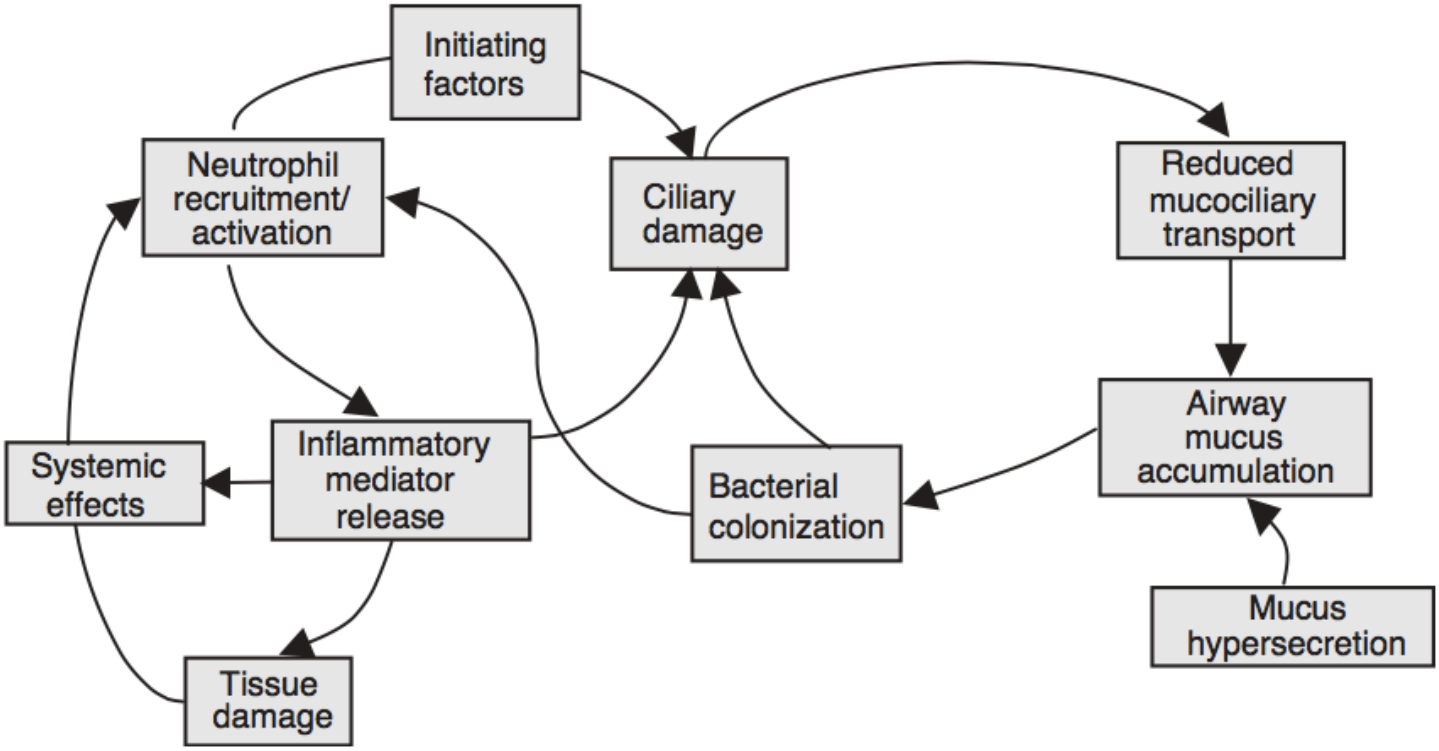
Inhaled Corticosteroids in the Treatment of COPD: The Risk of Developing Pneumonia



Source: Agusti, A. G. N. "COPD, a multicomponent disease: implications for management." *Respiratory medicine* 99.6 (2005): 670-682.

