TOWARDS CLINICAL PHENOTYPING IN COPD

Effects of inhaled corticosteroids in the GLUCOLD study

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Chapter 1

General introduction

COPD: importance of a highly prevalent disease

Patients with Chronic Obstructive Pulmonary Disease (COPD) have a lung disorder that limits daily activities and contributes considerably to emotional distress [1]. Most patients with COPD experience airway symptoms such as cough, sputum production and dyspnea at exertion. Subgroups of patients have periods with aggravation of symptoms, so-called exacerbations or "lung attacks". The presence of symptoms and exacerbations diminishes health status. According to the Global Initiative for COPD (GOLD) guidelines COPD is defined as "a common preventable and treatable disease that is characterised by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases". Exacerbations and comorbidities contribute to the overall severity in individual patients [2].

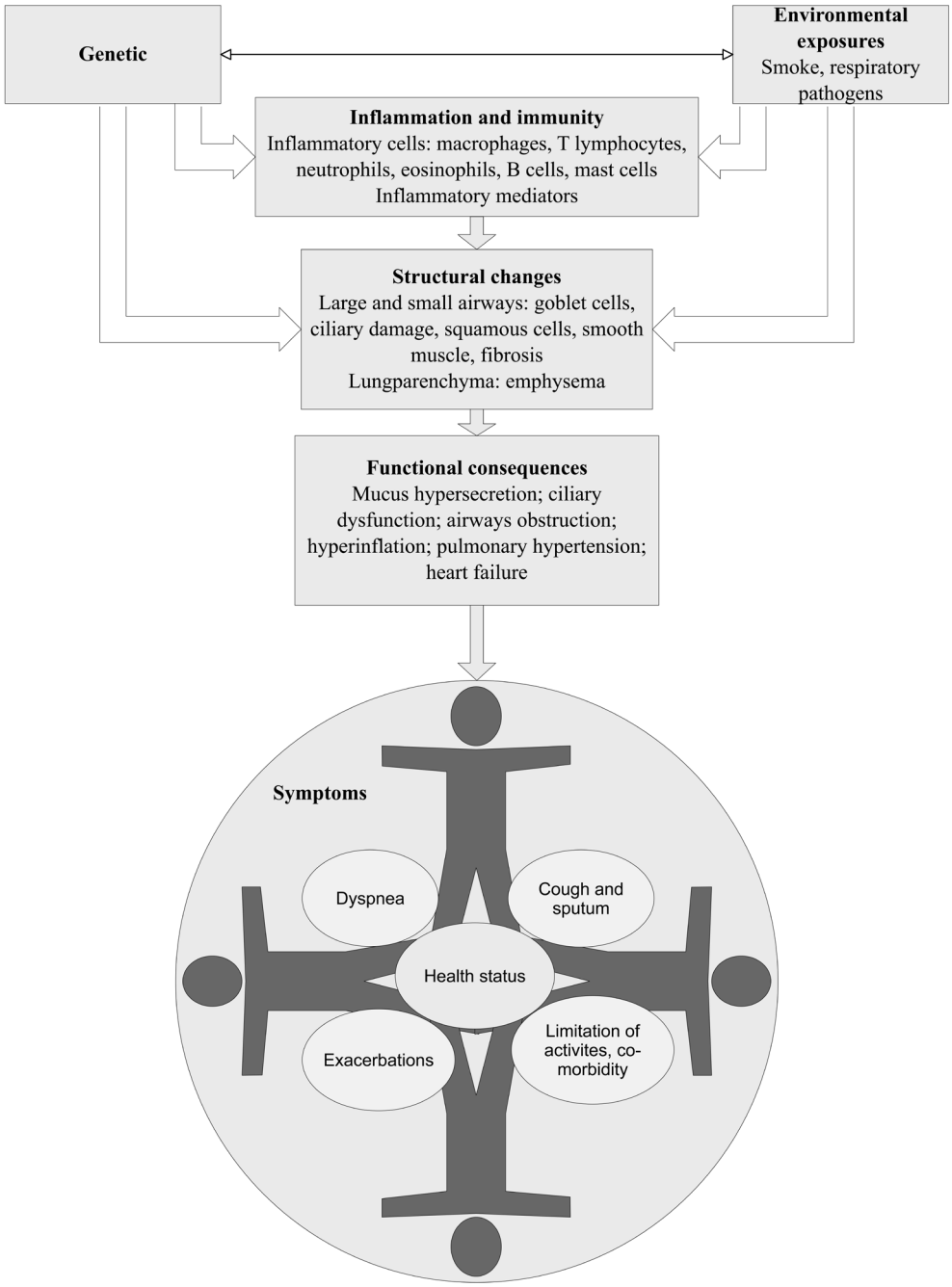
COPD is a highly common disease with a prevalence of 7 to 8% worldwide, whereas most European countries have a lower prevalence around 3 to 4% [3]. In The Netherlands, a standard general practice of 2300 patient takes care of around 60 patients with COPD [4]. In 2010 COPD moved from the fourth leading cause of death to the third in the United States [5]. The complexity of the disease, including many comorbidities, and aging of the population in the upcoming years will provide an enormous burden on the health care system. With advanced disease the health services will be used more intensively, because of exacerbations and hospitalizations. Currently, in The Netherlands many patients with COPD in general practice are not diagnosed and chronic care for these patients is still lacking in many places [6]. However, in recent years some important health policy changes have occurred and chronic care is slowly improving. This, for example, resulted in 'de Zorgstandaard COPD' which is a practical guideline for daily primary care practice in the Netherlands [7]. Also, the GOLD guidelines attempt to build a bridge between science and daily practice. The difficulties that have to be faced can be demonstrated when interpreting the scheme from chapter 2 of the GOLD guidelines for assessment of association between symptoms, spirometric classification, and future risk of exacerbations [2]. A three-dimensional graph is reduced to a two-dimensional scheme and it is not easy to classify patients correctly. Structured disease management may provide significant improvements for COPD patients, such as increased physical activity [8], improved quality of life [9], and reduced exacerbations [10], hospitalisation and emergency department visits [11]. This may provide a setting for a shift from a doctor's perspective towards tailored interventions based on a more patient centred perspective.

Patient centred perspective

From the patients' perspective the most important features of COPD are distressing symptoms, such as dyspnea, cough and sputum production and perceived limitation in daily activities (Figure 1). The distress can temporarily enhance during exacerbations. This can have a major impact on a patients' individual quality of life. Consequently, care providers seek to improve symptoms and improve functional status. In clinical trials health status measurement is a way of objectively measuring the impact of disease on a patients' daily life, health and well-being [12]. Disease-specific health status questionnaires such as the St. George Respiratory Questionnaire (SGRQ) [13], the Clinical COPD Questionnaire (CCQ) [14] and the COPD Assessment Test (CAT) [15] address airway symptoms, emotional and psychological distress and limitation of activities and daily disturbances.

The actual burden of COPD differs between patients. Some patients experience few symptoms, whereas others are completely handicapped or even bedridden by the disease. In addition, health status is only weakly associated with the underlying severity of airflow limitation [16-18]. Health status seems to be more closely associated with factors such as dyspnea, depression, anxiety and exercise tolerance than with forced expiratory volume (FEV_1) [19]. This may be due to the fact that COPD symptoms can be linked to different components of the disease. Breathlessness is an important characteristic of COPD and is mostly subjectively associated with exercise. The improvement in dyspnea by bronchodilators at rest is associated with improvements in forced inspiratory volume (FIV_1) rather than FEV_1 [20]. Static lung volumes are increased in COPD and the functional residual capacity rises even further at exercise, which is called dynamic hyperinflation. The improvement in dyspnea by a bronchodilator during exercise is also associated with inspiratory capacity rather than FEV_1 [21]. This dynamic hyperinflation may be associated with the underlying chronic inflammatory process and small airway disease. To date, no previous studies have investigated whether there is an association between health status and the severity of airway inflammation in COPD. Knowledge of the relationship between health status and the severity of airway inflammation might potentially provide clues for more tailored and patient-centred interventions in COPD.

Figure 1. Patient perspective on COPD



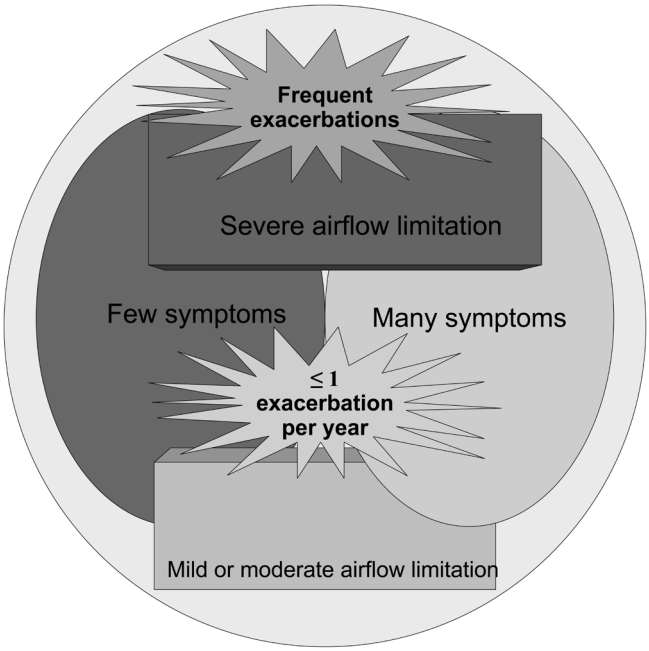
Clinical phenotypes

In patients with a disease as complex as COPD, effective care is only possible if we determine the individual profile. Insight in the underlying components of COPD is important to understand the disease in its full spectrum and to be able to influence prognosis and treatment. Ultimately, this will lead to tailoring treatment in individual patients [22]. Many patho-physiological abnormalities may determine different phenotypes, such as airway disease versus emphysema; rapid vs non-rapid decliners, frequent vs non-frequent exacerbators, chronic bronchitis vs non-chronic bronchitis. Early concepts of COPD were illustrated using a so-called 'Venn diagram' [23]. This diagram included overlapping components with chronic bronchitis, emphysema and subtypes of asthma with airways obstruction. More recent studies have shown that this diagram does not include all subgroups of patients with COPD [24]. Interestingly there are also patients with COPD without chronic bronchitis, emphysema or asthma [25]. Currently, the GOLD guidelines focus on symptoms, airflow limitation and future risk of exacerbations (Figure 2) [2]. However, it remains unclear whether these are the only important phenotypes. The group with few symptoms, severe airflow limitation and frequent exacerbations is probably small, whereas the group of patients with few exacerbations is probably larger with more heterogeneity. The latter could be due to the fact that patients frequently do not report to their physician [26]. However, the Evaluation of COPD Longitudinally to Identify Surrogate Endpoints (ECLIPSE) study showed that there may also be a specific phenotype that is more susceptible to exacerbations. From a clinical perspective it is important to identify specific phenotypes that have prognostic value for the effect of interventions on outcomes. A clinical phenotype can be defined as "a single or combination of disease attributes that describe differences between individuals with COPD as they relate to clinically meaningful outcomes" [27]. Taking into account the large heterogeneity of the disease, it can be expected that subgroups of patients may benefit from a given treatment. Hence, which clinical phenotypes are important in COPD?

Chronic bronchitis

One third of patients with COPD have symptoms of chronic cough and sputum production [28;29]. The presence of chronic bronchitis can be defined clinically as daily cough and sputum production for more than 3 months per year for > 1 year [30]. Patients with chronic bronchitis have lower health status, more severe breathlessness, more upper airway symptoms and fewer

Figure 2. Symptoms, spirometric classification and future risk on exacerbations (modified from GOLD [2])



exacerbations than patients without chronic bronchitis [31]. In addition, the presence of chronic bronchitis is associated with a steeper decline in FEV_1 [30;32] and mortality [33], even apart from GOLD stages [34]. This can have a major impact on the clinical well-being of these patients and clinicians often treat patients in order to relieve these symptoms. Still, there has been much debate as to whether chronic bronchitis is a specific phenotype that could be treated in order to slow down the progression of the disease. As early as 1950, 'The British Hypothesis' proposed that smoking caused mucus hypersecretion and worsened lung defences, leading to chronic infection, obstruction and emphysema [35]. However, Fletcher et al were unable to show an association between chronic mucus hypersecretion and the progression of COPD [36]. As a consequence, chronic bronchitis has long been considered as a benign condition with harmless symptoms.

Recent large epidemiological studies have found new evidence on the role of chronic bronchitis within COPD and thereby opinions are changing [37]. Inhaled noxious gases may damage the lung's innate defence system in early disease.

This includes reducing mucociliary clearance, producing an ineffective cough, disrupting the epithelial barrier, and initiating an acute inflammatory process in the lungs. In later stages there may be a shift from the innate to adaptive immune response [38]. The Copenhagen City Heart study showed that chronic mucus hypersecretion can be associated with accelerated decline in lung function in patients with more advanced disease [30]. At this stage the airways may become more colonised and infected which may provide a rationale for the link between chronic bronchitis and progression of the disease. The excess of sputum has been associated with the chronic inflammatory process that involves the epithelial lining of the airway lumen, bronchial glands and gland ducts of the central airways in patients with COPD. COPD patients with chronic bronchitis have increased numbers of neutrophils in the epithelium and more neutrophils, macrophages and $CD8^+$ cells in their bronchial glands as compared to asymptomatic non-COPD subjects [39]. This suggests that inflammatory cells and their mediators provide a major drive for mucus hypersecretion and subsequent symptoms of chronic bronchitis [40]. Hence, chronic bronchitis may reflect a specific phenotype in COPD and deserves specific attention not only to improve symptoms, but also to influence the progression of the disease.

Frequent exacerbations

Acute exacerbations can have enormous impact on patients with COPD. As an example the following patient (own experience in daily practice):

"A male patient of 65 years with moderate COPD who has increased coughing and sputum production for two weeks. His wife had ordered him to go see the general practitioner, but he wanted to wait and see whether his symptoms would wear off with time. During the weekend, he felt much worse. He now experiences dyspnea with just a little exertion. An unknown physician visits him at home. The general practitioner has limited information about the severity of the COPD and comorbidity of the patient and sends him to hospital where the patient subsequently stays for some days."

One can imagine the distress and major impact this experience brings to the patient and his family. In addition, patients with frequent exacerbations have an increased risk of death and a more rapid decline in lung function [28;41]. Particularly, patients with more advanced disease have more frequent and severe exacerbations. However, the ECLIPSE study showed that also patients with less severe airways obstruction can have frequent exacerbations, suggesting an independent susceptible phenotype [42]. Several studies have

reported prevention of exacerbation by treatment. A study with 233 patients with moderate COPD provided these patients with individualised action plans and ongoing support from a case manager. During 6 months of follow-up they showed that early detection and treatment of exacerbations may lead to accelerated recovery decreased impact of exacerbations on health status [43]. Treatment with inhaled corticosteroids with or without long-acting bronchodilators can reduce the frequency of exacerbations in patients with moderate to very severe COPD [44;45]. Interestingly, a recent study showed that daily azithromycin for one year, when added to usual treatment, can reduce the frequency of exacerbations in middle-aged patients with moderate to severe COPD [46]. However, significantly more patients using azithromycin reported hearing loss compared to placebo (25% vs 20%, *P*=0.04), and there was an increased prevalence of macrolide resistant colonisation of the airways, a drawback that has to be weighed against the beneficial effects. In conclusion, many studies have shown that patients with frequent exacerbations represent a specific phenotype in COPD, which has led for the GOLD guidelines to include the risk of future events such as exacerbations in the assessment of disease severity in order to guide therapy [2].

Accelerating decline in FEV_1

The progressive decline in lung function is an important characteristic of the disease. It is widely used as a composite objective measurement of the structural changes of the lung and describes severity of the lung. Current guidelines use FEV_1 level as an indicator of diagnosis, assessment of severity and treatment. In healthy individuals the lung function declines with 25-30 ml per year. In patients with COPD, this rate of decline is around 60 ml/year. While symptoms vary enormously between patients, a progressive loss of lung function is observed in all patients with COPD. It has to be noted that FEV_1 decline may be larger in early disease, and that the rate of decline differs amongst patients. It is noted that rapid decliners may reflect a specific phenotype where close monitoring is needed, since there is more to gain by adequate intervention. Still, not all patients experience a rapid progression of their disease [47;48]. Also, it is widely accepted that COPD is much more complex than being a disease based on the level or decline of FEV_1 and many other components influence the course of the disease [49;50]. Airway hyperresponsiveness has been associated with a more rapid decline in FEV_1, which may indicate a distinct phenotype [51]. However, it is not yet clear how these clinical and structural changes in COPD are associated

with the underlying inflammatory process in the airways. Novel approaches for addressing the complexity of COPD phenotypes are emerging, such as those based on a 'systems medicine' approach, in which composite biological phenotypes are integrated with clinical phenotypes by using a multi-scale 'systems medicine' approach [52;53].

Pathogenesis

The smaller airways (2mm diameter) are the major site of obstruction in COPD [54]. A recent study examined the relationship between small airway obstruction and emphysema in COPD [55]. Multidetector Computed Tomography (CT) and microCT was used in 78 patients with varying degrees of airflow limitation. This was applied in sections of isolated lungs from 12 patients who underwent lung transplantation and controls (donor lungs). The number of small airways was reduced in mild disease and was even further reduced in more advanced disease. Interestingly, it was suggested that the narrowing and loss of terminal bronchioles preceded the onset of emphysematous destruction. This may explain the increased resistance in patients with COPD. However, results should be interpreted with caution because of the cross-sectional design of the study. The pathogenesis of COPD is characterised by a complex inflammatory reaction to inhaled noxious gases [56]. Cigarette smoke is an important risk factor in developing COPD. Smoking causes an inflammatory response in the lungs. Still, only 20% of smokers develop COPD and it is suggested that the inflammatory response is enhanced or abnormally regulated in susceptible patients. In patients with established COPD, airway inflammation in sputum, biopsies and bronchoalveolar lavage (BAL) persists even after cessation of smoking [57].

In addition, the number of macrophages and neutrophils is similar in bronchial biopsies from current and ex-smokers with COPD [58]. However, percentages of macrophages with anti-inflammatory characteristics are higher in BAL from ex-smokers than current smokers, suggesting that smoking cessation may lead to a more anti-inflammatory phenotype. The mechanisms explaining this are still poorly understood. Important pathological phenotypes include chronic bronchitis, emphysema and small airways disease. Chronic bronchitis is linked to the presence of mucus hypersecretion which may be due to the inflammatory response in the submucosal glands and surface epithelium. Emphysema is due to the destruction of alveolar walls. The destruction of the tissue can be the result of 'insufficient tissue repair' [59]. Ultimately this leads to enlargement of the airspaces. Small airways (also referred to as peripheral airways) can

also be affected in COPD and are characterised as having an internal diameter <2mm. Inflammation in the small airways includes accumulation of neutrophils, macrophages, mast cells, $CD4^+$, $CD8^+$ cells and B cells. Tissue injury can exceed the ability to repair due to ongoing inflammation, a direct effect of cigarette smoke or 'exaggerated tissue repair' [54;60]. Tissue remodelling in the small airways can than be responsible for thickening of the small airways and fibrosis. Consequent narrowing of the airway lumen may lead to airflow limitation. Excessive mucus production and plugging of the small airways contributes to airway obstruction.

However, many different inflammatory responses are linked to multiple pathways and it is far from easy to classify the pathological process into specific phenotypes [61]. The underlying mechanisms comprise a balance or imbalance of exaggerated airway inflammation, protease/antiprotease imbalance, oxidative stress, necrosis and activation of programmed cell death (apoptosis). The enhanced inflammatory cell numbers may be cleared by endogenous protective mechanisms, such as apoptosis and subsequent phagocytosis of the apoptotic cells. Alternatively, airway inflammation may be resolved by transepithelial migration [62]. Leucocytes then migrate through tissue components and across the epithelial cells into the airway lumen. Mucus hypersecretion and mucociliary clearance can eliminate the inflammatory cells subsequently. It can be speculated that impaired endogenous protective mechanisms play a role in the susceptibility for COPD.

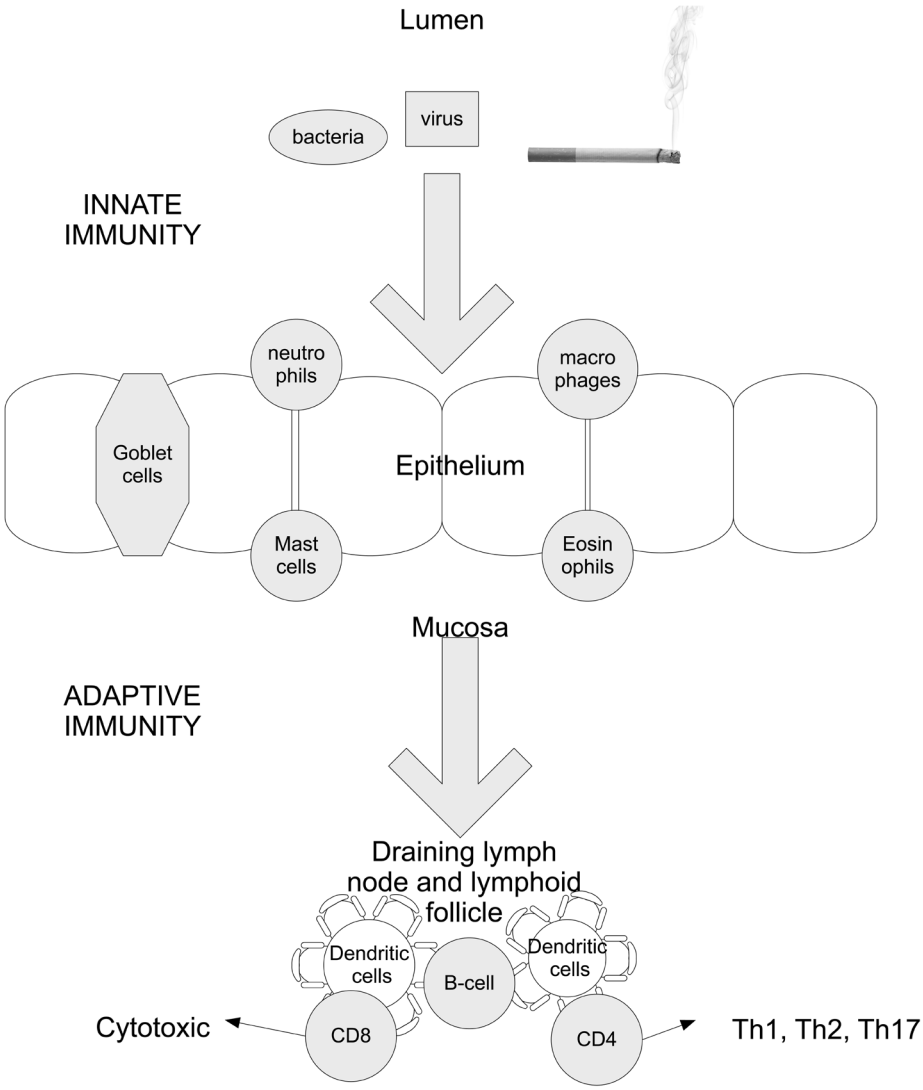
Airway inflammation plays a key role in COPD. The inflammatory process affects the whole respiratory tract and there are different ways to assess the severity and nature of the inflammatory process. One may use airway wall tissue from bronchoscopies, resected lung tissue or autopsy studies. Other approaches include indirect evaluation of airway inflammation with induced sputum samples and BAL. Even peripheral blood seems to be suitable for inflammatory characterisation in COPD when using modern techniques such as proteomics [63]. C-reactive protein (CRP) is raised in COPD and even further during exacerbations [64]. Fibrinogen is also raised in COPD and may be a more promising biomarker, because it shows less variability among patients with COPD. Techniques such as bronchoscopy, can provide reliable results in airway wall biopsies, but are invasive and provide large inconvenience for patients. Non-invasive techniques are therefore imperative and include exhaled breath volatile organic compounds, exhaled breath condensate and exhaled nitric oxide. Exhaled molecular components are associated with a specific inflammatory profile in COPD, suggesting that breath analysis can be

used as a phenotypic marker [65].

Inflammation and immunity

In COPD there is a chronic amplification of the normal inflammatory response of the lungs to noxious particles and gases (especially cigarette smoke) in susceptible patients [66] (Figure 3).

Figure 3. Innate and adaptive immunity



It is mainly characterised by neutrophils, macrophages and $CD8^+$ T lymphocytes [67]. In addition, $CD4^+$ T lymphocytes, mast cells and eosinophils may play a role. In response to injury of the lungs by infection or cigarette smoke, the instant non-specific innate immune system is triggered. Neutrophils are recruited to the airways by chemotactic factors such as interleukin (IL)-8 and leukotriene B_4 (LTB_4). The number of neutrophils is increased in induced sputum and BAL of COPD [68]. They may contribute to wound healing, but can also cause tissue injury. Activated neutrophils may cause mucous hypersecretion by the release of serine proteases such as elastase and matrix metalloproteinases (MMPs). In addition, production of neutrophil elastase contributes to the destruction of lung tissue leading to emphysema. Macrophage numbers are increased in the airway wall, lung parenchyma, BAL and sputum of patients with COPD [60]. They release inflammatory mediators such as tumor necrosis factor (TNF)-α, IL-8 and LTB_4, and may contribute to emphysema by e.g. release of MMP12. Eosinophils and mast cells are known to be important in asthma, but may also play a role in COPD. Some studies show an increase in eosinophils in COPD which may point to a specific phenotype [69;70]. In patients with moderate to severe COPD clinical exacerbation clusters can be determined. Especially bacteria- and sputum eosinophil-associated exacerbations can be identified using biomarker profiles which may be important for directing therapy [71]. Interestingly, a recent study showed that COPD patients with higher circulating eosinophils maintained their level of FEV_1 during a follow-up of 5 years. In a study from our centre comparing 16 patients with COPD with 15 patients without COPD inflammatory infiltrate was examined from tissue that was removed for lung cancer. The results showed that patients with COPD exhibited more mast cells in the epithelium, but not in the remainder of the airway wall [72]. In a recent study with endobronchial airway biopsies mast cells were more prominent in the lamina propria and the reticular basement membrane in COPD than in patients without COPD [73]. Epithelial cells may contribute to the inflammatory process by secreting a variety of inflammatory mediators such as TNF-α, transforming growth factor (TGF-β) and IL-8.

Dendritic cells link the innate immune system to the adaptive immune system which can recognize and remember specific pathogens in order to generate adaptive immunity. Adaptive immunity includes T lymphocytes ($CD4^+$, $CD8^+$) and B Lymphocytes. $CD8^+$ T lymphocytes are increased in the large [69;74] and small [75] airways of subjects with COPD. An important function of $CD8^+$ cells is to attack viruses by causing cell death of infected cells. However, T lymphocytes can also cause tissue destruction by direct cytolytic activity

by cytotoxic mediators such as granzyme or perforins. The specific role of $CD4^+$ cells in COPD is less clear. $CD4^+$ lymphocytes release pro-inflammatory cytokines such as TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, IL-2 (Th1), and IL-4, IL-13 and IL-5. $CD4^+$ cells are T helper cells and may provide help for B cell activation and support $CD8^+$ cells by maintaining their memory and ensuring their survival. In the GLUCOLD study, we found that the number of B cells in bronchial biopsies of central airways is higher in COPD than in patients without COPD [76]. In more advanced COPD the number of B cells follicles increased in the small airways in resected lung tissue [38]. However, the role of B cells is not yet clear. The accumulation of B cells may be due to stimulation by viruses or bacteria, and may serve a protective function to prevent infection. However, B cells may react to specific auto-antigens with autoantibodies against e.g. epithelial cells and elastin. This suggests that the increase in B cells may also point into the direction of an autoimmune process that may be involved in the pathogenesis of COPD [77], or it may be an epiphenomenon resulting from the extensive tissue injury.

Tissue repair and remodelling

Remodelling of the airways is associated with narrowing of the airway lumen, increased thickness of the airway wall and remodelling of the parenchyma [60]. An imbalance between matrix synthesis and degradation during the repair process may cause emphysema or fibrosis of the small airways. This may contribute to increased airway responsiveness, fixed airway obstruction and accelerated decline of lung function [78]. However, remodelling may also be protective. The increased thickness may be beneficial in decreasing the effect of the inflammatory response to specific antigens. In the lungs matrix proteins and antiproteinases maintain a balance in order to preserve the elasticity of the lung [79]. The increased airflow resistance in COPD is partly caused by early airway closure due to reduced elastic recoil. Reduction of decorin in the peribronchial area of severe COPD may contribute to loosening of collagen structures and alveolar attachments [80]. Elastic fibers containing elastin, provide physical recoil in de alveolar region of the lung and contribute to normal physiological function. Neutrophil elastase is a strong protease that can degrade mature elastin which leads to development of emphysema. In recent years many other proteases, such as serine and metalloproteinases, were shown to contribute to tissue injury during inflammatory processes. Various proteinase inhibitors restrict the activity of these proteinases, including the serine proteinase inhibitors alpha-1-antitrypsin and secretory leucocyte

protease inhibitor (SLPI), and the metalloproteinase inhibitor family of TIMPs.

Biomarkers

One of the most important challenges in current research is the use of biomarkers. A biomarker is any molecule or material (*e.g*. cells or tissue) that reflect the disease progress. Biomarkers are increasingly important in identifying specific phenotypes and act as surrogate outcomes in clinical trials. One example of a biomarker that clearly defines a subset of patients with COPD is the level of serum α1-antitrypsin, *i.e.* a specific subset of COPD with α1-antitrypsin deficiency. Many biomarkers are currently under investigation [81]. Studies such as the ECLIPSE study use proteomics and metabolomics techniques to examine for example blood samples, induced sputum, exhaled breath condensate, blood and urine [82]. A recent study showed the relationship between exhaled breath components and inflammatory cell counts in sputum in 12 patients with mild COPD and 16 patients with moderate COPD [65]. Exhaled markers were significantly associated with sputum neutrophils and eosinophils, which may offer a clinically relevant, non-invasive marker for patients with early COPD. It is likely that specific or composite molecular and cellular patterns will eventually be used as biomarkers for the phenotype, severity and progression of COPD [53].

Treatment

Smoking cessation is the most effective way to slow down the progression of the disease [83]. Therapy with tiotropium has been shown to improve health status exacerbations and mortality in patients with COPD in the Understanding Potential Long-Term Impacts on Function with Tiotropium (UPLIFT) study [84; 85]. Interestingly it reduced decline in FEV_1 in a subgroup of patients with earlier disease as based on GOLD stages [86].

Inhaled corticosteroids (ICS) are the mainstay of anti-inflammatory treatment in COPD and are prescribed to many patients. Treatment with ICS in patients with COPD results in short-term improvement in postbronchodilator FEV_1 [44;87;88]. Earlier large studies have not demonstrated that long-term ICS can exert a more permanent, disease modifying effect, as based on a decline in lung function [44;87;89;90]. However, it cannot be excluded that any effect on FEV_1 decline has been underestimated due to drop-out of patients with the most rapid FEV_1 decline [91] and/or the selection of COPD patients with prior usage of ICS [44]. Disease modification by ICS is plausible since long-term therapy improves airway hyperresponsiveness [90], which in itself constitutes a

risk factor for accelerated FEV_1 decline in COPD [51;92]. In a post-hoc analysis of the Towards a Revolution in COPD Health (TORCH) study the effects were studied of fluticasone 500 mcg plus salmeterol, either component alone or placebo. The rate of decline was reduced for all active treatments versus placebo, but similar among ICS-containing treatment arms and salmeterol alone [93].

At present, meta-analyses are still inconclusive regarding the benefits of long-term ICS on FEV_1 decline in patients with COPD [94-96], as well regarding any sustained beneficial effects on patient oriented outcomes, such as dyspnea [90] and health status [44]. Recent studies provide indirect support for the beneficial effects of ICS on FEV_1 decline in COPD showing that discontinuation resulted in a decrease in FEV_1 during one year follow-up, associated with worsening of dyspnea and an increase in exacerbation frequency [97-99]. However, it is as yet unclear whether longer duration of cessation of inhaled corticosteroid therapy leads to renewed accelerated decline in FEV_1 either or not associated with a relapse in airway hyperresponsiveness.

At present, long-acting $ß_2$-agonists (LABA) are prescribed to reduce dyspnea in COPD [2]. Their efficacy is presumed to run via reduction in airways obstruction and dynamic lung hyperinflation, although additional benefits on inflammatory and epithelial cells cannot be excluded [100]. Notably, combination therapy of LABA with ICS has been shown to provide additional improvements in health status in patients with moderate to severe COPD [45;101]. Consequently, the latest international guidelines recommend combination therapy for patients with FEV_1<50% and frequent exacerbations [2]. However, the vast majority of patients throughout the world has less severe COPD, and there is no evidence yet to support long-term combination therapy in patients with less advanced COPD.

Clinical and anti-inflammatory benefits of ICS in asthma are well established. In asthma $CD4^+$ T cells, eosinophils and mast cells are effectively reduced by ICS, which is accompanied with subjective and objective clinical improvement. The anti-inflammatory effects of ICS in COPD are less clear. The pattern of inflammation in COPD is different, but may be mediated, at least partially, by the anti-inflammatory efficacy of ICS. Short-term (2-3 months) treatment with ICS with or without LABA has been shown to differentially influence the numbers of macrophages, neutrophils and $CD8^+$ T cells in bronchial biopsies of patients with moderate to severe COPD [102-105]. A meta-analysis using data from several short-term studies showed overall reductions in sputum neutrophils, lymphocytes and epithelial cell counts [106]. Interestingly, studies

with a duration < 6 weeks had predominantly negative results, were studies with a duration > 6 weeks were mostly positive. So far, long-term anti-inflammatory effects by these interventions have not been reported.

Aims of the present study

This thesis describes studies directed at detailed phenotyping of COPD and effects of inhaled corticosteroid therapy and long-acting $ß_2$-agonist. The present studies include investigations on health status, the presence of chronic bronchitis, long-term effects of inhaled corticosteroid therapy and long-acting $ß_2$-agonist and prediction of effects of ICS in patients with moderate to severe COPD. This thesis is based on analyses from the Groningen Leiden Universities Corticosteroids in Obstructive Lung Disease (GLUCOLD) study.

The aims of the current thesis were to answer the following research questions:

1. To what extent are clinical symptoms and airway inflammation distinctive components of COPD?
2. Does airway inflammation contribute to impaired health status in COPD?
3. Does chronic bronchitis reflect an inflammatory sub-phenotype among COPD?
4. Does maintenance therapy with ICS have anti-inflammatory and clinical effects in COPD?
5. Can patients with specific phenotypes of COPD benefit more from ICS?
6. Does maintenance therapy with ICS have effect on day-to-day health status and peak-flow?

All studies were performed as part of the GLUCOLD project. The study was an investigator-initiated trial (registered as NCT00158847). The protocol was written by the GLUCOLD study group, and all procedures and analyses were carried out by the investigators only. The GLUCOLD study group applied for, and received grants from: the Netherlands Organization for Scientific Research (NWO) in a collaborative program with the Netherlands Asthma Foundation (grant 3.4.93.96.3), GlaxoSmithKline (The Netherlands), Stichting Astma Bestrijding (SAB), and the University Medical Center Groningen (UMCG) and the Leiden University Medical Center (LUMC).

The overall aims of the GLUCOLD study were to examine whether i) Long-term maintenance therapy with ICS with or without a LABA provides anti-inflammatory effects (primary outcome) in the airways of COPD patients; ii). Such effects are associated with clinical improvements; and iii) ICS discontinuation induces a flare-up of inflammation and clinical deterioration.

The GLUCOLD study was a double blind, randomised, controlled trial in

patients with COPD comparing the clinical, functional and pathological benefits of:

- Long-term maintenance therapy (2,5 year) with ICS
- Addition of long-term LABA to ICS
- Discontinuation of inhaled fluticasone after 6 months
- Placebo

Outline of the GLUCOLD study

The GLUCOLD study was investigator-initiated with a double-blind, parallel, four-arm, placebo-controlled, randomised design. Patients in this study were aged 45-75 years, they were all current or ex-smokers, with at least 10 pack-years of smoking (Table 1). The lung function levels were compatible with the GOLD guidelines stages II and III [2]. They were not allowed to have used ICS within 6 months prior to randomisation. Patients with asthma were carefully excluded by doctor's diagnosis and selfreported history, symptoms, treatment, or diagnosis of asthma. Almost all patients were recruited from family practices. Recruitment and follow-up was between 2000 and 2007. Patients were randomly assigned to (Figure 4):

1) short-term fluticasone propionate 500 µg twice daily, followed by placebo
2) long-term fluticasone 500 µg twice daily
3) combination of long-term fluticasone and salmeterol 500/50 µg twice daily
4) long-term placebo

Figure 4. Study design

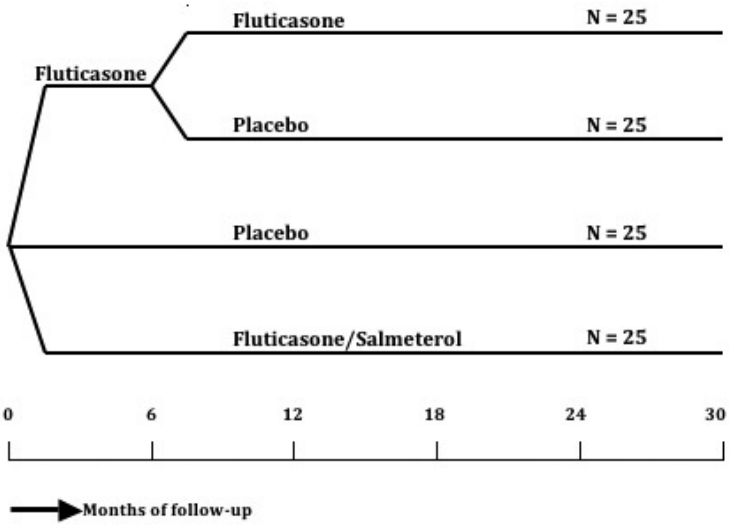


Table 1. In- and exclusion criteria

Inclusion criteria

1. Age: 45-75 years
2. > 10 packyears of smoking
3. At least one of the following symptoms: chronic cough, chronic sputum production, frequent exacerbations, or dyspnea on exertion
4. No course of oral corticosteroids during last 3 months, no maintenance treatment with inhaled or oral steroids during last 6 months
5. Postbronchodilator value (after 400 µg of inhaled salbutamol) of FEV_1 below the 90% confidence interval (90% CI) of the predicted FEV_1, and postbronchodilator FEV_1/IVC ratio below the 90% CI of the predicted FEV_1/IVC ratio [107]
6. Postbronchodilator FEV_1 > 1.3 liter and > 20% of predicted value.
7. Written informed consent

Exclusion criteria

1. Prior or concomitant history of asthma
2. Alpha-1 antitrypsin deficiency (SZ, ZZ, zero phenotype)
3. Other active lung disease except for mild bronchiectasis; bronchiectasis should not be the main reason for chronic cough and/or sputum production with additional mild obstruction.
4. Contra-indications for elective bronchoscopy, such as O_2 saturation <90%, abnormal coagulability, anti-coagulant therapy which cannot be temporarily withheld for performance of bronchoscopy, history of pneumothorax, uncontrolled angina pectoris.
5. Other diseases likely to interfere with the purpose of the study.
6. Inability to keep diary and to understand written and oral instructions in Dutch.

The predefined primary outcome was inflammatory cell counts in bronchial biopsies (Figure 5). Measurements of symptoms, health status, self-reported smoking status, medication compliance, and spirometry were made every 3 months. Bronchoscopy, sputum induction, and methacholine challenge were performed at 0, 6 and 30 months.

Figure 5. Measurements

Every 3 months: Questionnaires, SGRQ, CCQ, Postbronchodilator spirometry, Compliance with medication, 2 weeks diary (CCQ and PEF)

* Baseline, 6 and 30 months:

- Day 1: Venapunction: Hb, leucocytes, IgE, α1AT phenotype; Reversibility of FEV_1; N2 sb test; ECG
- Day 2: Chest X-ray; Questionnaires: Rand36, QOL-RIQ, EuroQOL, TTO, VAS; Body box: Raw, sGaw, TLC, RV; CO-diffusion capacity; PC_{20} Mch
- Day 3: eNO; 6 MWD; Sputum induction
- Day 4: Bronchoscopy

Outline of this thesis

Airway symptoms and inflammation

Chapter 2. Exploration of clinical symptoms and airway inflammation by factor analysis.

Chapter 3. Is health status in COPD associated with inflammation? In this chapter the association between health status and inflammatory cell counts in induced sputum and bronchial biopsies are investigated.

Chapter 4. Is there a phenotype of chronic bronchitis? Are COPD patients with chronic bronchitis characterised by a specific inflammatory cell profile in bronchial biopsies and sputum?

Effects of long-term inhaled corticosteroids and long-acting ß$_2$-agonists

Chapter 5. Do patients benefit from short- versus long-term therapy with inhaled corticosteroids and long-acting ß$_2$-agonists? The effects of 6- and 30-month treatment were assessed on health status, lung function and airways inflammation in induced sputum and bronchial biopsies.

Chapter 6. Which patients benefit the most from long-term therapy with ICS and long-acting ß$_2$-agonists? Long-term treatment with ICS was compared with placebo.

Chapter 7. Is there an effect of long-term therapy with inhaled corticosteroids and long-acting ß$_2$-agonists on day-to-day health status and peak expiratory flow?

Conclusions, general discussion and future implications

Chapter 8. A summary of the main results and conclusions of the different studies is presented. In addition, implications of these findings for clinical practice and future directions for research are discussed.

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Chapter 2

Dissociation of lung function and airway inflammation in Chronic Obstructive Pulmonary Disease (COPD)

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Abstract

Chronic obstructive pulmonary disease (COPD) is defined by progressive, irreversible airflow limitation and an inflammatory response of the lungs, usually to cigarette smoke. However, COPD is a heterogeneous disease in terms of clinical, physiological, and pathological presentation. We aimed to evaluate whether airflow limitation, airway responsiveness, and airway inflammation are separate entities underlying the pathophysiology of COPD by using factor analysis. A total of 114 patients (99 males/15 females, age 62 ± 8 years, 42 pack-years smoking, no inhaled or oral steroids >6 months) with irreversible airflow limitation (postbronchodilator FEV_1 63 ± 9% predicted, FEV_1/inspiratory vital capacity [IVC] 48 ± 9%) and symptoms of chronic bronchitis or dyspnea were studied in a cross-sectional design. Postbronchodilator FEV_1 and FEV_1/IVC, reversibility to inhaled β_2-agonists, diffusing capacity, provocative concentration of methacholine required to produce a 20% drop in FEV_1, total serum IgE, exhaled nitric oxide, and induced sputum cell counts (% eosinophils, % neutrophils) were collected. Factor analysis yielded 4 separate factors that accounted for 63.6% of the total variance. Factor 1 was comprised of FEV_1, FEV_1/IVC, and residual volume/total lung capacity. Factor 2 included reversibility, IgE, provocative concentration of methacholine required to produce a 20% drop in FEV_1, and diffusing capacity. Factor 3 contained exhaled nitric oxide and factor 4 included sputum % neutrophils and % eosinophils. We conclude that airflow limitation, airway inflammation, and features commonly associated with asthma are separate and largely independent factors in the pathophysiology of COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is a disease characterized by progressive airflow limitation, which is not fully reversible [1]. However, COPD has been recognized as a heterogeneous disorder [2], with components of chronic bronchitis, small airways disease, emphysema, and in some patients perhaps, features of asthma [3]. This is accompanied by pathophysiological characteristics, such as partial reversibility to bronchodilators, air trapping, impaired diffusing capacity, and airway hyperresponsiveness [4]. The presence and contribution of these features to the severity of COPD varies between patients and may reflect distinct pathophysiological mechanisms in development, clinical presentation, and course of the disease. It is increasingly recognized that such disease heterogeneity provides opportunities for targeted interventions [3;5].

