed this increase (chapter 5). Although all these studies and we found an increase in airway eosinophil counts during exacerbations, this does not necessarily mean that eosinophils are actively involved in the increase in respiratory symptoms during COPD exacerbations. Causality has not been found so far, but our study provided some contributing evidence for this: we found a correlation between the increase in eosinophils form stable phase to exacerbation and a decrease in FEV$_1$, although the relationship was weak. However, we did not find a relationship between improvements in FEV$_1$ and health status and decrease in sputum eosinophils during the trial treatment. This does not necessarily rule out a role for eosinophils during COPD exacerbations: The lack of association between change in sputum eosinophils and other treatment effects as airway obstruction and health status might be explained by the limited number of patients in our study, and a third of these patients did not receive steroid treatment, but placebo. Therefore we might not have had the power to show such a relationship. A second explanation might be that eosinophils are not involved in all COPD exacerbations, but in a subgroup. Papi et al showed that eosinophils were only increased in exacerbations with a viral infection(4), and our data might be suggestive for an increase in eosinophils in a subgroup as well (figure 3).
Figure 3: Increase in sputum eosinophils from a stable phase on varying types and dosages of inhaled steroids (visit 1), via steroid withdrawal for 2 months, to the beginning of a COPD exacerbation. Sputum eosinophil numbers are significantly increased during the exacerbation compared to the numbers at the inclusion visit. However, there is a large group of patients with COPD, in which sputum eosinophils are at a low numbers during an exacerbation, indicating that eosinophils increase only in a subgroup of patients.

The second reason to choose eosinophils as primary endpoint is based on reports showing that sputum eosinophils are responsive cells to steroids. Several studies reported that patients with COPD in a stable phase and high eosinophil levels in sputum had better outcomes in FEV$_1$ and symptoms when treated with steroids, either orally or inhaled (23-25). In COPD exacerbations, outcomes of steroid treatment depending on sputum eosinophil levels have not been tested. An interesting question would be whether patients with low eosinophil numbers in sputum benefit from steroid treatment at all, in stable phase or exacerbation. To study this, the large COPD exacerbation trials investigating the effects of steroid treatment, on which the evidence of steroid treatment during exacerbations is based, should be repeated, this time with measurement of the inflammatory characteristics of patients.

We could also have chosen airway neutrophil counts to assess anti-inflammatory effects of B/F during COPD exacerbations: airway neutrophil counts are increased during COPD exacerbations, as summarised in table 1 of the review (chapter 2). In chapter 5 we confirmed that neutrophil numbers are
increased during COPD exacerbations. Additionally, neutrophilic inflammation has been related to airflow limitation and severity of symptoms (4;26). Therefore, inhibition of neutrophil recruitment might be a strategy to treat COPD exacerbations. Leukotrienes are important neutrophil-recruiting cytokines during COPD exacerbations (27-29). Therefore inhibition of leukotriene activity could cause a reduction in neutrophils. Indeed, the treatment of patients with stable COPD with a leukotriene receptor antagonist resulted in reduction in neutrophils, and improvement in symptoms, quality of life and airway obstruction (30). Since we and others found that neutrophils are especially increased in exacerbations caused by bacterial infections, and neutrophils are known to be involved in the eradication of bacteria (27;31), the investigation of treatment of COPD exacerbation with “anti-neutrophil” therapy should be instituted under strict supervision and we would recommend anti-bacterial protection during such trials, to reduce the chance of bacterial overgrowth and pneumonia.

Future anti-inflammatory therapies in COPD exacerbations: inhalation of carbon monoxide?

In this thesis, we described two intervention trials with inflammation modifying drugs: budesonide/formoterol and inhaled carbon monoxide. We presumed that reduction of inflammation is beneficial and decreases symptoms. This was based on reports of trials in a stable phase, which showed that steroid therapy is beneficial in patients with a higher sputum eosinophil level (23;25), and that the reduction of eosinophils is the pathway by which steroids reduce airway obstruction in COPD (24). The effects of budesonide/formoterol were very encouraging, and when the indications of the treatment effects of our pilot study can be confirmed in a larger trial, this would shape the future of home treatment of COPD exacerbations.

