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**Author:** Snoeck-Stroband, Jiska Bouwien  
**Title:** Towards clinical phenotyping of COPD: effects of inhaled corticosteroids in the GLUCOLD study  
**Date:** 2012-09-12
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\textsubscript{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\textsubscript{1}) rather than FEV\textsubscript{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\textsubscript{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

- **Genetic**
- **Environmental exposures**
  - Smoke, respiratory pathogens

**Inflammation and immunity**
- Inflammatory cells: macrophages, T lymphocytes, neutrophils, eosinophils, B cells, mast cells
- Inflammatory mediators

**Structural changes**
- Large and small airways: goblet cells, ciliary damage, squamous cells, smooth muscle, fibrosis
- Lungparenchyma: emphysema

**Functional consequences**
- Mucus hypersecretion; ciliary dysfunction; airways obstruction; hyperinflation; pulmonary hypertension; heart failure

Symptoms
- Dyspnea
- Cough and sputum
- Exacerbations
- Limitation of activities, co-morbidity

Health status
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
Chapter 1  •  General introduction and aims of the studies

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.

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

Frequent exacerbations

Severe airflow limitation

Few symptoms

Many symptoms

≤ 1 exacerbation per year

Mild or moderate airflow limitation
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 then 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 FEV₁ 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 FEV₁ [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 FEV₁ decline has been underestimated due to drop-out of patients with the most rapid FEV₁ 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\textsubscript{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\textsubscript{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\textsubscript{1} decline in COPD showing that discontinuation resulted in a decrease in FEV\textsubscript{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\textsubscript{1}, either or not associated with a relapse in airway hyperresponsiveness.

At present, long-acting β\textsubscript{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\textsubscript{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 $\beta_2$-agonist. The
present studies include investigations on health status, the presence of chronic
bronchitis, long-term effects of inhaled corticosteroid therapy and long-acting
$\beta_2$-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 self-reported 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 FEV₁ below the 90% confidence interval (90% CI) of the predicted FEV₁, and postbronchodilator FEV₁/IVC ratio below the 90% CI of the predicted FEV₁/IVC ratio [107]
6. Postbronchodilator FEV₁ > 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 O₂ 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.
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 FEV₁; 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; PC₂₀ 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 β₂-agonists
Chapter 5. Do patients benefit from short- versus long-term therapy with inhaled corticosteroids and long-acting β₂-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 β₂-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 β₂-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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