Airway inflammation is thought to play an important role in the pathogenesis of COPD [6]. The cellular inflammatory response is characterized by an increase in neutrophils, macrophages, and CD8-positive T lymphocytes in small and large airways as well as in lung parenchyma [7]. The major cell type in induced sputum is the neutrophil, the quantity of which is associated with the severity of airflow limitation [8;9]. Although induced sputum does not cover all the inflammatory and structural changes of the lungs in patients with COPD, it does represent a noninvasive marker of inflammation that is potentially useful for disease monitoring. Sputum eosinophilia has also been observed in patients with stable COPD, but its relationship to airflow limitation is controversial [8;10;11]. It has been argued that sputum eosinophilia is related to concomitant features of asthma [12]. This link would indicate that the pathophysiological entities underlying the clinical phenotypes in COPD may be diverse and are still largely unknown.

The aim of this study was to objectively specify the heterogeneity of COPD by categorizing various functional and inflammatory features of COPD into separate, complementary domains without *a priori* assumptions. Factor analysis allows reducing multiple disease characteristics to a few independent factors, in which each factor groups associated parameters. Because this is essentially accomplished free of predetermined hypothesis on any interrelated parameters, this technique can be considered as a hypothesis-generating analysis.

Factor analysis has been applied previously in studies of patients with asthma [13-16] and COPD [17]. In patients with asthma, it has demonstrated that lung function, baseline airway hyperresponsiveness, and eosinophilic inflammation

in sputum are nonoverlapping dimensions [13], suggesting that evaluation of patients with asthma should include measurement of all these parameters. In COPD, factor analysis has been applied to study the relationship between dyspnea ratings, exercise capacity, lung function and hyperinflation [17-23]. However, these studies did not include inflammatory markers and were unable to study the interrelationships between airway inflammation and the functional features of COPD. Therefore, in this study, we performed factor analysis, including lung function indices and markers of inflammation in induced sputum and exhaled air, in 114 patients with clinically stable COPD. Some of the results of this study have been previously reported in the form of an abstract [24].

Material and Methods

Patients

Hundred fourteen patients (15 women) aged between 45 and 75 years with COPD, participating in a multi-center trial (Groningen Leiden Universities and Corticosteroids in Obstructive Lung Disease; GLUCOLD study), were included in this study. Patients were recruited from our outpatient clinics, as well as by advertisements in local newspapers, and by screening lung functions from patients in general practice in and around Leiden and Groningen, The Netherlands. All patients were current or ex-smokers and had a history of at least 10 pack years of smoking. They had irreversible airflow limitation (postbronchodilator FEV_1 and $FEV_1/IVC < 90\%$ confidence interval (CI) of the predicted value [25], $FEV_1 \geq 1.3$ liter and $> 20\%$ of the predicted value) and at least one of the following symptoms: chronic cough, chronic sputum production, or dyspnea on exertion. In choosing the 90% CI of predicted values as selection criterion, as opposed to percentage of predicted values *per se*, we followed the recommendation by the European Respiratory Society [25]. Patients with a prior or concomitant history of asthma, alpha-1 antitrypsin deficiency (SZ, ZZ, zero phenotype), or other active lung disease except for mild bronchiectasis were not permitted to the study. The patients did not use a course of oral or inhaled steroids during the last three months, and did not have maintenance treatment with inhaled or oral steroids during the last six months. Maintenance drug therapy of non-selective beta-antagonists, long-acting bronchodilators, methylxanthines, N-acetylcysteine or NSAID's was not permitted. Patients were allowed to use short acting β_2-agonists and ipratropium bromide as rescue medication. The patients were in clinically

stable condition and had no symptoms of respiratory tract infection for at least two weeks prior to the study. The Medical Ethics Committees of each center approved the study and all patients gave their written informed consent.

Study Design

The present study had a cross-sectional design, consisting of baseline measurements of the GLUCOLD Study. At visit 1 spirometry was performed before and after inhalation of salbutamol and blood was collected for measurement of total IgE. Body plethysmography, CO-diffusing capacity, and bronchial responsiveness to methacholine were obtained at a second visit. Finally, at the third visit exhaled NO was measured and hypertonic saline-induced sputum was collected. Inhaled bronchodilators were withheld for at least 8 hrs prior to visit day 1 and 2. All visits were performed within six weeks.

Lung Function

Spirometry was performed according to international guidelines [26]. A daily-calibrated pneumotachograph (Masterscreen Pneumo or Masterscreen IOS; Jaeger, Würzburg, Germany) was used throughout the study. First, 3 slow inspiratory vital capacity maneuvers (IVC, largest value used) were carried out. Second, maximally 8 forced expiratory vital capacity (FVC) maneuvers were performed to obtain at least 3 technically satisfactory expiratory flow-volume curves from which the forced expiratory volume in 1 second (FEV_1) and FVC did not deviate > 5% or 100 ml from the largest FEV_1 and FVC. From these curves, we used values of the curve with the largest sum of FEV_1 and FVC [26]. Reversibility of airflow limitation (ΔFEV_1) was measured 15 min after administration of 400 µg salbutamol per metered dose-inhaler connected to a spacer [27]. The response was expressed as change in FEV_1 as percentage of predicted value [27].

Total lung capacity (TLC) and residual volume (RV) were measured using a constant volume body plethysmograph (Masterscreen body or Masterlab body; Jaeger, Würzburg, Germany), using panting at 0.5 Hz [25].

The diffusing capacity (transfer factor) for carbon monoxide (TLCO) and TLCO per liter alveolar volume (K_{CO}) were measured using the single breath holding method with a rolling seal closed system (Masterscreen MS-CS-FRC, Masterlab transfer or Compactlab transfer; Jaeger, Würzburg, Germany) [28]. Reference values for all lung function measurements were obtained from Quanjer *et al* [25].

Methacholine challenge tests were performed according to the tidal breathing

method [29], using serial doubling concentrations methacholine-bromide (0.038 to 39.2 mg/ml) in saline. Methacholine was aerosolized (DeVilbiss 646, Somerset, PA) and inhaled by the 2-min tidal breathing method at 5-minute intervals until FEV_1 dropped by ≥ 20% (lowest of two FEV_1 values; at 30 and 90 seconds) from baseline. The response was expressed as the provocative concentration causing 20% fall in FEV_1 (PC_{20}).

Sputum induction and processing

Sputum was induced and processed according to a validated technique [30], with some modifications. Prior to sputum induction, 200 µg salbutamol was administered and baseline FEV_1 was recorded. Hypertonic sodium chloride aerosols (4.5 w/v %) were generated by a DeVilbiss Ultraneb 2000 ultrasonic nebulizer with a calibrated particle size (MMAD 4.5 µm) at maximal output (2.5 ml/min). The aerosols were inhaled via the mouth through a two-way valve (No. 2700; Hans-Rudolph, Kansas City, MO, USA), with the nose clipped. Subsequently, the patients inhaled hypertonic saline aerosols during 3 periods of 5 min and sputum was expectorated after each inhalation. Salbutamol was administered when FEV_1 dropped by > 10% from post-salbutamol baseline value and the procedure was interrupted when FEV_1 dropped by > 20%.

Whole sputum samples were processed. The sample was then mixed with an equal volume of dithiothreitol 0.1% w/v (Sputolysin, Calbiochem, USA) and placed in a shaking water bath at 37°C for 15 min. Then sputum was filtered through a nylon gauze (pore-size approximately 48 µm) and centrifuged (450 x g) for 10 minutes at 20oC. The cell pellet was then resuspended in phosphate-buffered saline (PBS) containing 1 % (w/v) human serum albumin (HSA), pH 7.4. Cell viability and total cell counts were performed by Trypan blue exclusion using a hemacytometer. Subsequently, cytocentrifuge slides were prepared (450 rpm, 6 minutes, 100 µl/slide, Cytospin 3, Shandon, Life Sciences International, Veldhoven, NL) and differential cell counts were performed on May-Grünwald-Giemsa-stained cytospins by a qualified technician. Differential leucocyte and cylindric epithelial cell counts were expressed as a percentage of nucleated cells excluding squamous cells. A sputum sample was considered adequate when the percentage squamous cells was less than 80% [30].

Exhaled nitric oxide

Exhaled NO (eNO) levels were determined according to a standardized procedure [31] with some modifications, using a chemiluminescence analyzer

(Sievers NOA 270B or ECO physics CLD 700 AL). Patients were asked not to smoke for at least 1 hour prior to the test. A slow vital expiratory capacity maneuver with a constant expiratory flow of 100 mL/sec against an expiratory resistance of 5 cm H_2O was performed. Expiratory NO concentration was sampled continuously from the center of the mouthpiece and the average concentration was determined during a period of 10 seconds. Three reproducible successive recordings were made at 30-s intervals, from which the mean values of exhaled NO were used in the analysis.

Peripheral blood measurements

Total serum IgE concentrations were measured by fluoroimmunoassay (FEIA) using the Pharmacia CAP system (Pharmacia Diagnostics, Uppsula, Sweden).

Statistical analysis

Mean values and SD were computed. When appropriate variables were logarithmically transformed before statistical analysis and presented as median with interquartile range.

Exploratory factor analysis included the following variables: postbronchodilator FEV_1 (%pred), postbronchodilator FEV_1/IVC (%), ΔFEV_1 (%pred), K_{CO} (%pred), PC_{20} (mg/ml), RV/TLC (%), eNO (ppb), sputum % neutrophils and % eosinophils and total serum IgE (IU/ml). The possibility to perform factor analysis was tested by Bartlett's test of sphericity. The Kaiser-Meyer-Olkin (KMO), a measure of sampling adequacy based on correlation and partial correlation, was also evaluated. A high KMO (maximum 1.0) indicates that data are likely to factor well since correlations between pairs of variables can be explained by the other variables (low partial correlation coefficients). Correlation coefficients were analyzed by principal component factor analysis and subsequent rotation according to the standard Varimax criterion [32]. In this type of analysis, the correlation between parameters is attributed to their common dependence on independent entities called "factors". The parameters are separated into independent subgroups, and the correlation of parameters within subgroups is due to their common factor. The coefficients that link parameters to factors are called "factor loadings", and are the correlation coefficient between parameters and factors. The number of factors is chosen to be as small as possible but large enough to account for most of the variation within the data. The number of factors was determined by the number of eigenvalues, an index of the proportion of variance explained by successive factors, whose magnitude was ≥ 1 on the Scree plot.

However, also the percentage of total variance explained by the factors was taken into account. The Varimax rotation procedure aims to increase the interpretability of the factors by rotation to a simple structure with optimal loadings, such that they are high or low. Ideally, each variable would have a high loading on one factor, whereas its loadings on other factors would be low. In the Varimax rotation this is technically achieved by maximalizing the variation within a factor. Finally, we conducted three additional factor analyses to determine the stability of the factor structures, and thus the robustness of our findings. First, we excluded outliers from the data set, defined as data outside the range of mean ± 3 x SD, and repeated the factor analysis. Next, the analysis was repeated with number of neutrophils and eosinophils per ml sputum, instead of % neutrophils and eosinophils. Since smoking is the main risk factor for development of COPD, an additional factor analysis was performed in which the number of pack years was added. Univariate correlations were evaluated using Pearson correlation coefficient. All analyses were performed with the Statistical Package of Social Sciences (SPSS 11.0).

Results

Patient characteristics

Patient characteristics of the 114 participants are presented in Table 1. The mean (SD) postbronchodilator FEV_1 was 63.0 (8.8) %pred, with a range from 40.8 to 77.7 %pred. This result indicates that all patients were classified as having moderate to severe COPD according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (GOLD stage II and III) [1]. The patients were heavy smokers with a median of 41.8 pack-years and most of them were current smokers (63.2%).

Table 1. Patient characteristics

	n	Mean (SD or IQR)
Clinical characteristics		
Sex, male/female	114	99 / 15
Age, yr	114	62 (8)
Smoking history, pack-years *	114	41.8 (31.2-54.8)
Current smoker, yes/no, n	114	72 / 42
IgE, IU/ml*	113	40 (11.5-125)
Lung Function		
Post FEV_1, %pred	114	63.0 (8.8)
Post FEV_1/IVC, %	114	48.2 (8.5)
ΔFEV_1, %pred	113	6.9 (4.9)
K_{CO}, %pred	112	75.9 (25.5)
PC_{20}, mg/ml †	110	0.60 (2.76)
RV/TLC, %	113	48.5 (8.8)
Airway inflammation		
eNO, ppm	92	13.1 (12.7)
Sputum eosinophils, % *	106	1.1 (0.3-2.2)
Sputum neutrophils, %	106	69.4 (16.0)
Sputum eosinophils, n (x 10^4/ml) *	106	1.4 (0.4-4.5)
Sputum neutrophils, n (x 10^4/ml) *	106	102 (47-229)

*Definition and abbreviations: ΔFEV_1 = change in FEV_1 as percentage of predicted (reversibility to salbutamol); eNO = exhaled nitric oxide; IQR = interquartile range (25^{th} and 75^{th} percentile"); IVC = inspiratory vital capacity; K_{CO} = diffusing capacity for carbon monoxide per liter alveolar volume; %pred = percentage of predicted; PC_{20} = provocative concentration of methacholine causing a 20% fall in FEV_1; RV = residual volume, TLC = total lung capacity. *Median (IQR). †Geometric mean ± doubling dose.*

Table 2. Varimax Rotated Factor-loading Matrix from Factor Analysis

	Factor 1	Factor 2	Factor 3	Factor 4
Postbr. FEV_1, %pred	**0.89**	-0.16	-0.09	-0.07
Postbr. FEV_1/IVC, %	**0.82**	0.04	-0.23	0.22
RV/TLC, %	**-0.59**	-0.36	-0.03	0.15
ΔFEV_1, %pred	-0.06	**-0.72**	0.08	0.11
IgE, IU/ml	-0.09	**0.61**	0.10	-0.07
PC_{20}, mg/ml	0.36	**0.51**	-0.35	0.14
K_{CO}, %pred	0.44	**0.49**	0.25	0.31
eNO, ppb	-0.09	0.10	**0.89**	-0.11
Sputum eosinophils, %	-0.09	0.02	0.19	**-0.84**
Sputum neutrophils, %	-0.18	-0.25	0.47	**0.54**
Eigenvalue	2.67	1.50	1.20	0.99
Total variance explained, %	26.7	15.0	12.0	9.9

Definition and abbreviations: ΔFEV_1 = change in FEV_1 as percentage of predicted (reversibility to salbutamol); eNO = exhaled nitric oxide; IVC = inspiratory vital capacity; K_{CO} = diffusing capacity for carbon monoxide per liter alveolar volume; %pred = percentage of predicted; PC_{20} = provocative concentration of methacholine causing a 20% fall in FEV_1; RV = residual volume, TLC = total lung capacity. Bold values represent the highest loadings of a variable.

Factor analysis

Bartlett's test of sphericity indicated a correlation between the presently used variables because the correlation matrix was statistically different from an identity matrix (approximate $\chi^2 = 165.864$, degrees of freedom = 45, $p<0.001$). The Kaiser-Meyer-Olkin value was 0.594. Factor analysis yielded 3 separate factors, explaining only 53.7% of the total variance in the data set when the eigenvalue 1 criterion was applied. Therefore, an additional factor analysis was performed with the same data in which 4 factors were selected. This resulted in an increase of total explained variance to 63.6%.

The correlations with the original variables obtained for each Varimax-rotated factor (called factor loadings) and the eigenvalues are displayed in Table 2. FEV_1, FEV_1/IVC, and RV/TLC loaded significantly on factor 1. Factor 2 included ΔFEV_1, total IgE, PC_{20}, and K_{CO}. eNO loaded on factor 3, whereas sputum % neutrophils and eosinophils loaded on factor 4. Interestingly, K_{CO} contributed also to factor 1, and % neutrophils contributed also to factor 3.

Additional Factor Analyses

Factor analysis without outliers in the data set resulted in a similar four-factor structure as the original one, accounting for 63.4% of the total variance, with

the exception that % neutrophils loaded on factor 3 with eNO and not on factor 4.

Factor analysis with number of neutrophils and eosinophils per ml sputum, instead of % neutrophils and eosinophils, did not change the contents of the factors essentially. Again four factors, accounting for 66.0% of the total variance, were found using the eigenvalue 1 criterion. Factor 1 was the same as in the original analysis. Factor 2 included numbers of neutrophils and eosinophils, both with a positive factor loading. Factor 3 contained the variables that originally loaded on factor 2: ΔFEV_1, total IgE, and PC_{20}. Finally, factor 4 included eNO and K_{CO}.

Factor analysis with inclusion of number of pack-years as an additional variable revealed four factors explaining 59.1% of total variance, according to the eigenvalue 1 criterion. All factors were similar to the original analysis described previously, with the exception that K_{CO} and PC_{20} switched from factor 2 to factor 1. Number of pack-years smoked loaded on factor 2 together with ΔFEV_1 and total IgE (Table 3).

Table 3. Varimax Rotated Factor-loading Matrix from Factor Analysis with pack years

	Factor 1	Factor 2	Factor 3	Factor 4
Postbr. FEV_1/IVC, %	**0.77**	-0.0002	-0.33	0.20
Postbr. FEV_1, %pred	**0.72**	-0.19	-0.24	-0.03
RV/TLC, %	**-0.68**	-0.05	-0.20	0.25
K_{CO}, %pred	**0.59**	0.25	0.37	0.24
PC_{20}, mg/ml	**0.59**	0.28	-0.13	-0.03
Pack-years	-0.22	**0.70**	-0.32	0.06
ΔFEV_1, %pred	-0.29	**-0.62**	-0.11	0.20
IgE, IU/ml	0.07	**0.61**	0.18	-0.05
eNO, ppb	-0.18	0.05	**0.84**	0.02
Sputum eosinophils, %	-0.23	0.09	0.16	**-0.74**
Sputum neutrophils, %	-0.28	-0.08	0.25	**0.68**
Eigenvalue	2.67	1.56	1.22	1.06
Total variance explained, %	24.2	14.2	11.1	9.6

Definition and abbreviations: ΔFEV_1 = change in FEV_1 as percentage of predicted (reversibility to salbutamol); eNO = exhaled nitric oxide; IVC = inspiratory vital capacity; K_{CO} = diffusing capacity for carbon monoxide per liter alveolar volume; %pred = percentage of predicted; PC_{20} = provocative concentration of methacholine causing a 20% fall in FEV_1; RV = residual volume, TLC = total lung capacity. Bold values represent the highest loadings of a variable.

Univariate correlations

The univariate relationships among physiological and inflammatory parameters (sputum neutrophils, eosinophils, and eNO) were as follows. ΔFEV_1 was not associated with inflammatory parameters; however, RV/TLC was associated with sputum % neutrophils (r=0.203, p=0.04), and postbronchodilator FEV_1, RV/TLC, and PC_{20} were associated with number of neutrophils/ml sputum (r=-0.246, p=0.01; r=0.213, p=0.03 and r=-0.338, p<0.001, respectively). Postbronchodilator FEV_1, PC_{20}, and FEV_1/IVC were associated with eNO levels (r=-0.203, p=0.05; r=-0.207, p=0.05 and r=-0.304, p=0.003, respectively). FEV_1/IVC was also related to sputum % eosinophils (r=-0.219, p=0.02), while postbronchodilator FEV_1, K_{CO}, PC_{20}, and FEV_1/IVC were also associated with number of eosinophils/ml sputum (r=-0.207, p=0.03; r=-0.204, p=0.04; r=-0.243, p=0.01 and r=-0.242, p=0.01, respectively).

Discussion

The aim of this study was to objectively specify the heterogeneity of COPD, by categorizing the various functional and inflammatory features of COPD into separate, complementary domains without *a priori* assumptions. Therefore, we performed a factor analysis using physiological and inflammatory data of 114 patients with moderate to severe COPD, not treated with inhaled steroids. This resulted in a four-factor structure, explaining 63.6% of the total variance. Factor 1 included: FEV_1, FEV_1/IVC and hyperinflation; factor 2: β_2-response, total serum IgE, airway hyperresponsiveness and K_{CO}; factor 3 included: eNO, and factor 4 included: sputum % neutrophils and eosinophils. These four factors indicate that airflow limitation, features commonly associated with asthma, and airway inflammation are separate, largely independent dimensions that characterize patients with COPD.

To our knowledge, this is the first study in patients with COPD combining functional parameters and markers of airway inflammation in a factor analysis. Previous studies have applied factor analysis on quality-of-life, symptoms scores, exercise capacity, and lung function parameters in patients with stable COPD, without evaluating inflammatory indices [17-23]. However, the latter have been part of factor analysis in recent asthma research [13;15;16]. In patients with mild to moderate asthma, Rosi and colleagues demonstrated by factor analysis that airway function, bronchial responsiveness with reversibility, and eosinophilic inflammation, as assessed in sputum, are independent dimensions [13]. Our current results demonstrate that airway function,

bronchial responsiveness with reversibility, and inflammation as assessed in sputum or exhaled are predominantly nonoverlapping dimensions in patients with COPD as well.

Interestingly, this study showed that measurements of airflow limitation, traditionally used to determine disease severity in COPD [33], and hyperinflation, a measure of air trapping, were combined in the first factor. According to the statistical method of factor analysis, these measurements represent an important, separate dimension in the assessment of patients with stable COPD. This result is consistent with some [17;23], but not all [21;22] previous factor analyses of lung function parameters in COPD. The second factor extracted from the data included reversibility of FEV_1, airway hyperresponsiveness, total serum IgE, and diffusing capacity. Similarly, Ries and colleagues reported that bronchodilator response and diffusing capacity of COPD patients were grouped into separate factors from expiratory flow rates and hyperinflation [17]. To our knowledge, there are no other studies in patients with COPD that have included these variables in a factor analysis. Finally, the third and fourth factor included eNO and sputum % neutrophils and eosinophils, respectively. This is a novel finding, illustrating the partial independency of these markers of inflammation from the traditionally used functional disease markers in COPD.

We included a large (n=114) group of patients with stable COPD, not using inhaled steroids for at least 6 months and without a clinical diagnosis of asthma. In terms of disease severity, patient characteristics included COPD patients of GOLD Stage II and III. Inclusion of very mild or very severe patients could have produced different results, and therefore our results potentially lack generalizability. In contrast to some other studies, we did not exclude patients with COPD who were partially reversible to a bronchodilator, because the selection of non-reversible patients only would have excluded a large group of patients with COPD [34-36]. Furthermore, it needs to be emphasized that this is a cross-sectional analysis of patients with stable COPD, and that the results do not account for exacerbations and other temporal events. Sputum cell counts and eNO were measured as noninvasive markers of inflammation. Obviously, this does not cover all the inflammatory and structural changes of the airways in COPD, but it does represent the markers that are potentially useful for disease monitoring.

Factor analysis is not an approach that is widely applied, presumably because of its complexity. A simple example that clarifies its value and interpretation has recently been described by Juniper and colleagues [14]. The purpose of

this procedure is to reduce a large set of variables and to clarify (absence of) relationships between various parameters without reference to a specific criterion. Factor analysis does not regroup variables that are highly correlated, but factors are created based on calculated estimates of shared variance among variables, with the restriction that the factors reflect independent sources of variation. This procedure allows the user to determine whether associations between parameters are attributable to noise of measurements. In clinical research, factor analysis allows the many parameters that characterize the disease to be reduced to a few, relatively independent factors. We applied standard procedures of exploratory factor analysis with respect to the number of variables used in the analysis, the selection of number of factors, and the factor rotation [32]. Additional factor analyses with replacement of % neutrophils and % eosinophils by cell counts per ml sputum, exclusion of outliers from the data set, and addition of the cumulative amount of smoking resulted in similar factor structures as the original analysis. This demonstrates the robustness of the current findings.

The disease heterogeneity in COPD in terms of lung function and inflammation suggests that distinct pathophysiological pathways contribute to COPD. In agreement with this concept, we observed that multiple functional and inflammatory characteristics were categorized into four independent dimensions. Interestingly, none of the parameters of factors 1 or 2 showed significant additional loadings on factors 3 or 4, and vice versa, which strengthens the independence of functional and inflammatory dimensions. One exception to this was K_{CO}, which loaded together with eNO in the additional factor analysis using number of sputum cells instead of cell percentages. The value of using factor analysis in mapping disease heterogeneity is illustrated by our finding that some of the parameters were found to provide complementary information (loading on different factors) despite the existence of mutual correlation in univariate analyses.

The first factor can be interpreted as irreversible airflow limitation. The fact that diffusing capacity also had modest loading on this factor confirms earlier findings that the destruction of lung tissue is associated with increased airflow limitation and hyperinflation [37]. Alternatively, diffusing capacity could also be a descriptor of the status of altered pulmonary circulation in COPD: another structural component of COPD. Hence, restructuring of airways as well as lung tissue seems to be an important mechanism resulting in airflow limitation. Interestingly, our data suggest that this process is greatly independent of neutrophilic and eosinophilic inflammation in the larger airways (grouped into

factor 4). Neutrophils are able to induce tissue damage through the release of serine proteases and oxidants. However, this is not a prominent feature of other pulmonary diseases where chronic airway neutrophilia is even more prominent, such as cystic fibrosis and bronchiectasis [38]. This suggests that other factors are involved in the generation of emphysema. In addition, increased neutrophil numbers are found in the airway lumen, but they are not a prominent feature of the airway wall or parenchyma in patients with COPD [7]. Furthermore, the presence and role of eosinophils in COPD are uncertain [38]. The observed increased levels of eosinophil cationic protein and eosinophil peroxidase in induced sputum from COPD patients suggest the eosinophils are degranulated [39], which may be the result of the high neutrophil elastase levels in COPD [40]. Our data supports this close relationship between neutrophils and eosinophils in COPD, but apparently, airflow limitation requires more than the presence of these granulocytes *per se*.

Parameters that are known to be associated with asthma predominantly grouped into the second factor. This may not be unexpected, since airway hyperresponsiveness, partial reversibility of airflow limitation, and increased serum IgE levels are not uncommon in COPD [41]. An alternative interpretation would be that this second factor represents risk factors for progression of COPD, because bronchodilator response, airway hyperresponsiveness, and serum IgE levels have been associated with lung function decline [42]. The finding that number of pack-years also loaded on factor 2 strengthens this concept, because smoking is known to be the main risk factor for progression of COPD [43]. Although factor 2 also included K_{CO}, its parallel loading on factor 1 suggests that gas exchange impairment is associated with diverse pathophysiology.

The current separation of airway hyperresponsiveness and FEV_1 into different factors supports epidemiological evidence that these disease characteristics provide complementary information on COPD [44], the PC_{20} in COPD not simply being a result of airflow limitation *per se*. However, the fact that PC_{20} also had moderate loading on the first factor and even highest loading the first factor in some of the additional factor analyses on, is in agreement with previous studies that suggest that PC_{20} is to some extent dependent on airway caliber in COPD [45]. Although it has been reported previously that partial reversibility of airflow limitation is associated with sputum eosinophilia and elevated eNO in COPD [34], our factor analysis suggests that these features are largely independent. This confirms a previous factor analysis in asthma [13] and again may challenge the concept that eosinophilic airways inflammation

is closely related to the "twitchiness" of the airways. Interestingly, we found that number of pack-years also loaded on the second factor with IgE and β_2-response, and not with airflow limitation or sputum neutrophils. A significant relationship between total IgE and the degree of tobacco smoking has been reported previously [46], suggesting that the increase in IgE may be partly due to tobacco smoking. Although, in this study, univariate analysis revealed significant correlations between eNO and FEV_1, FEV_1/IVC, and PC_{20}, eNO was extracted into a factor independent from functional parameters as well as sputum cell counts. This indicates that this exhaled marker might be a rather autonomic phenomenon and bodes ill for the use of eNO in monitoring of disease activity.

In conclusion, this analysis has categorized multiple disease features of COPD without *a priori* assumptions on their interrelationships. Our data suggest that airflow limitation, asthma-like components, eNO and sputum inflammatory cell counts offer separate and additive information about the pathophysiological condition of COPD patients. This confirms the complex heterogeneity of the disease and may change some of the current concepts on the distinct pathophysiological pathways involved. Accordingly, it needs to be examined whether the clinical evaluation of patients with COPD should include each of these complementary entities.

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Chapter 3

Airway inflammation contributes to health status in COPD:
a cross-sectional study

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Abstract

Background: Chronic obstructive pulmonary disease (COPD) is characterized by irreversible airflow limitation and airway inflammation, accompanied by decreased health status. It is still unknown which factors are responsible for the impaired health status in COPD. We postulated that airway inflammation negatively contributes to health status in COPD.

Methods: In 114 COPD patients (99 male, age: 62±8 yr, 41 [31-55] pack-years, no inhaled or oral corticosteroids, postbronchodilator FEV_1: 63±9 %pred, FEV_1/IVC: 48±9%) we obtained induced sputum and measured health status (St. George's respiratory questionnaire (SGRQ)), postbronchodilator FEV_1, hyperinflation (RV/TLC), and airway hyperresponsiveness to methacholine (PC_{20}). Sputum was induced by hypertonic saline and differential cell counts were obtained in 102 patients.

Results: Univariate analysis showed that SGRQ total and symptom score were positively associated with % sputum macrophages (r=0.20, p=0.05; and r=0.20, p=0.04, respectively). Multiple regression analysis confirmed these relationships, providing significant contributions of % sputum macrophages (B=0.25, p=0.021) and RV/TLC (B=0.60, p=0.002) to SGRQ total score. Furthermore, SGRQ symptom score was associated with % sputum macrophages (B=0.30, p=0.03) and RV/TLC (B=0.48, p=0.044), whilst SGRQ activity score was associated with % sputum macrophages (B=0.46, p=0.002), RV/TLC (B=0.61, p=0.015), and PC_{20} (B=-9.3, p=0.024). Current smoking and FEV_1 were not significantly associated with health status in the multiple regression analysis.

Conclusion: We conclude that worse health status in COPD patients is associated with higher inflammatory cell counts in induced sputum. Our findings suggest that airway inflammation and hyperinflation independently contribute to impaired health status in COPD. This may provide a rationale for anti-inflammatory therapy in this disease.

Background

Chronic obstructive pulmonary disease (COPD) is a major and growing cause of morbidity and mortality [1;2]. It is characterized by progressive and not fully reversible airflow limitation, as measured with the forced expiratory volume in one second (FEV_1). The airflow limitation is associated with a chronic inflammatory process in the airways and lung parenchyma in response to noxious particles or gases, in particular tobacco smoking [1;2].

In daily life COPD patients are bothered by airway symptoms such as dyspnea, cough and sputum production [2;3]. This is accompanied by a serious decrease of health status [4]. Several studies have attempted to link health status to the severity of airflow limitation in patients with COPD [4] and show that the relationship is at best a loose one. Even the largest study assessing health status by the St. George's respiratory questionnaire (SGRQ) provides only weak associations with the degree of airflow limitation, as measured by FEV_1 [5;6]. This suggests that other factors additionally contribute to the health status in COPD. One of those may be dynamic hyperinflation, *i.e.* increased residual volume and total lung capacity [7], possibly as a consequence of chronic inflammation and restructuring of the airways and/or parenchyma [8; 9].

The chronic inflammatory process in COPD is characterized by infiltration of the airways by neutrophils, macrophages and CD8-positive T cells [10;11]. Such features of inflammation in COPD are likely driven by various cellular pathways, including pro-inflammatory cytokines and mediators of oxidative stress [12;13]. These cytokines and mediators may not only be responsible for local airway inflammation but can also induce features of systemic inflammation in COPD [14-16]. The latter is assumed to be linked with impaired functional status in COPD [12], just as it has been shown in other chronic inflammatory conditions such as bronchiectasis, rheumatoid arthritis, chronic end-stage renal disease and inflammatory bowel syndrome [17;18]. Hence, it is not unlikely that the underlying local airway inflammation in COPD can drive impairment of health status as well [12].

We hypothesized that health status in COPD is affected by the severity of airway inflammation. The aim of our study was to test this hypothesis in a large cross-sectional study by assessing the relationship between airway inflammation, as measured by cell counts in induced sputum, and health status in COPD. In order to examine the independent effects of airway inflammation, the influence of clinical disease markers such as smoking, lung function, hyperinflation and airways hyperresponsiveness on health status was included.

Some of the results of this study has been previously reported in the form of an abstract [19].

Methods

Detailed information about subjects and methodology has been published previously [20]. In brief, 114 patients with COPD were included for the Groningen Leiden Universities Chronic Obstructive Lung Disease (GLUCOLD) Study. Patients (45-75 years, current or ex-smokers ≥10 pack-years) had at least one of the following symptoms: chronic cough, sputum production, or dyspnea on exertion. Postbronchodilator forced expiratory volume in one second (FEV_1) was > 1.3 liter and > 20% predicted and below the 90% confidence interval of the predicted FEV_1 [21]. Postbronchodilator FEV_1/IVC ratio was below the 90% confidence interval of the predicted FEV_1/IVC ratio. These lung function levels are compatible with GOLD stages II and III [2]. Patients were clinically stable for more than 2 months and free of common cold symptoms for 2 weeks before the measurements. They did not use a course of inhaled or oral corticosteroids during the past 3 months prior to randomisation and did not have maintenance treatment with these drugs during the past 6 months. Patients with considerable co-morbidity were excluded. Usage of short-acting bronchodilators was allowed during the study. Each center's local medical ethics committee approved the protocol and patients provided written informed consent.

This study represents a cross-sectional analysis of baseline data from the GLUCOLD Study. Health status was measured using the St. George's respiratory questionnaire [22]. This is a well-validated, standardized, self-administered questionnaire, specifically designed for respiratory diseases. It contains 50 items and is divided into three sections: symptoms (distress

Table 1. St. George's respiratory questionnaire (SGRQ): median scores (n=102)

	Median [IQR]
Total SGRQ score	32 [19-43]
Symptom SGRQ score	44 [34-55]
Activity SGRQ score	42 [23-54]
Impact SGRQ score	18 [8.0-30]

The SGRQ scores from 102 patients with adequate questionnaires and sputa. Data are presented as median [inter-quartile range (IQR)]. Higher SGRQ scores indicate worse health status: 0=best,100=worse.

caused by respiratory symptoms), activity (physical activities that cause or are limited by breathlessness), and impact (social and psychological effects of the disease). The total score and the three separate component scores were calculated. The scores range from zero to 100, where zero indicates best and 100 represents worst health status.

Sputum was induced and processed according to a validated technique [23]. After inhaling 200 µg salbutamol the patients inhaled hypertonic sodium chloride aerosols (4.5 w/v %) during 3 periods of 5 min. Whole sputum samples were processed within two hours from sputum induction. Differential cell counts were expressed as a percentage of nucleated cells, excluding squamous cells. A sputum sample was considered adequate when the percentage squamous cells was less than 80% [23].

Spirometry was performed, according to international guidelines [24], using the Quanjer reference values [21]. Total lung capacity (TLC) and residual volume (RV) were measured using a constant volume bodyplethysmograph [21]. Airway hyperresponsiveness was determined using the 2-minute tidal breathing method [25] and expressed as the provocative concentration causing a 20% fall in FEV_1 (PC_{20}). The diffusion capacity for carbon monoxide per liter alveolar volume (K_{CO}) was measured using the single breathholding method [26]. The associations of the SGRQ total, symptom, activity and impact scores with inflammatory cell counts and various other study variables were examined using Pearson's and Spearman's rank correlation. Differences between smokers and ex-smokers were analyzed with the Student *t* test and Mann Whitney U. Skewed data (pack-years, PC_{20}, % and numbers of inflammatory cells in sputum) were transformed when appropriate. Multiple linear regression analyses (ENTER method) were performed to assess the relation between health status (SGRQ total, symptom, activity and impact scores) and sputum inflammatory cell counts, independent of age, gender, current smoking, postbronchodilator FEV_1, RV/TLC, and PC_{20}. Probability values of ≤ 0.05 were considered significant. All analyses were performed using the Statistical Package for Social Sciences (SPSS)-12.

Results

Characteristics

A total of 114 patients were enrolled in the study. Patient characteristics have been published in extensive detail [20]. In short, most patients (87%) were middle-aged males (mean±standard deviation (SD) 62%±8). They had a median of 41 pack-years of smoking, 37% being ex-smokers. Patients had moderate to severe COPD as based on their postbronchodilator FEV_1 (mean±SD 63%±9 of predicted (pred)) and exhibited a wide range in RV/TLC (mean±SD 48±8) and PC_{20} (geometric mean, inter quartile range (IQR) 0.6 [0.17-2.40]). A total of 110 patients adequately completed the SGRQ and 102 from these were able to produce an acceptable sputum sample. Data from the 102 patients were used for all analyses. The median SGRQ scores were indicative of moderately impaired health status (Table 1). Number and differential counts of sputum cells are shown in Table 2.

Table 2. Inflammatory cells in induced sputum (n=102)

	Absolute numbers (10^4/ml)	Percentage
Total cell count	135.0 [76.8-311.3]	-
Neutrophils	99.2 [46.7-228.6]	72.6 [59.5-82.2]
Macrophages	32.3 [17.9-61.1]	22.8 [14.8-33.3]
Eosinophils	1.4 [0.3-4.8]	1.1 [0.3-2.2]
Lymphocytes	2.1 [1.0-6.8]	1.7 [1.2-2.3]
Epithelial cells	1.3 [0.6-3.8]	1.0 [0.3-2.3]

Data are presented as median [IQR]. Total cell count refers to the total number of non- squamous cells in sputum.

Univariate analysis

The total and symptom scores were positively associated with % macrophages (r=0.20, p=0.050; and r=0.20, p=0.041, respectively). The univariate relationship between the SGRQ scores and sputum inflammatory cell counts is shown in Table 3. The regression coefficient (B) in Table 3 represents the strenght of the association. Our results show that an increase in sputum macrophages of 1 % is associated with an increase of the mean total score of 0.22 point. In addition, figure 1 shows the effect-size of a higher percentage of sputum macrophages on the SGRQ scores. Patients with <15% sputum macrophages have a mean total score of 27. The total score is on average 5 points higher in patients with 15-45 % sputum macrophages, and 9 points higher in patients with >

Table 3. Association between sputum cell differential counts and health status assessed with SGRQ (n=102), results from linear regression analyses

		SGRQ total score	symptom score	activity sore	impact score
Total cell count †	B (95% CI)	-1.7 (-8.0 to 4.6)	-4.6 (-12 to 3.4)	-0.74 (-9.2 to 7.7)	-1.1 (-7.3 to 5.1)
% Neutrophils	B (95% CI)	-0.17 (-0.36 to 0.02)	-0.20 (-0.44 to 0.04)	-0.24 (-0.49 to 0.02)‡‡	-0.11 (-0.30 to 0.08)
% Macrophages	B (95% CI)	0.22 (<0.01 to 0.43)*	0.28 (0.01 to 0.56)*	0.28(-0.01 to 0.56)‡	0.15 (-0.07 to 0.36)
% Eosinophils #	B (95% CI)	0.22 (-4.0-4.5)	-0.73 (-6.13-4.67)	2.42 (-3.27-8.10)	-0.61(-4.80-3.58)

*The univariate association between SGRQ scores (dependent) and sputum cell counts (independent) was expressed by regression coefficient B with corresponding 95% confidence intervals (95% CI). The regression coefficient B represents the strength of the association. Our results show that an increase in sputum macrophages of 1 % is associated with an increase of the mean total score of 0.22 point, indicating that an increase in sputum macrophages of 20% is associated with an increase of the mean total score of 4.4 points, which exceeds the clinically relevant threshold of four units in SGRQ scores. †Total cell count was logtransformated, #% eosinophils were transformed using the square root. *p≤0.05, ‡p=0.061, ‡‡p=0.068.*

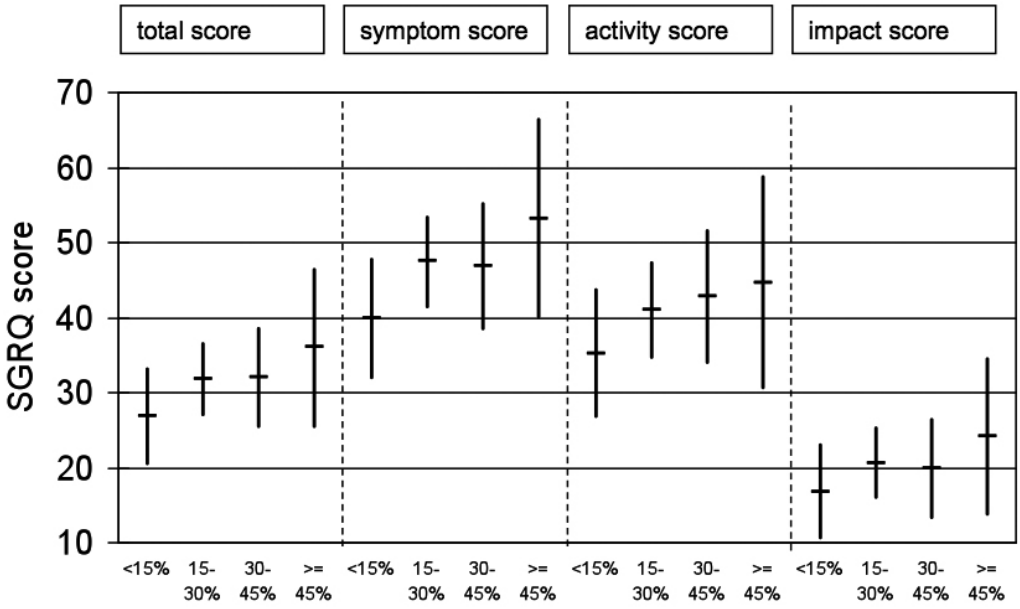
45 % sputum macrophages. A significant threshold of four units in SGRQ scores may be considered as clinically relevant [27]. The activity and impact scores were not significantly associated with sputum % macrophages (r=0.19, p=0.061; rs=0.14, p=0.16, respectively). No significant associations were found between all SGRQ scores and percentages of neutrophils, eosinophils, lymphocytes, epithelial cells, nor with absolute numbers of total sputum cells, neutrophils, macrophages, lymphocytes, and epithelial cells.

With regard to clinical and functional parameters, the total, symptom and impact scores were higher among smokers, as compared with ex-smokers (median total score=33.2 vs 25.8, p=0.040; median symptom score=46.8 vs 40.9, p<0.01; median impact score=19.7 vs 11.3, p=0.023, for smokers and ex-smokers, respectively). Higher symptom scores were associated with a larger amount of pack-years (r=0.29, p<0.01). Higher activity scores were associated with lower postbronchodilator FEV_1 (r=-0.24, p=0.017), increased lung hyperinflation, as assessed by RV/TLC ratio (r=0.25, p=0.012) and less hyperresponsiveness, as assessed by PC_{20} (r=-0.22, p=0.033). No associations were found between any of the SGRQ scores and CO-diffusion capacity, as assessed by K_{CO} (data not shown).

Multiple regression analysis

Multiple regression analysis confirmed the relationship between SGRQ total score and % sputum macrophages (B=0.25, p=0.021), with an explained variance of 14%. In this model there was a significant contribution of RV/TLC (B=0.60, p=0.002). Age, current smoking, gender, postbronchodilator FEV_1, or PC_{20} were not significantly associated with the total score in this model. The symptom domain also remained significantly associated with % sputum

Figure 1. Relationship between percentages macrophages in induced sputum (x-axis) and SGRQ scores (y-axis) (n=102). Results from linear regression analyses (B and 95% confidence interval)



macrophages in the multiple regression analysis (B=0.30, p=0.03), together with RV/TLC (B=0.48, p=0.044). Finally, multiple regression analysis showed a relationship between the activity score and % sputum macrophages (B=0.46, p=0.002), again with a significant contribution of RV/TLC (B=0.61, p=0.015) and also with PC_{20} (B=-9.3, p=0.024).

Discussion

This study demonstrates that health status in COPD is associated with inflammatory cell counts in induced sputum. The larger the percentage of sputum macrophages was, the more impaired a patient's health status was. This relationship was marginally modulated by the severity of hyperinflation and airways hyperresponsiveness.

These findings suggest that airway inflammation independently contributes to impaired health status in COPD.