Master Thesis

Name	**Corine Habraken**
Student number	**s1860356**
Date	**30-06-2015**
Supervisor	**Dr. H.I. Heijink**
Group	**EXPIRE**

Abstract

Inhaled corticosteroids (ICS) are widely used in the treatment of chronic obstructive pulmonary disease (COPD) due to their ability to improve quality of life and to reduce the frequency of exacerbations in a subset of patients. In other subsets of stable COPD patients, however, the use of ICS monotherapy does not appear to impede the progressive decline in lung function and has small and inconsistent effects on symptoms, quality of life, and the severity of exacerbations. Long-term use of ICS may cause systemic adverse effects, including diabetes, cataracts, and osteoporosis. Moreover, several clinical trials have suggested a link between the use of ICS, especially fluticasone propionate (FP), and an elevated risk of developing pneumonia. It remains uncertain whether this risk is similar for all ICS and whether it is dose-related, although the risk appears to be particularly increased using high doses and shorter durations. This thesis aims to provide an answer to the questions of whether ICS monotherapy and/or ICS/LABA combination treatment provide a beneficial addition to standard care in COPD, whether differences with regard to the effectiveness of monotherapy versus combination treatment exist and if differences between specific drugs and/or drug combinations exist, and whether this is the case for all patients or if subsets of patients should be distinguished. In order to answer these questions, currently available literature was reviewed.
As the use of ICS is associated with severe systemic side effects, high doses of ICS should only be prescribed to patients with severe COPD. Since ICS and LABA have additive effects over each other, combining treatment may lower the dose of the ICS needed in order to achieve a beneficial effect and thus reduce these adverse effects. Long-term treatment of COPD patients with budesonide/formoterol (FBC; formoterol/budesonide combination treatment) resulted in fewer exacerbations than long-term treatment with salmeterol/fluticasone (SFC; salmeterol/fluticasone combination treatment). Additionally, FP is thought to increase the occurrence of exacerbations and the risk of developing pneumonia. Therefore, the prescription of FBC appears favourable over the prescription of SFC for the treatment of COPD. As no studies comparing different doses of ICS/LABA combination treatment have yet been performed, this is an interesting topic for future research. In addition, treatment of COPD patients may be optimized by distinguishing several phenotypes of the disease and by adjusting treatment to the specific needs of the different subsets of COPD patients.

Table of Contents

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a progressive pulmonary disease that is characterized by mostly non-reversible limitation of airflow, affecting primarily middle-aged and elderly individuals.[1,2] COPD is an inflammatory disease that affects both the lungs and other organs, for instance resulting in an increased risk of atherosclerosis in patients.[3] In addition, cardiac disease and other comorbidities are associated with COPD, such as diabetes, rheumatoid arthritis, and osteoporosis.[4] Airflow limitation in COPD patients is caused by local inflammation, destroying the parenchyma and resulting in remodelling of the airways. During this process, alveolar attachments to the small airways are lost, decreasing elastic recoil in the lungs and resulting in a decreased ability of the airways to open during expiration.[1]

The main mechanisms resulting in limitation of airflow are narrowing of the small airways due to remodelling of the airways and emphysema with lung tissue destruction caused by persistent inflammation, protease-antiprotease imbalance, and oxidative stress.[5] In circa 90% of cases, COPD is caused by exposure of the lungs to cigarette smoke, although other environmental insults[1] such as wood smoke or occupational exposures,[5] also present a major risk factor,[1] Exposure to other risk factors in addition to tobacco smoke has a cumulative effect. However, genetic factors are also involved in the pathogenesis of COPD, as potential cumulative effects of environmental insults do not explain differences seen between smokers with COPD in the rate of forced expiratory volume in one second (FEV_1)-decline.[6]

Airway inflammation in COPD patients is dominated by neutrophils,[2] although macrophages and lymphocytes also play important roles in this disease.[7] Chronic exposure of the lungs to reactive oxygen species (ROS) due to smoking causes oxidative stress and injury, triggering the production of other ROS and lipid peroxidation.[5] Neutrophils are an important source of ROS, inflammatory cytokines, and enzymes capable of damaging tissue, thus playing an important role in mucus hypersecretion and the destruction of lung tissue in emphysema.[7] Additionally, neutrophils are thought to play a crucial role in the protease/antiprotease imbalance in the lungs of COPD patients, although this imbalance is likely the result of coordinated action between, amongst others, neutrophils and macrophages, which can activate or inactivate each other.[8] Macrophages secrete an array of compounds, including ROS, chemotactic factors, inflammatory cytokines, and matrix metalloprotease enzymes (MMPs).[7] MMPs are thought to facilitate leukocyte migration and infiltration into injured tissues,[9] thereby facilitating parenchymal destruction by $CD8^+$ T-cells and resulting in emphysema.[7] The pulmonary inflammation observed in COPD patients increases with disease severity.[10] Moreover, Sin et al. showed that the severity of COPD is associated with increased systemic inflammation.[3]