The use of inhaled carbon monoxide is not so close to clinical treatment of COPD exacerbation yet: we started to explore the effects of carbon monoxide in stable COPD population (chapter 7), which –as far as we are aware- was the first trial in humans to study the beneficial effects of carbon monoxide in COPD. We did not find significant effects on sputum neutrophil counts, the primary outcome parameter; although the median sputum neutrophil count was much lower (median 2.6 compared to 4.0 x10^6/ml) after CO treatment compared to placebo, the variability in our small study population was too large, resulting in insignificant p-values. We did find a trend of reduction in eosinophils by CO-inhalation. In vivo studies have shown that this anti-inflammatory effect of carbon monoxide is caused by inhibition of the mitogen activated protein (MAP)-kinase pathway. MAP-kinase pathways are a group of pathways which have in common that they are involved in the signal transduction from an external inflammatory stimulus to an inflammatory response of the cell, by activating intracellular transcription factors of pro-inflammatory cytokines (32).

To explore which anti-inflammatory mechanisms are affected by CO inhalation in humans, we performed additional measurements of the cytokines
8-isoprostane (figure 4), IL-1ß, IL-5 IL-6, IL-8, IL-10, vascular epithelial growth factor, and tumor necrosis factor-a. We were unable to confirm the effects on cytokines by CO found in *in vitro* and *in vivo* studies: none of these cytokine levels were significantly reduced by CO inhalation. Possible explanations for this lack of effects on these cytokines could be that the dose of CO was too low, or that our "inflammatory model", the ongoing inflammation in COPD patients who were not current smokers, provided insufficient levels of the inflammatory cytokines to measure effects of CO.

![Graph of 8-isoprostane levels](image)

**Figure 4.** Effects of carbon monoxide on the levels of 8-Isoprostane (urine).

Future studies on the therapeutic application of inhaled CO should investigate its effects in a larger population of patients, assessing the safety and optimal dosing schemes. Only when such studies show positive results, more severe indications as COPD exacerbations could be investigated with sufficient confidence.

*Improvements in treatment of COPD exacerbations in the near future.*

In the upcoming years, treatment of COPD exacerbations should be improved by various approaches. Both basic scientific research and clinical research is needed to achieve this.
Firstly, novel anti-inflammatory drugs should be developed for the treatment of COPD exacerbations. There is a need for novel drugs, since the effects of steroids in the treatment of exacerbations are modest. To make this possible, there is a need for models to test these. So far, there are no animal models resembling COPD exacerbations. The development of validated COPD exacerbation models in animals would be a step forward in the search for more efficient treatments of COPD exacerbations.

Secondly, as long as we do not have more efficient therapies in the treatment of COPD exacerbations, we need to improve the current treatment. An important step in this is the assessment of the optimal dose, and duration of steroid treatment. There is hardly any data on the optimal dose and duration of systemic steroid treatment in COPD exacerbations. Only one trial has assessed the difference between 2 week and 8 week steroid treatment (33). Shorter steroid schedules have to the best of our knowledge not been compared. Given the large number of COPD patients treated with steroids every year, this urgently calls for future investigations to provide evidence. There is also a marked paucity of data regarding the dose of systemic steroids. Clinical guidelines recommend prednisone 40 mg orally once/day for 10 days in patients with an acute exacerbation of COPD (34). This was based on a panel consensus judgment.

Thirdly, we need a better understanding of the course of COPD exacerbations. Future studies should further investigate the relation between cause of exacerbation, inflammatory mechanisms and susceptibility to treatment drugs. Previous studies have shown that the various causes of COPD exacerbations are associated with different inflammatory patterns. It is likely that the various causes of exacerbation also require specific treatment. For instance, future investigations need to assess whether exacerbations caused by bacteria with a neutrophilic inflammation pattern respond to steroid treatment at all. Perhaps these types of exacerbations should be treated with antibiotics and bronchodilators only. On the other hand, viral exacerbations with may induce with eosinophilia might respond reasonably well to steroid treatment, and might not benefit from treatment with antibiotics. Exacerbations caused by micro-aspiration might only respond to anti-reflux therapy. A split-up of exacerbations by its cause, and investigation of cause-specific treatment effects, might lead to a more efficient treatment of COPD exacerbations.

Research on COPD exacerbations and its treatment is still a starting field; however it is getting more and more attention in recent years. Much research effort is needed in the next years to improve both the understanding of COPD exacerbations and its treatment, but the gains will undoubtedly be large.
References