The novelty of this study is that we observed a relationship between health status and inflammatory cell counts in induced sputum in steroid naive, clinically stable patients with moderately severe COPD. In general, health status was markedly impaired, as indicated by a median SGRQ total score of 32 [5]. Interestingly, our data suggest that the inflammatory process is a stronger determinant of health status than physiological measures of hyperinflation or airflow limitation. After taking percentage sputum macrophages into account, only RV/TLC demonstrated a consistent association with the total score and subdomains of the SGRQ. This points towards an independent role of hyperinflation among the determinants of health status. Indeed, patients with a relatively high degree of hyperinflation are known to have increased breathlessness and reduced physical activities, which is even more pronounced during exercise [28;29]. This is likely to affect health status, especially with regard to the activities domain. In addition, we found some evidence of a contribution of airway hyperresponsiveness, which extends previous observations in the general population [30;31]. Although smoking is associated with health status, our results show that the relationship between health status and sputum percentages macrophages, within patients with COPD, is similar in smokers as compared with ex-smokers.

Our patient selection and methods seem to be appropriate for the current study. The sample size of 102 patients with a complete data set provided sufficient data for multivariate analysis. In general, some relationships might have been arisen by chance given the potential for multiple comparisons. However, the univariate associations between inflammatory cell counts and health status found in this study remained statistically significant when adjusted for other relevant parameters using multiple regression analysis, suggesting an independent and consistent role for inflammation with regard to health status in patients with COPD. Patients with clinically relevant co-morbidity were excluded. We reasoned that marked co-morbidity additionally affects disease-specific health status [32], which could potentially introduce confounders. We excluded all patients with maintenance therapy of inhaled corticosteroids during the last six months. Inhaled corticosteroids influence the inflammatory cell counts in induced sputum in patients with COPD [33;34], which easily might have disturbed any disease-related associations between the inflammatory process and health status.

How can we explain the observed positive association between the percentage macrophages in sputum and health status? In previous studies, neutrophils have been linked to the severity of COPD, as measured with FEV_1 [35]. In

a previous report from our study group Lapperre et el. categorized various functional and inflammatory features of COPD into separate complementary domains using a different statistical analysis, a so-called factor analysis. This revealed that FEV_1 and neutrophilic inflammation are complementary dimensions that characterize patients with COPD [20]. However, several studies suggest a central role for macrophages in inflammatory processes and structural changes in the lung of patients with COPD [36;37].

Chemokines, such as monocyte chemoattractant protein 1 (MCP-1) and its receptor C-C chemokine receptor 2 (CCR2), have been implicated in the recruitment of macrophages into the bronchiolar epithelium in COPD [38]. These macrophages can release a large variety of inflammatory cytokines such as tumor necrosis factor (TNF-α), IL-8, CXC-chemokines, $LTB_{4,}$ and reactive oxygen species that are likely to drive airway inflammation in COPD. Moreover they produce elastolytic enzymes, e.g. metalloproteinases [39;40] such as macrophage elastase (MME), that may degrade the extracellular matrix and thus contribute to the development of parenchymal damage and thereby to pulmonary emphysema in COPD [13;36;41].

The novelty of this study is that associations were observed between health status and local airways inflammation, whilst previous studies suggested associations between impaired health status and systemic inflammation in COPD [14;42]. Previously, it has been suggested that the systemic inflammatory response may be due to a overflow of pulmonary mediators from the airways [14]. However, Vernooy *et al* showed that soluble tumour necrosis factor receptor (sTNF-R) and IL8 in sputum and plasma were not correlated, suggesting that the inflammatory process in the local and systemic compartment are regulated differentially [43;44]. In the airways neutrophilic inflammation is associated with lower FEV_1 levels in COPD [45]. The role of airway macrophages may be linked to different pathophysiological processes as mentioned above. Environmental exposures such as tobacco smoke may promote macrophage-induced alveolar damage [46], leading to impaired alveolar-capillary gastransport and accompanying changes in health status. Interestingly, our results are suggestive of a distinct role for the differential cell counts rather than the total amount of macrophages. Taken together, we may speculate that the local and systemic inflammatory responses are partly differentially regulated, mutually determining the COPD phenotype. If so, this will be of major importance when developing effective interventions in this disease.

The percentage macrophages in sputum was associated with the SGRQ total

score (a summary measure of health status), as well as the SGRQ symptom score (severity of symptoms) and SGRQ activity score (physical activities that cause or are limited by breathlessness). As shown in figure 1, the differences in health status between patients with relatively higher and lower percentages of macrophages can be considered as clinically relevant, because they reached the clinically significant threshold of four units in SGRQ scores [27]. This suggests that airway inflammation in COPD is relevant for disease outcome in daily life. Inflammatory cell counts in sputum were not associated with the impact score. This score measures social and psychological effects of the disease, such as anxiety and coping, and it is plausible that this score is less influenced by the inflammatory component of the disease. It is important to notice that only a limited part of health status could be explained by the severity of airway inflammation. The likely reason for this is that a wide spectrum of disease processes potentially affects health status [4]. Furthermore, other factors such as coping or the presence and frequency of exacerbations might also play an additional role in its impairment in patients with COPD [47].

We observed a consistent and independent contribution of hyperinflation on health status in patients with mild to moderate COPD. This is in line with a previous study, where hyperinflation was associated with poor health status in very severe patients with COPD who were using long-term oxygen treatment [48]. Hyperinflation causes an increase in lung volume with a concomitant increase of work of breathing, functional impairment of inspiratory muscle function, and adverse effects on haemodynamics which all may contribute to dyspnea [49]. In a recent study in COPD tiotropium bromide significantly decreased the residual volume [7], which was correlated with a decrease in dyspnea. This is indicative of the clinical relevance of hyperinflation in COPD, and the more so because dyspnea appears to be an important factor influencing health status [50]. In addition, the activity score measures physical activities that on the one hand induce breathlessness, and on the other may become limited by this particular symptom. Therefore, the current associations between hyperinflation and various domains of health status are not unexpected.

In conclusion, we have observed that a worse health status in COPD is significantly associated with higher inflammatory cell counts in induced sputum, whereas only marginally additional contributions were found for lung function measures reflecting hyperinflation. Our observation that airway inflammation negatively affects health status of COPD patients may have clinical relevance.

At present, anti-inflammatory therapy with inhaled corticosteroids is a recommended treatment option in patients with advanced COPD [2]. This has been shown to reduce deterioration in health status [5;51]. If health status is partly driven by the local inflammatory process in COPD this may provide a rationale for the usage of anti-inflammatory therapy in COPD. It now needs to be examined whether the severity of airway inflammation predicts the benefits of long-term anti-inflammatory intervention on health status in COPD.

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Chapter 4

Chronic bronchitis sub-phenotype within COPD: inflammation in sputum and biopsies

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Abstract

Rationale: The presence of chronic bronchitis predicts more rapid decline of FEV_1 in patients with chronic obstructive pulmonary disease (COPD). The hallmark of COPD is airway inflammation. It was hypothesised that COPD patients with chronic bronchitis are characterised by a distinct inflammatory cell profile as measured in bronchial biopsies and sputum.

Methods: From 114 COPD patients (male/female ratio 99/15, mean±SD age 62±8 yrs, current smoking 63%, post-bronchodilator FEV_1 63±9% predicted, no steroids), with and without chronic bronchitis, inflammatory cell counts in bronchial biopsies and induced sputum were measured. Analysis was carried out by logistic regression.

Results: COPD patients with chronic bronchitis had lower eosinophil counts in biopsies and higher percentages of sputum eosinophils than patients without those symptoms, which remained after adjustment for smoking and sex. Patients with chronic bronchitis also showed higher percentages of macrophages and lower percentages of neutrophils in sputum, which could be explained by differences in smoking and sex.

Conclusion: It was concluded that chronic bronchitis reflects an inflammatory sub-phenotype among patients with COPD. The present results indicate a preferential distribution of eosinophils towards the airway lumen in those with chronic bronchitis. This may have implications for anti-inflammatory treatment of COPD patients with chronic bronchitis.

Introduction

Chronic obstructive pulmonary disease (COPD) is a leading cause of death and disability worldwide [1]. COPD is characterised by progressive and not fully reversible airflow limitation, as measured by the forced expiratory volume in one second (FEV_1). The airflow limitation is associated with a chronic inflammatory response of the airways and lung parenchyma to noxious particles or gases, in particular tobacco smoking. Nonetheless there is increasing evidence that COPD is a heterogeneous disease and that different phenotypes contribute, to a variable extent, to the severity of the disease. On average 34 % of patients with COPD suffer from chronic cough and sputum expectoration [2]. However, it is still unclear whether these coexisting symptoms of chronic bronchitis among patients with COPD are relevant for the progression and treatment of COPD.

Early epidemiological studies in the 1980s did not observe an association between clinical symptoms of chronic bronchitis and the progression of disease in patients with mild COPD, as measured by the annual decline of FEV_1 [3]. However, subsequent findings suggested that chronic sputum expectoration is associated with a low FEV_1 in patients with alpha-1 antitrypsin deficiency [4], and a steeper decline in FEV_1 in population based studies (including patients with COPD) [5;6]. In addition, chronic cough and sputum expectoration is associated with an increased risk in COPD-related mortality [7;8]. These follow-up studies suggest that the presence of chronic bronchitis is not just an innocent bystander, but might contribute to, or is a reflection of, the more rapid progression of COPD [9].

In COPD the inflammatory process is characterised predominantly by neutrophils, macrophages and $CD8^+$ cells in the airways [10]. The role of excessive mucus production in the pathophysiology in COPD is still controversial. Chronic mucus hypersecretion *per se* is associated with distinct pathological features, such as persistent epithelial goblet cell hyperplasia and submucosal gland hypertrophy in the airways [11]. However, no differences have been observed in total mucin content of the surface epithelium between COPD patients with and without symptoms of chronic bronchitis [12]. Goblet cells in the surface epithelium are the main producers of the mucin MUC5AC, whereas MUC5B is a characteristic product of the submucosal glands [13]. COPD patients with chronic bronchitis have increased numbers of neutrophils in the epithelium and more neutrophils, macrophages and $CD8^+$ cells in their bronchial glands, as compared with asymptomatic non-COPD subjects [14]. This suggests that inflammatory cells and their mediators provide a

major drive for mucus hypersecretion and subsequent symptoms of chronic bronchitis [11]. However, among patients with established COPD it is still unclear whether chronic bronchitis is featured by a distinct inflammatory cell profile in the airways. If so, the presence of chronic bronchitis in COPD may have therapeutic implications for current or future therapies [15].
Therefore, the hypothesis that COPD patients with concurrent clinical symptoms of chronic bronchitis are characterised by a distinct inflammatory cell profile in the airways was tested. This was addressed by measuring inflammatory cell counts in bronchial biopsies and induced sputum in well-characterised patients with COPD.

Methods

Detailed information about subjects and methodology has been published previously [16]. In brief, 114 patients with COPD were included for the Groningen Leiden Universities Chronic Obstructive Lung Disease (GLUCOLD) Study. Patients were 45-75 years, current or ex-smokers with a history of ≥10 pack-years and respiratory symptoms. Postbronchodilator forced expiratory volume in one second (FEV_1) was >1.3 l and >20% predicted and less than the upper limit of the 90% confidence interval of the predicted FEV_1 [17]. Postbronchodilator FEV_1/IVC ratio was below the 90% confidence interval of the predicted FEV_1/IVC ratio. Patients were clinically stable for >2 months before the measurements. They did not use a course of inhaled or oral corticosteroids during the previous 3 months prior to randomisation or maintenance treatment with these drugs during the past 6 months. The medical ethics committee of the Leiden University Medical Center (Leiden, The Netherlands) and the University Medical Center Groningen (Groningen, The Netherlands) approved the protocol, and patients provided written informed consent.

Design and definition of chronic bronchitis

This study represents cross-sectional data from the GLUCOLD study and contained four visits. Chronic bronchitis was considered to be present when subjects reported daily cough and sputum production for ≥3 months per year, for >1 year [5].

Pulmonary function tests

Spirometry was performed according to international guidelines [18], using the

reference values of Quanjer *et al* [17]. Total lung capacity and residual volume were measured using a constant volume bodyplethysmograph [17]. Airway hyperresponsiveness was determined using the 2-min tidal breathing method [19] and expressed as the provocative concentration causing a 20% fall in FEV_1.

Sputum induction and processing

Sputum was induced and processed according to a validated technique using the so called 'full sample' method [20]. After inhaling 200 µg salbutamol, patients inhaled hypertonic sodium chloride aerosols (4.5 weight/ volume %) during three periods of 5 min. Differential cell counts were expressed as a percentage of nucleated cells, excluding squamous cells. A sputum sample was considered adequate when the percentage squamous cells was ≤80%.

Table 1. Clinical characteristics of COPD patients with and without chronic bronchitis (CB)

	With CB	Without CB	p-value
Subjects n	53	60	
Age, yrs	61±8	62±7	0.28
Male / female	42 / 11	56 / 4	0.028*
BMI	25.1±4	25.3±4	0.87
Current smoking yes/no	40 / 13	31 / 29	0.009*
Pack-yrs	42 (34-56)	40 (28-53)	0.12
Post-bronchodilator FEV_1 L #	2.01±0.4	2.04±0.5	0.49
Post-bronchodilator FEV_1 % pred	63±8	63±10	0.54
Post-bronchodilator FEV_1/IVC %	49±8	47±9	0.34
Reversibility FEV_1 % pred	6.8±5	6.9±5	0.92
PC_{20} methacholine ¶ mg/ml	0.59 (0.15-2.72)	0.61 (0.17-2.07)	0.89
RV/TLC %	48.6±10	47.8±7	0.61
TLCO/VA % pred	73.8±25	77.2±26	0.49
TLCO/VA % pred	73.8±25	77.2±26	0.49

*Data are presented as mean±standard deviation (SD) or median (interquartile range), unless stated otherwise. BMI: body mass index; FEV_1 = forced expiratory volume in one second; % pred: % predicted; IVC: inspiratory vital capacity; PC_{20} methacholine = the provocative concentration of methacholine that causes a decrease in FEV_1 of 20%; RV: residual volume; TLC: total lung capacity; TLCO: transfer factor of the lung for carbon monoxide; VA: alveolar volume. #:adjusted for length; ¶geometric mean (IQR). *:p=<0.05.*

Bronchial biopsies

Fibreoptic bronchoscopy was performed using a standardised protocol and has been described in detail previously [21]. In brief, four paraffin embedded biopsies were cut in 4-µm thick sections. Haematoxylin eosin staining was used for evaluation and selection of the two morphological best biopsies per patient. Specific antibodies against T lymphocytes (CD3 and CD8: DAKO, Glostrup, Denmark; CD4, Novocastra, Newcastle upon Tyne, UK), macrophages

(CD68, DAKO), neutrophil elastase (NE, DAKO), mast cell tryptase (AA1, DAKO), plasma cells (CD138, IQ Products, Groningen, The Netherlands) and eosinophils (EG2, Pharmacia Diagnostics, Uppsala, Sweden) were used. Fully automated inflammatory cell-counting procedures were performed according to previously described validated methods [22]. The number of sub-epithelial positively staining inflammatory cells was counted within the largest possible area of maximal 125 µm deep beneath the basement membrane, per biopsy section, and expressed as the mean number of cells/0.1 mm^2 of the two biopsies.

Statistical analysis

Data were presented as mean±SD or medians (interquartile range). The differences between patients with and without chronic bronchitis were analysed using the unpaired t-tests for normally distributed continuous variables. Chi-squared tests were used for categorical data. Non-normally distributed data were log transformed. Multiple logistic regression analysis was used to investigate the independent association between chronic bronchitis and inflammatory cells. In this model the dependent variable was the presence of chronic cough and sputum expectoration, whereas independent variables were bronchial and sputum inflammatory cells, with additional adjustment for smoking habits and sex. Differences at p-values <0.05 were considered to be statistically significant (tested two-sided).

Table 2. Comparison of bronchial inflammatory cells in COPD patients with and without chronic bronchitis [#]

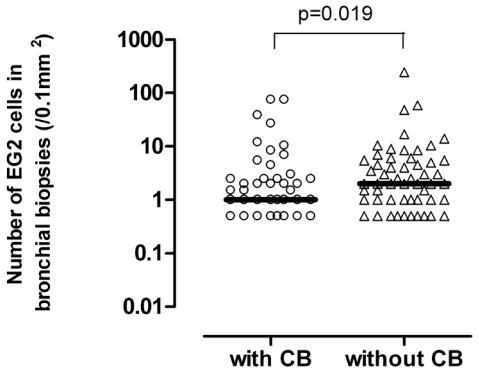
	With CB	Without CB	p-value
Subjects n	53	59	
CD3+ lymphocytes	124 (71-182)	121 (60-193)	0.95
CD4+ lymphocytes	45 (24-72)	48 (28-75)	0.61
CD8+ lymphocytes	18 (11-33)	23 (9.0-42)	0.48
CD4/CD8 %	2.04 (1.2-4.5)	2.08 (1.2-3.8)	0.96
CD8/CD3 %	0.16 (0.11-0.34)	0.20 (0.11-0.31)	1.00
CD4/CD3 %	0.37 (0.26-0.63)	0.43 (0.25-0.74)	0.51
EG2+ cells	1.0 (0.25-2.5)	2.0 (1.0-5.5)	0.019*
Neutrophils	3.0 (2.0-5.5)	5.5 (2.0-8.5)	0.080
Plasma cells	8.0 (3.5-15)	9.0 (4.0-14.5)	0.34
CD68+ cells	8.5 (4.3-11.8)	10 (5.0-14)	0.20
AA1+ cells	27 (21-34)	26 (18-35)	0.80

*Data are presented as median (interquartile range) of bronchial inflammatory cells (per 0.1 mm^2 of sub-epithelial area), unless stated otherwise. EG2: eosinophils. [#]: Data from one patient was excluded as biopsy specimens were not adequate for analysis. Data *: p=<0.05.*

Results

The patient characteristics of COPD patients with and without chronic bronchitis are shown in Table 1. Data on the presence of chronic bronchitis were available for 113 out of 114 patients with COPD. All patients had moderate to severe COPD as based on an average postbronchodilator FEV_1 of 63±9% of predicted and most of them were current smoking, middle-aged males. A minority of patients was mildly reversible to salbutamol, as based on FEV_1 (% pred) post minus pre-salbutamol. A total of 18 (16%) patients showed a change in FEV_1 that was >12 % pred and > 200 ml, whereas nine out of these 18 patients had chronic bronchitis. COPD patients with and without chronic bronchitis exhibited a wide range of hyperresponsiveness to methacholine, were slightly hyperinflated and were mildly impaired in carbon monoxide diffusion capacity per alveolar volume. Patients with chronic bronchitis were more likely to be current smokers than patients without these symptoms. Relatively more female patients reported chronic bronchitis. Other patient characteristics were similar between the two groups (Table 1).

Figure 1.



The number of eosinophils (EG2) in bronchial biopsies (per 0.1 mm^{-2} sub-epithelial area) in chronic obstructive pulmonary disease patients with and without chronic bronchitis (CB). The horizontal lines represent the median.

Bronchial inflammatory cell counts in COPD patients with and without chronic bronchitis

Data on the number of bronchial inflammatory cells were available for 53 patients and 59 patients with without chronic bronchitis (Table 2). Patients with chronic bronchitis had significantly fewer eosinophils in biopsies than patients without chronic bronchitis (p=0.019, Figure 1). Logistic regression analysis confirmed this association. After adjustment for smoking and sex, there was a statistically significant lower chance of 16% of having chronic bronchitis for each doubling in bronchial eosinophils (odds ratio (OR) 0.84 (95% CI 0.72-0.98): p=0.028). Patients with chronic bronchitis tended to have

Table 3. Comparison of nonsquamous sputum inflammatory cells in COPD patients with and without chronic bronchitis (CB) #

	With CB	Without CB	p-value
Subjects n	50	55	
Absolute numbers x10⁴/ml			
Total cell count	135.0 (78.6-283.9)	149.1 (73.8-313.0)	0.54
Neutrophils	90.0 (46.1-204.6)	110.0 (56.3-231.0)	0.31
Macrophages	32.6 (18.8-64.8)	29.6 (13.0-59.3)	0.45
Lymphocytes	2.2 (1.0-5.6)	2.1 (1.0-7.2)	0.94
Eosinophils	1.7 (0.48-4.9)	1.1 (0.2-3.3)	0.13
Epithelial cells	1.26 (0.69-3.39)	1.37 (0.43-3.82)	0.40
Basophils	0 (0-0)	0 (0-0)	-
Percentages			
Neutrophils	69.9 (55.0-97.5)	73.8 (65.8-83.7)	0.046*
Macrophages	23.0 (17.7-35.1)	21.3 (12.7-28.3)	0.039*
Lymphocytes	1.8 (1.2-2.4)	1.7 (0.8-2.2)	0.47
Eosinophils	1.4 (0 .5-2.3)	0.7 (0.2-1.7)	0.033*
Epithelial cells	1.0 (0.5-2.3)	1.3 (0.2-2.3)	0.32

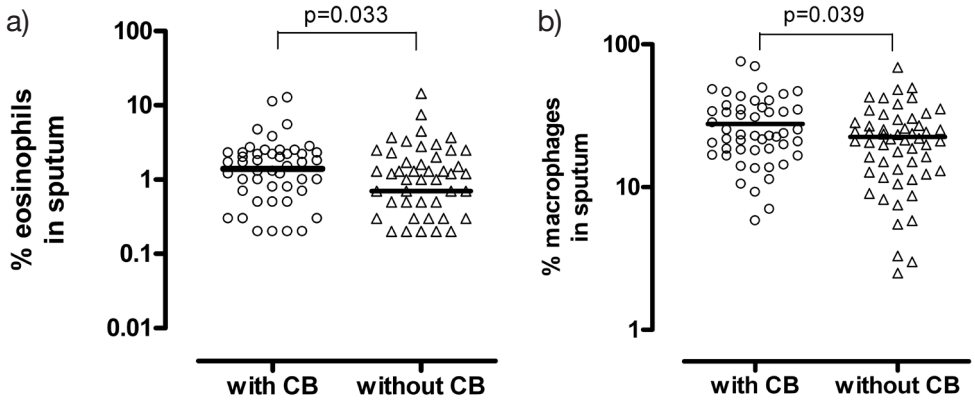
*Data are presented as median (interquartile range), unless stated otherwise. #:Sputa from 106 patients were available for analysis. *: p=<0.05.*

fewer neutrophils in biopsies than patients without chronic bronchitis, but this difference was not statically significant (p=0.080). The remaining inflammatory parameters in bronchial biopsies were similar between the two groups.

Sputum inflammatory cells in COPD patients with and without chronic bronchitis

Table 3 shows the numbers and percentages of inflammatory cells in induced sputum for COPD patients with and without chronic bronchitis. COPD patients with chronic bronchitis had significantly higher percentages of sputum eosinophils (p=0.033) than COPD patients without these symptoms (Figure 2a). After adjustment for smoking and sex, each doubling in sputum % eosinophils was borderline significantly associated with a 24% higher chance of having chronic bronchitis (OR=1.24 (95% CI 0.99-1.54); p=0.057). When using a sputum eosinophil percentage of > 3% as threshold, sputum eosinophils had a specificity of 87% in identifying patients with chronic bronchitis. COPD patients with chronic bronchitis had significantly higher percentages of macrophages (p=0.039; Figure 2b), and lower percentages of sputum neutrophils (p=0.049) than COPD patients without those symptoms. After adjustment for smoking and sex, these differences lost statistical significance (OR 1.45 (95% CI 0.94-2.24); p=0.097 and 0.96, (0.91-1.01), p=0.11, for macrophages and neutrophils respectively). No differences between patients

Figure 2.

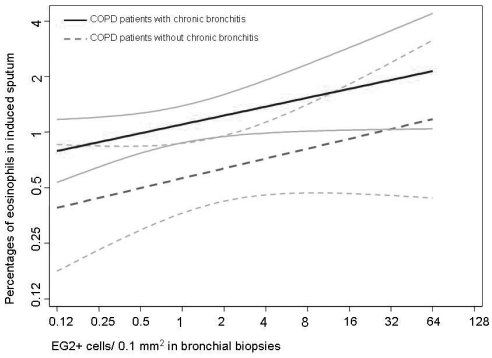


Percentages of a) eosinophils and b) macrophages in induced sputum in chronic obstructive pulmonary disease patients with and without chronic bronchitis (CB). The horizontal lines represent the median.

with or without chronic bronchitis were found for percentages of lymphocytes and epithelial cells, or numbers of total cell counts, neutrophils, macrophages, lymphocytes, and epithelial cells in induced sputum.

Relation between sputum and bronchial inflammatory cell counts

Figure 3.



Relationship between the percentages of eosinophils in induced sputum (y-axis) and eosinophils (per 0.1 mm^{-2} sub-epithelial area) in bronchial biopsies (x-axis) in chronic obstructive pulmonary disease patients with and without chronic bronchitis.

Percentages of eosinophils in sputum were positively associated with eosinophil counts in biopsies within the chronic bronchitis groups as well as within the group of patients without chronic bronchitis. Interestingly, the percentages of sputum eosinophils were doubled in patients with chronic bronchitis compared to patients without chronic bronchitis for a given number of eosinophils in the bronchial biopsies (b=2.03, p=0.01, Figure 3).

Discussion

This study shows that chronic bronchitis reflects an inflammatory sub-phenotype among patients with moderate to severe COPD, which is characterised by a distinct inflammatory cell profile as measured in a large sample of induced sputum specimens and bronchial biopsies. More specific, clinical symptoms of chronic bronchitis in COPD are associated with a distinct distribution of bronchial and sputum eosinophils. In addition, patients with chronic bronchitis had higher percentages of macrophages and lower percentages of neutrophils in their sputum. The latter could be explained by differences in current smoking habits and sex distribution between the two groups. No significant differences were found between the two groups with regard to other inflammatory cells in biopsies or sputum. Taken together, clinical symptoms of chronic bronchitis in COPD appear to represent an inflammatory sub-phenotype, which may have implications in anti-inflammatory treatment in clinical practice.

The present study demonstrates for the first time that chronic bronchitis among patients with manifest COPD is characterised by a partially distinct inflammatory cell profile of eosinophils, macrophages and neutrophils. Comparison between the present findings on eosinophils and studies in literature is difficult as outlined hereafter, since either chronic bronchitis within COPD was not addressed as a separate entity [14;23] or different tissues were used [24]. Furthermore, in most of these studies lower numbers of patients were investigated [14;24]. Therefore, the present observation of a preferential distribution of eosinophils towards the airway lumen in COPD patients with chronic bronchitis extends a previous report [14], where no differences were observed in the number of eosinophils in the submucosa of the airway wall (resected lung tissue) from smokers with chronic bronchitis (COPD) as compared to non-smoking controls (non-COPD). Furthermore, it has recently been found that smokers with chronic bronchitis (COPD) have similar percentages of sputum eosinophils as compared to non-smoking controls (non-COPD) [23]. Together, these studies and the present authors' observations show that comparison of COPD patients with and without chronic bronchitis reveals different inflammatory sub-phenotypes within COPD, whereas comparison of COPD patients with chronic bronchitis and nonsmoking controls may reflect the inflammatory process associated with the development of COPD.

The present authors observed that COPD patients with chronic bronchitis had relatively higher percentages of macrophages and lower percentages

of neutrophils in sputum, which was mainly due to differences in current smoking habits between the two groups. These associations with smoking are in line with results from Willemse *et al* [23]. Remarkably, no differences were found with regard to T lymphocytes, neutrophils, macrophages or mast cells in bronchial biopsies between COPD patients with and without chronic bronchitis. This extends the findings by Saetta *et al*., where inflammatory cells in the submucosa were similar between subjects with chronic bronchitis (COPD) and non-smoking controls (non-COPD) [14]. However, again this may reflect the different populations and tissues examined.

To the present authors' knowledge, the present study is one of the largest using induced sputum as well as bronchial biopsies in well-characterised steroid-naïve patients with COPD. Nonetheless, some limitations must be mentioned. There is overlap of data in biopsies and sputum between both groups. Yet, this is in line with results from other studies examining similar parameters in different and smaller groups of patients [14;25]. More importantly, for a given number of eosinophils in the bronchial biopsies the percentages of sputum eosinophils were doubled in patients with chronic bronchitis compared to patients without these symptoms. The presence of chronic bronchitis, as based on clinical symptoms only, may be biased due to different awareness by sex, through retrospective selection of the patients, or the influence of recurrent exacerbations. The present results, however, were corrected for sex, and it needs to be emphasized that symptoms of chronic bronchitis have been associated with hypersecretion of mucus from enlarged bronchial glands and inflammatory cells in resected lung tissue since the early 1950s [14;26;27]. Only 9 patients experienced exacerbations (symptoms plus prednisone) in the year prior to our study and we believe that the influence of exacerbations on the presence of chronic bronchitis was limited. Furthermore, chronic symptoms of cough and sputum production have been associated with a more rapid decline in FEV_1 and increased COPD-related mortality [5;8]. Therefore, despite the fact that chronic bronchitis is indeed likely to be a continuum, the currently used definition is supported by clinical and pathological data.

It is possible that we did not investigate the right anatomic region when studying bronchial biopsies and that peripheral lung tissue is needed to investigate the total airway wall, therefore allowing the use of other parameters such as the Reid's index (*i.e.* the ratio of the thickness of the mucous gland layer to the thickness of the wall between the epithelium and cartilage). However, previous studies showed that patients with both chronic bronchitis and fixed airway obstruction had the same Reid's index compared to controls, whereas scores

of inflammation were better associated with mucus hypersecretion [14;27]. It may also be argued that the distribution of inflammatory cells obtained with different techniques (*i.e.* induced sputum and bronchial biopsies) is difficult to interpret. A previous study [28], however, showed fairly good agreement between the number of eosinophils in different compartments in the airways in patients with chronic bronchitis. It is noteworthy that, although not significant, the differences in absolute numbers of total cells, eosinophils, macrophage and neutrophils in sputum between patients with and without chronic bronchitis demonstrated the same trend and magnitude as the differences in percentages between both groups. In addition, inflammatory cell numbers may not represent cell activity, an aspect that requires more in-depth analysis. However this was beyond the scope of the present study.

How can these results be interpreted? Mucus hypersecretion, which is the hallmark of clinical chronic bronchitis, is the result of mucin production, secretion and clearance [29]. Inflammatory mediators such as neutrophil elastase, are important secretagogues for mucin-producing cells. In COPD, both cigarette smoke and neutrophil elastase are main determinants of not only mucin production and secretion but also of clearance, by impaired ciliary activity and dehydration of the airway surface layer [29]. Nevertheless, the present study shows that chronic bronchitis is related to eosinophils in biopsies and sputum. Increased numbers of eosinophils in sputum that have migrated through the epithelial layer may contribute to mucus hypersecretion through the action of transforming growth factor (TGF-α) [30] or by stimulating degranulation of mucus-producing cells through the release of inflammatory mediators, including cysteinyl leukotrienes [31]. Therefore, the present findings of a preferential distribution are in line with a role of eosinophils in mucus hypersecretion. This is further supported by other studies showing increased sputum eosinophils during COPD exacerbations [32] and a positive correlation between airway eosinophilia and increased sputum production in asthma [33]. A decrease in the number of eosinophils in the airway wall, especially around the glands, may also contribute to mucus hypersecretion. Eosinophils are a major cellular source of TGF-α [34] and Baraldo *et al*. showed that impaired TGF-α signalling is associated with bronchial gland enlargement [35]. Therefore a lower number of eosinophils around the bronchial glands may lead to bronchial gland enlargement and a subsequent rise in mucus in the airway lumen, due to a decreased local TGF-α availability. Another explanation for our findings is that the same mechanism is involved in eosinophil recruitment into the airway lumen as well as in mucus hypersecretion. T-helper cell type

2 cytokines may be involved in such mechanisms since the expression of interleukin (IL)-4 and -13 is higher in patients with chronic bronchitis [36] and these cytokines are involved in the regulation of both eosinophil influx [37] and mucin production [38]. Based on the current information, we can not discriminate between these two explanations for the altered distribution of eosinophils in COPD patients with chronic bronchitis that we observed.

The present results also showed that patients with chronic bronchitis had higher percentages of macrophages in sputum, which was mainly explained by current smoking. This may indicate that sputum macrophages may act as an intermediary variable in the causal pathway of chronic bronchitis. Activated by cigarette smoke, macrophages might contribute to mucus hypersecretion directly *via* release of pro-inflammatory cytokines such as IL-1ß and indirectly by neutrophil-chemotactic factors such as leukotriene B_4 and IL-8 [39]. Neutrophils in bronchial biopsies would be expected to be related to the presence of chronic bronchitis. Neutrophil elastase is thought to stimulate both the release and production of mucin. The present study showed no differences in neutrophils between patients with and without chronic bronchitis. One explanation might be that the submucosal glands, thought to be responsible for the largest amount of mucus in the large airways [11], are more important in defining the sub-phenotype of chronic bronchitis.

What are the implications of this study? Previous studies showed that treatment with steroids may reduce numbers of sputum eosinophils in patients with COPD [40;41], whereas sputum eosinophilia in COPD may be predictive of a clinical response to steroid treatment [42;43]. Distinct inflammatory cell profiles may require different (anti-inflammatory) interventions. Therefore, this and other novel anti-inflammatory strategies [44] may need to be examined in COPD patients with and without chronic bronchitis.

We concluded that clinical symptoms of chronic bronchitis reflect a distinct sub-phenotype among patients with manifest COPD, as based on inflammatory cells in induced sputum and bronchial biopsies. The present results indicate a preferential distribution of eosinophils towards the lumen. This may have implications for current and future treatment strategies [41-44] in chronic obstructive pulmonary disease patients with clinical symptoms of chronic bronchitis.

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Chapter 5

Effect of fluticasone with and without salmeterol on pulmonary outcomes in chronic obstructive pulmonary disease: a randomized, controlled trial

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Abstract

Background: Inhaled corticosteroids (ICS) and long-acting ß$_2$-agonists (LABAs) are used to treat moderate to severe chronic obstructive pulmonary disease (COPD).

Objective: To determine whether long-term ICS therapy, with or without LABAs, reduces inflammation and improves pulmonary function in COPD.

Design: Randomised, placebo-controlled trial. (ClinicalTrials.gov registration number: NCT00158847)

Setting: 2 university medical centers in The Netherlands.

Patients: 114 steroid-naïve current or former smokers with moderate to severe COPD.

Measurements: Cell counts in bronchial biopsies and sputum (primary outcome); methacholine responsiveness at baseline, 6 and 30 months; and clinical outcomes every 3 months.

Intervention: Random assignment by minimization method to receive fluticasone propionate, 500 µg twice daily, for 6 months (n=31) or 30 months (n=26); fluticasone , 500 µg twice daily, and salmeterol, 50 µg twice daily, for 30 months (single inhaler; n=28); or placebo twice daily (n=29).

Results: 101 Patients were greater than 70% adherent to therapy. Fluticasone therapy decreased counts of mucosal $CD3^+$ cells (-55% [95% CI, -74% to -22%]; $P = 0.004$), $CD4^+$ cells (-78% [CI, -88 to -60%]; $P < 0.001$), $CD8^+$ cells (-57% [CI, -77% to -18%]; $P = 0.010$), and mast cells (-38% [CI, -60% to -2%]; $P = 0.039$] and reduced hyperresponsiveness ($P = 0.036$) versus placebo at 6 months, with effects maintained after 30 months. Fluticasone therapy for 30 months reduced mast cell count and increased eosinophil count and percentage of intact epithelium, with accompanying reductions in sputum neutrophil, macrophage, and lymphocyte counts and improvements in FEV_1 decline, dyspnea, and quality of life. Reductions in inflammatory cells correlated with clinical improvements. Discontinuing fluticasone therapy at 6 months increased counts of $CD3^+$ cells (120% [CI, 24% to 289%]; $P = 0.007$), mast cells (218% [CI, 99% to 407%]; $P < 0.001$), and

plasma cells (118% [CI, 9% to 336%)]; *P* = 0.028) and worsened clinical outcome. Adding salmeterol improved FEV_1 level.

Limitations: The study was not designed to evaluate clinical outcomes. Measurement of primary outcome was not available for 24% of patients at 30 months.

Conclusions: ICS therapy decreases inflammation and can attenuate decline in lung function in steroid-naïve patients with moderate to severe COPD. Adding LABAs does not enhance these effects.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by a progressive decrease in lung function, accompanied by worsening respiratory symptoms and health status [1]. These clinical features are associated with airway inflammation (such as that resulting from neutrophils, macrophages, lymphocytes, and mast cells) [2-5] and alterations of the bronchial epithelium (such as that resulting from squamous cell metaplasia or goblet and basal cell hyperplasia) [6].

Current guidelines [1] recommend treating patients who have severe COPD and frequent exacerbations with inhaled corticosteroids (ICSs) and adding long-acting $ß_2$-agonists (LABAs) for *P*atients with moderate to severe COPD. Regular ICS treatment leads to clinical benefits in terms of symptoms, exacerbation rates, and initial improvements in FEV_1 [7-10]. However, withdrawal of ICS treatment results in deterioration of clinical outcome [11;12]. Combining a LABA with an ICS provides additional clinical improvements [13;14]. A recent analysis of the TORCH (Towards a Revolution in COPD Health) study suggests that prolonged therapy with ICS and LABA attenuates FEV_1 decline in COPD [15], in contrast to previous studies [13;14;16-19].

The clinical benefits of ICS therapy for COPD, with or without a LABA, may be at least partially mediated its anti-inflammatory efficacy. Short-term treatment of COPD (2-3 months) with ICS reduced the number of bronchial mast cells but not $CD8^+$ cells, neutrophils, or macrophages [20;21]. Combination therapy with ICS and LABAs for 3 months provided more anti-inflammatory effects than ICS monotherapy by reducing bronchial $CD8^+$ cells and macrophages [22]. No long-term anti-inflammatory effects have been reported for these interventions. Our goal was to link pathological and clinical efficacy during 30-month treatment.

We hypothesized that:

1) long-term maintenance therapy with ICS provides anti-inflammatory effects (primary outcome) in the airways of patients with COPD;
2) such effects are associated with clinical improvements;
3) discontinuing ICS therapy induces a flare-up of inflammation and clinical deterioration; and
4) adding a LABA to ICS therapy provides no further anti-inflammatory effects.

Methods

Our study is investigator-initiated, with a double-blind, parallel, 4-group, placebo-controlled, randomised design.

Setting and participants

The GLUCOLD (Groningen Leiden Universities Chronic Obstructive Lung Disease) project [23] enrolled patients with COPD who were aged 45 to 75 years, were current or former smokers, had smoked for 10 or more pack-years, and had lung function levels compatible with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages II and III [1]. Exclusion criteria were asthma and receipt of ICS within 6 months before random assignment. We determined the presence of asthma on the basis of a physician ‘s diagnosis or selfreported history, symptoms, treatment, or diagnosis of asthma. Patients were clinically stable and were allowed to continue taking short-acting bronchodilators. We determinec smoking status on the basis of self-reports and gave standard clinical advice to quit smoking in accordance with Dutch national guidelines. We recruited almost all patients from family practices by electronically selecting patients aged 45 to 75 years who did not have an International Classification of Primary Care code for asthma (R96). Their general practitioner sent them a letter asking for participation in research. A telephone interview revealed 4617 potentially eligible patients, who received spirometry. In addition, were recruited patients by advertising in local newspapers. We performed chest radiography and electrocardiography to rule out important comorbid conditions. Recruitment and follow-up was between 2000 and 2007. Both centers’ ethics committees approved the study, and all patients provided written informed consent.

Random assignment and interventions

We randomly assigned patients to receive 1 of 4 regimens: fluticasone propionate, 500 µg twice daily, for the first 6 months followed by placebo, twice daily, for 24 months; fluticasone, 500 µg twice daily, for 30 months; fluticasone, 500 µg twice daily, and salmeterol, 50 µg twice daily, in a single inhaler for 30 months; or placebo, twice daily, for 30 months. Study medications were individually numbered, and we used Diskus dry-powder inhalers (GlaxoSmithKline, Zeist, The Netherlands) with 60 doses per inhaler; all active treatment medication and placebo were identical in appearance. The placebo consisted of lactose monohydrate (also included in other treatment groups). At entry, an independent randomisation center provided patient

and medication numbers by using a minimization procedure that balanced treatment groups for center, sex, smoking status, FEV_1/IVC (<60% or ≥ 60%), and methacholine PC_{20} (the provocative concentration of methacholine that causes a 20% decrease in FEV_1) (<2 mg/mL or ≥ 2 mg/mL).

Outcomes and Measurements

Our predefined primary outcome was inflammatory cell counts in bronchial biopsies and induced sputum. We performed fiberoptic bronchoscopy, biopsy processing, and quantification as described elsewhere [24]. We stained paraffin-embedded biopsy sections with Periodic acid-Schiff/Alcian blue to identify goblet cells, epithelial intactness, and squamous metaplasia as described elsewhere [25]. We performed immunohistochemistry by using specific antibodies against T lymphocytes (CD3, CD4, and CD8), macrophages (CD68), neutrophil elastase, mast cell tryptase (AA1), eosinophils (EG2), plasma cells (CD138), and proliferating cells (Ki-67). We expressed subepithelial cells as number of cells per 10^{-7} m^2 by fully automated image analysis [26]. We used the full sample method [23] to perform sputum induction.

Secondary outcomes included postbronchodilator spirometry and hyperresponsiveness to methacholine PC_{20}, assessed by using standardized procedures [23], dyspnea score by the modified Medical Research Council (MRC) dyspnea scale (range, 1 to 5); and health status by the St. George's Respiratory Questionnaire (SGRQ) (range, 0 to 100; 100 = maximum disability) [27] and Clinical COPD Questionnaire (CCQ) (range, 0 to 6; 6 = worst) [28].

Follow-up Procedures

We measured symptoms, health status, self-reported smoking status, medication adherence, and spirometry every 3 months. We checked adherence by counting the doses on the inhalers. We performed bronchoscopy, sputum induction, and methacholine challenge at baseline and at 6 and 30 months.

Statistical analysis

We based our sample size on the latest data released in 2002 [29] regarding the standard deviation (0.77) of the fluticasone-induced short-term change in submucosal CD8 cell count in patients with COPD. A 2-fold difference in change from baseline to 6 months and from 6 to 30 months in the fluticasone group versus placebo should be detectable with 80% power with 20 patients per treatment group. Because this was an efficacy trial, per-protocol analysis included all available data from randomly assigned patients who adhered to

their therapy regimen (using ≥70% of the prescribed dose), including data from patients who did not complete follow-up.

We used linear mixed-effects models with a random intercept at the patient level to analyze the data and assumed that data were missing at random. We used STATA, version 9.0 (StataCorp, College Station, Texas) for analysis. The linear mixed models included the main effect of treatment (3 indicators), the main effect of time (2 indicators), and the interaction of treatment and time. For outcomes with 3-month measurements, we replaced the time effect with terms that allowed a shift or linear change in the average outcome during the first 6 months and a subsequent linear change in the average outcome after 6 months. Because of the considerable number of model parameters and the sample size, we did not include center, age, or sex as covariates in the baseline model. We performed a post hoc analysis to adjust for smoking status at baseline and during the study. We present the effects as adjusted means in the figures and as percentage of change in estimates, CIs, and *P* values in the text.

We analyzed correlations between statistically significant treatment effects on inflammatory outcomes and lung function by using the Spearman correlation coefficient (Rs). Data are presented as means (SDs) or medians (interquartile ranges). We considered 2-sided P values less than 0.05 to be statistically significant.