Epithelial cells and alveolar macrophages (AMs) provide the first line of defence against inhaled, potentially harmful, environmental agents. They are thought to play an important role in the aberrant inflammatory response to cigarette smoke, as they both have the capacity to recruit inflammatory cells to the airways by secreting chemoattractants. In addition, the epithelium can direct the migration of inflammatory cells through the expression and induction of cell-surface molecules[11] such as α-integrins and intercellular adhesion molecule (ICAM)-1, which play a role in the recruitment of inflammatory cells to the epithelium.[12] A major role in airway inflammation in COPD is played by elevated levels of interleukin (IL)-6 and -8, and tumour necrosis factor (TNF)-α, which mediate various pro-inflammatory processes important in airway inflammation. Whilst TNF-α is mainly secreted by AMs, both epithelial cells and AMs produce IL-6 and -8. TNF-α acts as an activator of neutrophils, T-cells, and macrophages and, like IL-6, induces the production of acute-phase proteins. IL-6 induces the activation and differentiation of T-cells.[11] IL-8 is known to act as a chemoattractant for neutrophils, T-cells,[13] and primed eosinophils.[14]

Upon chronic exposure to smoke, accumulation of macrophages, neutrophils, and CD8+ T-cells in the lungs occurs. Being localized to sites of alveolar destruction,[15] the inflammatory mediators and enzymes released by these cells are known to interact with airway structural cells, lung parenchyma and lung vasculature, inducing structural changes and amplifying the

inflammatory process.[16] The proteinases released by macrophages and neutrophils are capable of activating each other, of inhibiting their endogenous inhibitors, and of cleaving components of the extracellular matrix.[5] For example, the potent elastolytic enzyme neutrophil elastase[17] is also capable of inhibiting the tissue inhibitors of MMPs (TIMPs), enabling MMPs to cleave components of the extracellular matrix, elastin fibres and collagen.[5] Strong evidence supports the notion that severe deficiency of α_1-antitrypsin, the main inhibitor of neutrophil elastase, plays an important role in the pathogenic mechanism involved in emphysema. In patients with this deficiency, only 15-20% of normal anti-elastase protection in the interstitium of the lungs and alveolar space is present.[17] The elastin fragments and collagen-derived peptides that are generated are known to act as chemotactic factors for monocytes; the precursor for macrophages and neutrophils. Therefore, chemotactic peptides play an important role in macrophage and neutrophil accumulation and the destruction of pulmonary tissue.[5] Although only a small part of COPD patients suffer from this deficiency, it illustrates the relevance of the interaction between genes and environment leading to COPD.[16] Other examples of genetic factors that may play a role in COPD are a SNP in *MMP12* that has been shown to protect lung function and reduce the risk of COPD in adult smokers, and a SNP in *MMP9* that is associated with the development of emphysema induced by smoking.[5]

Treatment

Upon the diagnosis of moderate COPD, the Global Initiative on Obstructive Lung Disease (GOLD) and the National Institute of Health and Clinical Excellence (NICE) guidelines recommend the use of long-acting β_2 agonists (LABA) or long-acting muscarinic agonists (LAMA) in addition to the use of short acting bronchodilators for the treatment of COPD. Upon progression to severe COPD, i.e. in patients whose FEV_1 is lower than 50% of the predicted value, who remain symptomatic in spite of long-acting bronchodilator treatment, and who have two or more exacerbations that require antibiotic or oral corticosteroid treatment within one year, the addition of inhaled corticosteroids (ICS) or another long-acting bronchodilator is recommended.[1,18] ICS have fewer adverse effects than oral corticosteroids.[2] However, the use of ICS remains a controversial issue in the treatment of COPD, as evidence of a reduction of the predominantly neutrophilic inflammation seen in COPD is lacking.[2,19] Moreover, the chronic use of ICS has been implicated in the elevated risk of pneumonia seen in patients. This especially holds true for therapy including fluticasone,[4] suggesting that different ICS have different pharmacological effects.[20] See figure 1 for an overview of currently used medications for COPD and their function in treating different aspects of this disease.