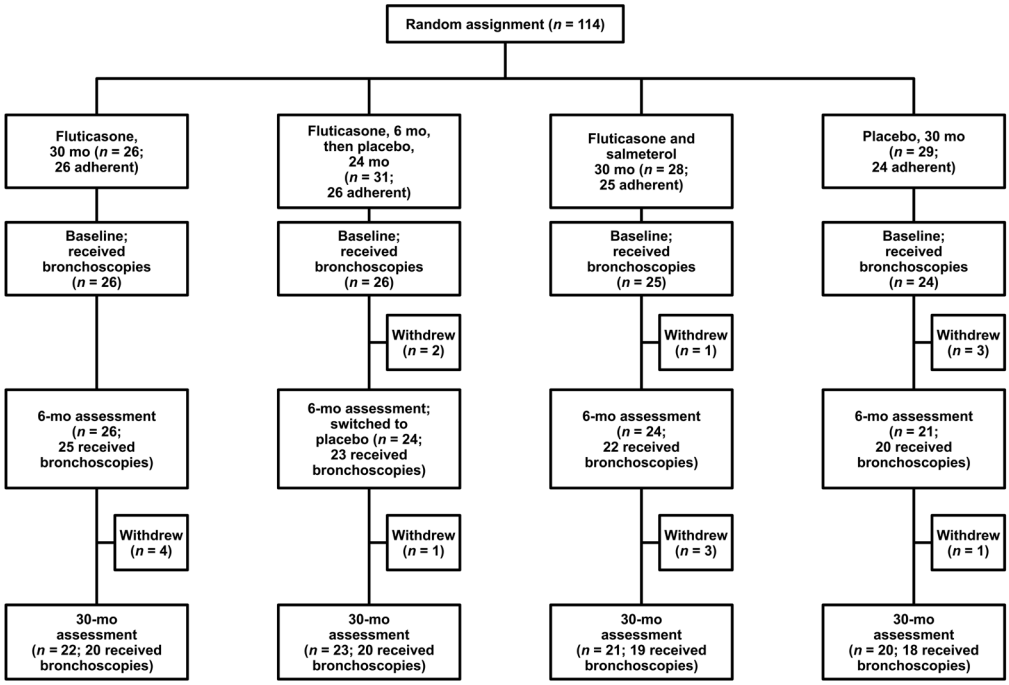
Role of the funding source

This was an investigator-initiated trial. The study was funded by the Netherlands Organization for Scientific Research, Netherlands Asthma Foundation, GlaxoSmithKline of The Netherlands, University Medical Center Groningen, and Leiden University Medical Center. The funding sources had no role in the design, conduct, and analysis of the study or in the decision to submit the manuscript for publication.

Results

Of the 114 randomly assigned patients, we analyzed 101 adherent patients (**Figure 1**). Mean postbronchodilator FEV_1 was 63% predicted (SD, 9%) (91 patients were GOLD stage II and 10 were GOLD stage III) and geometric mean methacholine PC_{20} was 0.6 mg/mL (SD, 2.6 doubling dose). Seven patients had ever received a short course of corticosteroids and only 5 had ever received ICS maintenance therapy.

Figure 1. Study flow diagram



Total number of patients randomised and compliant (>70% medication use) per treatment group. At each stage of the study (0, 6 and 30 months) the numbers are listed of those who underwent bronchoscopy amongst the number of patients remaining in the study. Definition of abbreviations: n = number.

Baseline patient characteristics were similar among the 4 treatment groups (**Table 1**). Sputum and biopsy inflammatory cells counts did not differ.

The amount of missing data, including missing data due to dropouts, for each study measure were 12% for FEV_1, 13.9% for methacholine PC_{20}, 12.5% for MRC score, 13.9% for SGRQ score, 14.7% for CCQ score, 12.5% for bronchial inflammatory cells, 14.2% for epithelial features, and 14.2% sputum cells.

Short-term therapy with ICS

Fluticasone therapy decreased counts of bronchial $CD3^+$ cells (-55% [CI, -74% to -22%]; $P = 0.004$), $CD4^+$ cells (-78% [CI, -88% to -60%]; $P < 0.001$), $CD8^+$ cells (-57% [CI, -77% to -18%]; $P = 0.010$), and mast cells (-38% [CI, -60% to -2%]; $P = 0.039$) at 6 months compared with placebo (**Figure 2** and **Table 2**]. This was accompanied by an increase in methacholine PC_{20} (1.5 doubling dose [CI, 0.1 to 3.0]; $P = 0.036$) (**Figure 3, B**) and CCQ mental score (0.2 point [CI, 0.01 to 0.4 points]; $P = 0.037$) compared with placebo. We found no other

*Table 1. Patient characteristics at baseline **

Characteristics	Placebo, 30 mo	Fluticasone 6 mo, Then Placebo, 24 mo	Fluticasone, 30 mo	Fluticasone plus salmeterol, 30 mo	P value †
Patients, n	24	26	26	25	
Clinical					
Men/women, n/n	20/4	22/4	23/3	22/3	0.94
Age, y	59 (8)	64 (7)	62 (8)	62 (8)	0.31
Current smoker/ not current smoker, n/n	17/7	14/12	16/10	17/8	0.61
Median smoking history (range), pack-years	42 (34-54)	41 (29-57)	44 (31-55)	47 (31-56)	0.62
Lung Function					
Prebronchodilator FEV_1, % predicted	54.1 (8.3)	56.8 (11)	56.6 (9.9)	55.0 (11)	0.742
Postbronchodilator FEV_1, % predicted	61 (8.3)	65 (8.6)	64 (9.1)	61 (9.4)	0.41
Change in FEV_1, % predicted ‡	7.1 (4.5)	7.3 (5.3)	7.1 (4.0)	6.2 (6.3)	0.87
Postbronchodilator FEV_1/IVC, %	47 (9.0)	51 (8.3)	49 (9.0)	46 (8.4)	0.157
Geometric mean methacholine PC_{20}, mg/mL §	0.7 (2.0)	0.7 (3.2)	0.4 (2.4)	0.7 (2.7)	0.64
K_{CO}, % predicted	65 (19)	79 (29)	77 (22)	74 (27)	0.188
Symptoms and health status					
MRC dyspnea score ‖	2.7 (0.8)	2.5 (0.6)	2.6 (0.6)	2.9 (1.0)	0.53
SGRQ total score ¶	33.5 (18.5)	25.7 (15.2)	32.9 (10.9)	28.1 (13.2)	0.27
CCQ total score ¨	1.77 (1.3)	1.16 (0.6)	1.26 (0.6)	1.43 (0.7)	0.35

CCQ = Clinical COPD [chronic obstructive pulmonary disease] Questionnaire; IVC = inspiratory vital capacity; K_{CO} = transfer factor for carbon-monoxide; methacholine PC_{20} = provocative concentration of methacholine that causes a 20% decrease in FEV_1; MRC = Medical Research Council; SGRQ = St. George's Respiratory Questionnaire.

** Values are means (SDs) unless otherwise indicated.*

† By analysis of variance or Kruskall-Wallis tests between groups.

‡ Reversibility in FEV_1 by 400-µg inhaled salbutamol.

§ Methacholine PC_{20} values are expressed as mean doubling doses.

‖ Range of 1 to 5 (a higher score indicates more dyspnea).

¶ Range of 0 (best) to 100 (worst).

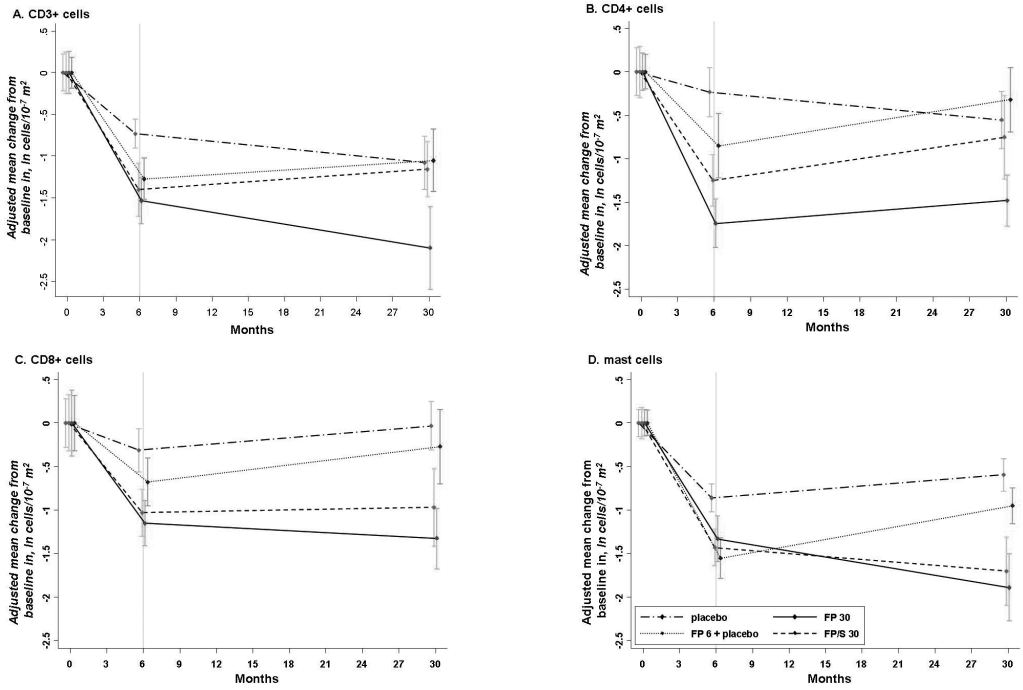
¨ Range of 0 (best) to 6 (worst).

statistically significant effects of 6 months of fluticasone therapy. The change in FEV_1 after 6 months did not significantly differ between patients who were randomly assigned to continue fluticasone therapy and those assigned to switch to placebo.

Long-term continuation of ICS therapy

Continuing fluticasone therapy from 6 to 30 months maintained the reduction in $CD3^+$, $CD4^+$ and $CD8^+$ cell counts (**Figure 2** and **Table 2**) after 30 months, compared with placebo. This was accompanied by a further -56% change in mast cell count (CI, -73% to -29%; *P* = 0.001), a 125% increase in eosinophil count (CI, 2% to 399%; *P* = 0.046), and a 101% increase in the percentage of intact epithelium in bronchial biopsies (CI, 10% to 268%; *P* = 0.024) after 30 months (**Figure 2** and **Tables 2 and 4**). In addition, the 30-month fluticasone group had lower counts of sputum neutrophils (-58% [CI, -82% to -1%); *P* = 0.047), macrophages (-57% [CI, -81% to -3%]; *P* = 0.041), and lymphocytes

Figure 2. Pathological outcomes



Adjusted mean change in log-transformed bronchial cell numbers (/10^{-7} m^2 lamina propria) over time during treatment with fluticasone (500 µg bid) for 30 months (FP30), fluticasone (500 µg bid) for 6 months (FP6), the combination of fluticasone/salmeterol (500/50 µg bid) for 30 months (FP/S) and placebo for 30 months in patients with COPD. Error bars represent 95% confidence intervals (CI). Data of bronchial $CD3^+$ cells (2A), $CD4^+$ cells (2B), $CD8^+$ cells (2C), and mast cells (2D) are presented.

*Table 2. Bronchial inflammatory cell counts at baseline and after 6 and 30 months**

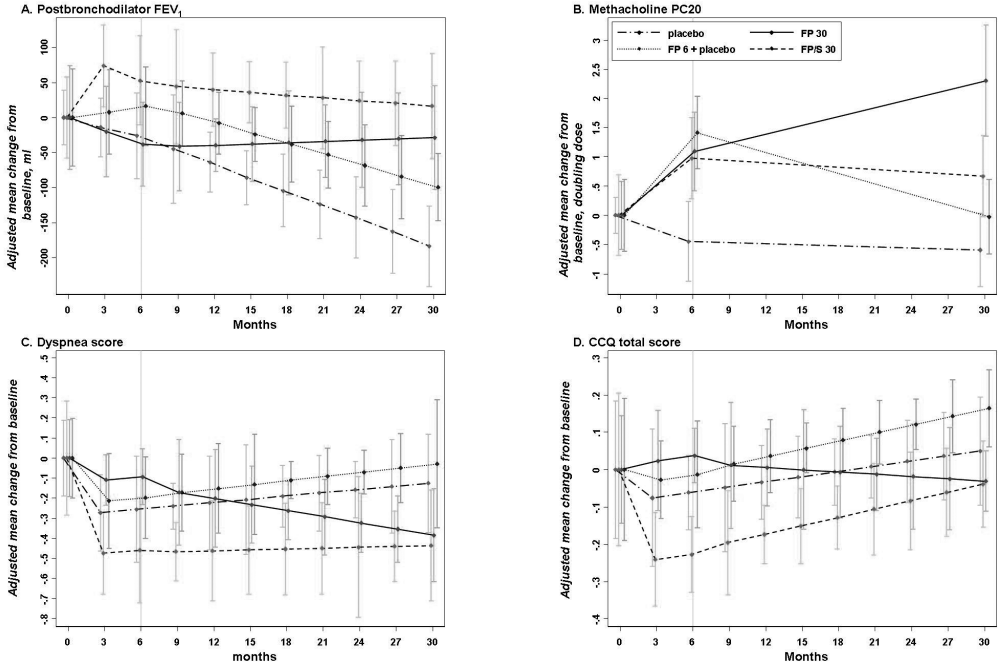
	Placebo, 30 mo			Fluticasone, 6 mo, then Placebo, 24 mo			Fluticasone, 30 mo			Fluticasone Plus Salmeterol, 30 mo		
	Baseline	6 mo	30 mo	Baseline	6 mo	30 mo	Baseline	6 mo	30 mo	Baseline	6 mo	30 mo
Patients, n	24	20	18	26	23	20	25	24	19	25	22	19
$CD3^+$ cells	135	57	50	111	28	38	124	21	12	118	35	36
	(76-197)	(37-84)	(24-79)	(69-180)	(21-45)	(19-89)	(63-192)	(16-33)	(5.5-38)	(74-191)	(17-54)	(15-53)
$CD4^+$ cells	44	33	24	34	11	27	68	10	22	48	11	15
	(21-66)	(18-67)	(11-42)	(24-67)	(6.5-19)	(12-57)	(43-100)	(6.0-19)	(6.5-26)	(26-82)	(6.9-25)	(11-57)
$CD8^+$ cells	19	14	22	17	5.5	11	23	6.8	4.0	23	8.8	7.5
	(10-33)	(9.0-23)	(12-33)	(6.9-29)	(3.0-9.0)	(4.3-19)	(11-41)	(3.3-9.5)	(2.0-9.5)	(16-52)	(6.3-19)	(4.0-24)
Neutrophils	4.0	3.0	5.3	5.0	7.0	9.8	2.5	5.5	13	5.0	6.3	7.5
	(2.1-8.0)	(1.5-10)	(2.9-15)	(1.5-9.0)	(3.0-11)	(5.6-22)	(1.5-5.0)	(2.6-12)	(8.5-24)	(3.0-8.0)	(3.0-18)	(4.0-24)
Eosinophils	1.0	0.5	1.0	2.0	0.5	5.5	1.5	0.5	2.5	1.5	0.3	1.0
	(0.5-5.8)	(0-2.2)	(0.4-3.4)	(0.5-7.5)	(0-1.0)	(1.1-11)	(0.5-3.3)	(0-1.4)	(1.0-8.5)	(0.5-2.5)	(0-3.3)	(0-5.0)
Plasma cells	7.8	2.0	5.5	11	2.0	6.3	8.0	2.0	1.0	6.5	1.3	4.0
	(3.5-17)	(1.5-11)	(2.0-12)	(7.4-14)	(1.0-3.0)	(1.6-13)	(2.8-15)	(0.6-3.4)	(1.0-3.0)	(4.0-18)	(0.4-2.5)	(1.0-7.5)
Macrophages	8.3	5.3	4.0	9.3	3.5	5.3	10	4.0	3.0	9.5	4.8	4.0
	(4.1-10)	(2.6-12)	(2.9-15)	(4.5-12)	(2.0-7.5)	(2.3-14)	(5.0-23)	(2.5-7.9)	(0.5-8.5)	(5.5-12)	(1.9-12)	(0.5-21)
Mast cells	24	11	14	31	6.0	12	22	8.0	2.5	26	7.0	5.0
	(17-32)	(8.5-13)	(9.6-18)	(23-41)	(3.0-9.0)	(7.5-16)	(16-34)	(3.0-10)	(0.5-4.5)	(17-32)	(4.4-8.8)	(1.5-10)

** Cell counts are expressed as medians (25^{th}-75^{th} percentiles) count/10^{-7} m^2 of subepithelium*

(-52% [CI, -76% to -5%]; $P = 0.035$) at 30 months than did the placebo group (**Table 3**).

The rates of FEV_1 decline from 6 to 30 months were -79 mL/y (CI, -112 to -46 mL/y) for the placebo group, -62 mL/y (CI, -93 to -31 mL/y) for the 6-month fluticasone group, 7.3 mL/y (CI, -21 to 35 mL/y) for the 30-month fluticasone group, and -16 mL/y (CI, -46 to 15 mL/y) for 30-month fluticasone and salmeterol group. Fluticasone significantly diminished annual FEV_1 decline over the last 2 years of the study compared with placebo (difference, 86 mL/y [CI, 43 to 129 mL/y]; $P < 0.001$) (**Figure 3, A**). The improvement in methacholine PC_{20} by fluticasone compared with placebo that we observed during the first 6 months was maintained during the following 2 years (**Figure 3, B**). In addition, maintaining fluticasone therapy reduced dyspnea scores more than placebo over the last 2 years of the study (-0.2 point/y [CI, -0.3 to -0.06 point/y]; $P = 0.003$) (**Figure 3, C**), and significantly improved SGRQ activity score (-3.1 points/y [CI, -5.5 to -0.7 points/y]; $P = 0.012$) and CCQ total score (-0.1 point/y [CI, -0.2 to -0.01 points/y]; $P = 0.036$), symptom score (-0.1 points/y [CI, -0.3 to -0.02 points/y]; $P = 0.026$), and functional score (-0.1 points/y [CI, -0.2 to -0.01]; $P = 0.027$) (**Figure 3, D**).

Figure 3. Clinical outcomes



Adjusted mean change ± 95% CI over time during treatment with fluticasone (500 µg bid) for 30 months (FP 30), fluticasone (500 µg bid) for 6 months (FP 6), followed by placebo (as indicated by the vertical line), the combination of fluticasone/salmeterol (500/50 µg bid) for 30 months (FP/S 30) and placebo (bid), in patients with moderately severe COPD. Changes in PC_{20} are expressed as mean doubling doses. Data are presented for forced expiratory volume in one second (FEV_1) (3A), log-transformed provocation concentration of methacholine causing a 20% fall in FEV_1 (PC_{20}) (3B), Medical Research Council dyspnea score (3C) and Clinical COPD Questionnaire (CCQ) (3D).

Discontinuation of ICS therapy

Discontinuing fluticasone therapy after 6 months increased $CD3^+$ cell count by 120% (CI, 24% to 289%; $P = 0.007$), mast cell count by 218% (CI, 99% to 407%; $P < 0.001$), and plasma cell count by 118% (CI, 9% to 336%; $P = 0.028$) at 30 months versus continuing therapy (**Figure 2** and **Table 2**). Bronchial epithelial parameters and sputum inflammatory cells did not change significantly (**Tables 3** and **4**).

Discontinuing fluticasone therapy after 6 months worsened subsequent FEV_1 decline compared with continuing therapy during the last 2 years of follow-up (difference in slope, -70 mL/y [CI, -111 to -28 mL/y]; $P = 0.001$) (**Figure 3, A**), with an accompanying deterioration in methacholine PC_{20} (-2.6 doubling dose [CI, -4.1 to -1.2 doubling dose]; $P < 0.001$) (**Figure 3, B**). Stopping fluticasone therapy also worsened dyspnea scores by 0.2 points/y (CI, 0.08 to 0.3 points/y; $P = 0.001$) (**Figure 3, C**), SGRQ total score by 1.7 points/y (CI, 0.19 to 3.2

points/y; *P* = 0.028) and activity score by 2.9 points/y (CI, 0.6 to 5.3 points/y; *P* = 0.015), and CCQ total score by 0.1 point/y (CI, 0.04 to 0.2 points/y; *P* = 0.003) and symptom score by 0.2 points/y (CI, 0.1 to 0.3 points/y; *P* < 0.001), compared with continuing therapy (data not shown).

Addition of LABAs to ICS therapy

At 6 months, combination treatment provided no additional anti-inflammatory effects compared with fluticasone alone; however, at 30 months, $CD3^+$ cell count had increased by 126% (CI, 27% to 303%; *P* = 0.006) and plasma cell count by 144% (CI, 21% to 393%; *P* = 0.013) (**Figure 2** and **Table 2**), and eosinophils in bronchial biopsies had changed by -55% (CI, -79% to -1%; *P* = 0.047). Salmeterol had no additional effect on bronchial epithelial parameters or sputum inflammatory cells (**Table 3** and **4**).

At 6 months, combination therapy increased postbronchodilator FEV_1 (96 mL [CI, 16 to 176 mL]; *P* = 0.018) (**Figure 3, A**) and improved dyspnea scores (-0.4 points [CI, -0.7 to -0.04 points]; *P* = 0.027) (**Figure 3, C**) more than fluticasone alone. Improved FEV_1 was maintained during prolonged combination therapy without further alteration of FEV_1 decline, compared with fluticasone alone, but the dyspnea score increased after 30 months (0.1 points/y [CI, 0.01 to 0.3 points/y]; *P* = 0.029). During the first 6 months, combination therapy resulted in a change of -0.3 points (CI, -0.5 to -0.07 points; *P* = 0.007) in CCQ total score, -0.3 points (CI, -0.6 to -0.04; *P* = 0.028) in symptom score, and -0.3 points (CI, -0.6 to -0.08 points; *P* = 0.008) in functional score (**Figure 3, D**). The minimal clinically important difference of 0.4 was not reached [30]. During the subsequent 24 months, combination therapy did significantly worse than

*Table 3. Sputum inflammatory cell counts at baseline and after 6 and 30 months**

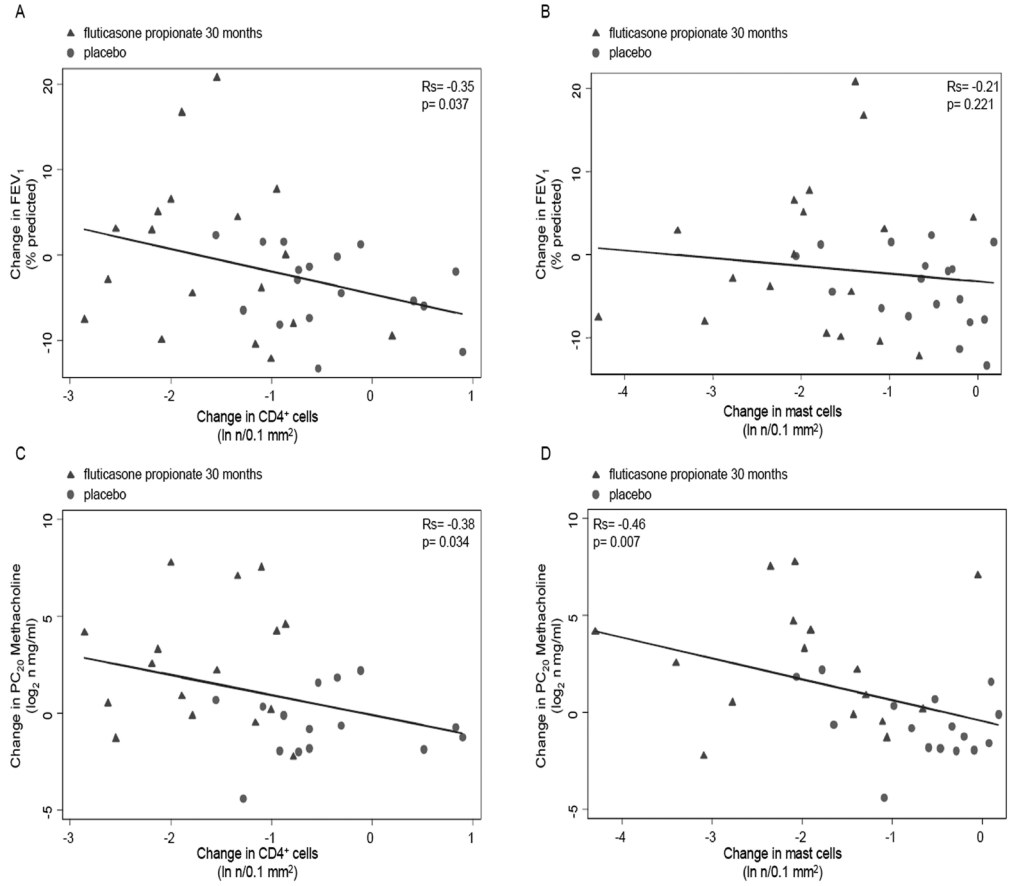
	Placebo, 30 mo			Fluticasone, 6 mo, Then Placebo, 24 mo			Fluticasone, 30 mo			Fluticasone Plus Salmeterol, 30 mo		
	Baseline	6 mo	30 mo	Baseline	6 mo	30 mo	Baseline	6 mo	30 mo	Baseline	6 mo	30 mo
Total cell count, $x10^6$ cells/mL)	168	62	107	117	101	95	175	95	58	136	114	55
	(77-235)	(41-212)	(18-268)	(53-380)	(80-320)	(57-164)	(101-316)	(53-178)	(23-74)	(78-247)	(60-201)	(17-160)
Neutrophils, %	72	74	70	73	67	75	66	68	71	72	74	75
	(54-80)	(54-81)	(50-85)	(63-82)	(56-79)	(61-79)	(50-77)	(47-78)	(45-79)	(61-81)	(64-81)	(65-81)
Eosinophils, %	0.9	0.8	1.0	1.3	1.0	1.0	1.2	0.8	0.8	1.3	0.8	0.8
	(0.3-2.2)	(0.2-1.3)	(0.2-1.8)	(0.5-2.6)	(0.5-1.6)	(0.5-1.9)	(0.3-2.2)	(0.2-1.9)	(0.5-1.8)	(0.2-2.3)	(0.4-1.3)	(0.3-2.0)
Macrophages, %	22	23	22	22	20	20	29	25	19	23	19	19
	(16-36)	(16-39)	(11-31)	(13-27)	(14-34)	(15-28)	(19-37)	(17-37)	(14-38)	(17-32)	(14-31)	(16-29)
Lymphocytes, %	1.8	1.7	1.8	1.8	1.5	2.0	2.2	2.0	1.9	1.3	2.0	1.7
	(1.3-3.0)	(1.0-3.2)	(1.2-3.7)	(1.5-2.2)	(1.2-2.3)	(1.2-3.3)	(1.2-3.1)	(1.2-2.9)	(1.1-2.3)	(0.8-2.4)	(0.7-2.8)	(1.2-2.5)

** Data are expressed as medians (25^{th} - 75^{th} percentiles)*

fluticasone alone on these outcomes, with a change of 0.1 point/y (CI, 0.04 to 0.2 points/y; *P* = 0.003) in total score, 0.1 point/y (CI, 0.03 to 0.3 points/y; *P* = 0.013) in symptom score, and 0.1 point/y (CI, 0.03 to 0.2 points/y; *P* = 0.012) in functional score.

We analyzed the data by using a model that also included individual variances of the slopes and obtained similar results.

Figure 4. Correlation between pathological and clinical outcomes



Upper panel. Correlation of changes (30 months minus baseline) in postbronchodilator forced expiratory volume in one second (FEV_1, % predicted) with changes in log-transformed $CD4^+$ cell numbers ($/10^{-7}$ m^2) (4A) and changes in log-transformed mast cell numbers ($/10^{-7}$ m^2) (4B) in the lamina propria of bronchial biopsies in COPD patients treated with fluticasone propionate 30 months or placebo. Lower panel. Correlation of changes (30 months minus baseline) in log-transformed provocative concentration of methacholine causing a 20% fall in FEV_1 (PC_{20}) with changes in log-transformed $CD4^+$ cell numbers ($/10^{-7}$ m^2) (4C) and changes in log-transformed mast cell numbers ($/10^{-7}$ m^2) (4D) in the lamina propria of bronchial biopsies in COPD patients treated with fluticasone propionate 30 months or placebo.

*Table 4. Bronchial epithelial features at baseline and after 6 and 30 month**

	Placebo, 30 mo			Fluticasone, 6 mo, Then Placebo, 24 mo			Fluticasone, 30 mo			Fluticasone Plus Salmeterol, 30 mo		
	baseline	6 mo	30 mo	baseline	6 mo	30 mo	baseline	6 mo	30 mo	baseline	6 mo	30 mo
Intact epithelium, %	23	22	12	30	21	15	20	20	16	29	29	25
	(18-35)	(10-30)	(3-20)	(18-42)	(10-30)	(4-27)	(14-33)	(14-28)	(10-31)	(16-47)	(24-38)	(20-50)
Squamous-cell metaplasia, % of epithelium	0	0	0	0	0	0	1.1	0	0	0	0	0
	(0-30)	(0-0)	(0-0)	(0-8.4)	(0-0)	(0-8.4)	(0-21)	(0-0)	(0-0)	(0-24)	(0-0)	(0-0)
Squamous-cell metaplasia, % of patients	24	20	24	26	26	26	25	27	25	25	26	25
PAS/AB-positive area, %	15	17	9.9	8.4	14	5.0	9.2	20	9.1	10	17	13
	(5.9-20)	(8.2-26)	(4.3-27)	(3.3-20)	(6.1-20)	(1.0-12)	(3.9-15)	(7.9-32)	(4.6-23)	(4.7-16)	(8.0-24)	(4.9-23)
Ki-67$^+$ cells, per mm of basement membrane	15	6	3.4	9.9	5.4	33	12	4.1	5.4	9.2	7.3	11
	(3.6-23)	(0.5-28)	(1.6-8.6)	(3.7-34)	(1.2-30)	(9.3-67)	(0.2-39)	(0.4-12)	(1.3-13)	(5.6-33)	(1.5-25)	(6.2-36)

** PAS/AB = periodic acid-Schiff/Alcian blue.*
Data are expressed as medians (25th - 75th percentiles).

Smoking and treatment effects

During the study, 3 patients started smoking and 13 patients stopped smoking (balanced among groups). All above results remained statistically significant when adjusted for smoking status throughout the study, except for the reduction in sputum lymphocyte numbers by long-term fluticasone therapy.

Relation of treatment effects with pathology and lung function

Analyses of patients that received either fluticasone or placebo for 30 month showed that decreases in CD4$^+$ cells were associated with improvements in predicted postbronchodilator FEV_1 (Rs, -0.35; P = 0.037) (Figure 4). Improvements in methacholine PC_{20} were associated with reductions in CD3$^+$ cells (Rs, -0.36; P = 0.041), CD4$^+$ cells (Rs, -0.38; P = 0.034), and mast cells (Rs, -0.46; P = 0.007), and increases in Percentage intact epithelium (Rs, 0.40; p = 0.024) (Figure 4).

Discussion

Our study shows that 2.5-year maintenance therapy with ICS in COPD reduces bronchial T lymphocyte and mast cell numbers and increases eosinophils and the integrity of bronchial epithelium, with an accompanying reduction in sputum cell counts. These effects are associated with a reduced rate of FEV_1 decline and improvements in airway hyperresponsiveness, dyspnea, and health status. Stopping ICS therapy at 6 months leads to relapse of bronchial inflammation and hyperresponsiveness, dyspnea, and poorer health status, with acceleration of FEV_1 decline. Combination therapy with ICS and a long-acting $ß_2$-agonist does not provide further anti-inflammatory effects compared with fluticasone alone but improves the level of FEV_1 without further influencing FEV_1 decline. Our findings indicate that a subphenotype of patients with COPD who are steroid-naïve and have moderate airway obstruction and airway hyperresponsiveness are sensitive to long-term ICS therapy. These prolonged effects on inflammation and lung function do not imply causality but suggest that disease modification can be achieved in particular phenotypes of patients with COPD.

We observed differential effects of ICS on inflammatory cell counts. Although smoking may reduce corticosteroid responsiveness [31], our data show that at least part of the inflammation in COPD remains sensitive to this treatment. The contribution of $CD8^+$ cells to inflammation and the relevant antigen-specific triggers in COPD are still unknown. $CD4^+$ cells may contribute to activation and memory formation of $CD8^+$ cells, as well as provide help for B cells [32]. Mast cells and their secreted enzymes can drive various processes relevant to inflammation and remodeling [33]. Although in vitro studies suggest that corticosteroids are less effective in inhibiting activation of mast cells than activation of T-cells [34], our data indicate corticosteroids can have selective anti-inflammatory effects in COPD.

The observed increase in intact epithelium by ICS has also been found in persons with asthma [35]. Corticosteroid-induced changes in epithelial integrity and inflammation correlated with improvements in methacholine PC_{20}, which supports the notion that airway hyperresponsiveness in COPD can be a marker of disease activity [36;37].

The clinical novelty of our findings is that anti-inflammatory effects observed with long-term ICS treatment associate with reduced FEV_1 decline in COPD. Previous short-tem studies that investigated patients with COPD and similar degrees of airway obstruction [20;21;38] have shown anti-inflammatory effects of ICS in COPD. We show that these beneficial

effects are maintained during long-term treatment up to 30 months. The detrimental effects of discontinuing ICS therapy on bronchial inflammation are also novel. Previous short-term studies of the combination of a LABA and ICS demonstrated anti-inflammatory effects versus placebo [39] or additional reductions of bronchial $CD8^+$ cells and macrophages versus ICS alone [22]. Our data suggest that this is not a long-lasting additional effect; we observed a slight increase in $CD3^+$ and plasma cells. The attenuated FEV_1 decline in our patients with COPD contrasts with large COPD trials from the 1990s [7-9]. The more recent TORCH study [15] did show reductions of FEV_1 decline in patients with COPD who received therapy ICSs, LABAs, or both. Our results suggest that the improvement in the level of FEV_1 in the combination group might be due to a residual bronchodilator effect of salmeterol and not further disease modification. Discrepancies between the previous trials and our study may be due to differences in study samples, which may provide a clinical message.

Our study comprised a common subset of patients with COPD. First, by choosing steroid-naïve patients, we aimed to exclude patients with unknown previous benefits from ICS at baseline and avoid the problem of selective dropouts in the placebo group. Second, our patients had predominantly moderate degrees of airway obstruction and most demonstrated airway hyperresponsiveness or modest reversibility of FEV_1. Recent studies [10;40] show that these characteristics, previously attributed to asthma alone, can also be components of COPD. This COPD phenotype may be particularly sensitive to ICS, similar to the documented beneficial effects of smoking cessation [37]. Of note, the decrease in postbronchodilator FEV_1 in the placebo group was quite similar to that observed in previous studies [8;10;15]. We were particularly careful to exclude patients with a previous or concurrent diagnosis of asthma by carefully taking histories, checking family practice medical records, and obtaining clinical judgments from chest physicians. Furthermore, most patients had low numbers of eosinophils in sputum and biopsies (similar to those reported by Bourbeau and colleagues [22]), had smoked for many years, and had a mean reversibility of FEV_1 to salbutamol of only 7% of predicted value, and most (83%) were nonreversible according to European Respiratory Society criteria - yet all adhered to the GOLD criteria. This is consistent with the patient characteristics of short-term COPD studies that show benefits with ICS therapy [22;39]. Airway hyperresponsiveness was similar to that in the Lung Health COPD study [10], which measured long-term changes in airway hyperresponsiveness. Finally, our post hoc analysis showed that actual smoking throughout the study was unlikely to be a major

confounder. Taken together, our findings suggest that ICS therapy, when given for the first time and for longer duration to steroid-naïve patients with relatively moderate disease, has the potential to change the clinical course of COPD.

Our study has limitations. First, only 77 of 101 analyzed patients had biopsies at 30 months because patients dropped out or were unwilling to have another bronchoscopy. This might have resulted in selection bias; however, lost-to-biopsy rates were similar among treatment groups. Second, our study was not powered to examine clinical outcomes. Nevertheless, the primary and secondary outcome parameters were all pre-specified. According to international standards on clinical investigations [41], the secondary outcomes point toward a clinically relevant treatment benefit, given our positive findings in the primary outcome. In addition, the positive findings on FEV_1 decline are consistent with the symptomatic benefit we observed [42]. Third, because this was an efficacy trial, we used data from adherent patients. As expected, the placebo group had more nonadherent patients, which may have led to underestimate the treatment effect. Fourth, the pathologic changes in COPD are not uniformly distributed among central and peripheral airways [43;44]. We inevitably focused on the central airways. Fifth, despite its beneficial effects, long-term ICS treatment has potentially meaningful adverse effects, such as increased frequency of pneumonia [14]. Our sample size was too small to draw conclusions on this.

Our study should lead to subsequent analyses of the benefits of inhaled steroids in COPD. Histological outcomes need to include inflammatory and epithelial cell activity and aspects of airway wall remodeling and fibrosis. Studies are also needed to determine the best inflammatory and clinical predictors of steroid efficacy in COPD. Finally, our results indicate a need to study the cost benefit of changing disease progression by using maintenance ICS therapy.

In conclusion, long-term maintenance therapy with ICS can reduce inflammation in bronchial biopsies and sputum in COPD. This is mirrored by attenuated lung function decline, airway hyperresponsiveness, dyspnea, and improved quality of life. Adding a LABA provided supplementary benefit for lung function but did not further alter the course of FEV_1 decline. Clinicians who are treating patients recognize that COPD is a heterogeneous disease that includes various phenotypes [45]. Our observations indicate that progressive decline in lung function can be attenuated in steroid-naïve patients with moderate COPD, a long history of smoking, and airway hyperresponsiveness. The observed treatment response by this particular subphenotype of COPD

underscores the potential of tailored therapy in COPD to achieve clinical benefit.

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Langdurige behandeling met inhalatiesteroïden heeft gunstig effect bij matig ernstige COPD*

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en de GLUCOLD studie groep

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Samenvatting

Doel: De effecten bepalen van een langdurige behandeling met inhalatiesteroïden, met en zonder langwerkende ß$_2$-agonisten, op luchtwegontsteking en longfunctie bij COPD.

Opzet: Gerandomiseerd, placebogecontroleerd onderzoek in 2 Nederlandse centra (http://clinicaltrials.gov/ct2/show/NCT00158847).

Methode: 114 steroïdnaïeve patiënten met matig tot ernstig COPD (rokers en ex-rokers) werden dubbelblind behandeld met: fluticasonpropionaat 500 µg 2 dd gedurende 6 (n = 31) of 30 maanden (n = 26), of met fluticasonpropionaat 500 µg 2 dd en salmeterol 50 µg 2 dd gedurende 30 maanden (n = 28), of met placebo 2 dd (n = 29). Celtellingen in bronchiale biopten en geïnduceerd sputum dienden als primair resultaat. Luchtweghyperreactiviteit werd gemeten voor randomisatie, na 6 en 30 maanden; klinische parameters werden elke 3 maanden bepaald.

Resultaten: 101 Patiënten waren meer dan 70% therapietrouw. Na 6 maanden fluticasongebruik was het aantal lymfocyten ($CD3^+$, $CD4^+$ en $CD8^+$) en mestcellen in de luchtwegwand verminderd (allen $p < 0,005$) en de hyperreactiviteit verbeterd ($p < 0,05$) ten opzichte van placebo. Deze effecten bleven behouden na 30 maanden. Langdurige behandeling met fluticason gedurende 30 maanden verminderde het aantal mestcellen en verhoogde het percentage intact epitheel in de bronchusbiopten. In het sputum verminderde het aantal neutrofielen, macrofagen en lymfocyten. Deze veranderingen gingen gepaard met een verminderde achteruitgang van de longfunctie en een verbetering van de kortademigheid en de kwaliteit van leven. Na staken van fluticasongebruik na 6 maanden verhoogde het aantal $CD3^+$-cellen, mestcellen en plasmacellen significant en verslechterde het klinische resultaat. Toevoeging van salmeterol aan de behandeling verbeterde het niveau van het geforceerde expiratoire 1-secondevolume (FEV_1).

Conclusie: Behandeling met inhalatiecorticosteroïden kan de luchtwegontsteking en de versnelde afname van de longfunctie verminderen bij steroïdnaïeve patiënten met matig tot ernstig COPD. Het toevoegen van langwerkende ß$_2$-agonisten versterkte deze effecten niet.

Introductie

Kortdurende behandeling met inhalatiecorticosteroïden (ICS) heeft gunstige effecten bij chronische obstructieve longziekte (COPD). De effecten van langdurige ICS-behandeling zijn echter nog onduidelijk.

COPD wordt gekenmerkt door een progressieve afname van longfunctie gecombineerd met luchtwegklachten en verslechtering van de gezondheidsstatus [1]. De aandoening wordt in verband gebracht met ontsteking in de luchtwegwand [2-4].

De huidige richtlijnen adviseren om patiënten met ernstige COPD en frequent optredende exacerbaties te behandelen met ICS in combinatie met langwerkende $ß_2$-agonisten (LABA's) [1]. ICS-behandeling leidt tot verbetering van symptomen, vermindering van exacerbatiefrequentie en tot initiële verbetering van longfunctie [5-8]. Bij staken van ICS-behandeling verslechtert het klinisch beeld [9]. Indien de behandeling bestaat uit een combinatie van ICS en een LABA verbetert het klinisch beeld meer dan bij behandeling met ICS alleen [10;11].

Een recent onderzoek suggereert dat langdurige behandeling met ICS of LABA de progressieve afname van het geforceerde expiratoire 1-secondevolume (FEV_1) kan remmen bij COPD [12]. Dit is in tegenstelling tot eerdere onderzoeken, waaruit bleek dat langdurige behandeling met ICS of LABA bij COPD geen effect had op de afname van FEV_1 [13-16].

De klinische effecten van ICS- en LABA-behandeling bij COPD kunnen gedeeltelijk berusten op anti-inflammatoire werking. Behandeling met ICS gedurende 2-3 maanden verminderde het aantal mestcellen, maar niet het aantal $CD8^+$-cellen, neutrofielen of macrofagen in bronchusbiopten [17;18]. Combinatiebehandeling met ICS en LABA gedurende 3 maanden gaf meer anti-inflammatoire effecten dan ICS als monotherapie, [19] maar deze effecten werden niet op de lange termijn bestudeerd.

Het doel van dit onderzoek was om de effecten van ICS op zowel pathologische als klinische kenmerken van COPD te onderzoeken tijdens een langdurige behandeling van 30 maanden.

Patiënten en methoden

Setting en deelnemers

Het 'Groningen Leiden Universities Corticosteroids in Obstructive Lung Disease'(GLUCOLD)-project had een dubbelblinde, parallelle, placebogecontroleerde, gerandomiseerde opzet met 4 groepen [20].

Rokers en ex-rokers met > 10 pakjaren en COPD werden ingesloten (leeftijd:

45-75 jaar). Hun longfunctie was matig tot ernstig verstoord [1]. Exclusiecriteria waren: een voorgeschiedenis van astma, of huidige symptomen, diagnose of behandeling daarvan of -voor, en ICS-behandeling in de 6 maanden voorafgaande aan het onderzoek. Standaard werd geadviseerd om te stoppen met roken. Patiënten werden geworven in huisartsenpraktijken. De ethische commissies van beide centra in Leiden en Groningen keurden het onderzoek goed; alle patiënten gaven schriftelijke toestemming.

Randomisatie van interventies

Patiënten werden gerandomiseerd in 4 behandelgroepen: (a) fluticasonpropionaat 500 µg 2 dd gedurende 6 maanden, gevolgd door placebo 2 dd gedurende 24 maanden; (b) fluticasonpropionaat 500 µg 2 dd gedurende 30 maanden; (c) fluticasonpropionaat 500 µg 2 dd en salmeterol 50 µg 2 dd gedurende 30 maanden; (d) placebo 2dd gedurende 30 maanden. Actieve medicatie en placebo werden geïnhaleerd via identieke Diskus-poederinhalatoren (GlaxoSmithKline, Zeist, Nederland). Minimisatie (het gelijk verdelen van patiënten met hun prognostische factoren over de verschillende behandelarmen; elke nieuwe patiënt wordt gerandiomiseerd op basis van de verdeling van prognostische factoren die op dat moment aanwezig is) vond plaats voor centrum, geslacht, rookstatus, de verhouding FEV_1: inspiratoire vitale capaciteit (IVC) (< 60 of ≥ 60%) en de provocatiedosis methacholine die nodig was om het FEV_1 20% te laten dalen (PD_{20}- methacholine) (< 2 of ≥ 2 mg/ml).

Onderzochte parameters

De primaire uitkomstmaat was het aantal inflammatoire cellen in bronchusbiopten en in geïnduceerd sputum [20;21]. De details over bronchoscopie, biopsieverwerking en kwantificatie zijn beschreven in een eerder artikel met baselinegegevens [22]. Spirometrie na luchtwegverwijding en hyperreactiviteit (PD_{20}-methacholine) waren secundaire uitkomstmaten [20]. Kortademigheid werd gemeten met een aangepaste 'Medical research council(MRC)-kortademigheidschaal'; gezondheidsstatus met de 'St. George's respiratory questionnaire' (SGRQ) en 'Clinical COPD questionnaire' (CCQ) [23]. In de CCQ is een verandering in score van 0,4 punten ten opzichte van baseline de kleinste verandering die klinisch significant genoemd kan worden [24].

Follow-up

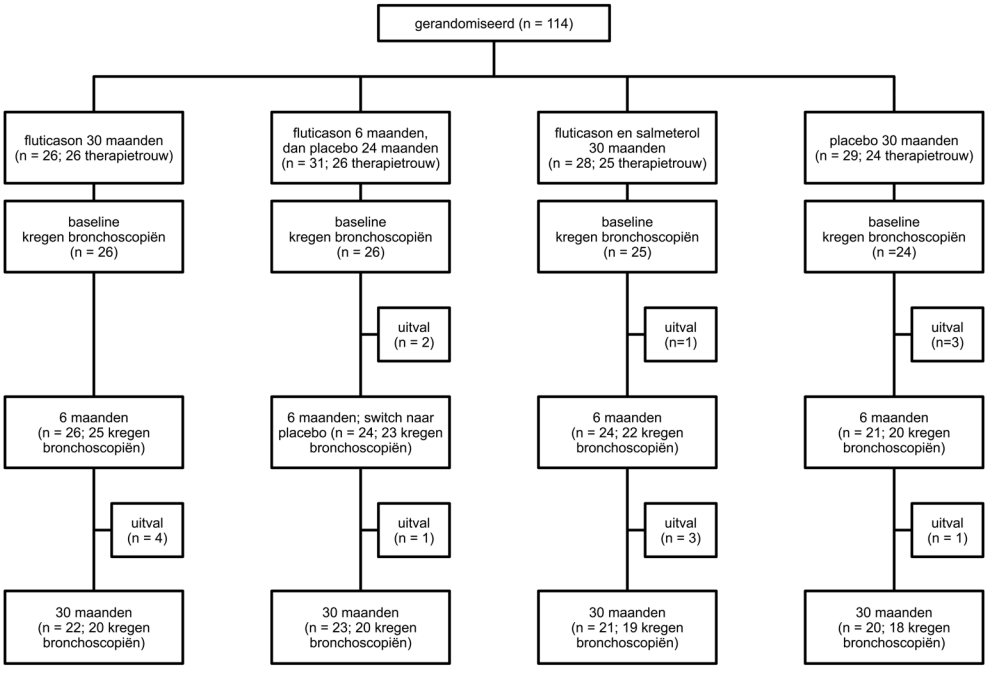
Elke 3 maanden werden symptomen, gezondheidsstatus, rookstatus, therapietrouw (tellen van aantal doses) gemeten en spirometrie verricht. Bronchoscopie, sputuminductie en methacholineprovocatie werden bij de start uitgevoerd, en na 6 en 30 maanden.

Statistische analyse

De steekproefgrootte werd gebaseerd op fluticason-geïnduceerde kortetermijnverandering van de submucosale $CD8^+$-celaantallen bij COPD [25]. Bij een aantal van 20 patiënten per behandelgroep is een halvering van $CD8^+$-celaantallen in de fluticasongroep in vergelijking tot placebo met een onderscheidingsvermogen ('power') van 80% detecteerbaar [25]. Omdat het een onderzoek naar de werkzaamheid betrof, werd een per-protocolanalyse uitgevoerd bij alle compliante patiënten (> 70% van de voorgeschreven doses gebruikt).

Met lineaire 'mixed effect'-modellen (STATA versie 9.0; StataCorp, Texas,

Figure 1.



Stroomdiagram van een studie naar effecten van langdurige behandeling met inhalatiecorticosteroïden bij matig ernstig COPD. Per behandelgroep is weergegeven het totaal aantal patiënten dat gerandomiseerd werd en dat therapietrouw (>70% van de voorgeschreven dosis ingenomen) was. Voor elke fase van de studie (0, 6 en 30 maanden) zijn de aantallen patiënten vermeld die een bronchoscopie ondergingen.

*Tabel 1. Studie naar effecten van langdurige behandeling met inhalatiecorticosteroïden bij matig ernstig COPD; karakteristieken van 101 therapietrouwe patiënten bij aanvang van de studie**

Karakteristieken	Behandeling, duur				P-waarde †
	Placebo	Fluticasonpropionaat		Fluticasonpropionaat en salmeterol	
	30 maanden (n = 24)	6 maanden, daarna 24 maanden placebo (n = 26)	30 maanden (n = 26)	30 maanden (n = 25)	
klinisch					
man / vrouw; n / n	20 / 4	22 / 4	23 / 3	22 / 3	0,94
leeftijd; jaren	59 (8)	64 (7)	62 (8)	62 (8)	0,31
huidige roker / niet-roker; n/n	17 / 7	14 / 12	16 / 10	17 / 8	0,61
pakjaren roken; mediaan (bereik)	42 (34-54)	41 (29-57)	44 (31-55)	47 (31-56)	0,62
longfunctie					
FEV_1; % voorspeld					
vóór luchtwegverwijding	54,1 (8,3)	56,8 (11,0)	56,6 (9,9)	55,0 (11,0)	0,74
na luchtwegverwijding	61 (8,3)	65 (8,6)	64 (9,1)	61 (9,4)	0,41
verandering in FEV_1; % voorspeld ‡	7,1 (4,5)	7,3 (5,3)	7,1 (4,0)	6,2 (6,3)	0,87
FEV_1 / IVC na luchtwegverwijding; %	47 (9,0)	51 (8,3)	49 (9,0)	46 (8,4)	0,16
geometrisch gemiddelde PD_{20}-metacholine; mg/ml	0,7 (2,0)	0,7 (3,2)	0,4 (2,4)	0,7 (2,7)	0,64
K_{CO}; % voorspeld	65 (19)	79 (29)	77 (22)	74 (27)	0,19
luchtwegklachten en gezondheidsstatus					
MRC-dyspneu-score §	2,7 (0,8)	2,5 (0,6)	2,6 (0,6)	2,9 (1,0)	0,53
SGRQ-score; totaal ‖	33,5 (18,5)	25,7 (15,2)	32,9 (10,9)	28,1 (13,2)	0,27
CCQ-score; totaal ¶	1,77 (1,3)	1,16 (0,6)	1,26 (0,6)	1,43 (0,7)	0,35

FEV_1 = geforceerde expiratoire 1-secondevolume; IVC = inspiratoire vitale capaciteit; PD_{20}-methacholine = de provocatieve concentratie van methacholine die een afname van 20% in FEV_1 veroorzaakt; KCO = diffusiecapaciteit voor CO; MRC = 'Medical research council'; SGRQ = 'St. George's respiratory questionnaire'; CCQ = 'Clinical COPD questionnaire'.

** Waarden zijn gemiddelden (SD) tenzij anders vermeld.*

† Variantieanalyse of kruskal–wallistoets tussen groepen.

‡ Reversibiliteit in FEV_1 na inhalatie van 400 µg salbutamol.

§ Bereik van 1 tot 5 (een hogere score betekent meer dyspneu).

‖ Bereik van 0 (best) tot 100 (slechtst).

¶ Bereik van 0 (best) tot 6 (slechtst).

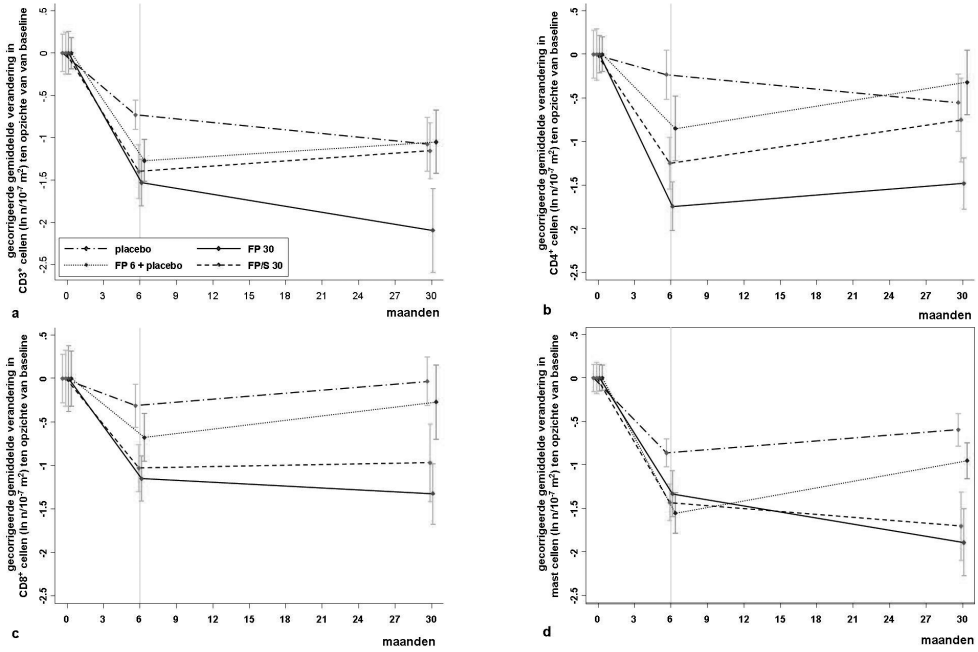
VS) werd het effect van de behandeling, tijd en de interactie van behandeling en tijd geanalyseerd [20]. Een post-hocanalyse onderzocht effecten van de rookstatus. Correlaties tussen statistisch significante behandeleffecten op inflammatoire cellen en longfunctie werden geanalyseerd met de spearman-rangcorrelatiecoëfficiënt. De gegevens worden weergegeven als gemiddelden (SD) of medianen (interkwartielbereiken); een 2-zijdige p-waarde < 0,05 werd als statistisch significant beschouwd.

Resultaten

Van de 114 geïncludeerde patiënten werden 101 therapietrouwe patiënten geanalyseerd (Figuur 1). Van hen hadden 7 ooit een korte kuur met orale corticosteroïden gehad en 5 hadden ooit ICS gebruikt. De

patiëntkarakteristieken bij aanvang van het onderzoek waren vergelijkbaar tussen de 4 behandelgroepen (Tabel 1), evenals het aantal inflammatoire cellen in het sputum en de bronchusbiopten.

Figuur 2.



Uitkomsten van pathologisch onderzoek in een studie naar de effecten van langdurige behandeling met inhalatiecorticosteroïden bij matig ernstig COPD. De grafieken tonen de aangepaste gemiddelde verandering in loggetransformeerde celaantallen in bronchusbiopten (per 10^{-7} m^2 lamina propria) tijdens behandeling met fluticasonpropionaat 500 µg 2 dd gedurende 30 maanden, fluticasonpropionaat 500 µg 2 dd gedurende 6 maanden, de combinatie van fluticasonpropionaat/salmeterol 500/50 µg 2 dd gedurende 30 maanden en placebo gedurende 30 maanden. 'Error bars' geven het 95%-betrouwbaarheidsinterval weer. Weergegeven zijn de gegevens van (a) bronchiale $CD3^+$-cellen, (b) $CD4^+$-cellen, (c) $CD8^+$-cellen en (d) mestcellen.

ICS-behandeling

6 maanden

Behandeling met fluticason verminderde, in vergelijking tot placebo, het aantal bronchiale $CD3^+$-cellen met 55% ($p = 0,004$), $CD4^+$-cellen met 78% ($p < 0,001$), $CD8^+$-cellen met 57% ($p = 0,010$) en het aantal mestcellen met 38% ($p = 0,039$; Figuur 2). Tegelijkertijd nam bij fluticasonbehandeling de PD_{20}-methacholine toe vergeleken met placebobehandeling (1,5 verdubbelende dosering van methacholine; $p = 0,036$) (Figuur 3B), evenals de score van een onderdeel van de CCQ over het mentale welzijn (toename met 0,2 punten, $p = 0,037$).

30 maanden

Tijdens de behandeling met fluticason van 6 tot 30 maanden bleef de afname van het aantal $CD3^+$-, $CD4^+$- en $CD8^+$-cellen in bronchusbiopten behouden vergeleken met placebo (zie Figuur 2). Het aantal mestcellen nam verder af (56%, p = 0,001), het aantal eosinofielen nam toe (125%, p = 0,046) evenals het percentage intact epitheel (101%, p = 0,024). Daarnaast verminderde in het sputum het aantal neutrofielen (58%, p = 0,047), macrofagen (57%, p = 0,041) en lymfocyten (52%, p = 0,035) na 30 maanden, vergeleken met placebo.

Ten opzichte van de gemiddelde FEV_1-waarde na 6 maanden, daalde de waarde na 30 maanden met 79 ml/jaar in de placebogroep, met 62 ml/jaar in de 6-maanden-fluticasongroep en met 16 ml/jaar in de groep met 30 maanden combinatietherapie. In de 30-maanden-fluticasongroep steeg de gemiddelde FEV_1-waarde met 7,3 ml/jaar. Vergeleken met placebo, verbeterde het gebruik van fluticason de jaarlijkse FEV_1-afname van 6 tot 30 maanden met 86 ml/jaar (p < 0,001; Figuur 3a).

De verbetering van PD_{20}-methacholine door fluticasonbehandeling in de eerste 6 maanden, vergeleken met placebo, bleef behouden tijdens de volgende 2 jaar (zie Figuur 3b). Gedurende de laatste 2 jaar van het onderzoek verbeterde fluticasongebruik de 'kortademigheidsscore' (p = 0,003; Figuur 3c), de 'SGRQ-activiteitenscore' (p = 0,012), de totale score van de CCQ (p = 0,036) en van de 'CCQ-symptoomscore' (p = 0,026) en 'CCQ-functionele score' (p = 0,027; Figuur 3d).

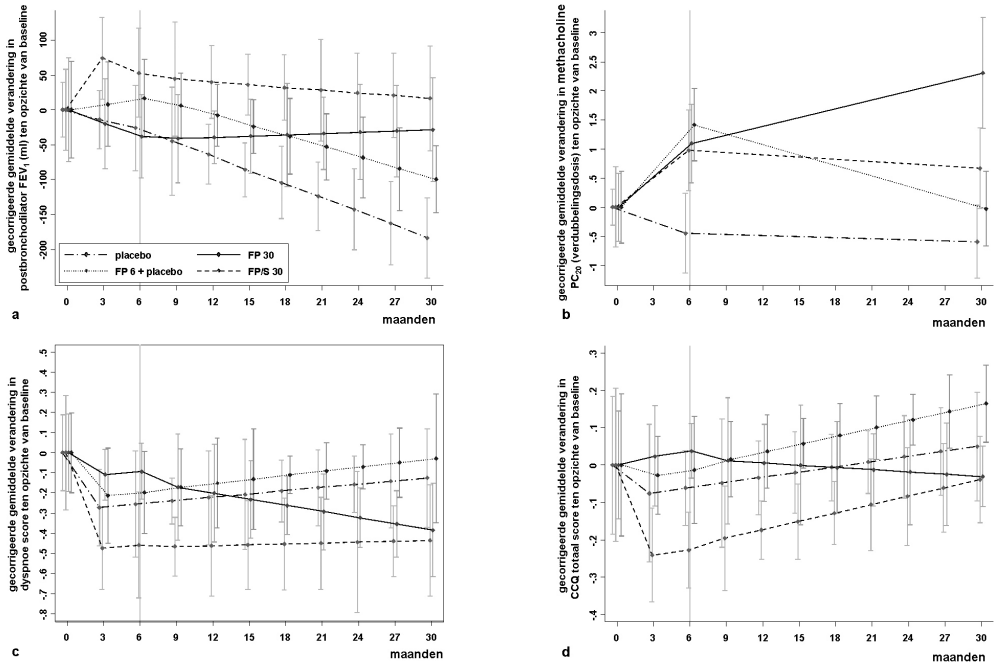
Staken van ICS-behandeling

Staken van fluticasonbehandeling na 6 maanden had geen effect op de aantallen inflammatoire cellen in het sputum en op de epitheelparameters in bronchusbiopten. Wel resulteerde het staken na 6 maanden fluticasonbehandeling in een toename van het aantal $CD3^+$-cellen (120%, p = 0,007), mestcellen (218%, p < 0,001) en plasmacellen (118%, p = 0,028) in bronchusbiopten na 30 maanden, vergeleken met de biopten van patiënten die 30 maanden fluticason hadden gebruikt (zie Figuur 2).

De FEV_1-afname van 6 tot 30 maanden was groter na stoppen dan bij continueren van fluticasongebruik (verschil van 70 ml/jaar; p = 0,001; Figuur 3a). De PD_{20}-methacholine verslechterde met 2,6 verdubbelingsdoses (p < 0,001; Figuur 3b). De kortademigheidscore daalde met 0,2 punten/jaar (p = 0,001; Figuur 3c), evenals de volgende scores: 'SGRQ totale score' (met 1,7 punten/jaar; p = 0,028), 'activiteitenscore' (met 2,9 punten/jaar; p = 0,015), 'CCQ totale score' (met 0,1 punt/jaar; p = 0,003) en de 'symptoomscore' (met

0,2 punten/jaar; p < 0,001) (gegevens niet getoond).

Figuur 3.



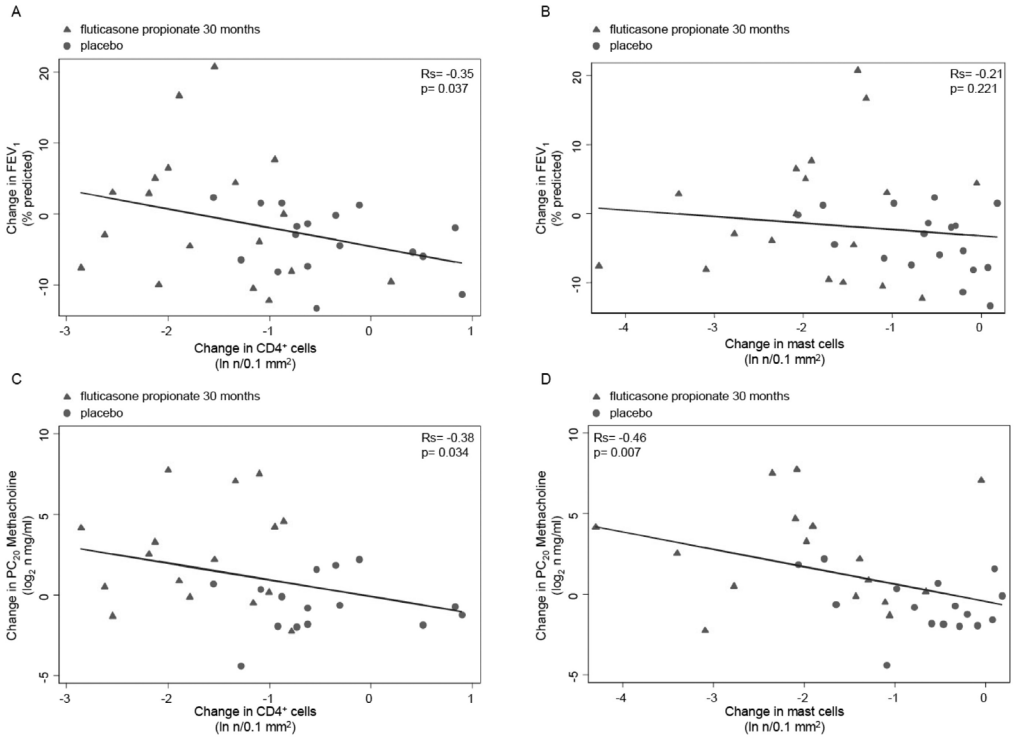
Klinische uitkomsten in een studie naar effecten van langdurige behandeling met inhalatiecorticosteroïden bij matig ernstig COPD. De grafieken tonen de aangepaste gemiddelde verandering met 95%-betrouwbaarheidsintervallen, tijdens de behandeling met fluticasonpropionaat 500 µg 2 dd gedurende 30 maanden, fluticasonpropionaat 500Mg 2 dd gedurende 6 maanden gevolgd door placebo, de combinatie van fluticasonpropionaat/salmeterol 500/50Mg 2 dd gedurende 30 maanden en placebo. Gegevens zijn gepresenteerd als (a) geforceerde expiratoire 1-secondevolume (FEV_1), (b) loggetransformeerde provocatieve concentratie van methacholine die een afname van 20% in FEV_1 veroorzaakt (PD_{20}-metacholine; veranderingen hierin zijn weergegeven als gemiddelde verdubbelingsdosis), (c) 'Medical research council'-dyspneu-score en (d) 'Clinical COPD questionnaire' (CCQ).

Combinatie ICS en langwerkende ß$_2$-agonisten

Combinatiebehandeling gedurende 6 maanden gaf geen extra anti-inflammatoire effecten vergeleken met fluticasonbehandeling alleen. Na 30 maanden nam het aantal $CD3^+$-cellen en plasmacellen toe (126%; p = 0,006 respectievelijk 144%; p = 0,013; Figuur 2) en het aantal eosinofiele cellen af (55%; p = 0,047). Behandeling met salmeterol had geen extra anti-inflammatoir effect op de aantallen cellen in het sputum [20].

Na 6 maanden combinatiebehandeling verbeterde de gemiddelde FEV_1-waarde met 96 ml (p = 0,018; Figuur 3a) en de kortademigheidscores met 0,4 punten (p = 0,027; Figuur 3c), meer dan bij fluticasonmonotherapie. Deze initiële verbetering van het FEV_1 werd behouden tijdens de voortgezette combinatiebehandeling, zonder verdere beïnvloeding van de FEV_1-

Figuur 4.



Correlatie tussen verandering in pathologie en klinische uitkomsten bij COPD-patiënten behandeld met fluticason 30 maanden of placebo. Weergegeven zijn correlaties van veranderingen (30 maanden minus baseline) in geforceerde expiratoire 1-secondenvolume (FEV_1, % van voorspeld) na luchtwegverwijdering, met (a) veranderingen van loggetransformeerde $CD4^+$-celaantallen (per 10^{-7} m^2 lamina propria van bronchus biopten; Spearman rangcorrelatiecoëfficiënt (Rs): -0,35; p: 0,037) en (b) met veranderingen in loggetransformeerde mestcelaantallen (per 10^{-7} m^2 lamina propria van bronchusbiopten; Rs: -0,21; p: 0,221). Daaronder zijn weergegeven correlaties van veranderingen (30 maanden minus baseline) in loggetransformeerde provocatieve concentratie van methacholine die een afname van 20% in FEV_1 veroorzaakt (PD_{20}) met (c) veranderingen van loggetransformeerde $CD4^+$-celaantallen (per 10^{-7} m^2 lamina propria van bronchus biopten; Rs: -0,38; p: 0,034) en met (d) veranderingen in loggetransformeerde mestcel aantallen (per 10^{-7} m^2 lamina propria van bronchusbiopten; Rs: -0,46; p: 0,007).

daling, vergeleken met alleen fluticason. De kortademigheidscore nam na 30 maanden toe met 0,1 punt (p = 0,029). Tijdens de eerste 6 maanden combinatiebehandeling verbeterde de 'totale CCQ-score' (p = 0,007), 'symptoomscore' (p = 0,028) en 'functionele score' (p = 0,008) (Figuur 3d). Het minimale klinisch significante verschil van 0,4 werd niet bereikt [24]. Tijdens de daarop volgende 24 maanden verslechterde de 'totale CCQ-score' (p = 0,003), 'symptoomscore' (p = 0,013) en 'functionele score' (p = 0,012) in de groep met combinatiebehandeling.

Roken

Alle bovenstaande resultaten bleven significant na correctie voor rookstatus, behalve het aantal lymfocyten in het sputum na 30 maanden fluticasonbehandeling.

Relatie pathologie en longfunctie

Analyse van patiënten die gedurende 30 maanden fluticason of placebo gebruikten, toonde dat verbetering van de FEV_1-waarden correleerde met afname van $CD4^+$-cellen ($p = 0,037$; Figuur 4). Een verbetering in PD_{20}-methacholine correleerde met afname van $CD3^+$-cellen ($p = 0,041$), $CD4^+$-cellen ($p = 0,034$) en mestcellen ($p = 0,007$) en met toename van het percentage intact epitheel ($p = 0,024$; Figuur 4).

Beschouwing

Ons onderzoek bij COPD toonde aan dat gedurende een ICS-behandeling van 2,5 jaar het aantal bronchiale T lymfocyten en mestcellen verminderde en het aantal eosinofielen en de bronchiale epitheliale integriteit toenam, tegelijk met afname van celaantallen in het sputum. Deze effecten gingen gepaard met een minder snelle FEV_1-afname en met verbetering van luchtweghyperreactiviteit, kortademigheid en gezondheidsstatus. Het stopzetten van de ICS-behandeling na 6 maanden deed deze positieve effecten teniet. De combinatiebehandeling van ICS en LABA bood geen extra anti-inflammatoire effecten vergeleken met fluticasonmonotherapie. Combinatiebehandeling gaf een initiële verbetering van FEV_1 die ook op lange termijn bleef bestaan. Echter, langer doorgaan met behandeling gaf geen verdere beïnvloeding van de daling van FEV_1ten opzichte van alleen inhalatiecorticosteroïden. Onze bevindingen gaven aan dat een subgroep van COPD-patiënten met een matige tot ernstige luchtwegobstructie, die steroïdnaïef zijn en hyperreactieve luchtwegen hebben, goed reageerde op langdurige ICS-behandeling.

Hoewel roken de reactie op behandeling met corticosteroïden kan verminderen [26], toonden onze gegevens aan dat een deel van de ontsteking bij COPD, ondanks roken, toch gevoelig is voor ICS. De bijdrage van $CD8^+$-cellen aan de ontsteking en de relevante antigeen-specifieke triggers bij COPD zijn nog altijd niet bekend. $CD4^+$-cellen kunnen bijdragen aan de activering en geheugenvorming van $CD8^+$-cellen, evenals B-celactivatie [27]. Mestcellen en hun uitgescheiden enzymen kunnen diverse processen aansturen die relevant zijn voor luchtwegontsteking en remodelering [28].

De klinische nieuwswaarde van onze bevindingen is dat anti-inflammatoire effecten bij COPD tijdens 2,5 jaar behandeling met ICS samengaan met een verminderde FEV_1-achteruitgang. Eerder kortdurend onderzoek bij matig tot ernstig COPD toonde reeds anti-inflammatoire effecten van ICS aan [17;18;29]. Ons onderzoek liet zien dat deze gunstige effecten ook op de lange termijn behouden bleven en dat er nadelige effecten op luchtwegontsteking kunnen ontstaan als ICS-behandeling wordt gestopt.

De gevonden vermindering van de FEV_1-afname bij fluticasonbehandeling staat in contrast met eerdere onderzoeken [5-8]. Daarentegen liet het 'TOwards a Revolution in COPD Health'(TORCH)-onderzoek [16] ook vermindering van FEV_1-afname zien bij COPD-patiënten die werden behandeld met ICS, LABA's of beide. Onze resultaten suggereren dat de verbetering van het FEV_1-niveau in de combinatiegroep kan worden veroorzaakt door een luchtwegverwijdend effect van salmeterol en niet door een verdere verandering van de onderliggende ontstekings- en remodeleringprocessen. Verschillen tussen eerdere onderzoeken en ons onderzoek kunnen het gevolg zijn van verschillen in selectie van patiëntenpopulatie.

Door steroïdnaïeve COPD-patiënten te selecteren, sloten we patiënten uit die al gunstige effecten van ICS-behandeling hadden bij aanvang van het onderzoek, en vermeden we dat deze zouden uitvallen in de placebogroep. Onze patiënten hadden overwegend matig ernstige luchtwegobstructie en de meeste patiënten hadden luchtweghyperreactiviteit en geringe reversibiliteit van de obstructie. Hyperreactiviteit past ook bij COPD [8;30] en de reversibiliteit was, met gemiddeld 7%, vergelijkbaar met eerder gepubliceerd interventie-onderzoek bij COPD [6;8;16]. We willen benadrukken dat de diagnose 'astma' bij de patiënten zorgvuldig werd uitgesloten, gebaseerd op het klinische oordeel van longartsen, en raadpleging van huisartsdossiers.

Ons onderzoek heeft enkele beperkingen: (a) 77 van de 101 geanalyseerde patiënten ondergingen na 30 maanden een bronchoscopie; het percentage uitvallers was echter vergelijkbaar tussen de behandelgroepen; (b) de onderzoeksgrootte van de studie was relatief klein om veranderingen in achteruitgang van de longfunctie vast te stellen. Des te opvallender is onze waarneming dat de ICS-geïnduceerde verbeteringen in luchtwegontsteking samenhangen met verbeteringen in longfunctie, hyperreactiviteit en luchtwegklachten; (c) dit was een onderzoek naar werkzaamheid van behandeling, reden waarom we alleen gegevens van therapietrouwe patiënten gebruikten. Zoals verwacht, waren er meer therapieontrouwe patiënten in de placebogroep, waardoor het behandeleffect eerder onderschat dan overschat

is; (d) wij onderzochten biopten van de centrale luchtwegen, terwijl de perifere luchtwegen een belangrijke rol spelen bij COPD [31].
Voor de dagelijkse praktijk is het belangrijk om bij deze patiënten met matig ernstig COPD te bepalen wat de beste voorspellers zijn voor een gunstige respons op ICS-behandeling. Daarnaast is het belangrijk om de kosteneffectiviteit te bestuderen van langdurige ICS-behandeling in een pragmatisch onderzoek.

Conclusie

Onze bevindingen suggereren dat behandeling met inhalatiecorticosteroïden (ICS), wanneer deze voor het eerst en langdurig gegeven worden aan steroïdnaïeve COPD-patiënten met een relatief matig ernstig ziektestadium en met aanwezigheid van luchtweghyperreactiviteit, de potentie heeft om het klinische beloop van COPD gunstig te beïnvloeden. Het verminderde de ontstekingsparameters in bronchusbiopten en sputum, wat weerspiegeld werd in verminderde achteruitgang van longfunctie, minder hyperreactiviteit en kortademigheid, en verbetering van de kwaliteit van leven. Het toevoegen van een langwerkende $ß_2$-agonist verbetert de longfunctie, maar verandert de progressie van de aandoening niet. De waargenomen respons op de behandeling bij dit specifieke subfenotype van COPD onderstreept de mogelijkheid voor een behandeling op maat bij COPD en geeft daarmee een nieuw klinisch perspectief.

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Chapter 6

Towards predicting long-term benefits of inhaled steroids by phenotypic markers in moderate to severe COPD:
a randomised controlled trial

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Submitted

Abstract

Background: In subgroups of patients with COPD the decline in lung function has been reduced by long-term inhaled corticosteroid (ICS) treatment. We aimed to identify which clinical, physiological and/or non-invasive inflammatory characteristics predict effects of inhaled corticosteroids on lung function decline in COPD and on inflammatory cell counts in bronchial biopsies.

Methods: Analysis was performed in 50 steroid-naive compliant patients with moderate to severe COPD (postbronchodilator forced expiratory volume in one second (FEV_1) compatible with GOLD stages II-III, 30-80% of predicted), age 45-75 years, >10 packyears smoking and without asthma. Patients were treated with fluticasone propionate (500 µg bid) or placebo for 2.5 years. Postbronchodilator FEV_1, dyspnea and health status were measured every 3 months, lung volumes, airway hyperresponsiveness (PC_{20}), induced sputum and bronchial biopsies at 0, 6 and 30 months. A linear mixed effect model was used for analysis.

Results: Predictors of attenuated FEV_1 decline by fluticasone treatment compared to placebo included: fewer packyears smoking, preserved diffusion capacity, limited hyperinflation and lower inflammatory cell counts in induced sputum. Predictors of long-term reduction in $CD3^+$ cells in bronchial biopsies were ex-smoking and increased inflammatory cell counts in sputum.

Conclusions: Long-term benefits by inhaled corticosteroids on lung function decline in patients with moderate to severe COPD are most pronounced in patients with less advanced disease and less signs of emphysema. These data generate novel hypotheses on phenotype-driven therapy in COPD.

Background

Accelerated decline in lung function is the hallmark of chronic obstructive pulmonary disease (COPD). Recently, three studies provided the first evidence that the decline in postbronchodilator forced expiratory volume in one second (FEV_1) can be reduced by long-term inhaled corticosteroid (ICS) treatment during one to three years follow-up [1-3]. This was unexpected, since most big trials had not shown such benefit previously [4]. There appears to be considerable heterogeneity in treatment effects amongst COPD patients [1; 2; 3]. Hence, implementation of long-term ICS therapy in daily practice is difficult since this requires predictive, phenotypic patient characteristics to determine who should receive this long-term treatment [5;6].

Few clinical features have been reported to predict ICS response in COPD. A relatively large bronchodilator response was shown to predict the short-term (months) ICS response [7-10]. Reports on long-term predictors (years) are, however, sparse. Current smoking may reduce long-term ICS responses [11-14], as well as a higher number of packyears smoked, reflecting the cumulative effect of smoking on lung disease [11]. In daily practice, physicians are tempted to use lung function reversibility as a guide for prescribing ICS. However, conflicting results exist whether reversibility can predict long-term steroid effects [11;15;16]. Furthermore, many COPD patients demonstrate increased airway hyperresponsiveness, a factor related to accelerated FEV_1 decline [17-19]. This possibly reflects a distinct phenotype of COPD that may exhibit enhanced ICS treatment response [15;20;21].

The progressive decline of FEV_1 in COPD is associated with an inflammatory process in the intrapulmonary airways [2]. Such airway inflammation is characterised by neutrophils, macrophages, $CD8^+$ cells and mast cells [22]. Inhaled corticosteroids may decrease CD3, CD4, CD8 and mast cells in bronchial biopsies [2]. Eosinophil and T-cell markers have been reported as predictors of short-term lung function responses to ICS treatment in COPD [23;24]. However, there are no data available to our knowledge on the predictive value of these and other inflammatory cells for long-term effects of ICS treatment on the accelerated FEV_1 decline present in COPD.

We postulated that clinical, physiological and/or non-invasive inflammatory characteristics can serve as predictors of long-term beneficial effects of inhaled corticosteroids on lung function decline in steroid-naive patients with moderately severe COPD. Secondly, we postulated that such features can also predict steroid effects on inflammatory cell counts in bronchial biopsies. The present *a priori* planned analysis was meant to be hypothesis generating

in order to reveal a COPD phenotype that will more likely benefit from anti-inflammatory therapy with respect to disease progression.

Methods

The present study focuses on detailed phenotyping of patients with moderate to severe COPD. For this purpose, 50 patients with COPD, who received 2.5 years of treatment with inhaled corticosteroids or placebo in the GLUCOLD study (Groningen Leiden Universities and Corticosteroids in Obstructive Lung Disease) were included in the analysis. The original cohort also included patients who stopped inhaled corticosteroids after 6 months and patients with the combination therapy of inhaled corticosteroids and long-acting $ß_2$-agonist. For this analysis we excluded these patients because we focused on long-term effects of steroid therapy as such without adding long-acting $ß_2$-agonists. A precise description of patient characteristics and methods can be found in previous reports [2;25]. Briefly, patients had irreversible lung function that was compatible with the Global initiative for chronic Obstructive Lung Disease (GOLD) stages II and III [26], and had ≥ 10 packyears smoking. Asthma was excluded by doctor's diagnosis and self-reported symptoms, treatment or diagnosis of asthma. Participants had not been treated with inhaled and oral corticosteroids within 6 and 3 months of trial entry respectively, and 27/30 patients were steroid-naive before entry of the study. The vast majority of patients were recruited between 2000 and 2003 from general practices and all patients had been clinically stable for at least 8 weeks before the measurements. The study was performed complying with the Helsinki declaration and approved by local ethics committees and all subjects gave written informed consent.

The GLUCOLD study was a prospective longitudinal, randomised, double blind, placebo-controlled two-centre trial. Patients were randomly assigned to receive either fluticasone propionate (500 µg bid) or placebo for 2.5 years. Study medication included Diskus® dry powder inhalers (GlaxoSmithKline, The Netherlands), and active treatment and placebo were identical in appearance. Randomisation was performed by an independent randomisation centre using a minimisation procedure balancing treatment groups for a number of variables (centre, gender, current smoker, FEV_1/IVC < or ≥ 60%, PC_{20} methacholine < or ≥ 2 mg/ml). Measurements of symptoms and lung function were made every 3 months, whilst lung volumes, airway hyperresponsiveness, peripheral blood collection, induced sputum and bronchial biopsies were obtained at baseline,

6 and 30 months. Compliance was checked by counting the doses on the Diskus® inhalers.

Spirometry was performed before and after inhalation of a short-acting bronchodilator, total lung capacity (TLC) and residual volume (RV) using a constant volume body plethysmograph and airway hyperresponsiveness to methacholine with the two-minute tidal breathing method [25]. Patients were asked about dyspnea according to the American Thoracic Society guidelines [27] using the modified Medical Research Council (MRC) dyspnea scale (1=no dyspnea to 5= dyspnea at rest) and the presence of chronic bronchitis (daily cough and sputum production for at least 3 months a year, for more than one year) [28]. Health status was measured using the St. George's Respiratory Questionnaire (SGRQ) [29] and the Clinical COPD Questionnaire (CCQ) [30].

Sputum induction and processing were performed according to a validated technique using the so called 'full sample' method [2;25]. Cell counts were expressed as both total and differential counts. Fibreoptic bronchoscopy was performed using standardized procedures and sections from bronchial biopsies were analysed as previously described [31]. In brief, four paraffin embedded biopsies were cut in 4-µm thick sections and stained with antibodies against T lymphocytes (CD3, DAKO, Glostrup, Denmark; CD4, Novocastra, Newcastle upon Tyne, UK; CD8, DAKO), macrophages (CD68, DAKO), neutrophil elastase (NE, DAKO), mast cell tryptase (AA1, DAKO), plasma cells (CD138, IQ Products, Groningen, The Netherlands) and eosinophils (EG2, Pharmacia Diagnostics, Uppsala, Sweden) [25]. Fully automated inflammatory cell counting procedures on digitized images were performed [32].

Statistical analysis

Long-term (2.5 years) decline in postbronchodilator FEV_1 served as the primary end-point. T lymphocytes in bronchial biopsies were included as secondary endpoints, since the GLUCOLD trial [2] was powered to assess the effect of inhaled corticosteroids on $CD8^+$ cell counts in bronchial biopsies. Because this was an efficacy trial, per-protocol analysis included all available data from randomised patients who were compliant with study medication (using ≥70% of the prescribed dose), including data from patients who did not complete follow-up. Potential clinical predictor variables were *a priori* selected according to a tailored strategy developed for small datasets [33]. Guidelines suggest that one candidate predictor can be studied for every 10 patients [34]. Since the current exploratory study population consisted of 50 patients we selected 5 clusters (with high baseline within-cluster correlation) of potential predictors:

(1) packyears [11]; (2) presence of chronic bronchitis; (3) lung function (baseline FEV_1, reversibility of FEV_1,[15]) PC_{20},[17;21] RV/TLC; (4) TLCO; and (5) absolute and differential cell counts in induced sputum [24;35]. Although continuous variables might provide more information we dichotomised variables based on median values in order to allow clinically meaningful interpretation of the results.

A set of 2 linear mixed effect models [36] was used to identify predictors of 1) long-term (2.5 years) ICS effect on postbronchodilator FEV_1 by inhaled corticosteroids; and 2) long-term effects on $CD3^+$, $CD4^+$, $CD8^+$ in bronchial biopsies. These outcomes were selected based on their significant long-term changes by inhaled steroids [2;25]. For each potential predictor, the following variables were included in the model: time, treatment (placebo coded as 0; fluticasone coded as 1), predictor (reference coded as 0; index coded as 1), their interaction (treatment*predictor) and their interaction terms with time, using FEV1 or inflammatory cells as the dependent variable. Thus, the interaction term (treatment*predictor*time) reflects the additional value of the predictor to the effect of treatment with inhaled fluticasone compared to placebo on longitudinal changes in FEV_1 or inflammatory cells. Index category

Table 1. Patient characteristics at baseline

Characteristic	Fluticasone (n=26)	Placebo (n=24)
Age, yrs	62 (8)	59 (8)
Sex , m/f	23/3	20/4
Current smoking / ex-smoking	16/10	17/7
Dyspnea ‡	2.6 (0.8)	2.7 (0.8)
SGRQ total score †	29 (13)	33 (19)
CCQ total score §	1.3 (0.6)	1.7 (1.3)
Postbr. FEV_1, % pred	63 (8)	61 (8)
Reversibility, mL	3.2 (2.1)	3.6 (2.5)
Geom. mean PC_{20} methacholine (DD), mg/ml ‖	0.9 (2.8)	0.5 (2.4)
RV/TLC, %	47 (9)	47 (7)
TLCO, % pred	70 (20)	59 (16)
Median IgE (IQR), IU	28 (12-128)	51 (21-119)

Characteristics of the study population per treatment group at baseline. Data represent mean (SD). ‖ PC_{20} methacholine is expressed as geometric mean (standard deviation in doubling dose (DD)). ‡range 1 to 5 (higher scores indicate more dyspnea); †range 0 (best) to 100 (worst score); §range 0 (best) to 6 (worst score).

Definition of abbreviations: postbr. = postbronchodilator; FEV_1 = forced expiratory volume in one second; % pred = percentage of predicted value; PC_{20} methacholine = the provocative concentration of methacholine that causes a decrease in FEV_1 of 20%; RV/TLC = residual volume/total lung capacity; TLCO = diffusion capacity of the lung for carbon monoxide.

is defined relative to median value, and represents the more favourable outcome by fluticasone. Reference category is complementary. Because of the limited number of patients, we tested these variables univariately in the model. The statistical analyses were performed with STATA 11.0 (StataCorp; College Station TX, USA). Differences at p-values ≤0.05 (tested 2-sided) were considered significant.