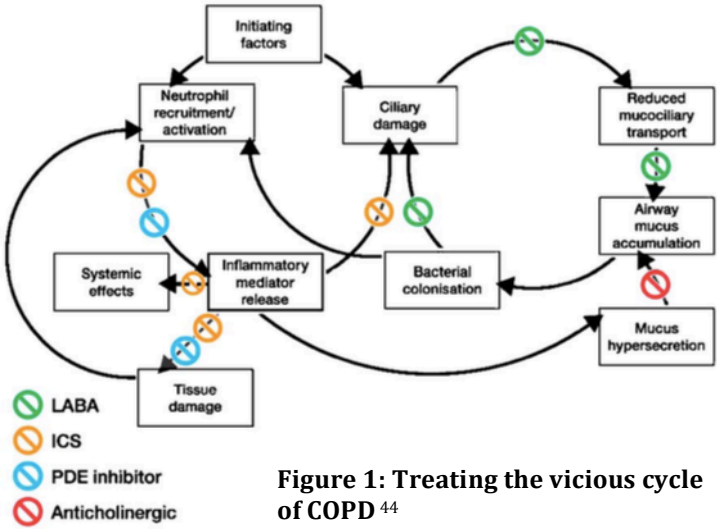


Figure 1: Treating the vicious cycle of COPD[44]

Exacerbations

The clinical course of COPD is likely influenced by the frequency of exacerbations, which are defined as events in the natural course of the disease that are characterized by a change in the patient's baseline dyspnoea, cough, and/or sputum production beyond normal day-to-day variations. Exacerbations are acute in onset, and changes in regular medication may be necessary.[21] Exacerbations of COPD are associated with increased airway inflammation, declined lung function, and increased mortality.[22] Exacerbations are often caused by infections, either viral or bacterial, and inhalation of irritants.[1] However, often a specific cause cannot be

identified and many exacerbations are related to non-adherence to medication intake. [1,21] Exacerbations may be treated in the outpatient setting or, in case of severe exacerbation, in hospital. Successful outpatient care generally comprises increased doses and/or frequencies of the intake of bronchodilators and the initiation of systemic corticosteroid treatment. Antimicrobials are administered during severe exacerbations when patients show clinical signs of bacterial infection, characterized by increased dyspnoea and increased sputum volume and purulence. [16,21] The number of previous exacerbations and disease severity are predictive factors of exacerbation frequency, and intervention may affect both the severity and frequency of exacerbations. [23]
It has been suggested that, in addition to neutrophilic inflammation, eosinophilic inflammation of the airways may play a role in the development of especially more severe exacerbations. Increased numbers of eosinophils have been found in sputum and bronchial biopsies acquired during exacerbation, [24] and increased mortality has been linked to blood eosinophilia. [22] Tdhe positive effects of corticosteroids on the treatment and prevention of COPD may therefore be due to modulation of eosinophilic inflammation, since the effects of corticosteroids on neutrophilic airway inflammation are unclear. [22] Additionally, a subset of COPD patients with eosinophilic inflammation, during stable disease or exacerbation, has been identified, [25,26] and it is speculated that corticosteroid treatment is most effective in this subset of patients. [19] For instance, it was shown that patients with eosinophilia in sputum showed a greater improvement in FEV_1 and health status following oral prednisolone treatment than placebo. [19]

The main objective of this thesis is to review available literature on the use of ICS monotherapy and ICS/LABA combination therapy in COPD and their potential effects on the risk of developing pneumonia. The aim is to provide an answer to the questions of whether these treatments provide a beneficial addition to standard care in COPD, whether differences with regard to the effectiveness of monotherapy versus combination treatment exist and if differences between specific drugs and/or drug combinations exist, and whether this is the case for all patients or if subsets of patients should be distinguished.
Several clinical trials, including the Towards a Revolution in COPD Health (TORCH) [27] and Investigating New Standards for Prophylaxis in Reduction of Exacerbations (INSPIRE) [28] trials, have found a relation between the use of FP and an increased risk of pneumonia. This suggests that differences exist between different ICS and that the use of some ICS is preferable over the use of others. Since it was shown that the addition of a LABA resulted in fewer exacerbations than monotherapy, it is hypothesized that combination treatment may be more effective in the treatment of COPD than monotherapy. [29] Moreover, since the eosinophilic subset of patients is known to respond well to corticosteroid treatment, [19] it is possible that more subsets can be distinguished that would benefit from personalised treatment.