*Table 2. Values of predictors per stratum per treatment**

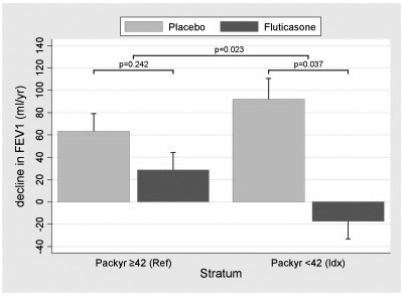
	Fluticasone (n=26)		Placebo (n=24)	
Predictor	**Index #**	**Reference ▫**	**Index**	**Reference**
Median smoking history (IQR), packyears	31 (25-34)	51 (47-62)	35 (31-41)	55 (47-63)
Median number of years smoking, (IQR), yrs	37 (32-39)	47 (45-54)	38 (33-40)	48 (46-54)
Postbr. FEV_1, % pred	69 (3.4)	54 (6.3)	69 (3.4)	58 (6.1)
Geom. PC_{20} methacholine (DD), mg/ml ‖	2.3 (1.6)	0.05 (1.5)	2.4 (2.3)	0.10 (1.6)
RV/TLC, %	39 (5.1)	55 (8.2)	41 (4.1)	51 (3.3)
TLCO, % pred	83 (17.1)	54 (7.9)	74 (7.7)	48 (12.5)
Median IgE (IQR), IU	13 (9-24)	144 (84-244)	17 (6-25)	113 (31-201)
Gmean sputum total cell count (GSD), 10^4/ml ‡	62 (2.1)	272 (1.6)	86 (1.7)	392 (1.8)
Sputum cell percentages				
Neutrophils, %	51 (1.3)	80 (1.1)	51 (1.3)	79 (1.1)
Macrophages, %	16 (1.6)	40 (1.4)	17 (1.3)	39 (1.3)
Eosinophils, %	0.3 (2.4)	2.4 (1.6)	0.4 (2.4)	2.4 (1.7)
Lymphocytes, %	2 (1.2)	4 (1.2)	2 (1.3)	4 (1.3)

*#Index category is defined relative to median value, and represents the more favourable outcome by fluticasone. For example: decline of FEV_1 by fluticasone is diminished (=favourable outcome) in patients with a lower number of packyears (=index category). ▫Reference category is complementary to the index category. *Values are mean (standard deviation (SD)), unless stated otherwise. ‖ PC_{20} methacholine is expressed as geometric mean (standard deviation in doubling dose (DD)), ‡sputum cells as Gmean (GSD). Definition of abbreviations: postbr. = postbronchodilator; FEV_1 = forced expiratory volume in one second; % pred = percentage of predicted value; PC_{20} methacholine = the provocative concentration of methacholine that causes a decrease in FEV_1 of 20%; RV/TLC = residual volume/total lung capacity; TLCO = diffusion capacity of the lung for carbon monoxide.*

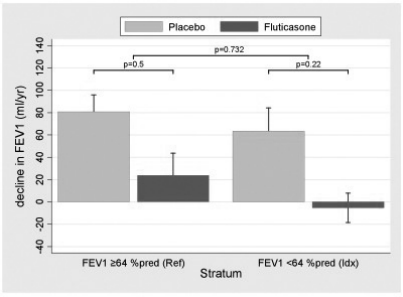
Results

Data from 26 patients with fluticasone and 24 patients with placebo treatment for 30 months were used for the present analysis (Table 1). In short, most patients were middle-aged males, two-thirds were current smokers and many had been heavily smoking for many years. The vast majority of patients could be classified as moderately severe COPD, as based on mean (SD) postbronchodilator FEV_1 of 63 (9) % predicted. They were mildly impaired in health status, slightly reversible with moderately severe hyperresponsiveness. Only 3 patients ever used inhaled corticosteroids before entry of the study.

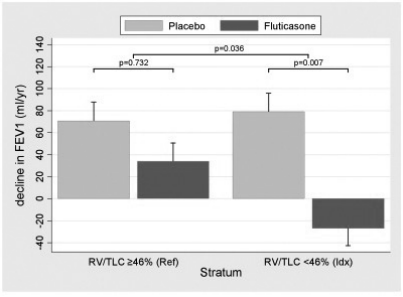
Figure 1. Long-term predictors of FEV_1 decline by fluticasone treatment



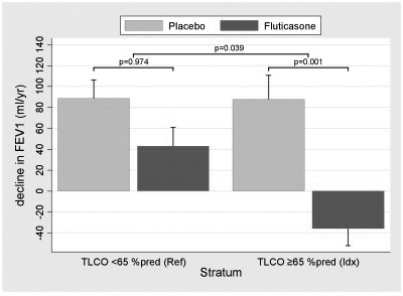
A) Packyears



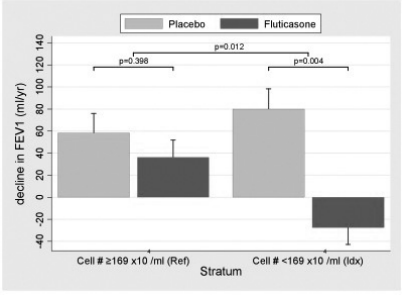
B) baseline FEV_1



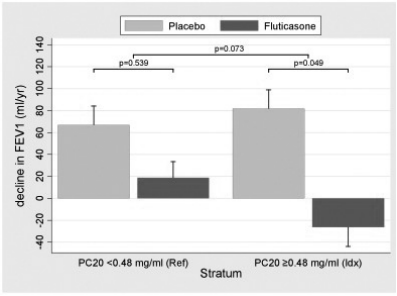
C) RV/TLC



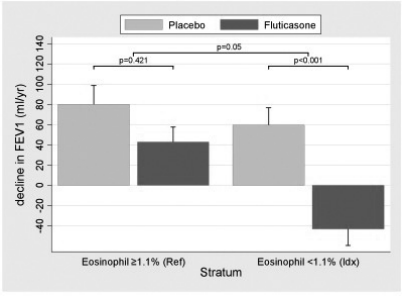
D) TLCO



E) Sputum total cell count



F) PC_{20} methacholine



G) Sputum percentage eosinophils

Predictors of the decline of FEV_1 by 2.5 year fluticasone treatment (ml) and placebo, stratified by median values of the predictor. A) packyears smoking; B) baseline FEV1 pred; C) RV/TLC; D) TLCO; E) total number of cell counts in induced sputum and F) PC_{20} methacholine; G) percentage of eosinophils in sputum by 30 months treatment with fluticasone compared to placebo.
[#]Index category (Idx) is defined relative to median value, and represents the more favourable outcome by fluticasone. For example: decline of FEV_1 by fluticasone is diminished (=favourable outcome) in patients with a lower number of packyears (=index). Reference category (Ref) is complementary.

Predictors of changes in FEV_1 decline by fluticasone treatment over 30 months

Long-term predictors of effects of ICS on FEV_1 decline are shown in Table 2 and Figure 1. In patients with fewer packyears fluticasone improved the accelerated decline in FEV_1 significantly more than in patients with more packyears, the difference being +75 ml/yr, 95%CI +10/+139, p=0.023, (Figure 1A). Baseline FEV_1 was not a predictor of ICS effect on FEV_1 decline (Figure 1B). In patients with lower RV/TLC, long-term treatment with fluticasone improved FEV_1 decline significantly more than in patients with higher RVTLC (+69 ml/yr, 95%CI +4/+134, p=0.037) (Figure 1C). Finally, higher CO-diffusion capacity (+78 ml/yr, 95%CI +4/+153, p=0.040) (Figure 1D) and lower total sputum cell counts (+85 ml/yr, 95%CI +19/+152, p=0.012) (Figure 1E) were also predictors of ICS effect on FEV_1 decline. Comparable but non-significant trends were found in patients with a shorter duration of smoking (Table 3), less airway hyperresponsiveness (Figure 1F) and a lower percentage of eosinophils in induced sputum (Figure 1G).

Table 3. Predictors of attenuation of long-term FEV_1 decline by fluticasone treatment

Predictor	Index category#	Difference in change of FEV_1 ml/yr	95% CI	P value
Packyears, yrs	<42	+75	+10/+139	0.023
Number of years smoking, yrs	<42	+63	-0.7/+127	0.052
Postbr. FEV_1, % pred	<64	+12	-56/+81	0.73
PC_{20} methacholine, mg/ml	≥0.48	+60	-6/+126	0.074
RV/TLC, %	<46	+69	+4/+134	0.036
TLCO, % pred	≥65	+78	+4/+153	0.039
IgE, IU	<36	+75	+11/+140	0.022
Sputum total cell count, 10^4/ml	<169	+85	+19/+152	0.012
Sputum cell percentages				
Neutrophils, %	<69	+44	-23/+111	0.20
Macrophages, %	>25	-34	-102/+34	0.33
Eosinophils, %	<1.1	+66	-0.2/+132	0.051
Lymphocytes, %	<3	-7	-74/+61	0.85

Definition of abbreviations: postbr. = postbronchodilator; FEV_1 = forced expiratory volume in one second; % pred = percentage of predicted value; PC_{20} methacholine = the provocative concentration of methacholine that causes a decrease in FEV_1 of 20%; RV/TLC = residual volume/total lung capacity; TLCO = diffusion capacity of the lung for carbon monoxide. #Index category is defined relative to median value, and represents the more favourable outcome by fluticasone. Reference category is complementary.

Predictors of changes in T lymphocytes in bronchial biopsies by fluticasone over 2.5 years

Ex-smokers had a larger decrease in numbers of $CD3^+$ cells in bronchial biopsies with long-term fluticasone (-48% 95%CI -9/-70, p=0.021), as compared to current smokers. Higher number of total cell counts in induced sputum predicted a larger decrease in numbers of $CD3^+$ cells in bronchial biopsies by treatment with fluticasone (-48%, 95%CI -11/-70, p=0.018). Reduction in $CD3^+$ cells was more prominent in patients with lower percentages of macrophages and higher percentages of neutrophils in sputum (-53% 95%CI -17/-73, p=0.009; -55%, 95%CI -22/-74, p=0.004). There were no significant predictors of change in $CD4^+$ and $CD8^+$ cells in bronchial biopsies with long-term fluticasone.

Discussion

This study demonstrates that clinical and inflammatory phenotypes can partly predict efficacy of long-term inhaled corticosteroid use with respect to preventing FEV_1 decline in steroid-naive patients with moderate to severe COPD. Patients with fewer packyears smoked, preserved diffusion capacity of the lung, limited hyperinflation, and lower number of total cells in induced sputum benefitted most from inhaled corticosteroids. In addition, the inflammatory background, expressed by higher number of total cells and neutrophils in induced sputum, appeared to be predictive of a more effective reduction of the number of $CD3^+$ in bronchial biopsies by treatment with fluticasone at 30 months. These data indicate that features of less advanced disease (fewer signs of emphysema and less hyperinflation) predict a better course of lung function with treatment of inhaled corticosteroids in patients with moderate to severe COPD. This suggests that COPD patients expressing milder and/or earlier stages of the disease or a subtype of COPD patients in whom airway obstruction is accompanied by less degrees of emphysema can benefit from anti-inflammatory therapy, which favors a differential approach in the treatment of COPD.

The long-term efficacy of fluticasone in patients with less packyears smoked is in line with a previous study on long-term effects of budesonide in patients with mild COPD who continued smoking [11], in which patients with <36 packyears smoked showed more benefit from budesonide. The present study extends these findings by showing, for the first time, that less impaired diffusion capacity (and less hyperinflation) predict a better effect of

fluticasone therapy on FEV_1 decline. It has been shown previously that short-term treatment with a combination of inhaled corticosteroids and long-acting $ß_2$-agonist reduces lung hyperinflation in patients with severe COPD [37]. Treatment at an earlier stage of the disease is supported by recent concepts [38]. Interestingly, reversibility of FEV_1 was not predictive of decline in FEV_1, which suggests that steroid responsiveness in COPD may be different from that seen in asthma. Remarkably, long-term effects on decline in FEV_1 were more pronounced in patients with lower number of sputum cell counts. Notably, eosinophil counts were not predictive of steroid-effects. This seems to contrast with previous studies showing benefit on FEV_1 from steroids in patients with more eosinophilic inflammation [23;24;35]. However, the latter results were found in patients with more advanced COPD (FEV_1 being around 38% predicted) [23] who were followed < 1 year [23;24;35]. Taken together, our results consistently point towards the notion that long-term treatment with inhaled corticosteroids can be predicted by clinical and inflammatory features in particular phenotypes suggestive of less advanced COPD.

To our knowledge this is the first study showing that efficacy of long-term inhaled corticosteroid use on FEV_1 decline can be predicted by clinical and inflammatory features in steroid-naive patients with moderate to severe COPD. Obviously, there are some limitations to our study. The limited sample-size only allowed univariate analyses of the predictors. However, the fact that the long-term treatment effects on FEV_1 decline over 2.5 years were consistently predicted by a number of indicators of less advanced disease supports the plausibility of our findings. We did not assess different cut-off values that are known in clinical research in order to avoid multiple testing and data driven decisions [33]. Instead, we used specific cut-off values based on medians of predictor variables. The continous character of a variable maximises preditive capacity. However, by using medians the clinical interpretation may improve. We only included patients who used ≥70% of the prescribed fluticasone dose in our analysis. This strategy was chosen because our study was designed as an efficacy trial. However, data from patients who did not complete follow-up were included in the analysis. For this analysis we used data from a subgroup of patients from the original randomised controlled trial. We excluded the salmeterol-fluticasone propionate arm of the original protocol, because we found no long-term effect of adding salmeterol on lung function decline and excluding this arm simplified the analysis.

How can we interpret these data? Glucocorticosteroids are highly effective in other chronic inflammatory lung diseases such as asthma. In contrast, it has

been suggested that patients with other inflammatory diseases such as COPD are more steroid-resistant [39]. Indeed, the big trials during the past decade have not observed efficacy by inhaled steroid on the decline of FEV_1 in COPD [4]. However, our results suggest that certain phenotypes of COPD, including patients with fewer packyears smoked, less hyperinflation, better CO-diffusion capacity and lower inflammatory load are characterised by preserved, long-term sensitivity to steroids. Multiple distinct mechanisms contribute to steroid resistance. Excessive activator protein-1 found in asthma reduces interaction from glucocorticosteroid receptor with transcriptional coactivator molecules resulting in steroid resistance [40]. In COPD, advanced oxidative stress reduces histone deacetylase-2 (HDAC2) so that inflammation might become less sensitive to anti-inflammatory effects of steroids [41]. The latter has been associated with the effect of current smoking [42], and indeed our results show a reducing effect on the number of $CD3^+$ cells in bronchial biopsies in ex-smokers. Our observation also suggests that fewer packyears rather than current smoking status predict a better efficacy of long-term inhaled steroids on decline of FEV_1 This suggests that a relatively higher cumulative amount of smoking contributes to the irreversibility of the damage. In addition, our findings are in keeping with the notion that advanced inflammation in COPD with mechanical changes of the airways, as indirectly reflected by hyperinflation, may contribute to relative glucocorticosteroid resistance. The same holds for damage of the airways and lung tissue due to progressive loss of elastic recoil, as reflected by reduced CO-diffusion capacity that is compatible with emphysema. Taken together, our data raise the hypothesis that treatment with inhaled corticosteroids at an earlier stage of the disease or in patients with less emphysema and/or less hyperinflation, may provide a window of opportunity to slow down the progression of the disease.

These data have implications for clinical care. We studied COPD patients with mainly moderate disease severity. Even though most studies on treatment predictors have been performed in more severe disease, the majority of patients in day-to-day care have less advanced COPD. Subgroups of patients with COPD such as those with more emphysema have been shown to display accelerated decline of FEV_1 [43]. Our study generates the hypothesis that treating these patients as early as possible before damage has occurred could be of major importance and may slow down the disease. Taken together, our data raise the point that certain subgroups such as patients with less advanced disease or those with a particular subphenotype of COPD with less emphysema might benefit from a differential therapeutic approach. This

novel hypothesis requires external validation in larger subsets of patients with moderate to severe COPD, before our results can be generalized into daily practice. This approach would allow extending current recommendations in COPD guidelines by offering a promising perspective for subgroups of patients with COPD towards the potential of slowing down the progression of the disease

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Chapter 7

Self-monitoring day-to-day health status and peak-flow:
effect of long-term treatment with inhaled corticosteroids and long-acting $ß_2$-agonists in COPD

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Abstract

Aims: It is unknown to what extent daily patient-monitored outcomes are responsive to long-term therapy in COPD. This study assessed the long-term effects of inhaled corticosteroids with or without long-acting $ß_2$-agonists on day-to-day symptoms and Peak Expiratory Flow (PEF) in mostly steroid-naive patients with moderate to severe COPD.

Methods: In a double blind, placebo controlled study 114 patients received short-term (6 months) or long-term (30 months) fluticasone propionate 500 µg twice daily (bid), long-term fluticasone/salmeterol 500 µg/50 µg bid (single inhaler), or placebo bid. The clinical COPD questionnaire (CCQ) was used to measure day-to-day health status. 2-Week diary cards with daily CCQ and PEF were collected every three months.

Results: Short-term addition of salmeterol to fluticasone treatment improved mean daily total and functional CCQ score (-0.2, p=0.008; -0.3, p=0.002, respectively) and improved mean morning PEF from baseline (10.6 l/min, p<0.002). Long-term treatment with inhaled corticosteroids improved the mean daily total and functional CCQ score (-0.09/yr, p=0.003; -0.12/yr, p<0.001 respectively) and improved change in morning PEF from baseline (2.46 l/min/yr, p=0.052). Stopping inhaled corticosteroids after 6 months worsened the mean daily total, symptom and functional CCQ score (0.09/yr, p=0.003; 0.14/yr, p=0.001; 0.07/yr, p=0.043). More patients had a relevant worsening of the CCQ total score (OR 10.4, p=0.024) and less patients had a relevant improvement in morning PEF change (OR 15.0, p=0.009). Addition of long-acting $ß_2$-agonists to inhaled corticosteroids provided short-term benefits.

Conclusions: Long-term therapy with inhaled corticosteroids improves daily reported general and functional health status in a group of patients with mostly steroid-naive, moderate to severe COPD. Stopping inhaled corticosteroids worsens health status and addition of long-acting $ß_2$-agonists provides initial benefits. Part of self-monitored outcomes were clinically relevant. This study indicates modest benefit of long-term combination therapy on self-monitored outcomes in COPD.

Introduction

The current raise in disease prevalence of Chronic Obstructive Pulmonary Disease (COPD) in a greying society will enhance the burden to society enormously (www.goldcopd.org) [1]. Patients with COPD experience themselves day-to-day symptoms and limitations in physical activity, with an adverse impact on health status [2]. Currently the emphasis in healthcare is shifting from physician-based to patient-centred care and self-monitoring of respiratory symptoms provides the opportunity to engage patients in their care [3;4]. Therefore, regular assessments made by the patients themselves are needed, which may better reflect treatment impact from the patient perspective whilst providing direct feedback in a standardized way.

Patient diary cards and brief questionnaires can be used as direct measures for self-monitoring. Diary cards assess daily questions on respiratory symptoms and lung function. The clinical COPD questionnaire (CCQ) is a short practical tool that can be used to evaluate the effect of treatment on symptoms [5;6]. The weekly CCQ has been shown to be able to discriminate between stable state of COPD and exacerbations, and may be used in early detection of exacerbations [7]. Self-monitoring can be used to assess treatment effects. However, the responsiveness of the daily CCQ to long-term treatment in stable COPD is as yet unknown.

Large epidemiological studies did not show long-term effects of inhaled corticosteroids on physiological outcome in COPD, such as the decline of forced expiratory volume in one second (FEV_1) [8;9]. However, COPD is a heterogenous disease and in certain subgroups of patients long-term therapy with inhaled corticosteroids (ICS) can improve dyspnea and FEV_1 decline in patients with moderate to severe COPD[10], whilst discontinuation worsens dyspnea and FEV_1 decline [11]. Hence, self-monitoring may provide relevant additional signals of treatment benefits during long-term treatment of COPD patients. In addition, self-monitoring may strengthen the adherence to chronic medications when immediate and clinically important impact on symptoms and lung function can be demonstrated [12].

We hypothesised that maintenance treatment with inhaled corticosteroids with and without $ß_2$-agonists improves self-monitored day-to-day health status and lung funtion in patients with moderate to severe COPD. To that end, we examined effects of 2.5 years versus 6-month treatment of fluticasone propionate with and without salmeterol, as well as cessation of fluticasone on day-to-day health status and Peak Expiratory Flow (PEF) in COPD. In addition, the number of patients with a minimally important clinical change in outcome was explored.

Methods

We conducted a 2.5 year prospective longitudinal, randomised, double blind, placebo-controlled two-centre trial, called the Groningen Leiden University Obstructive Lung Disease (GLUCOLD) study. The methodology has been described in detail previously [11;13]. Briefly, patients had irreversible lung function loss that was compatible with the Global initiative for chronic Obstructive Lung Disease (GOLD) stages II and III, and had ≥ 10 pack years smoking [1]. Asthma was excluded by doctors diagnosis and self-reported symptoms, treatment or diagnosis of asthma. Participants with inhaled and oral corticosteroids within 6 and 3 months of trial entry were not included in the study. Seven patients ever used a short-term course of corticosteroids and only five patients ever used maintenance therapy with inhaled corticosteroids. The vast majority of patients were recruited from general practices between 2000 and 2003 and all patients had been clinically stable for at least 2 months before entry. The study was approved by local ethics committees and all subjects gave written informed consent.

Patients were randomly assigned to receive either 500 µg fluticasone propionate (FP) twice daily for 2.5 years, 500 µg FP twice daily for 6 months, 50 µg FP/salmeterol (S) twice daily for 2.5 year or placebo (P). Study medication included Diskus® dry powder inhalers (GlaxoSmithKline, The Netherlands), and active treatment and placebo were identical in appearance. Randomisation was performed by an independent randomisation centre using a minimisation procedure balancing treatment groups for a number of variables (centre, gender, current smoker, FEV_1/IVC < or ≥ 60%, PC_{20} methacholine < or ≥ 2 mg/ml). Regular visits with measurements of symptoms and lung function were made every 3 months. Methacholine challenge was performed at 0, 6 and 30 months. Compliance was checked by counting the doses on the Diskus® inhalers.

Patients completed 2-weeks diary cards every three months prior to the visit. Records were reviewed at each visit. Amongst validated disease-specific health status questionnaires such as the clinical COPD Questionnaire (CCQ) and the COPD assessment Test (CAT) [14], we have chosen to use the daily CCQ measure day-to-day health status. The CCQ is a 10-item health status questionnaire from which the following scores were assessed: total, symptom, functional and mental scores (0=best score, 6=worst score). Daily Peak Expiratory Flow (PEF) measurements were performed three times in the morning and three times in the evening. The best of three measurements in the morning was used for analysis. Patients were asked not to use rescue medication 6 hours prior to the measurement. The minimal important difference (MID) of the CCQ that represents a clinically relevant change in health status is 0.4 points [15]. There is no validated MID for PEF measurements in patients with COPD. However, the Understanding Potential Long-term Impacts on Function with Tiotropium (UPLIFT) trial showed that two-third of patients with COPD experienced a clinically meaningful reversibility of 15% PEF at baseline. [16] This is comparable to the MID known in patients with asthma [17]. Therefore, a change of 15% was defined as clinically relevant (*i.e.* minimal important difference) PEF.

Spirometry was measured after 400 mcg salbutamol, according to standardized guidelines [13].

Statistical analysis

The power of the GLUCOLD study was based on the primary outcome and included the standard deviation (0.77) of the fluticasone-induced short-term change in submucosal $CD8^+$ cell counts in COPD patients [11;18]. Because this was an efficacy trial, per-protocol analysis included all available data from randomised patients who were compliant with study medication (using ≥70% of the prescribed dose), including data from patients who did not complete follow-up. A linear mixed effects model was used for analysis of mean CCQ scores and PEF values. The linear mixed models included the main effect of treatment (3 indicators), the main effect of time (2 indicators) and the interaction of treatment and time. Treatment effects were assessed from: a. long-term treatment with inhaled fluticasone vs placebo, b. discontinuation of fluticasone after 6 months vs its continuation, and c. the additional treatment effect of adding salmeterol to fluticasone vs fluticasone alone. Apart from analysis of the outcome parameters as continuous variables, patients were classified in the following 4 categories by health status and PEF for clinical

interpretation: 1) number of patients with a (relevant) improvement that reached the minimal important difference (MID); 2) the number of patients with a (non-relevant) improvement lower than the MID; 3) number of patients with a (non-relevant) worsening lower than the MID; 4) number of patients with a (relevant) worsening that reached the MID. A logistic random effects model was used for analysis. All analyses were performed with STATA 11.

Results

From 114 patients, 101 patients were adherent. Data from 88 patients who performed diary cards at baseline were analysed. 82 Patients had moderate COPD at baseline with GOLD stage II and 6 patients had severe COPD, stage III (Table 1).

*Table 1. Baseline characteristics per treatment group**

	Placebo, 30 mo	Fluticasone 6 mo, then followed by placebo, 24 mo	Fluticasone 30 mo	Fluticasone plus salmeterol, 30 mo
Mean daily CCQ ‡ score				
- total score	1.42 (1.2)	0.95 (0.5)	1.10 (0.5)	1.21 (0.7)
- symptom score	1.82 (1.0)	1.49 (0.6)	1.67 (0.6)	1.68 (0.7)
- functional score	1.38 (1.3)	0.85 (0.7)	1.01 (0.6)	1.18 (0.9)
- mental score	0.71 (1.3)	0.089 (0.2)	0.13 (0.3)	0.36 (0.6)
Stable mean PEF † , l/min	271 (66)	337 (88)	333 (112)	305 (83)
GOLD §				
- stage II, n	19	19	23	21
- stage III, n	2	2	1	1

Values are mean±standard deviation (SD); definition of abbreviations: mo=months; ‡Clinical COPD Questionnaire with range 0 (best) to 6 (worst score); †Peak Expiratory Flow (PEF); §the Global initiative for Chronic Obstructive Lung Disease (GOLD).

Short-term effects of ICS with or without salmeterol on clinical control and lung function

Short-term addition of salmeterol to fluticasone treatment improved mean daily total and functional CCQ score compared with placebo (-0.2/6months, p=0.008; -0.3, p=0.002, respectively) and improved mean morning PEF from baseline (10.6 l/min per 6 months, p<0.002, Table 2, Figure 1). A lower CCQ score indicates improved health status. Figure 2 shows the number of patients with a (relevant) improvement or worsening of health status and lung function during follow-up. Fewer patients had a relevant worsening of the CCQ total,

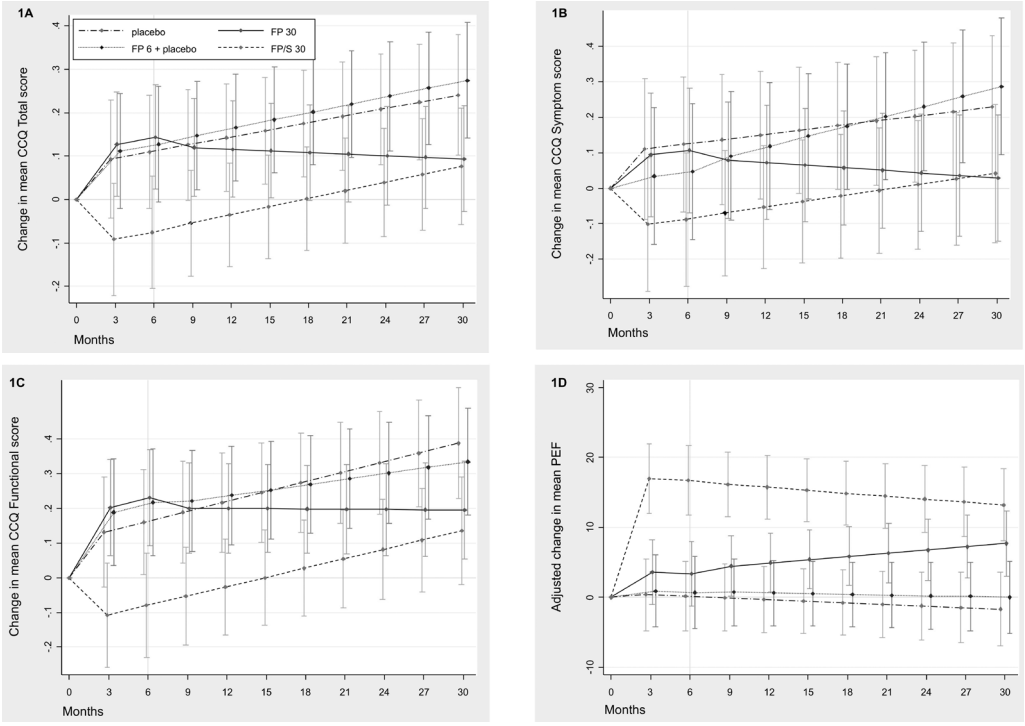
Table 2. Effects of inhaled corticosteroids with or without long-acting ß$_2$-agonists on clinical control and PEF

Change from baseline	Effect of long-term continuation with fluticasone	Effect of cessation with fluticasone at 6 mo	Addition of short-term salmeterol	Addition of long-term continuation with salmeterol
	coeff, 95% CI, *P* value	coeff, 95% CI, *P* value	coeff, 95% CI, *P* value	coeff, 95% CI, p-value
Mean daily CCQ ‡ score				
- total score	-0.09, -0.15 to -0.03, 0.003	0.09, 0.03 to 0.15, 0.003	-0.22, -0.39 to -0.06, 0.008	0.11, 0.05 to 0.17, <0.001
- symptom score	-0.08, -0.17 to 0.001, 0.053	0.14, 0.06 to 0.22, 0.001	-0.21, -0.45 to 0.03, 0.087	0.11, 0.03 to 0.2, 0.008
- functional score	-0.12, -0.19 to -0.06, <0.001	0.07, 0.002 to 0.13, 0.043	-0.30, -0.49 to -0.11, 0.002	0.12, 0.05 to 0.18, 0.001
- mental score	-0.04, -0.09 to 0.01, 0.13	0.02, -0.03 to 0.07, 0.38	-0.12, -0.26 to 0.03, 0.12	0.07, 0.02 to 0.12, 0.007
Stable mean PEF †, l/min	2.46, to -0.02 to 4.94, 0.052	-2.42, -4.92 to 0.09, 0.058	10.6, 3.9 to 17.3, 0.002	-1.23, -3.7 to 1.25, 0.33

Definition of abbreviations: ‡Clinical COPD Questionnaire; †Peak Expiratory Flow (PEF).

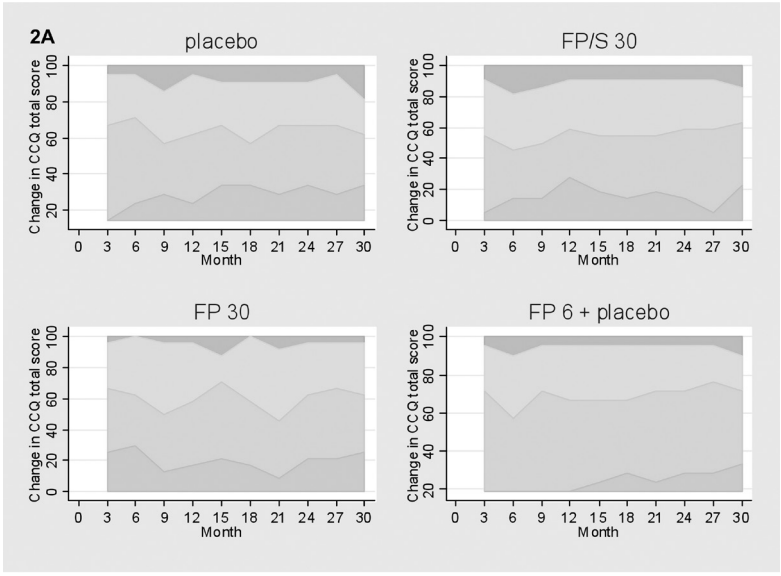
functional and mental score by adding salmeterol to short-term fluticasone (OR 11.1, p=0.014; OR 12.6, p=0.007; OR 15.4, p=0.021, respectively, Figure 2). More patients had a relevant worsening of PEF with short-term therapy with fluticasone as compared to placebo (OR 53, p=0.010, Figure 2).

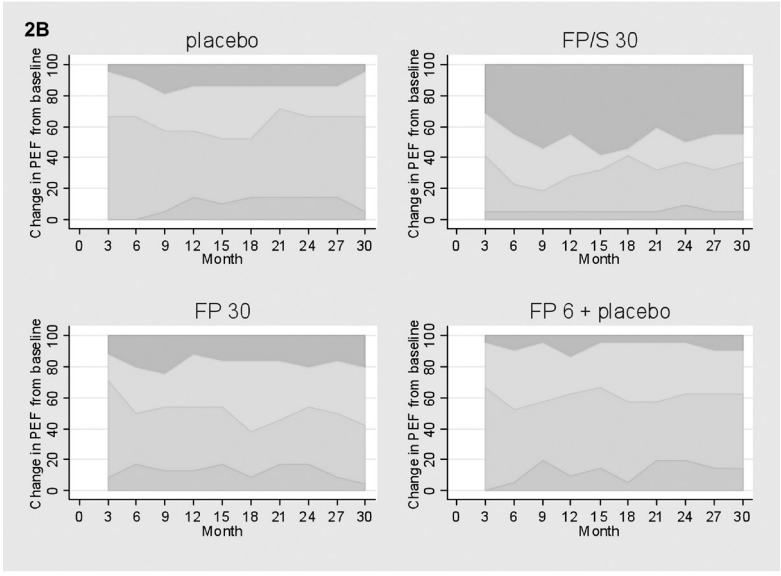
Figure 1. Change in CCQ score and PEF by fluticasone



Adjusted mean change ± 95% CI over time during treatment with fluticasone (500 µg bid) for 30 months (FP 30), fluticasone (500 µg bid) 6 months (FP 6), followed by placebo, the combination of fluticasone/salmeterol (500/50 µg bid) 30 months (FP/S 30) and placebo (bid), in patients with moderate to severe COPD. Data are presented for: A) mean Clinical COPD Questionnaire (CCQ) Total score; and B) mean CCQ Symptom score; C) mean CCQ Functional score and D) Peak Expiratory Flow (PEF, l/min).

Figure 2. A) Number of patients with a relevant improvement or worsening of total CCQ score and B) relevant change in PEF from baseline





Relevant changes over time are presented during treatment with fluticasone (500 µg bid) for 30 months (FP 30), fluticasone (500 µg bid) for 6 months (FP 6), followed by placebo, the combination of fluticasone/salmeterol (500/50 µg bid) for 30 months (FP/S 30) and placebo (bid), in patients with moderate to severe COPD. Data are presented for: A) total Clinical COPD Questionnaire (CCQ) score; and B) Peak Expiratory Flow (PEF). Four categories are shown: 1) relevant improvement depicted in upper part; 2) improvement that is not clinically relevant (upper middle part); 3) worsening that is not clinically relevant (lower middle part); 4) relevant worsening (lower part).

Long-term effects of ICS with or without salmeterol on clinical control and lung function

Long-term continuation with fluticasone improved mean daily total and functional CCQ score after 2.5 years, compared to placebo (-0.09/yr, p=0.003; -0.12, p< 0.001, Figure 1) and improved change (non-significant) in morning PEF from baseline (2.46 l/min/yr, p=0.052, Figure 1). Fewer patients had a relevant (non-significant) worsening of the CCQ functional score by using long-term therapy with fluticasone (OR 5.4, p=0.054, Figure 2). Cessation of fluticasone at 6 months worsened the mean daily total, symptom and functional CCQ score at 2.5 years versus long-term fluticasone (0.09/yr, p=0.003; 0.14/yr, p=0.001; 0.07/yr, p=0.043, Figure 1) and worsened change (non-significant) in morning PEF from baseline (-2.42/yr, p=0.058). More patients had a relevant worsening of the CCQ total score by stopping fluticasone (OR 10.4, p=0.024) and less patients had a relevant improvement in morning PEF change from baseline (OR 15.0, p=0.009, Figure 2). Long-term continuation of salmeterol increased (worsened) the CCQ score on the dimensions total (0.11/yr, p<0.001), symptom (0.11/yr, p=0.008), functional (0.12/yr, p=0.001) and mental (0.07/yr, p=0.007) compared to fluticasone alone (Figure 1).

Discussion

The findings of the present study show that long-term treatment with inhaled corticosteroids improves patient-reported day-to-day health status and functional performance in steroid-naive patients with moderate to severe COPD. Stopping fluticasone treatment at 6 months worsens daily patient-reported health status, symptoms and functional performance. Addition of a long-acting $ß_2$-agonist to ICS provides short-term benefit on health status, functional performance and PEF, but does not provide an additional long-term benefit. The observed effects on health status and PEF partly reached the minimal important difference (MID) of 0.4 points for the CCQ and 15% change from baseline for PEF. These long-term effects on daily reported health status point to some benefits for patients in daily symptoms and lung function.

The novelty of this study is that COPD patients with stable disease benefited from long-term inhaled corticosteroids as reflected in self-monitored daily health status and lung function. In asthma, self-monitoring of asthma control including symptoms and PEF is recommended in combination with an action plan common in clinical trials and it has been shown that this may enhance adherence to monitoring the disease [12;17]. In addition, in clinical trials such

as the Gaining Optimal Asthma control (GOAL) study PEF data were used to measure effects of ICS and combination therapy on asthma control [19]. However, it is likely that the monitoring approach that has been found useful in asthma cannot simply be extrapolated to COPD [20].

Our findings with patient-monitored outcomes in COPD demonstrate that COPD seems to be a treatment responsive disease. Indeed, controlled trials have provided a rationale for treatment in specific subgroups of patients with COPD [1]. The combination of ICS and long-acting $ß_2$-agonist has provided positive effects on 3-monthly measured health status and lung function [10;11]. Inhaled corticosteroids decrease exacerbations in more severe disease and improve quality of life [8]. Three studies reported benefits from long-term combination therapy in specific groups of patients [10;11;21] We extended these findings by showing positive effects on patient centred, daily reported health status and PEF.

Although the present study showed positive effects of inhaled corticosteroids on day-to-day health status and PEF, there are some limitations. A relatively small number of patients was enrolled in the study, since sample size was based on inflammatory cell counts in bronchial biopsies as primary outcome of the study. Although long-term treatment with ICS was effective at the patient level, there was a limited number of patients where effects reached the MID. First, this may be COPD-specific, due to the notion that COPD patients are generally older than asthma patients, having more complex multi- and/or co-morbidity and are more readily adapting to their disease due to the slow disease progression. Second, it may be explained in part by differences in populations. The original MID for the CCQ was established in patients with more advanced disease, and who were admitted to the hospital for an exacerbartion of COPD [15]. Third, the MID can be determined by different methods, which may influence the MID [22;23]. In addition, the individual patient is the only one who can judge whether there is an improvement or worsening that is clinically relevant [24;25]. Finally, prior to the study, patients were mostly steroid-naïve. Since many patients in daily practice use combination therapy, this may hamper generalisation of the results. However, because of the present study design, the observed effects of ICS were not biased by carry-over effects from previous treatment.

How can we interpret our findings? Long-term self-monitoring has been shown to positively influence the impact of exacerbations on health status, and decrease respiratory symptoms of exacerbations [26]. Therefore, one might argue that self-monitoring should be part of disease management in COPD.

These programs include many aspects such as education about the disease, optimisation of evidence based medication and self-management. In patients with relatively mild disease monitoring patients with integrated disease management improved health status and exercise capacity [27;28]. Disease management programs include non-medical and medical interventions. Interestingly, we found that long-term ICS improved daily reported health status and functional impairment in moderate to severe stable COPD. This could promote regular physical activity, which is associated with higher CO-diffusion capacity, muscle strength and exercise capacity as well as lower levels of systemic inflammation [29]. This suggests that long-term ICS provide modest benefit at the patient level in certain COPD patients.

In conclusion, long-term ICS improves day-to-day health status, functional impairment and PEF in a patients with mostly steroid-naïve, moderate to severe COPD. Stopping ICS worsens health status and addition of long-acting $ß_2$-agonist provides initial benefits. Part of the observed effects were clinically relevant. This study suggests that the benefits of long-term effects of combination therapy on clinical health status and PEF in moderate to severe COPD is modestly experienced at the patient level.

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Chapter 8

Conclusions, general discussion and implications

Introduction

Chronic obstructive pulmonary disease (COPD) is highly prevalent and one of the most common causes of chronic morbidity and mortality world-wide [1]. The WHO (World Health Organisation) predicts an increase in COPD-related deaths in the up-coming years [2]. In the past, COPD has been viewed as an irreversible and untreatable disease. However, in the most recent decades, research has focused increasingly on COPD and current research offers a more optimistic point of view.

COPD is a heterogeneous disease characterised by airway symptoms, impaired quality of life, enhanced airflow limitation, emphysema and extra-pulmonary co-morbidities that vary interindividually in expression and severity. Airway inflammation is widely accepted to be the central feature of COPD, leading to destruction of lung parenchyma, goblet cell hypertrophy and tissue remodelling. Because of the complexity of the disease, it is important that the goal for therapy focuses on these multiple components.

This thesis focuses on clinical phenotyping in COPD and effects of therapy on various components of the disease including: health status, chronic bronchitis, the effects of inhaled corticosteroid and long-acting bronchodilator drugs on airway inflammation, clinical phenotypes and the decline in FEV_1, predictors of the effects of therapy and monitoring of daily self-reported symptoms and lung function. The Groningen Leiden Universities and Corticosteroids in Chronic Obstructive Lung Disease (GLUCOLD) study was a prospective longitudinal, randomised, double blind, placebo-controlled two-centre trial. Patients were randomly assigned to receive either fluticasone propionate (500 µg bid) or placebo for 2.5 years. The main conclusions are presented below, followed by a general discussion and directions for future research.

Conclusions

Clinical symptoms and airway inflammation

- Airflow limitation, other features previously associated with asthma, *i.e.* bronchodilator reversibility, total serum IgE and airway hyperresponsiveness and airway inflammation are distinctive, largely independent components of COPD (**Chapter 2**).
- Airway inflammation independently contributes to impaired health status in COPD: the larger the percentage of sputum macrophages, the more impaired health status was (**Chapter 3**).
- Chronic bronchitis reflects an inflammatory sub-phenotype among patients with moderate to severe COPD that is characterised by a distinct inflammatory cell profile in sputum and bronchial biopsies. The presence of chronic bronchitis is associated with a differential distribution of eosinophils in the airway wall and sputum, and higher percentages of macrophages in sputum (**Chapter 4**).

Long-term therapy with inhaled fluticasone propionate, with and without salmeterol

- Long-term inhaled steroid therapy can provide prolonged anti-inflammatory efficacy in COPD, associated with attenuated decline in lung function. Our results suggest that disease progression may be slowed in subgroups of steroid-naïve patients with moderate to severe COPD (**Chapter 5**).
- Patients with fewer pack years smoking, preserved diffusion capacity of the lung, limited hyperinflation, and lower number of total cells in induced sputum benefited most from inhaled corticosteroids. In addition, higher number of total cells and neutrophils in induced sputum, appeared to be predictive of a more effective reduction of the number of $CD3^+$ in bronchial biopsies by treatment with fluticasone during 30 months. This suggests that COPD patients expressing milder and/or earlier stages of the disease or a subtype of COPD patients in whom airway obstruction is less determined by emphysema can benefit from anti-inflammatory therapy, which favours a differential approach in the treatment of COPD (**Chapter 6**).
- Long-term treatment with inhaled corticosteroids improves patient-reported day-to-day general and functional health status in patients with moderate to severe COPD, whereas stopping fluticasone at 6 months worsens daily health status, symptoms, functional performance and peak

expiratory flow (PEF) Combination of ICS and a long-acting ß$_2$-agonist improves initial additional positive effects on health status, functional performance and PEF. Part of self-monitored outcomes were clinically relevant (**Chapter 7**).

Heterogeneity of the disease

It is widely recognized that COPD is a complex syndrome with irreversible airflow limitation that is associated with airways inflammation, but is also characterised by different pulmonary and extra-pulmonary components. Previous large epidemiological studies examined COPD patients as a distinct, but single disorder. However, it is now time to look further than just regarding all COPD patients as having the same disease and to focus on assessing the complexity of the syndrome of COPD. Remarkably, in June 2012 a PubMed-based literature search produced 1059 hits when searching for papers on "COPD phenotype or subgroup" amongst the 35,942 papers that are dealing with COPD. The data in the present thesis provides good evidence that COPD phenotyping is clinically relevant, strongly suggesting that phenotyping will improve current clinical practice and individualised treatment options [3-10].

In chapter 2 we have examined the heterogeneity in COPD by factor analysis. This analysis was used to categorise various clinical and inflammatory characteristics into specific independent domains of the disease without *a priori* assumptions. Airflow limitation, features previously associated with asthma (bronchodilator reversibility, total serum IgE and airway hyperresponsiveness) and airway inflammation were distinctive, largely independent components of COPD. Previous studies focused primarily on clinical features [11;12], whereas we extended these findings by distinguishing a separate domain for airway inflammation. Interestingly, there was a distinct domain for 'asthma-like' characteristics. These features have been suggested to be linked to the progression of the disease. However, in chapter 6 we have seen that these characteristics were not predictive of the effects of therapy on the decline in FEV_1, indicating that these characteristics may reflect different components in COPD than in asthma. This fits with modern views on the phenotypic characteristics that are discriminating (and sometimes are shared) between asthma and COPD [13].

Health status

The most important features from the patients' perspective are worsening of airway symptoms, limited physical activity and psychological distress,

resulting in decreased health status. Patients seek medical help with their clinical physician to feel better and clinicians will probably try to positively influence the distress with life-style advises or medical therapy. So, why should we bother about time-consuming measurements such as lung function, airway hyperresponsiveness, hyperinflation or even worse, invasive measurements such as induced sputum or bronchial biopsies? Reason for this is that clinical meaningful outcomes such as symptoms in COPD cannot easily be used as surrogate markers for the underlying components of the disease. It is well-known that health status is only marginally associated with FEV_1 and the effect of therapy on health status can be influenced by many other aspects of the disease such as older age [14]. If medical therapy is able to modify the underlying process of the disease, and hence can slow down the progression of the disease this will be an important reason to provide therapy, even in the absence of distressing symptoms.

One of the main components in COPD is airway inflammation. In our study, we examined whether there is an association between health status and inflammatory cell counts in induced sputum and bronchial biopsies (Chapter 3). Health status as measured with the SGRQ, was associated with higher inflammatory cell counts in induced sputum. Worse total SGRQ scores as well as the subdomains “symptom” and “activity” scores were associated with higher percentages macrophages in sputum. Patients with <15% macrophages had a total score of 27 and this score was worse by >4 points in patients with >15% macrophages, which exceeds the clinical threshold in SGRQ that is considered to be clinically relevant [15]. Interestingly, the inflammatory process was a stronger determinant of health status than other physiological measurements. Only hyperinflation showed a consistent independent influence on health status. Expiratory airflow limitation in COPD increases the time that lungs need to fully empty. With increasing respiratory rate this will lead to dynamic hyperinflation, a major drive for dyspnea. Neutrophils are considered as an important inflammatory cell in COPD, which can be linked to the severity of the disease. However, macrophages also may play an important role in the lungs of patients with COPD. Macrophages can be recruited to the bronchiolar epithelium and release a number of inflammatory cytokines such as tumour necrosis factor (TNF-α) or produce elastolytic enzymes that eventually may contribute to the development of emphysema and the progression of the disease, which could possibly influence health status. Only part of health status could be explained by this type of airway inflammation. This indicates that a distinct pathological phenotype contributes to health status.

Few long-term studies reported effects of therapy on health status. In the Inhaled Steroids in Obstructive Lung Disease (ISOLDE) study health status was measured with the SGRQ during 3 years. Usage of ICS positively influenced health status in patients with moderate to severe COPD. In addition, the combination of ICS and a LABA can improve health status [16] where long-term tiotropium can slow down decline of health status [17]. Controversial effects in studies can be due to differences in population characteristics such as baseline exacerbation frequency or different drop-outs [14].
Our study showed that in our subgroups of patients with moderate to severe COPD long-term maintenance therapy with ICS improved SGRQ activity and CCQ total, symptom and functional scores, whereas stopping ICS worsened SGRQ total and activity score, and both CCQ total and symptom scores (Chapter 5). Adding a LABA to ICS improved improved short term CCQ total, symptoms and functional scores. However, during the subsequent 24 months, combination therapy did significantly worse on these outcomes compared with fluticasone alone. In conclusion, health status is an overall measure of impairment that is associated with a distinct inflammatory profile and can be influenced by long-term ICS therapy in subgroups of patients with COPD.
We observed positive effects on daily reported health status and lung function in patients with stable disease (Chapter 7). Long-term monitoring in combination with therapy in COPD has been shown to decrease the impact of exacerbations on health status, and decrease symptoms of exacerbations [18]. However, the number of patients with a minimal important difference was limited, which may hamper adherence to chronic medication in moderately severe COPD.

Chronic bronchitis

Despite the fact that 30% of patients with COPD have symptoms of chronic bronchitis, it is still controversial whether this points to a benign disorder or a relevant sub-phenotype. It is not yet clear how a normal lung changes into a lung where mucus production is exaggerated, contributing to obstruction of the upper and lower airways. Aiming at the underlying airway inflammation might be of therapeutic value. This is not an easy approach since the pathology in COPD is complex. It might be considered as a ‘burning process’ driven by inflammatory cells, pro-inflammatory cytokines and oxidative stress. The lungs react with an innate and adaptive response to clear this burning process. Inability to restrict this innate and adaptive immune response may contribute to remodelling, increasing mucous glands and mucus-producing cells in

the surface epithelium. Inflammatory mediators such as neutrophil elastase are important secretagogues for mucin producing cells. The exaggerated mucin production and defective clearance is responsible for the mucus hypersecretion and presence of chronic bronchitis.
In this thesis the presence of chronic bronchitis was associated with a distinct inflammatory cell profile in the airways, as measured in a large sample of induced sputum and bronchial biopsies (Chapter 4). Remarkably, patients with chronic bronchitis had significantly fewer eosinophils in biopsies and higher percentages of sputum eosinophils than COPD patients without these symptoms. This is suggestive of a preferential distribution of eosinophils towards the airway lumen in COPD patients with chronic bronchitis. In chapter 4 we conclude that the clinical symptoms of chronic bronchitis reflect a distinct sub-phenotype among patients with manifest COPD, as based on inflammatory cells in induced sputum and bronchial biopsies. This may be important when trying to tackle the underlying inflammation in order to reduce symptoms in patients. However, effects of treatment with ICS with or without salmeterol was not different between COPD patients with and without chronic bronchitis (Chapter 5) and the presence of chronic bronchitis was not a predictor of the effect of therapy on the accelerated decline in FEV_1 in our study (Chapter 6). Therefore, according to our data it is still controversial whether chronic bronchitis can be considered as a relevant phenotype within COPD.

Long-term therapy with inhaled fluticasone propionate, with and without salmeterol

It is well-known that ICS can suppress the inflammatory process effectively in asthma. A potential similar effect on airways inflammation in COPD was the reason to prescribe this therapy on great scale in these patients. However, the effects in large studies of COPD are much more modest than in asthma. Current guidelines recommend maintenance treatment with ICS only in patients with advanced disease and a history of recurrent exacerbations. However, two recent studies suggested that prolonged therapy with ICS and a long-acting $ß_2$-agonist can attenuate FEV_1 decline in COPD.
The results of our study show that long-term maintenance therapy with fluticasone improves the rate of FEV_1 decline in the steroid-naïve patients with moderate to severe COPD included in our study (Chapter 5). This is accompanied by sustained improvement of airway hyperresponsiveness and reduced dyspnoea. Adding salmeterol to fluticasone provides a sustained

improvement in FEV_1-level and dyspnoea, but does not further improve the rate of FEV_1 decline and slightly worsens health status.

Effects on clinical measurements

Previous studies showed initial improvement in FEV_1, but could not demonstrate benefit on the subsequent annual FEV_1 decline in COPD [19-22]. We did not find an initial improvement in FEV_1, yet we believe that we were able to detect longstanding improvements in FEV_1 decline due to differences in study design. The vast majority of our patients was completely steroid-naïve, whereas over 50% of patients used steroids in previous studies [16;21;23-25]. By choosing steroid-naïve patients, we aimed to avoid patients with unknown previous benefits by inhaled steroids at baseline, and to limit drop-outs on placebo treatment. Furthermore, we used relatively high inhaled steroid doses in patients with predominantly mild to moderate COPD, who were examined in only two centers in which we took great care to apply the same methodology. The observed improvements in airway hyperresponsiveness and dyspnoea corroborate results from a previous study, showing that ICS can reduce airway hyperresponsiveness and dyspnoea after 9 and 33 months [22]. Discontinuation of ICS led to relapse of these disease features in our study, extending previous findings where stopping ICS led to a prompt deterioration in FEV_1 [24] and corroborating that inclusion of steroid naive patients may have been a decisive contribution to the differential effect between ICS and placebo. In keeping with previous reports, addition of salmeterol to fluticasone provided a consistent improvement in the level of FEV_1, yet we could not show further modification of long-term decline in FEV_1 [16;23]. Short-term addition of salmeterol improved daily reported health status and peak expiratory flow, which reached minimal important differences. Therefore, patients may actually experience the benefits in daily life. This suggests that addition of a LABA to ICS can be provided in order to relieve instant symptoms, but does not affect disease progression.

Effects on inflammatory cell counts

Our study shows that 2.5-year maintenance therapy with ICS in COPD reduces bronchial T lymphocyte and mast cell numbers, whilst increasing the number of eosinophils and integrity of bronchial epithelium, which was accompanied by a reduction in sputum cell counts. Previous short-tem studies investigating COPD patients have shown different anti-inflammatory effects of ICS in COPD [26-28]. 2-Month treatment in a small sample of patients with COPD showed

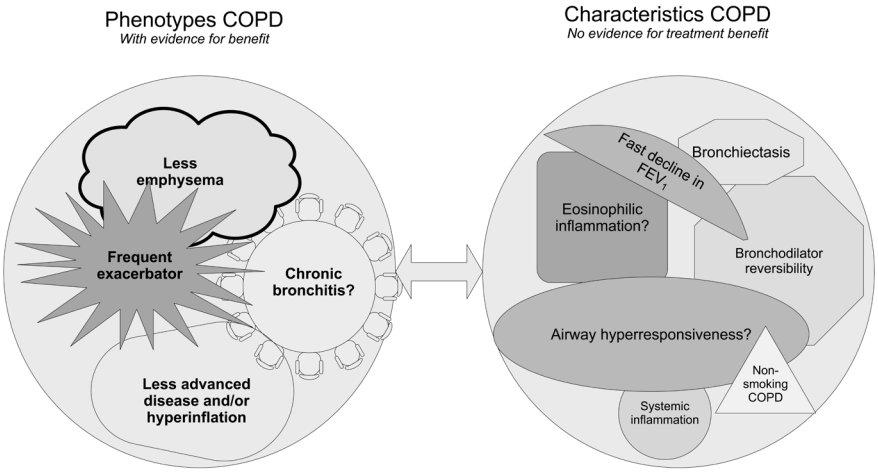
a reduction in sputum neutrophils and total cells by high dose budesonide in patients with moderate disease [29]. 3-Month treatment with the combination therapy of ICS and LABA reduced IL-8 and MMP-9, but did not statistically influence total number of cells in induced sputum compared with tiotropium in older patients with a FEV_1 around 55% of predicted and relatively poor quality of life (SGRQ total score of around 45) [30]. 3-Month treatment with ICS alone reduced subepithelial mast cells, but not CD8, CD68 or neutrophils in bronchial biopsies compared to placebo in 30 patients with an FEV_1 around 45% of predicted [31]. 13 Week treatment with the combination of 2 times daily 250/50 fluticasone and salmeterol reduced eosinophils in sputum and CD8, CD4, CD45 in bronchial biopsies, but not CD68 macrophages in 140 patients with moderate to severe COPD [32]. In addition, combination therapy reduced differential neutrophils and total eosinophils. This study did not include an ICS or LABA alone treatment group. 3-Month combination treatment with SFP (50/500, twice daily) suppressed $CD8^+$ T cells and CD68 macrophages in bronchial biopsies compared to placebo, but failed to find a result with fluticasone alone [33]. One possible explanation for the less clear effect of ICS on suppression of inflammation in COPD as compared to asthma may be the reduced expression of the enzyme histone deacetylase-2, which is required by ICS to switch off activated inflammatory genes in more advanced disease [34]. However, our study showed that beneficial effects in subgroups of patients with more moderate disease can be maintained during long-term treatment up to 30 months. Interestingly, the positive findings on inflammatory effects are consistent with positive effects on lung function decline and symptoms, which points towards a clinically relevant treatment benefit.

Who will benefit? Towards clinical phenotyping

In this thesis, the most important clinical question to be answered is who will benefit from ICS with or without LABA. The patient with COPD sitting in front of you when being a GP or pulmonologist is bothered by his or her symptoms and doctor and patient have to jointly decide whether to start with therapy. This is not an easy question and this thesis may add a small piece to the puzzle. Clinical phenotyping focuses on differences between individuals with COPD with regard to the effects of therapy on outcome. Figure 1 shows an example of visualising clinical phenotypes according to treatment effects. This is a dynamic model where, based on new evidence, phenotypes can switch between the left and right circle. This figure does not contain information on clinical phenotypes with an effect on clinical outcome, because we focused on

the effects of therapy, as it is important for daily practice.

Figure 1. Clinical phenotypes in COPD: a dynamic model



Chapter 6 shows that in our subgroup of patients with COPD, patients who had fewer packyears smoked, preserved diffusion capacity of the lung, limited hyperinflation, and lower number of total cells in induced sputum benefited most from inhaled corticosteroids. In addition, a higher number of total cells and neutrophils in induced sputum predicted a more effective reduction of the number of $CD3^+$ in bronchial biopsies by treatment with fluticasone at 30 months. This may suggest that earlier stages of the disease or a subtype of COPD patients in whom airway obstruction is accompanied with less severe emphysema predict a better course of lung function with treatment of ICS in patients with moderate to severe COPD. This extends findings from Lee *et al*, showing that short-term treatment response to ICS was smaller in patients with an emphysyma-dominant phenotype compared to the obstruction-dominant phenotype and moderate to severe COPD [35].

The severity in COPD is currently based on the level of FEV_1, symptoms and future risk of exacerbations [1]. However, it is the question whether to focus solely on these features [36]. In additon, it unclear whether early stages of disease should be treated or should we wait till there is more advanced disease? COPD is a disease with early (small) airway narrowing with continuos presence of inflammation where later in advanced disease tissue repair contributes to airway remodeling including irreversibe fibrosis [37]. Results from the Trial of Inhaled Steroids and Long-acting $ß_2$-agonists (TRISTAN) study showed that clinical benefits were not restricted to more advanced

disease as based on the level of FEV_1 and it is important also to focus on more mild disease where is much more to gain [38]. In our study, patients with less emphysema benefited more from ICS. The ECLIPSE study showed that emphysema as assessed with CT was associated with more rapid decline in FEV_1 in patients with more severe disease (FEV_1 48% of predicted) [39]. In a recent study 279 patients with COPD (GOLD stage I:26%, II:45%, III:24%, IV:5%) were followed for five years [40]. Emphysema severity as measured by either $\%K_{CO}$ of CT scan was independently associated with a rapid decline in FEV_1. This supports our findings that a clinical phenotype defined by emphysema should be considered in clinical trials with COPD or in daily practice, given its possible consequences for therapy.

Patients with asthma are more sensitive to the anti-inflammatory actions of corticosteroids than patients with COPD. Interestingly, in COPD several characteristics associated with asthma, such as bronchodilator responsiveness, airway hyperresponsiveness and eosinophilic inflammation have been reported to predict beneficial effects of ICS. However, in our study patients with asthma were carefullly excluded by doctor's diagnosis and self-reported symptoms, treatment or diagnosis of asthma. It is interesting whether the presence of asthmatic characteristics in COPD could point to a specific phenotype. Previous studies showed more benefit in COPD patients with more bronchodilator responsiveness [41-43]. However, these results have not been confirmed by others [21]. In 32 patients with mild to moderate COPD, manitol hyperresponsiveness was significantly associated with eosinophil numbers and soluble markers in sputum [44]. Because of the high specificity and low sensitivity this suggests that mannitol hyperresponsiveness can be used as a guidance for individualised treatment. However, this has to be confirmed in clinical trials. Reversibility in our study was not a predictor of clinical outcome and suggests that the underlying mechanisms contributing to reversibility in COPD may be different from reversibility in asthma. A previous study did not find improvement in FEV_1 after 6 weeks of treatment with ICS of patients with COPD who where hyperresponsive to adenosine 5'-monophosphate, which is an indirect stimulus [45]. We extended these findings by showing that airway hyperresponsiveness was no predictor of the long-term beneficial effects of ICS. Sputum eosinophils have been predictive of a larger increase of FEV_1, health status and exacerbations in studies with a duration up to one year. 82 patients with severe COPD (FEV_1 around 38% of predicted) were treated according to their symptoms or to both symptoms and eosinphilic inflammation. Intriguingly, guidance by eosinophilic inflammation led to a reduction in the

frequency of hospital admissions [46]. In our subgroup of patients we did not find such effect with maintenance therapy with ICS for 2.5 years. Rather than specific characteristics of asthma our data point to the direction that in certain subgroups of COPD such as patients with less advanced disease or a subphenotype with less emphysema, ICS may be beneficial. This may aid in the process of unraveling wich clinical phenotypes are important in COPD (Figure 1). Current guidelines advise long-term treatment only in patients with advanced disease and frequent exacerbations. External validation of our results in larger subsets of patients with moderate to severe COPD is necessary to be able to generalise our results into daily practice. This would allow extending current recommendations in COPD guidelines by offering a more optimistic perspective for subgroups of patients with COPD.

Limitations

We believe that the strength of our study is that it was designed to investigate the potential benefits of continuation of inhaled corticosteroids, with and without long-acting $ß_2$-agonists, in patients with stable, moderate to severe COPD who in the vast majority had not been treated with these drugs in the past. We examined long-term treatment and its discontinuation in the same study, including a placebo-arm. It has been shown that discontinuation of previously prescribed ICS in COPD leads to increased exacerbation rate [23], which may have increased the number of drop-outs in the placebo-groups of other studies. By including steroid-naïve patients in the present study, such selective drop-out was avoided, and this may explain the significant benefits of ICS on lung function decline as observed in the present study.

Still, some limitations apply to our study. For clinical endpoints, the number of patients was relatively low due to our primary aim to examine bronchial inflammation. This is the likely explanation for observing only small effects on health status. In addition, due to practical reasons, we did not include a 5th arm investigating salmeterol alone, but based on previous studies it can be anticipated that salmeterol has no disease modifying effect by itself [25;47]. This was an efficacy trial. Therefore, only data from patients using more than 70% of the prescribed dose were analysed. This may have underestimated the (detrimental) treatment effects in the placebo group due to expected drop-outs in this group. The sample size of our study was too small to investigate adverse events. Several studies reported a higher probability of having pneumonia as an adverse event among patients using ICS. The TORCH trial reported a higher risk on pneumonia with long-term fluticasone [48]. This

was supported by an updated meta-analysis showing a significant increased risk on pneumonia with ICS (RR 1.57, 95% CI1.41-1.75) [49]. It could be that usage of ICS leads to local immunosuppressive effects inducing an increased susceptibility to infections, especially in patients with more advanced disease. A detailed profile of the underlying inflammatory pathology is difficult to obtain. Pathological changes in COPD appear in central and peripheral airways and in the parenchyma, whereas in the lungs of smokers the inflammatory cells are not uniformly distributed [50]. The small airways play an important role in the pathogenesis of COPD [51] and by using bronchial biopsies of the larger airways and induced sputum, we focused only on the central airways.

Conclusion

Many COPD studies performed in the past were large epidemiological trials directed at clinical data or specifically directed at the inflammatory process in small samples with a small duration. However, the patient with COPD in front of the doctor experiences symptoms as a result of the complex pathogenesis of the disease. Physicians are trained to look at the patient as a whole. This should include understanding the patient and his disease perception, the symptoms and limitations belonging to the disease, but also the underlying components of the multiple pathways leading to progression of the disease [52]. Besides impairment in lung function other factors such as hyperinflation, emphysema, cough and sputum, and persistent inflammation should be evaluated, also in patients with less advanced COPD. Only then, we can put a step forward towards clinical phenotyping directed at individualised therapy [53]. The unique setting of the GLUCOLD study has provided the cooperation between multiple departments resulting in detailed phenotyping of patients with predominantly moderate COPD, including clinical as well as inflammatory data from induced sputum and bronchial biopsies. By showing that certain subgroups of COPD such as patients with less advanced disease or a subphenotype with less emphysema can benefit from inhaled corticosteroids, our results may offer an indication for a promising perspective for subgroups of patients with COPD towards the potential of slowing down the progression of the disease in the future.

Current implications in daily practice

Patient-centred care is the solution for improvement of quality of care and costs for chronic disease. Primary care may provide a setting where patient-centred care can be implemented. GPs can deliver integrated care with

other health care providers. Continuity of nearby presence of care can help the patient with integrating personal goals in daily life. So, how can we build bridges between evidence based medicine and practical daily care. It is increasingly known that diagnoses such as COPD are strongly related to other chronic diseases such as cardiovascular disease [54] and in the future we may go beyond specific diagnoses towards fingerprints of inflammation and/or RNA/DNA. However, we are not there yet and have to take one step at a time. Interpreting current guidelines is difficult since many patients with COPD have been and still are excluded from large randomised controlled trials [55]. So how can we start with implementing current knowledge in daily care? Is it sufficient to treat patients ad hoc? Do we rely on symptoms, exacerbations and FEV_1 or should we pro-actively focus on clinical phenotyping, including extended assessment in early disease? With increasing costs in society in the nearby future, the latter may be difficult to obtain with the enormous amount of patients with asthma and COPD. More simply, we cannot measure everything in every patient at any moment. It will be an enormous challenge to examine which patients with mild or more advanced disease will need early extended assessment in order to improve care (and reduce costs in the end).

The authors' perspective

How can we implement current evidence into daily practice? At this moment, I work as a general practitioner in a large primary care practice in The Hague. Around 1,000 patients (from a total of 10,500 registered persons) have a diagnosis of asthma and/or COPD. Together with lung physicians, we have started implementing integrated care. The novelty in our project is that caretakers from first and secondary care communicate in a structured and efficient way, in order to decide which patient needs further assessment using innovative e-health instruments. With current political movements in the Netherlands, the future will show whether programs like this will be stimulated, facilitated or stopped by the government. For now, a specialised lung nurse will make a detailed assessment of the patients' disease using questionnaires and spirometry within the primary care practice. Teleconsultation will be used to improve communication and feedback between primary and secondary care. If necessary, patients will have a secondary assessment in the hospital, with the intention of returning to primary care within 3 months of time. Few patients will actually be referred to a hospital. In addition, patient-centered care will be enhanced by usage of an internet based self-management programme, www.PatientCoach.nl. This programme was developed in Leiden for patients with

chronic disease and provides the possibility to optimise disease management, including the possibility of communication between the patient and the caretaker as well as scientific evaluation. Registration with the programme is integrated in the electronic registration system of the primary care physician. Currently, we are performing an implementation study with PatientCoach for patients with moderate asthma in primary care (IMPlementation strategies of internet-based Asthma Self-management Support in usual carE (IMPASSE) Trial). Patients with asthma can monitor their asthma symptoms and lung function at home with a consequent medication advice according to an algorithm based on current guidelines. In addition, we have started the Pulmonary Rehabilitation of Asthma and COPD: a Trial of sustained Internet-based Self-management Support (PRACTISS) study. The aim of this study is to assess the cost-effectiveness of sustaining self-management support via PatientCoach as compared to usual care in patients with 1) severe asthma who have completed pulmonary rehabilitation in Davos and 2) COPD patients who have completed pulmonary rehabilitation in the Rijnlands Rehabilitation Centre in The Netherlands.

The simplest reason for taking all of these efforts is to improve chronic care and reduce the enormous distress that patients experience from their chronic disease, accompanied with a decrease in costs. By structuring basic daily care we may try to find a way not just to measure everything in every one, but to focus on clinical phenotyping, setting personal goals and individualised care. By performing research with new topics such as biomarkers or e-health and integrating primary and secondary care, patients may benefit. This may lead eventually towards integrated disease management, individualised care, improved quality of life, and possibly slowing down the progression of chronic disease.

Abbreviations

α1AT	Alpha-1 Antitrypsin
BAL	Bronchoalveolar Lavage
Body box	Plethysmography
CCQ	Clinical COPD Questionnaire
Chest X-ray	Chest radiograph
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-Reactive Protein
CT	Computed Tomography
DNA	Deoxyribonucleic Acid
ECG	Electrocardiogram
ECLIPSE	Evaluation of COPD Longitudinally to Identify Surrogate Endpoints
FEV_1	Forced Expiratory Volume in one second
FIV_1	Forced Inspiratory Volume in one second
GM-CSF	Colony-Stimulating Factor
GLUCOLD	Groningen Leiden Universities Corticosteroids in Obstructive Lung Disease study
GOLD guidelines	Global Initiative for COPD guidelines
Hb	Hemoglobine
ICS	Inhaled Corticosteroids
IgE	Immunoglobuline
IL-8	Interleukin (IL)-8
IMPASSE	IMPlementation strategies of internet-based Asthma Self-management Support in usual carE trial
ISOLDE	Inhaled Steroids in Obstructive Lung Disease
K_{CO}	transfer factor for carbon-monoxide
LABA	Long-Acting $ß_2$-Agonist
LTB_4	Leukotriene B_4

LUMC	Leiden University Medical Center
MMPs	Matrix Metalloproteinases
6 MWD	6-Minute Walk test
NWO	Netherlands Organization for Scientific Research
N2 sb test	single-breath nitrogen test
PEF	Peak Expiratory Flow
PC_{20} methacholine	the provocative concentration of methacholine that causes a decrease in FEV_1 of 20%
PRACTISS	Pulmonary Rehabilitation of Asthma and COPD: a Trial of sustained Internet-based Self-management Support study
TNF-α	Tumor Necrosis Factor-α
TGF-ß	Transforming Growth Factor
QOL-RIQ	Quality of Life for Respiratory Illness Questionnaire
Rand36	Short Form Health Survey
RNA	Ribonucleic Acid
SAB	Stichting Astma Bestrijding
SGRQ	St. George Respiratory Questionnaire study
SLPI	Leucocyte Protease Inhibitor
TIMPs	Tissue Inhibitor of Metalloproteinases
TRISTAN	Trial of Inhaled Steroids and Long-acting $ß_2$-agonists study
TORCH	Towards a Revolution in COPD Health study
UMCG	University Medical Center Groningen
VAS	Visual Analogue Scale
TLC	Total Lung Capacity
CO-diffusion capacity	Carbon-monoxide-diffusion capacity

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Chapter 9

Nederlandse samenvatting

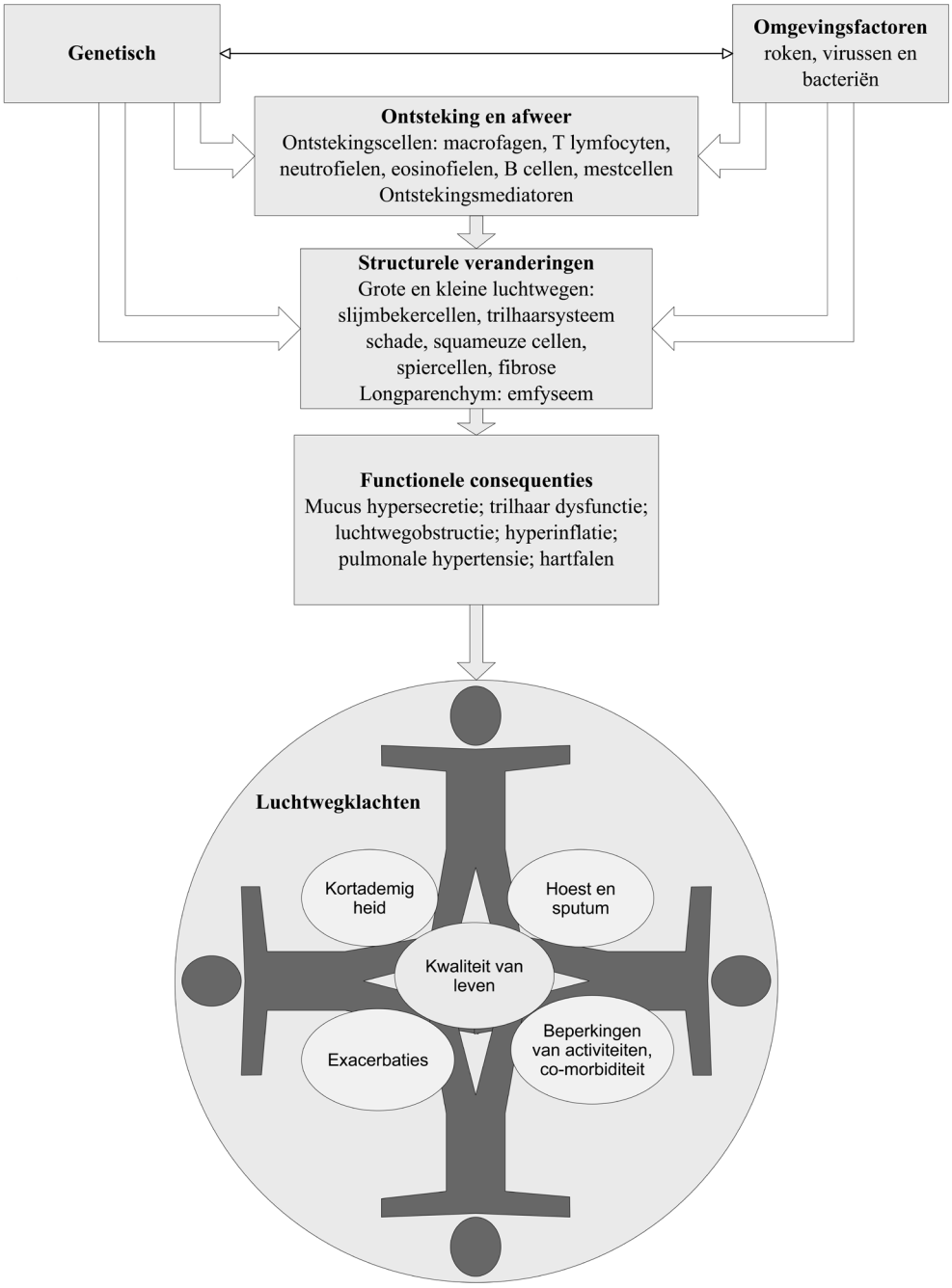
In dit proefschrift staat onderzoek naar behandeling met geïnhaleerde ontstekingsremmers (inhalatiecorticosteroïden) bij patiënten met een chronisch obstructieve longziekte (COPD) centraal. In het eerste deel van deze samenvatting wordt ingegaan op de recente inzichten op het gebied van COPD. In het tweede deel wordt de inhoud van het proefschrift stapsgewijs besproken.

COPD

Patiënten met COPD hebben een verminderde functie van de longen. COPD is een Engelse afkorting voor Chronic Obstructive Pulmonary Disease. Het is een verzamelnaam voor onder andere chronische bronchitis en longemfyseem. Chronische bronchitis is een term die door artsen gebruikt wordt voor patiënten die meer dan 3 maanden per jaar gedurende meer dan één jaar hoesten en daarbij slijm (mucus) opgeven. Bij longemfyseem zijn diep in de longen de longblaasjes en het longweefsel beschadigd waardoor de 'rek' uit de longen is gehaald. COPD komt veel voor: in elke huisartspraktijk in Nederland zijn er gemiddeld 60 patiënten die deze aandoening hebben. Het merendeel van de patiënten met COPD heeft matig tot ernstig COPD en wordt door de huisarts behandeld. De meest kenmerkende klachten zijn hoesten, slijm opgeven en benauwdheid. Voor de term "benauwdheid" worden ook veel andere omschrijvingen gebruikt, zoals 'minder lucht', 'kortademigheid', 'kort van adem' of 'benauwd gevoel op de borst', en in het Gronings "achter de poest". Het treedt vooral op bij inspanning. Alleen bij patiënten met ernstig COPD komt ook benauwdheid in rust voor. Indien er in korte tijd een verergering van klachten ontstaat, noemen we dat een 'exacerbatie'. Een exacerbatie wordt vaak veroorzaakt door infecties in de luchtwegen, meestal optredend in de wintermaanden.

Een belangrijk kenmerk van COPD is de verminderde functie van de longen. Dit wordt in medische termen luchtwegobstructie genoemd. Om de diagnose COPD te stellen wordt hiervoor de functie van de longen gemeten met een longfunctiemeter. Voor deze meting moet de patiënt rustig en diep inademen en dan zo hard en lang mogelijk uitademen. De diagnose COPD kan pas worden gesteld als de longfunctie na inname van specifieke medicijnen die de luchtwegen open zetten, zogeheten luchtwegverwijders, nog steeds verminderd is. Bij COPD zijn de longen onherstelbaar beschadigd en de longfunctie zal naarmate de patiënt ouder wordt verminderen. De weerstand bij uitademen is bij patiënten met COPD verhoogd. De lucht kan er wel goed in, maar niet goed uit. Om te begrijpen wat hier gebeurt, zou je een wasknijper

Figuur 1. Patiënten perspectief



op je neus kunnen zetten en dan door een rietje ademen. Dit geeft een idee hoe iemand met COPD zich voelt.

Patiëntenperspectief

Voor de patiënt zijn luchtwegklachten en beperkingen in het dagelijkse leven het belangrijkst (Figuur 1). De klachten en exacerbaties die met de aandoening gepaard gaan kunnen leiden tot een minder goede kwaliteit van leven. Hierbij kun je denken aan meer moeite om sport te beoefenen, te spelen met de kleinkinderen of trappen te lopen. Bij ernstiger COPD kunnen ziekenhuisopnames erg belastend zijn. Bij wetenschappelijk onderzoek is kwaliteit van leven gemeten met specifieke vragenlijsten. Hierbij bleek dat kwaliteit van leven maar matig is gerelateerd aan de onderliggende longfunctie. Er zijn dus patiënten met mild COPD met veel klachten en patiënten met ernstig COPD met weinig klachten. Dit kan komen doordat kwaliteit van leven ook beïnvloed wordt door andere factoren als depressie. Er zijn weinig studies die de relatie tussen kwaliteit van leven en onderliggende ontsteking van de luchtwegen hebben onderzocht.

Risicofactoren

Het roken van sigaretten is de belangrijkste risicofactor voor het krijgen van COPD. Eén op de vijf mensen die veel rookt of gerookt heeft zal COPD ontwikkelen. Van de patiënten die COPD hebben gekregen heeft ongeveer 90% gerookt.

Verschillende fenotypes

De ene patiënt met COPD is de andere niet. Er zijn patiënten die vooral last hebben van chronische bronchitis, hoesten en slijm opgeven, maar ook patiënten die vooral last hebben van periodes met een verergering van hun klachten, die we exacerbaties noemen. De verschillende types van de aandoening kunnen we beschrijven als fenotypes. Maar wat is nu precies een fenotype? Er bestaan meerdere definities, maar voor de praktijk is het belangrijk om naar een verzameling kenmerken te zoeken die klinisch relevant zijn voor bijvoorbeeld de behandeling. Een klinisch fenotype kan worden gedefinieerd als “een enkel of een combinatie van kenmerk(en) die het verschil beschrijft tussen individuen onderling en gerelateerd is aan klinisch belangrijke uitkomsten zoals kwaliteit van leven of effect van behandeling”. Een klinisch fenotype wordt bepaald door een combinatie van genetische factoren (genotype), omgevingsfactoren en klinisch relevante uitkomsten voor een

bepaalde aandoening. Een klinisch fenotype is hiermee bij een patiënt in de tijd vaak enigszins variabel. Als bijvoorbeeld omgevingsfactoren veranderen, kan het zijn dat een patiënt nu wel binnen een fenotype valt, of zelfs binnen een ander fenotype dan daarvoor. Welke fenotypen zijn van belang voor COPD?

Chronische bronchitis

Ongeveer een derde van de patiënten met COPD heeft chronische bronchitis. Dit kan klinisch worden gedefinieerd als aanwezigheid van dagelijks hoesten en slijm opgeven gedurende tenminste 3 maanden per jaar gedurende meer dan 1 jaar. Het is evident dat hoesten en slijm opgeven patiënten erg kunnen hinderen in hun dagelijks leven. Er is daarnaast veel discussie of chronische bronchitis een speciaal fenotype is dat behandeld kan worden om de achteruitgang van de aandoening af te remmen. Al in de jaren ‘50 werd gedacht dat roken de slijmproductie kon bevorderen, waardoor langdurige infectie aanwezig was met als gevolg beschadiging van de longen (Britse hypothese). Echter, dit kon niet worden aangetoond in andere studies en lang werd toen gedacht dat hoesten en slijm onschuldige klachten waren. In meer recente studies werd een verband gevonden tussen veel slijm opgeven en achteruitgang van de longfunctie. Bij patiënten met chronische bronchitis werden meer ontstekingscellen gevonden bij slijmproducerende cellen in de luchtwegen wat kan duiden op een onderliggende oorzaak. Mogelijk zijn ontstekingscellen de motor achter de vermeerderde slijmproductie met bijbehorende hoestklachten. Dit zou betekenen dat chronische bronchitis niet zo maar een onschuldig fenotype is bij COPD, maar dat de aanwezigheid of de mate van chronische bronchitis van belang kan zijn voor de ontwikkeling van de aandoening.

Frequente exacerbaties

Een exacerbatie is een tijdelijke verergering van luchtwegklachten. Dit kan enorme impact hebben op patiënten. Stel je bijvoorbeeld de volgende patiënt voor (eigen ervaring uit de praktijk): de heer T is een man van 65 jaar. Hij heeft matig ernstig COPD en is verkouden. Na een week gaat dit niet over zoals bij veel mensen het geval is, maar krijgt hij alleen maar meer klachten. Hij krijgt verhoging, gaat meer hoesten en is kortademig bij zeer geringe inspanning. De huisarts komt langs en constateert een behoorlijke verergering van zijn COPD (exacerbatie). Hij besluit de man naar het ziekenhuis te sturen, waar deze een aantal dagen moet blijven om weer op te knappen. Het is goed voor te stellen dat deze periode veel impact heeft op de man en zijn familie.

Het hebben van veel exacerbaties lijkt een risicofactor te zijn voor overlijden en een versnelde achteruitgang van de longfunctie. Het zijn vooral patiënten met ernstig COPD die meer frequente en ernstiger exacerbaties hebben. Echter, de 'Evaluation of COPD Longitudinally to Identify Surrogate Endpoints' (ECLIPSE) studie heeft laten zien dat patiënten met zowel mild als ernstig COPD veel exacerbaties kunnen hebben, wat duidt op een apart fenotype. Er zijn verschillende studies die laten zien dat je exacerbaties kunt voorkomen. Door het opstellen van een individueel actieplan worden exacerbaties eerder ontdekt en vroegtijdige behandeling kan leiden tot een vermindering van duur en ernst van de exacerbatie. Behandeling met inhalatiecorticosteroïden al dan niet met langwerkende luchtwegverwijders kan het aantal exacerbaties doen verminderen. In nationale en internationale richtlijnen wordt daarom geadviseerd patiënten met frequente exacerbaties te behandelen met inhalatiecorticosteroïden. Interessante resultaten van een recente studie laten zien dat een onderhoudsbehandeling met antibiotica het aantal exacerbaties kan doen verminderen bij patiënten met matig ernstig COPD. Deze studie liet echter ook bijwerkingen zien zoals 5% meer gehoorsverlies bij de onderzochte patiënten. Samenvattend zijn er verschillende studies die laten zien dat het hebben van frequente exacerbaties op een apart fenotype duidt, waardoor het risico op exacerbaties in de recente internationale richtlijnen is opgenomen als leidraad bij het behandelen van patiënten met COPD.

Versnelde achteruitgang van longfunctie

De versnelde achteruitgang van de longfunctie is een hoofdkenmerk van COPD. Het wordt wereldwijd gebruikt als een objectieve meting om de ernst van de aandoening te beschrijven. Bij gezonde personen daalt de longfunctie met de leeftijd ongeveer 25-30 ml per jaar. Bij patiënten met COPD is dit meer, en daalt de longfunctie met gemiddeld 60 ml per jaar. In het begin hebben de longen veel reserve en kunnen patiënten met COPD weinig last hebben van de verminderde longfunctie. Echter, bij een deel van de COPD-patiënten daalt de longfunctie veel sneller. Mogelijk duidt dit op een apart fenotype waar winst behaald kan worden met intensiever controleren (monitoren) en behandelen.

Naast de longfunctie zijn er meer objectieve kenmerken van COPD. Er zijn patiënten die last hebben van hyperreactiviteit (prikkelbaarheid van de luchtwegen). Hyperreactiviteit is geassocieerd met de versnelde achteruitgang van de longfunctie wat mogelijk duidt op een apart fenotype. Een ander kenmerk is hyperinflatie, waarbij er sprake is van een toename van lucht in de longen die een patiënt niet meer uit kan ademen. Dit wordt versterkt door obstructie of vernauwing van de luchtwegen en/of emfyseem. Bij inspanning zal hyperinflatie toenemen waardoor patiënten vaak meer benauwd worden. Bij andere patiënten staat emfyseem op de voorgrond. De diverse uitingen en verschillen in het onderliggend ziektebeeld bieden mogelijkheden voor verschillen in behandeling.

Ontsteking in de longen

De kleine luchtwegen (minder dan 2 mm in doorsnede) zijn de belangrijkste plaats voor luchtwegobstructie bij COPD. Een recente studie onderzocht de relatie tussen obstructie in deze kleine luchtwegen en emfyseem. Met een Computer Tomografy (CT) zijn longen onderzocht bij 78 patiënten die een transplantatie hadden ondergaan. Een belangrijk resultaat was dat het aantal kleine luchtwegen was verminderd bij patiënten met mild COPD en verder afgenomen bij patiënten met ernstig COPD. Dit suggereert dat de vernauwing en verlies van de kleine luchtwegen voorafgaat aan het ontstaan van emfyseem.

Pathogenese

Pathogenese kan worden gedefinieerd als het ontstaan van ziekte door opeenvolgende veranderingen van structuur en functie van cellen, weefsel of organen door de effecten van microbiële, chemische of fysische invloeden. De pathogenese bij COPD wordt gekenmerkt door een complexe ontstekingsreactie ten gevolge van het inhaleren van schadelijke stoffen. Het roken van sigaretten veroorzaakt een ontstekingsreactie in de longen. Echter, ongeveer 20% van de rokers ontwikkelt COPD en het lijkt erop dat de ontstekingsreactie bij deze patiënten versterkt is of abnormaal gereguleerd doordat ze er extra gevoelig voor zijn. Bij patiënten met COPD blijft een groot deel van de ontstekingsreactie ook actief nadat patiënten stoppen met roken.