Inhaled Corticosteroids

Enhanced chronic inflammatory responses induce structural changes with mucus hypersecretion and narrowing of the small airways of COPD patients, which result in limitation of airflow that is persistent and usually progressive. [16] ICS are the most effective anti-inflammatory agents used to treat diseases of the airways, such as asthma, as they are capable of suppressing the inflammatory response by exerting their effects on inflammatory cells and pathways involved in disease. [18] The use of anti-inflammatory drugs has little to no effect on the rate of decline in lung function of COPD patients, [30] although it may reduce the frequency of exacerbations, [30] especially when treatment is combined with an inhaled LABA. [27] ICS are usually prescribed to patients with more severe disease. [18]

Mechanism of Action

Corticosteroids, also known as (glucocortico)steroids, or glucocorticoids are capable of influencing the inflammatory response by acting through several mechanisms [31] dependent and independent of DNA-binding. [18] Mechanisms dependent of DNA-binding include *trans*-activation (induction) and *trans*-repression (suppression) of gene transcription. Examples of genes undergoing *trans*-activation include the inhibitor of nuclear factor (NF)-κB (IκB)-α pathway, mitogen-activated protein kinase (MAPK) phosphatase (MKP)-1 and the expression of anti-inflammatory or inhibitory cytokines, such as IL-10 and IL-12. Genes undergoing *trans*-repression upon corticosteroid treatment include genes that encode inflammatory cytokines such as IL-6 and TNF-α, chemokines such as chemokine (C-C motif) ligand (CCL)1 and CCL-5, and inflammatory enzymes and peptides (e.g. endothelin-1). [31]

Corticosteroids exert their effects by diffusing across the cell membrane and binding to the cytoplasmic glucocorticoid receptors (GRs) of target cells. [31] Almost all cell types express GRs and their density varies from 200 to 30 000 per cell. [10] Upon binding to the ligand, GRs are activated and dissociate from their chaperone proteins, such as heat shock protein-90 (HSP-90). Subsequent translocation to the nucleus involves nuclear import proteins importin-α and importin-13. [31] GRs dimerise in the promoter region of corticosteroid-responsive genes and bind to glucocorticoid response elements (GREs). This way, structures are formed that allow for enhanced or, occasionally, repressed gene transcription. [31] The specific ligand, number of GREs, and the position of the GREs in relation to the transcriptional start site influence the magnitude of the transcriptional response to corticosteroids. [10]

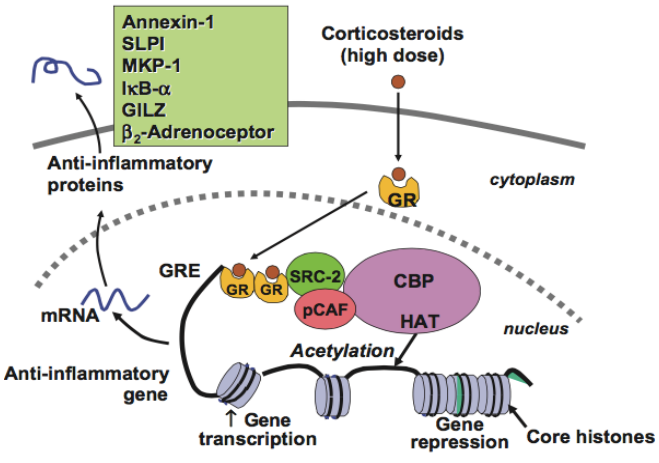


Figure 2: [31] **Mechanisms of *trans*-activation by the GR.** Upon binding of corticosteroids to the cytoplasmic glucocorticoid receptors (GRs), GRs translocate to the nucleus, where they bind glucocorticoid response elements (GREs) in the promoter region of steroid-sensitive genes. Additionally, direct or indirect binding of co-activator molecules with intrinsic histone acetyltransferase (HAT) activity, such as CREB-binding protein (CBP), occurs. Subsequently, lysines on histone H4 are acetylated, leading to the activation of genes that encode anti-inflammatory proteins such as mitogen-activated kinase phosphatase (MKP)-1. [31]

Trans-Activation

DNA is tightly packed around a protein core consisting of nucleosomes, forming a chromatin structure. Nucleosomes consist of an octamer of two of each core histone proteins H2A, H2B, H3, and H4, and are surrounded by 146 base pairs of DNA. Gene expression and repression are induced by enzymatic modifications of core histones. Core histones may undergo post-translational modifications such as acetylation, methylation, ubiquitination, or

phosphorylation of specific residues within the N-terminal tails, i.e. lysine, arginine, and serine, affecting gene expression. [10] Corticosteroid-responsive genes are activated through an interaction between the DNA-bound GR and transcriptional co-activator molecules, such as cAMP response element-binding protein (CREB)-binding protein (CBP) and steroid receptor coactivator-1 (SRC-1), which induce acetylation of core histones, in particular H4, through their intrinsic histone acetyltransferase (HAT) activity (figure 2). Chromatin remodelling engines are then recruited to tagged histones and subsequent association of RNA polymerase II results in activation of the gene.