Pathologische fenotypes

Er zijn verschillende pathologische fenotypes bekend. Chronische bronchitis is gekoppeld aan een overproductie van slijm, wat mogelijk aangestuurd kan

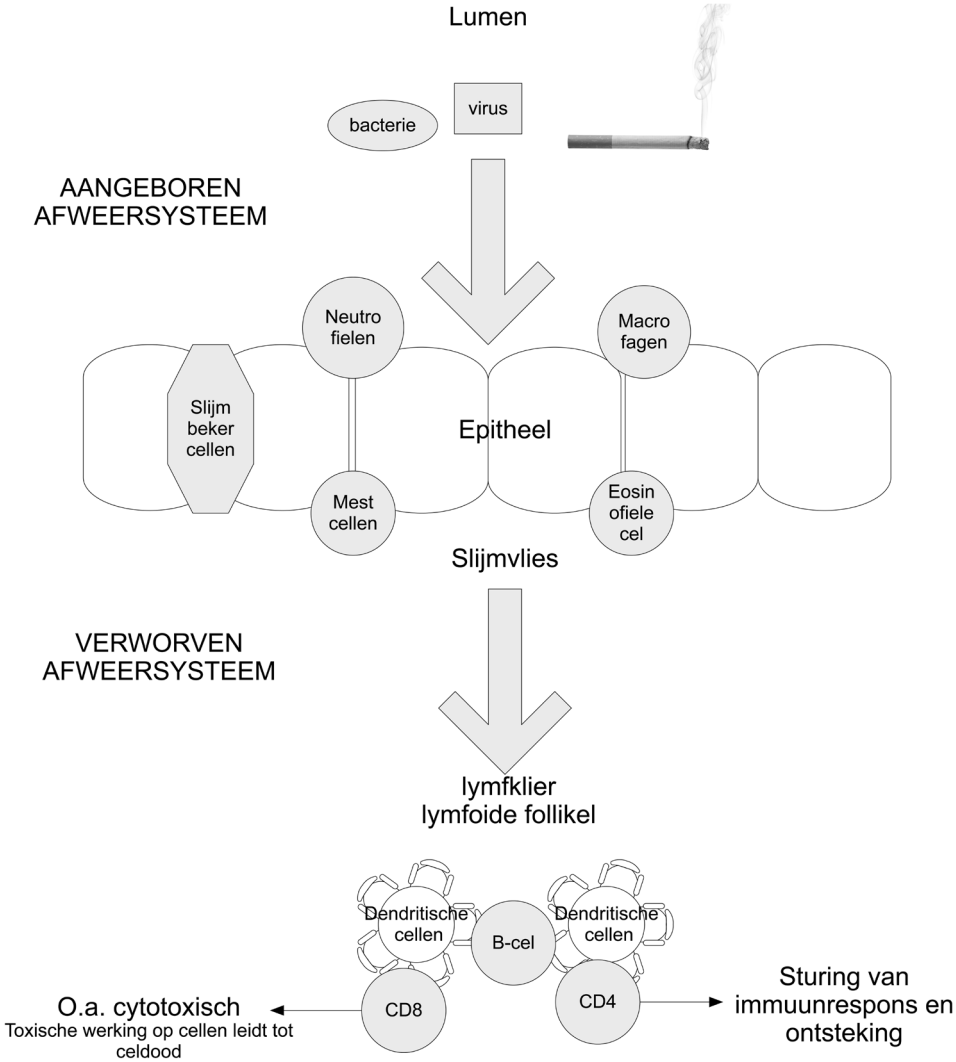
worden door een ontstekingsreactie in de slijmklieren en bekleding van de luchtwegen. Emfyseem wordt veroorzaakt doordat de luchthoudende blaasjes in de longen kapot gaan. Dit kan deels komen door onvoldoende herstel van het longweefsel. De kleine luchtwegen zijn vaak als eerste aangedaan en lijken bij vroege stadia van COPD in aantal te verminderen. Er is in onderzoek een toename gevonden van verschillende ontstekingscellen. Het weefsel kan kapot gaan als direct gevolg van de ontsteking en afbraak van het elastische longweefsel, waardoor bij het uitademen de (kleine) luchtwegen eerder dichtklappen. Daardoor blijft lucht achter in de longen, hetgeen wij hyperinflatie noemen. Daarnaast kan het weefsel beschadigd worden door een overdreven herstel van het longweefsel. Als gevolg van deze beschadigingen kan er een structurele verandering van het longweefsel optreden, ook wel verbindweefseling of fibrose genoemd. De luchtwegen verdikken en vernauwen, wat bij kan dragen aan de verminderde longfunctie. Overproductie van slijm en slijmproppen draagt verder aan bij aan de obstructie. Echter, de verschillende onderliggende mechanismen maken het erg moeilijk om de verschillende pathologische processen in fenotypes onder te verdelen.

Luchtwegontsteking is het basiskenmerk van COPD. De ontsteking komt voor in de gehele longen en er zijn verschillende manieren om dit te meten. In het GLUCOLD onderzoek hebben patiënten bronchoscopieën ondergaan. Hierbij wordt met een flexibel slangetje in de luchtwegen gekeken en een hapje (biopt) uit het longweefsel genomen. Dit weefsel kan worden bewerkt en onderzocht. Het is een belastend onderzoek en het wordt door ons als onderzoekers zeer gewaardeerd dat patiënten met COPD in ons onderzoek hieraan mee wilden werken. Een andere manier om ontsteking te onderzoeken is een slijmonderzoek. Ook dit is vrij belastend en wordt in de praktijk alleen in sommige ziekenhuizen verricht. Minder belastend onderzoek is bloedonderzoek. Recent onderzoek laat zien dat er ook nieuwe technieken zijn die belangrijke resultaten laten zien bij COPD. Met een speciaal apparaatje (‘elektronische neus’) kan de uitgeademde lucht worden gemeten en onderzocht. Deze uitgeademde lucht van patiënten met COPD is gerelateerd aan een specifiek ontstekingsprofiel wat suggereert dat het gebruikt kan worden om een fenotype vast te stellen bij COPD.

Ontsteking en afweer

Bij COPD is bij daarvoor gevoelige patiënten met COPD sprake van een abnormale toename van de ontstekingsreactie van de longen als gevolg van schadelijke stoffen (Figuur 2).

Figuur 2. Aangeboren en verworven afweer



Bij iemand die schadelijke stoffen inhaleert of een luchtweginfectie doormaakt komen prikkelende stoffen in de luchtwegen terecht. Deze botsen als eerste op de luchtwegwand en het aangeboren afweersysteem. Dit is de eerste verdedigingslinie. Als de stofjes door de luchtwegwand heen komen, ontstaat een golf van reacties van het afweersysteem. De reactie is gunstig voor het

opruimen van de infectie, maar kan zo sterk zijn dat er schade (ontsteking) ontstaat. De ontsteking zelf heeft ook weer als doel om de infectie of het schadelijke stofje te bestrijden. Belangrijke cellen in dit proces bij COPD zijn neutrofiele granulocyten, macrofagen en $CD8^+$ T lymfocyten. Daarnaast zijn er aanwijzingen dat $CD4^+$ T lymfocyten, mestcellen en eosinofiele cellen een rol spelen.
Neutrofiele granulocyten gaan naar delen in de longen toe om wonden te helen. Geactiveerde neutrofielen kunnen slijmproductie stimuleren. Helaas kan een teveel aan neutrofielen ook schade berokkenen en kunnen afgescheiden stofjes zoals elastase emfyseem bevorderen. Een andere belangrijke cel is de macrofaag. Het aantal macrofagen is toegenomen in de luchtwegen en in het slijm van patiënten met COPD. Ook de macrofaag kan schadelijke stofjes afscheiden die bijdragen aan het ontstaan van emfyseem. Eosinofielen en mestcellen spelen een belangrijke rol bij patiënten met astma, maar lijken ook een rol te spelen bij COPD. Eosinofiele cellen spelen bijvoorbeeld een rol bij exacerbaties en het sturen van behandeling. Epitheel cellen zijn oppervlakkige cellen in de luchtwegwand en scheiden diverse stofjes af die van invloed zijn op de ontsteking. Na de eerste reactie van het aangeboren afweersysteem zijn dendritische cellen verantwoordelijk voor het stimuleren van het verworven afweersysteem. Het verworven afweersysteem kan specifieke stofjes herkennen en onthouden om de afweer goed te reguleren. Belangrijke ontstekingscellen zijn hierbij T lymfocyten ($CD4^+$ en $CD8^+$) en B lymfocyten. Een belangrijke functie van $CD8^+$ cellen is het aanvallen van virussen door celdood van geïnfecteerde cellen. De rol van $CD4^+$ is wat minder duidelijk. Deze cellen scheiden diverse stofjes af en zijn de zogenaamde T helper cellen. Ze ‘helpen’ door het activeren van de B lymfocyten en ondersteunen activiteit van de $CD8^+$ cellen. In de GLUCOLD studie werd gevonden dat patiënten met COPD meer B lymfocyten hebben dan patiënten zonder COPD. De rol van B lymfocyten is nog niet geheel opgehelderd. Een toename kan worden gestimuleerd door virussen of bacteriën en dient mogelijk als bescherming tegen infecties. Recente studies hebben aangetoond dat mestcellen meer voorkomen in de luchtwegen bij patiënten met COPD dan bij gezonde mensen.

Weefselherstel en remodelling

Het gevolg van bovenstaande mechanismen is dat de longen zich proberen te herstellen of gaan reorganiseren (remodelling). Als er geen goede balans is tussen schade en herstel leidt dit tot onherstelbare schade aan de longen. Reorganisatie van weefsel veroorzaakt vernauwing van de luchtwegen,

verdikking van de wanden en verandering van het longweefsel (parenchym). Het is de vraag of de reorganisatie altijd schadelijk is. Mogelijk geeft bijvoorbeeld een verdikking van de luchtwegen een betere bescherming tegen agressieve stoffen van buiten af. De schade die ontstaat bij de longblaasjes geeft een verslapping van het weefsel. Elastase (afkomstig van de neutrofiel) is een bijzonder stofje dat elastische vezels af kan breken waardoor de rek uit de longen gaat wat leidt tot emfyseem.

Behandeling

Een van de peilers bij de behandeling is het verbeteren van leefgewoonten. En dan vooral het stoppen met roken, omdat dit een duidelijk positief effect heeft op de klachten, het aantal exacerbaties en de achteruitgang in longfunctie. In het verleden werd COPD gezien als een onbehandelbare aandoening. De aandoening kan nog altijd niet worden genezen, maar in de afgelopen jaren wordt toch iets positiever tegen de behandeling aangekeken. Er zijn voor patiënten met mild tot matig ernstig COPD twee belangrijke soorten medicijnen. Er zijn kort- en langwerkende luchtwegverwijders. Deze medicijnen kunnen de luchtwegen meer open zetten en daarmee de patiënt meer lucht geven. Daarnaast zijn er medicijnen die inhalatiecorticosteroïden oftewel ontstekingsremmers worden genoemd. Dit zijn medicijnen die zoals de naam al zegt de ontsteking in de luchtwegen zouden moeten remmen. Bij patiënten met astma werkt dit inderdaad zo. Patiënten met astma hebben ook een obstructie in de luchtwegen, maar deze is in tegenstelling tot bij COPD, wel herstelbaar. Bij patiënten met COPD ligt het iets ingewikkelder. Vier grote studies uit de jaren ‘90 lieten zien dat ontstekingsremmers de achteruitgang van de longfunctie niet af konden remmen. In de afgelopen jaren hebben diverse studies een positief effect laten zien van inhalatiecorticosteroïden al dan niet met langwerkende luchtwegverwijders op kwaliteit van leven en exacerbaties bij patiënten met COPD. Een aantal studies liet zien dat inhalatiecorticosteroïden een goed effect hebben bij patiënten met veel exacerbaties. Momenteel is dit dan ook de reden dat deze medicijnen alleen worden geadviseerd bij patiënten met frequente exacerbaties. Echter, er zijn heel erg veel patiënten die minder dan twee exacerbaties per jaar hebben. In de dagelijkse praktijk krijgen toch ook heel veel patiënten uit deze grote groep deze medicijnen. Het is een uitdaging aan de wetenschap om te onderzoeken of er meer fenotypes zijn die baat hebben bij de behandeling met inhalatiecorticosteroïden.

Het doel van de studie

Deze studie doet onderzoek naar gedetailleerde fenotypering van COPD en effecten van behandeling met inhalatiecorticosteroïden al dan niet met langwerkende luchtwegverwijders. Het is gebaseerd op analyses van de "Groningen Leiden Universities Corticosteroids in Obstructive Lung Disease" (GLUCOLD) studie.

De onderzoeksdoelen van het huidige proefschrift zijn:

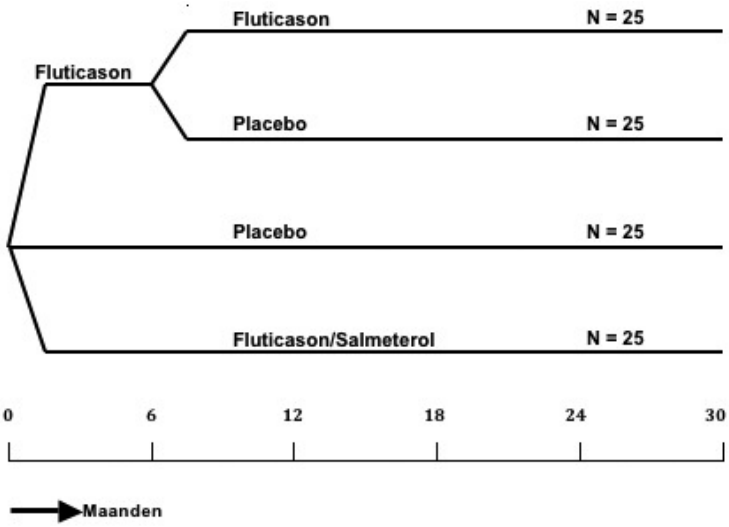
1) Zijn klinische symptomen en luchtwegontsteking verschillende componenten van COPD?
2) Draagt luchtwegontsteking bij aan verminderde kwaliteit van leven?
3) Is chronische bronchitis een apart fenotype?
4) Heeft onderhoudshandeling met ontstekingsremmers een effect op de chronische ontsteking en symptomen bij COPD?
5) Kan het effect van ontstekingsremmers al dan niet met langwerkende luchtwegverwijders voorspeld worden?
6) Heeft onderhoudsbehandeling met ontstekingsremmers al dan niet met langwerkende luchtwegverwijders een effect op de dagelijkse kwaliteit van leven en longfunctie bij patiënten met COPD?

Wat was de opzet van de GLUCOLD studie?

De GLUCOLD studie is door wetenschappelijke onderzoekers geïnitieerd, uitgevoerd en geanalyseerd. De patiënten in de studie werden toegelaten als ze 45-75 jaar oud waren, (ex)-rokers en longfuncties tussen de 30 en 80% hadden vergeleken met gemiddelde personen van gelijke leeftijd of geslacht. Patiënten mochten geen astma hebben. De metingen vonden plaats tussen 2003 en 2007. Patiënten werden ingedeeld in de volgende vier behandelgroepen (Figuur 3):

1) Kortdurend (6 maanden) geïnhaleerde ontstekingsremmers (inhalatiecorticosteroïden, fluticasone), gevolgd door placebo. Een placebo is een nep medicijn.
2) Langdurend (2,5 jaar) inhalatiecorticosteroïden
3) Combinatie van inhalatiecorticosteroïden met langwerkende luchtwegverwijders (salmeterol)
4) Langdurend placebo

Figuur 3. Opzet van de studie



In eerste instantie is het effect op ontstekingsparameters in longweefsel (biopten) onderzocht (Figuur 4). Daarnaast zijn elke 3 maanden luchtwegklachten gemeten, kwaliteit van leven, rookstatus, bloedonderzoek, therapietrouw en longfunctiemetingen. Op 0, 6 en 30 maanden zijn bronchoscopiën, slijmonderzoek en hyperreactiviteitsmetingen verricht*.

Figuur 4. Metingen

Conclusies van dit onderzoek

Klinische symptomen en ontsteking

- Luchtwegobstructie, hyperreactiviteit en ontsteking zijn aparte, grotendeels onafhankelijke componenten van COPD (**Hoofdstuk 2**).
- Ontsteking in de luchtwegen draagt onafhankelijk bij aan de verminderde kwaliteit van leven bij patiënten met COPD (**Hoofdstuk 3**).
- Chronische bronchitis reflecteert een fenotype gebaseerd op een specifiek ontstekingsprofiel, waarbij eosinofiele cellen in de luchtwegen lijken te verschuiven van de wand naar het lumen en er een hoger percentage macrofagen in het sputum is (**Hoofdstuk 4**).

Langdurig behandelen met geïnhaleerde ontstekingsremmers al dan niet met lang werkende luchtwegverwijders

- Langdurig behandelen met geïnhaleerde ontstekingsremmers heeft in deze studie bij deze patiënten met COPD een remmend effect op de ontstekingscellen. Dit is geassocieerd met een gunstig effect op de achteruitgang van de longfunctie. Langwerkende luchtwegverwijders verbeteren de longfunctie op de korte termijn, een effect dat op de lange termijn wordt behouden. Onze resultaten suggereren een effect van inhalatiecorticosteroïden op de voortgang van de aandoening bij bepaalde groepen patiënten met matig ernstig COPD die daarvoor merendeels niet behandeld waren met ontstekingsremmers (**Hoofdstuk 5**).
- Patiënten die minder hebben gerookt, een betere zuurstof opname capaciteit hebben in de longen, minder hyperinflatie en een lager totaal aantal cellen in hun slijm hebben een grotere kans om te profiteren van inhalatiecorticosteroïden. Dit suggereert dat patiënten met milder COPD, een eerder stadium of een bepaald fenotype van de ziekte waarbij COPD minder wordt bepaald door emfyseem meer profijt kunnen hebben van geïnhaleerde ontstekingsremmers (**Hoofdstuk 6**).
- Langdurig behandelen met geïnhaleerde ontstekingsremmers verbetert de dagelijkse symptomen, terwijl stoppen na 6 maanden dagelijkse kwaliteit van leven en longfunctie benadeelt. Langwerkende luchtwegverwijders geven een kortdurend effect **(Hoofdstuk 7)**.

Heterogeniteit van COPD

'De ene patiënt met COPD is de andere niet'. In hoofdstuk 2 hebben we de diversiteit van patiënten met COPD onderzocht. De GLUCOLD studie is een

studie waarbij we patiënten met COPD zeer gedetailleerd in kaart hebben gebracht. Er is gekeken naar klachten van de patiënt en kwaliteit van leven, maar ook naar complexe mechanismen die de onderliggende aandoening COPD bepalen. Al deze verschillende kernmerken zijn in een bepaalde analyse gehergroepeerd om te kijken of er een onderlinge samenhang is. Hier bleek dat longfunctie, ontsteking, en kenmerken die eerder aan astma werden toebedeeld aparte componenten zijn. Dit suggereert dat het mogelijk is fenotypes te definiëren binnen de grote groep patiënten met COPD.

Kwaliteit van leven

Het belangrijkste voor de patiënt met COPD zijn niet de afwijkingen aan de luchtwegen en het longweefsel, maar de luchtwegklachten en beperkingen die hij of zij in het dagelijks leven ervaart en die een verminderde kwaliteit van leven veroorzaken. Dus, waarom zouden we ingewikkelde en belastende metingen als broncoscopiëen en slijmonderzoek verrichten? Het blijkt uit onderzoek dat luchtwegklachten geen directe afspiegeling zijn van de ontsteking van de onderliggende aandoening. Uiteindelijk is het natuurlijk wel de onderliggende aandoening die op de lange termijn voor voortgang van de ziekte en voor de uiteindelijke klachten zorgt. Daarbij is ontsteking een belangrijk aangrijpingspunt voor behandeling om de voortgang van de ziekte af te remmen. Daarom is het van belang niet alleen naar klachten, maar ook naar de onderliggende mechanismen van de aandoening te kijken. Veel studies kijken of naar klachten of naar ontsteking, maar weinig studies kijken naar het geheel. In de GLUCOLD studie is nauwkeurig onderzocht of er een verband is tussen de klachten die belangrijk zijn voor de patiënt en de onderliggende ontsteking (**Hoofdstuk 3**). De resultaten lieten zien dat kwaliteit van leven was gerelateerd aan meer ontstekingscellen in slijm. Symptomen en activiteiten waren gerelateerd aan hogere percentages macrofagen in slijm. Deze resultaten suggereren dat een speciaal pathologisch fenotype van invloed kan zijn op de kwaliteit van leven bij patiënten met matig ernstig COPD.

Langdurig behandelen met inhalatiecorticosteroïden verbeterde diverse aspecten van kwaliteit van leven in ons onderzoek, terwijl stoppen met deze medicijnen kwaliteit van leven verslechterde. Toevoegen van langwerkende luchtwegverwijders aan ontstekingsremmers verbeterde in eerste instantie de kwaliteit van leven, maar op de lange termijn verslechterde de kwaliteit van leven weer. Langdurig behandelen met ontstekingsremmers gaf vergelijkbare effecten op kwaliteit van leven gemeten door patiënten thuis (**Hoofdstuk 7**). Niet voor alle patiënten was dit een klinisch relevante verbetering.

Chronische bronchitis

Ongeveer een derde van alle patiënten met COPD heeft chronische bronchitis (langdurig hoesten en slijm). Het is nog altijd niet duidelijk hoe een gezonde long kan veranderen in een COPD-long die veel meer dan de normale hoeveelheid slijm produceert. Je zou de cellen van een COPD-long kunnen vergelijken met een smeulend vuurtje. Soms is het rustig, soms flakkert het vuur op. Het kan worden gestimuleerd door prikkelende stoffen als rook of uitlaatgassen, maar ook de long zelf doet mee. Deze stimuleert de afweer om het vuur te bestrijden. Bij de COPD-long wordt de afweer overdreven gestimuleerd. De balans tussen schade en herstel is weg. Er komen meer slijmcellen, meer slijmproductie en een minder effectief schoonmaaksysteem van al dat slijm dat de luchtwegen verstopt. De patiënt blijft daarom hoesten om het slijm maar kwijt te raken. Echter, veel is nog onduidelijk over de precieze werking van dit proces.
In de huidige studie was chronische bronchitis gerelateerd aan een specifiek ontstekingspatroon van de luchtwegen (**Hoofdstuk 4**). Het bleek dat patiënten met chronische bronchitis minder eosinofiele cellen in het longweefsel hadden, terwijl er meer eosinofiele cellen in slijm werden gevonden. Dit kan interessant als aangrijpingspunt voor specifieke therapie. In de groep patiënten van de GLUCOLD studie hebben we een effect gevonden van langdurig behandelen met geïnhaleerde ontstekingsremmers. Echter, er was geen verschil in effect tussen patiënten met en zonder chronische bronchitis (**Hoofdstuk 5**). Ook hebben we onderzocht of we konden voorspellen bij wie deze therapie werkt. Hiervoor hebben we onderzocht bij wie er een langdurig effect bestond van de ontstekingsremmers op de achteruitgang van de longfunctie. Het hebben van chronische bronchitis bleek in deze studie geen voorspeller te zijn. Het is algemeen bekend dat het hebben van chronisch bronchitis een belangrijk fenotype is als je naar kwaliteit van leven kijkt. In onze groep patiënten bleek het echter geen fenotype te zijn dat relevant is voor de huidige vormen van therapie.

Langdurig behandelen met inhalatiecorticosteroïden al dan niet met langwerkende luchtwegverwijders

Bij een groot deel van de patiënten met astma kunnen ontstekingsremmers de ontsteking afremmen waardoor patiënten minder klachten hebben en de voortgang van de aandoening kan worden afgeremd. Omdat astma en COPD beide obstructieve longziekten zijn met ontsteking als basiskenmerk van de ziekte werd ook bij COPD op grote schaal ontstekingsremmers voorgeschreven. Echter, bij COPD zijn de effecten minder duidelijk dan bij

astma. De huidige richtlijnen adviseren alleen inhalatiecorticosteroïden voor te schrijven bij frequente exacerbaties. Grote studies begin jaren 2000 lieten zien dat er geen effect was op de versnelde achteruitgang van de longfunctie. Twee recente studies nuanceren dit enigszins. In de GLUCOLD studie hebben we een aantal positieve klinische effecten gevonden van de behandeling met inhalatiecorticosteroïden al dan niet met langwerkende luchtwegverwijder. Bij patiënten die na 6 maanden doorbehandeld werden met ontstekingsremmers verminderde de achteruitgang van de longfunctie op de lange termijn. Dit werd begeleid door een aanhoudend effect op de hyperreactiviteit en vermindering van kortademigheid. Deze lange termijneffecten waren geassocieerd met effecten op ontsteking, gemeten in biopten en slijm. Stoppen met ontstekingremmers had een nadelig effect op de longfunctie, hyperreactiviteit, kortademigheid en kwaliteit van leven en de ontstekingscellen in de luchtwegen en het slijm. Toevoegen van een langwerkende luchtwegverwijder aan de ontstekingsremmer gaf een verbetering van de longfunctie en klachten op de korte termijn, echter doorgaan gaf geen extra effecten ten opzichte van ontstekingsremmers alleen.

Maar hoe kun je nu de verschillen verklaren tussen de resultaten van deze studie en eerdere studies waar geen effecten werden gevonden? Mogelijk komt dit door verschillen tussen de onderzochte patiëntengroepen. De personen in onze studie hadden bijvoorbeeld de afgelopen 6 maanden geen ontstekingremmers gebruikt en de meesten hadden deze medicijnen zelfs nooit eerder gebruikt. In andere studies gebruikte meer dan 50% van de patiënten eerder ontstekingsremmers. In de GLUCOLD studie is een hoge dosering ontstekingsremmers gebruikt wat mogelijk meer effect kan opleveren. Het additionele effect van langwerkende luchtwegverwijders op de korte termijn in onze studie komt overeen met resultaten van andere studies. Onze studie liet echter zien dat langer doorbehandelen geen extra effect gaf op de aandoening.

Langdurig behandelen verminderde het aantal T cellen en mestcellen, terwijl het aantal eosinofielen vermeerderde en het oppervlakte epitheel verbeterde. Dit werd begeleid door een afname in slijmcellen. Andere studies laten verschillende effecten zien, zoals minder eosinofiele cellen in slijm, minder $CD8^+$ en $CD4^+$ cellen, of minder macrofagen in longweefsel. Een mogelijke verklaring waarom de ontsteking bij patiënten met COPD resistent kan zijn voor ontstekingsremmers is een afname histone deacetylase-2 in meer ernstig COPD. Resultaten van de GLUCOLD studie suggereren dat er bepaalde effecten van inhalatiesteroïden mogelijk zijn bij een deel van de patiënten

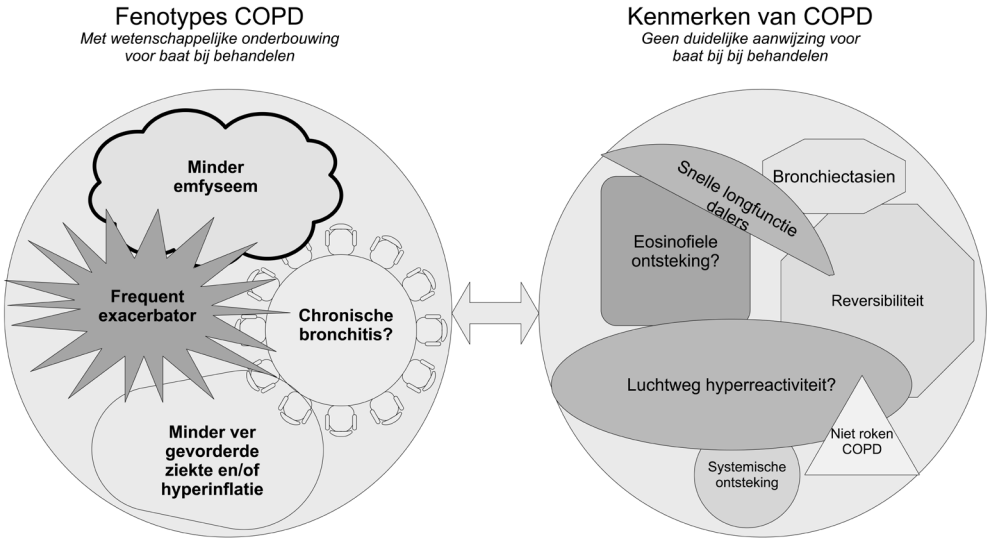
met minder ernstig COPD. Het is interessant dat de effecten op klinische uitkomsten parallel lopen aan effecten op ontsteking in de luchtwegen en slijm.

Wie heeft wel baat bij de behandeling en wie niet?

"Towards clinical phenoptyping"

In dit proefschrift is de belangrijkste vraag welke patiënt met COPD baat heeft bij gebruik van inhalatiecorticosteroïden. Het is duidelijk dat niet iedere patiënt met COPD baat heeft bij deze medicijnen. Daarom is het belangrijk op zoek te gaan naar specifieke fenotypes, oftewel groepen patiënten met een gezamenlijk kenmerk, die hier wel beter van worden. Een fenotype staat niet vast. Patiënten kunnen in de loop van de tijd tot een bepaalde groep gaan horen zoals de groep met kenmerken van chronische bronchitis (hebben van veel hoesten en slijm). Anderzijds kan de wetenschap nieuwe inzichten geven waarbij bepaalde fenotypes toch baat hebben bij een bepaalde behandeling. In onderstaande Figuur 5 wordt dit schematisch weergegeven.

Figuur 5. Fenotypes in COPD: een dynamisch model



In de GLUCOLD studie kwam naar voren dat er een beter effect was op de achteruitgang van de longfunctie bij patiënten die minder gerookt hebben, met longen die beter zuurstof op kunnen nemen, minder hyperinflatie (vergrootte luchthoudendheid) hebben en minder ontstekingscellen in slijm hebben. Dit suggereert dat het beter werkt in een eerder stadium van de aandoening of bij

patiënten die minder last hebben van emfyseem ('rek uit de longen'). Hierbij is het belangrijk om te weten dat dit gold voor patiënten uit deze studie met matig ernstig COPD waarvan de meesten nooit eerder inhalatiecorticosteroïden hadden gebruikt.
Momenteel wordt de ernst van COPD gebaseerd op de longfunctie, luchtwegklachten en op toekomstig risico op exacerbaties. Echter, het is de vraag of dit de enige kenmerken zijn waar je op kunt varen. Het is de vraag of je moet wachten met behandelen tot er veel en onherstelbare veranderingen / beschadigingen zijn opgetreden in de longen of dat je eerder wilt behandelen om erger te voorkomen. Een andere grote studie, de 'Trial of Inhaled Steroids and Long-acting $ß_2$-agonists' (TRISTAN) studie liet zien dat het inderdaad belangrijk is om ook al in een eerder stadium in te grijpen met behandeling zodat er meer winst te behalen valt op de lange termijn. Onze studie liet zien dat patiënten met minder emfyseem meer baat hadden bij behandeling met inhalatiecorticosteroïden. De ECLIPSE studie liet zien dat emfyseem gemeten met een CT-scan geassocieerd was met een snellere achteruitgang van de longfunctie. Dit geeft aan dat het een fenotype is dat belangrijk kan zijn bij COPD. Inhalatiecorticosteroïden werken beter bij astma dan bij COPD. In onze studie zijn patiënten met astma nauwkeurig uitgesloten van deelname. Kenmerken van astma zijn bijvoorbeeld reversibiliteit (directe verbetering van de longfunctie na toedienen van een luchtwegverwijder), hyperreactiviteit (prikkelbaarheid van de longen), eosinofiele cellen in slijm. Deze kenmerken leken in onze studie onderling samen te hangen (**Hoofdstuk 2**). Je kunt je afvragen of de medicijnen beter werken bij COPD patiënten die ook enkele kenmerken hebben van astma. In onze studie waren astmakenmerken als reversibiliteit, hyperreactiviteit of eosinofiele cellen in slijm geen voorspellers van het effect op de snelle achteruitgang van de longfunctie. Onze resultaten wijzen eerder op een beter effect bij patiënten met een eerder stadium van COPD of fenotypes met minder emfyseem. Deze inzichten zouden opnieuw moeten worden getest in grotere groepen patiënten om te kunnen zeggen dat het voor alle patiënten met COPD geldt. Als onze resultaten worden bevestigd door andere studies zouden de richtlijnen verder uitgebreid kunnen worden met een potentieel optimistisch perspectief voor een deel van de patiënten met COPD.

Beperkingen van de GLUCOLD studie

In de wetenschap moet je kritisch naar resultaten kijken. Er zijn altijd beperkingen te bedenken, de wereld is niet zwart wit en er kan nooit een bewijs worden

gevonden voor een hypothese. De belangrijkste beperking in onze studie is mijns inziens de generaliseerbaarheid naar de praktijk. In de opzet van de studie wilden we primair het effect onderzoeken van inhalatiecorticosteroïden op de ontstekingscellen in slijm en biopten bij patiënten met matig ernstig COPD. Om dit goed te kunnen onderzoeken wilden we patiënten includeren die tevoren minstens 6 maanden geen inhalatiecorticosteroïden hadden gebruikt. In de praktijk bleken echter, ondanks de conservatieve richtlijnen met betrekking tot inhalatiecorticosteroïden, veel patiënten deze medicijnen toch in te nemen waardoor ze niet met het onderzoek mee mochten doen. Daardoor was het moeilijker om een grote groep patiënten te vinden die met ons onderzoek mee konden doen. Ook was het totaal aantal metingen in de GLUCOLD studie en de belasting van 3 bronchoscopiën per persoon een reden voor mensen om niet mee te willen doen.

Maar de studie heeft ons inziens ook hele sterke punten. De GLUCOLD studie is uniek om een aantal redenen. Het is de grootste biopten studie ter wereld, waarbij ontsteking is onderzocht op 3 momenten gedurende 2,5 jaar. Astma werd nauwkeurig uitgesloten. De patiënten zijn heel goed in kaart gebracht. Hierbij is aandacht gegeven aan veel klinische kenmerken als luchtwegklachten, kwaliteit van leven, longfunctie, maar ook aan meer ingewikkelde kenmerken als hyperreactiviteit, hyperinflatie, ontstekingscellen in slijm en luchtwegweefsel in biopten gedurende een lange onderzoeksperiode. Alle data zijn nagekeken, dubbel ingevoerd en gecheckt op inconsequenties. Het is ook belangrijk om te weten dat de studie is geïnitieerd, ontworpen, uitgevoerd en opgeschreven door wetenschappers zonder inmenging van de industrie (die de studie wel financieel heeft ondersteund, maar geen invloed had op de analyse).

Het is een belangrijke vraag in hoeverre je met resultaten van 114 patiënten een uitspraak kan doen over alle patiënten met matig ernstig COPD. Voor het aantonen van effecten op luchtwegklachten en kwaliteit van leven is het fijn als je een grote groep patiënten hebt. Als je geen effect kunt vinden kan dit komen doordat er te weinig mensen onderzocht zijn. Echter, als je wel effecten vindt, zoals in dit onderzoek beschreven, zijn de effecten sterk genoeg om zelfs in een kleine groep aantoonbaar te zijn. Daarbij is het van belang dat de effecten bij elkaar pasten en elkaar niet tegenspraken. Desondanks moet elke wetenschapper kritisch blijven en zal er meer onderzoek moeten volgen om onze resultaten te bevestigen. Pas daarna kunnen de huidige richtlijnen worden aangepast.

Conclusies

De patiënt met de chronische longziekte COPD die naar de dokter gaat, wil over het algemeen minder klachten en een voortschrijding van de aandoening voorkomen. Dokters zijn getraind om naar de patiënt en zijn aandoening als geheel te kijken. Dit is nog niet zo gemakkelijk. COPD is een zeer heterogene en complexe aandoening met kenmerken van klachten, ontsteking en schade in de longen. Sommige patiënten hebben veel klachten en toch maar 'mild' COPD, maar ook kan het zijn dat patiënten weinig klachten hebben bij ernstig COPD. Dit houdt in dat we niet alleen af moeten gaan op de klachten die een patiënt heeft, maar verder moeten kijken naar de onderliggende aandoening. Naast luchtwegklachten zouden we bij een deel van de patiënten ook de longfunctie moeten meten, hyperinflatie of zelfs ontsteking op een zo min mogelijk invasieve manier, ook bij patiënten in een eerder stadium van de ziekte. De unieke setting van de GLUCOLD studie heeft hier een stap in gezet door matig ernstige COPD patiënten zeer gedetailleerd in kaart te brengen en te onderzoeken wat de effecten van behandeling zijn. Hierbij blijken in de onderzochte onderzoeksgroep bepaalde patiënten met COPD met minder gevorderd ziektebeeld of fenotypes met minder emfyseem meer baat te hebben van inhalatiecorticosteroïden. Dit geeft een hoopvol perspectief voor een deel van de patiënten met COPD op het doen afremmen van de voortgang van de aandoening in de toekomst.

Wat betekent dit voor de dagelijkse praktijk?

De patiënt staat centraal. De huisartspraktijk is een van de mogelijkheden om dit te realiseren. Een huisarts staat dicht bij de patiënt en kan als een spin in het web fungeren om de patiënt te begeleiden bij een chronische aandoening. Met wetenschap zijn we op zoek naar antwoorden die betrekking hebben op ziekte en behandeling. De vraag is: hoe kunnen we een brug slaan tussen wetenschap en wat belangrijk is voor de patiënt? Wellicht zullen we ons in de toekomst minder richten op specifieke diagnoses, maar meer op unieke vingerafdrukken van bijvoorbeeld ontsteking, het mRNA of DNA. Echter, zover zijn we nog lang niet. Hoe kunnen we de huidige kennis uit de wetenschap dan toepassen in de praktijk? Is het voldoende om patiënten ad hoc te behandelen op basis van luchtwegklachten? Of moeten we verder kijken en ons voordeel doen met kennis uit de wetenschap en meer gebruik maken van profielen zoals die nu onderzocht worden in meerdere studies. Eerder behandelen zou schade kunnen voorkomen. Vandaar dat voorspelling van gunstige effecten van behandeling bij COPD voor de praktijk zo belangrijk is. Deze twee zaken

lijken vanzelfsprekend, maar het is nog wel de vraag hoe je dit moet realiseren. Meten is weten, maar het is eenvoudigweg veel te kostbaar om alles te meten bij alle patiënten. Het is een enorme uitdaging voor de wetenschap, zorgverleners en patiënten samen om precies te weten welke patiënten in een vroeg stadium van de ziekte veel metingen nodig hebben of behandeling vergen om erger te voorkomen.

Perspectief van de auteur

Het is een uitdaging om niet alleen met deze nieuwe informatie over COPD en de behandeling in de praktijk te werken, maar ook in het algemeen om bij te dragen aan het slaan van een brug tussen de nieuwste resultaten van wetenschappelijk onderzoek en de dagelijkse (huisartsen)praktijk. Op dit moment werk ik als huisarts in een huisartsenpraktijk in Den Haag en als onderzoeker verbonden aan het LUMC in Leiden. In deze huisartsenpraktijk hebben ongeveer 1000 patiënten een diagnose astma of COPD (op een totaal van 10.500 ingeschreven patiënten). Samen met longartsen willen we graag niet alleen de huidige behandeling, maar ook de organisatie van de behandeling van deze patiënten nog verder verbeteren. Hiervoor zijn we een nieuw samenwerkingsproject gestart, waarbij ook de Zorggroep een rol speelt. Hierbij willen we schotten weghalen tussen eerste en tweede lijn en goed uitzoeken welke patiënt bij welke behandelaar hoort. Zoveel als mogelijk zullen we hierbij gebruik maken van fenotypering, maar ook van innovatieve e-health instrumenten. De toekomst zal moeten uitwijzen of dergelijke projecten worden tegengehouden of juist gestimuleerd en gefinancierd door de overheid of zorgverzekeraars. In het huidige project zal een longverpleegkundige vragenlijsten afnemen en longfuncties meten. Via teleconsultatie wordt advies gevraagd aan de longarts. Het is hierbij de bedoeling patiënten beter in kaart te brengen, zodat ze beter behandeld kunnen worden en alleen patiënten die het echt nodig hebben worden verwezen naar het ziekenhuis. Daarnaast zullen patiënten de mogelijkheid hebben hun astma/COPD te monitoren met behulp van een zelfmanagement programma via internet, genaamd www.PatientCoach.nl. Dit programma is ontwikkeld in Leiden om patiënten beter te kunnen begeleiden, is geïntegreerd met registratiesystemen van de huisarts om beter te kunnen communiceren en is tevens bedoeld voor wetenschappelijk onderzoek.

Momenteel wordt het programma geïmplementeerd bij huisartsen in de regio Leiden en Den Haag bij patiënten met matig ernstig astma (IMPLementation strategies of internet-based Asthma Selfmanagement Support in usual

care (IMPASSE). Patiënten kunnen hierbij hun eigen luchtwegklachten en longfunctie thuis monitoren met zo nodig advies om hun medicatie aan te passen volgens de huidige richtlijnen. Daarnaast zijn we gestart met de “Pulmonary Rehabilitation of Asthma and COPD: a Trial of sustained Internet-based Self-management Support (PRACTISS) studie”; het doel hierbij is om patiënten met astma in Davos en COPD in het Rijnlands Revalidatiecentrum na revalidatie thuis verder te begeleiden.
Naast bovenstaande werkzaamheden neem ik sinds kort deel aan de kerngroep en de wetenschapscommissie van de landelijke “COPD & Astma Huisartsen Advies Groep” (www.cahag.nl).
De meest eenvoudige reden om al deze moeite te doen is zorg voor chronische longziekten te verbeteren en de enorme impact die dit voor patiënten heeft in hun dagelijkse leven te verminderen, gecombineerd met zo laag mogelijke kosten. Door de zorg slim te structureren, willen we niet alles meten bij iedereen, maar ons richten op fenotypering van patiënten en het stellen van individuele doelen. Met behulp van wetenschap op nieuwe terreinen met min of meer eenvoudige hulpmiddelen als een ‘elektronische neus’ of e-health zal dit in de toekomst verder moeten worden onderbouwd. Uiteindelijk kan dit leiden naar geïntegreerde zorg, individuele zorg, verbeterde kwaliteit van leven, efficiëntere kostenbesteding en mogelijk het doen afremmen van de voortgang van chronische aandoeningen als COPD.

Curriculum Vitae

Jiska Snoeck-Stroband is geboren op 5 oktober 1971 te Delft. Zij groeide op in Bilthoven als oudste in een gezin met 3 kinderen. In 1990 deed ze eindexamen Gymnasium aan het Nieuwe Lyceum te Bilthoven. Aansluitend begon zij de studie Geneeskunde aan de Rijksuniversiteit te Leiden. Tijdens de studie heeft ze een klinische stage Orthopedie gedaan in de praktijk van dr. C. de Bree in Neukirchen, Duitsland en een stage bij huisarts M.H.M. de Meijer in Cadzand in Zeeland. Na het doctoraal examen heeft zij gedurende een half jaar een klinische stage Interne Geneeskunde verricht onder begeleiding van Dr G. Geyskes in het ziekenhuis Nieuw Nickerie in de republiek Suriname. In 1998 behaalde ze haar artsexamen.

Hierna startte ze haar promotieonderzoek bij de afdelingen Public health en Eerstelijnsgeneeskunde, Longziekten en Medische Besliskunde in het Leids Universitair Medisch Centrum (LUMC) te Leiden. Gedurende de daarop volgende jaren bekwaamde ze zich in het verrichten van onderzoek middels het volgen van symposia en congressen. Daarnaast volgde ze diverse epidemiologische cursussen in het LUMC, te Schiermonnikoog, aan het VU Medisch Centrum in Amsterdam en de Erasmus Universiteit van Rotterdam.
Van 2003 tot 2008 volgde zij de opleiding Huisartsgeneeskunde in het LUMC te Leiden. Aansluitend heeft ze een inventarisatie gemaakt van ‘Evidence Based Medicine’ in de huisartsopleiding in Nederland aan de afdeling Public health en Eerstelijnsgeneeskunde te Leiden.

Vanaf 2008 heeft ze verschillende functies en betrekkingen. Ze werkt parttime als huisarts in huisartspraktijk ‘Akelei’ te Den Haag (http://www.akeleihuisartsenzorg.nl). Tevens werkt ze als onderzoeker bij de afdeling Medische Besliskunde in het LUMC te Leiden. Hier werkt ze als coördinator van multicenteronderzoek en begeleider van fellows bij diverse promotieonderzoeken naar astma en COPD, onder andere naar de ontwikkeling van een internetbased programma voor zelfmanagement bij astma en COPD (www.PatientCoach.nl). In de regio ’s-Gravenhage houdt ze zich bezig met astma en COPD. Ze neemt deel aan de werkgroep COPD van de Transmurale Stichting te Den Haag (http://www.transmuralezorg.nl). Met de afdeling Longziekten van het HAGA ziekenhuis en de zorggroep ELZHA werkt ze samen om de zorg van astma en COPD te verbeteren. Jiska neemt deel aan de COPD en Astma Huisartsen Advies Groep (CAHAG) en

zal zich de komende jaren meer gaan richten op taken van de kerngroep en wetenschapscommissie.

Zij is getrouwd met Jean-Philippe, werkzaam bij de ING als compliance officer en samen hebben zij 3 kinderen gekregen, Sebastiaan (2005), Duco (2008) en Philippine (2010).

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Met groot enthousiasme hebben velen bijgedragen aan het tot stand komen van dit proefschrift. Iedereen die hieraan mee heeft gewerkt, heel hartelijk bedankt. Enkelen wil ik hier persoonlijk bedanken.

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Pieter, heel hartelijk dank voor al je ondersteuning. Heel fijn hoe je open staat voor elke vorm van discussie en voor de huisartsgeneeskunde in het bijzonder.
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José, sinds lange tijd ben je mijn vriendin en net als Therese mijn paranimf, maar dan wel officieel. Ik vind het heel bijzonder dat we zo lange tijd samen onderzoek hebben gedaan. Ik heb genoten van de congressen die we samen hebben meegemaakt en de vriendschap die we met Thérèse delen.

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