GR's mechanism of action is similar to that of other transcription factors; it increases gene transcription by acting on chromatin remodelling and the recruitment of RNA polymerase II to the site of local DNA unwinding due to acetylation of lysines. [10] Corticosteroids exert part of their anti-inflammatory effects through the activation of genes, such as those that encode β_2-adrenergic receptors (β_2-AR) and MKP-1, which inhibits MAPK pathways.

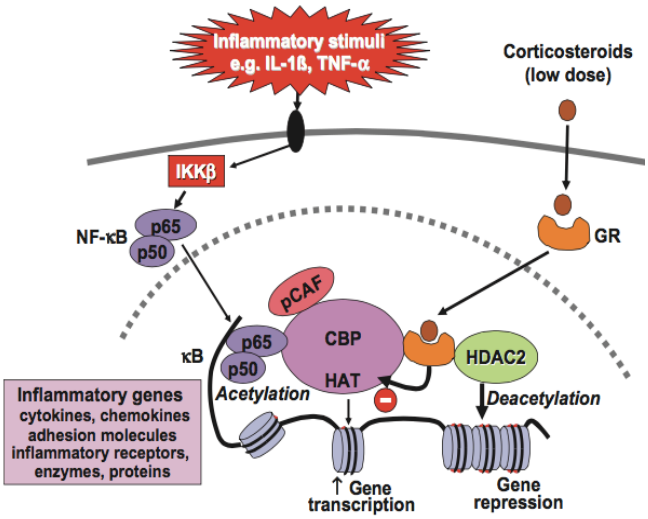


Figure 3: [31] **Mechanisms of *trans*-repression by the GR.** Inflammatory stimuli, such as interleukin (IL)-1β or tumour necrosis factor (TNF)-α, activate inflammatory genes, resulting in the activation of inhibitor of IκB kinase-β (IKKβ). IKKβ activates the transcription factor nuclear factor κB (NF-κB). A dimer of p50 and p65 of NF-κB proteins then translocates to the nucleus, where it binds to specific recognition sites and to co-activators with intrinsic histone acetyltransferase (HAT activity), such as CREB-binding protein (CBP). This results in the acetylation of histone H4 and thus in increased expression of multiple inflammatory proteins. Upon activation by corticosteroids, GRs translocate to the nucleus, where they bind to co-activators to directly inhibit HAT activity and to recruit histone deacetylase (HDAC)-2, which suppresses activated inflammatory genes by reversing histone acetylation.[31]

Trans-Repression

Whilst increased gene transcription is associated with increased acetylation of histones, reduced transcription and gene silencing are correlated with hypoacetylation induced by histone deacetylases (HDAC). [10] In fact, repression of inflammatory genes represents the major anti-inflammatory mechanism exerted by corticosteroids. This inhibitory effect likely occurs mainly through an interaction between activated GRs and pro-inflammatory transcription factors like NF-κB. [31]

As mentioned before, activation of cytoplasmic GRs results in translocation to the nucleus. In the nucleus, monomeric GR can bind directly or indirectly to the transcription factors activating protein (AP)-1 and NF-κB. Thereby, the ability of these transcription factors to switch on gene expression is inhibited. [10,32] Alternatively, dimerised GRs can inhibit inflammatory gene expression by binding to a GRE that overlaps the DNA-binding site for a pro-inflammatory transcription factor or by binding to the start site of transcription. [10,32] Another mechanism through which inflammatory gene expression can be repressed by corticosteroids is through the recruitment of histone deacetylase-2 (HDAC2) to the activated inflammatory gene complex by activated GRs, which results in the suppression of activated inflammatory genes by reversing acetylation of histones (figure 3). [31]

Post-Transcriptional Modifications

Stimulation of cells by inflammatory mediators may stabilize unstable messenger RNA (mRNA) that is usually degraded rapidly by certain RNAses, as is the case for several pro-inflammatory genes including TNF-α. [31] Exposure to corticosteroids can reverse this effect, which results in

rapid degradation of mRNA and a reduction in the secretion of inflammatory proteins. This process is thought to take place through increased expression of proteins that destabilize the mRNA of inflammatory proteins. An example of this is the zinc finger protein tristetraprolin, which is capable of binding the 3' AU-rich untranslated region of mRNAs. [31,33]

ICS Treatment in COPD

The effects of corticosteroid treatment on lower airway inflammation are controversial, as a clear clinical response is lacking and both treatment with inhaled or oral corticosteroids fail to reduce the numbers of inflammatory cells and the amount of cytokines, chemokines, and/or proteases in COPD patients' induced sputum or airway biopsies. [10] The pulmonary inflammation seen in COPD patients is relatively resistant to corticosteroid treatment and long-term treatment with a high dose of ICS increases the risk of several long-term side effects such as osteoporosis, diabetes, cataracts, hypertension, and pneumonia. The GOLD guidelines therefore currently state that high doses of ICS are only suitable for symptomatic patients with severe to very severe disease, i.e. with an FEV_1 <50% of predicted, and those who experience frequent exacerbations. [34] However, only circa 10% of patients meets these criteria, yet currently approximately 80% of patients with a clinical diagnosis of COPD receive treatment with a high dose of ICS, increasing the risk of the mentioned side effects in these patients.

Differences Between Fluticasone Propionate and Budesonide

Several ICS are currently available, including fluticasone propionate (FP), budesonide (BUD), beclomethasone dipropionate (BDP), ciclesonide (CIC), flunisolide (FLU), and mometasonefuroate (MF). FP and MF have the highest binding affinity for the GR, followed by BUD and the rest. BDP and FLU have high systemic bioavailability, which is low for BUD and negligible FP, MF, and CIC. Due to their high liposolubility, FP and CIC have a large volume of distribution, whilst BUD, MF, and BDP have intermediate volumes of distribution. Calculations of equivalent doses used in clinical trials are based on the binding affinity and the percentage of lung delivery obtained with different administration forms, such as metered-dose inhalers (MDI) or dry powder inhalers (DPI). [18] Currently, the only combination therapies containing an ICS and a LABA licenced for the treatment of COPD are salmeterol/fluticasone (SFC; salmeterol/fluticasone combination treatment) and formoterol/budesonide (FBC; formoterol/budesonide combination treatment). Therefore, the focus of this thesis will lie on the ICS fluticasone and budesonide.

Although both SFC and FBC contain an ICS and a LABA, the pharmacokinetic and pharmacodynamics properties of the components differ. The bioavailability and clearance, the volume of distribution, and the rate at which the drug is taken up in the airways determine the clinical efficacy and safety of these components. For instance, BUD is less lipophilic than FP and therefore dissolves in the airway mucus more easily and is taken up quicker by the airway tissue and into the systemic circulation. Due to its lipophilic properties, FP is retained in the lumen of the airways, increasing its chance of being removed from the airways by mucociliary clearance and cough. Therefore, the marked airflow obstruction seen in patients with severe COPD leads to greater proximal deposition of the inhaled drugs and, thus, greater mucociliary clearance, [35] resulting in lower drug penetration and deposition due to higher airway resistance. [18] Studies in patients with asthma and airflow obstruction showed that the systemic exposure to BUD is less affected by lung function than that of FP, [35] suggesting that higher doses of FP are needed to achieve the same effect. More importantly, other consequences of FP's higher lipophilicity are its larger volume of distribution and higher retention time in the lungs irrespective of patients' lung function. As a result, FP remains in the mucus for a longer period and requires more time to dissolve than BUD, [18] resulting in slower uptake into the systemic circulation. [35] In turn, the local activity of FP is high and its duration of action is long due to its pharmacological properties. BUD's long duration of action, which has been shown during clinical trials, results from a

different mechanism, namely active intracellular esterification and deposition, with lipolysis of the drug resulting in prolonged release.[18] Additionally, Borchard et al. showed that transport of BUD by human bronchial epithelial cells (Calu-3 cells) is concentration-dependent, taking place throughout the entire 10-hour measurement period. Additionally, it was shown that release of BUD to the apical side was almost twice as high as basolateral release (6.2% versus 3.2%, respectively). FP was released from the cells quickly and the release of FP did not differ significantly between the apical and basolateral compartments. It was therefore concluded that BUD is retained within airway epithelial cells by conjugation to fatty acids, increasing the duration of its pharmacological effects. FP does not undergo this conjugation, and its prolonged duration of action is therefore ascribed to its lipophilicity and retention in the airway mucus.[36] BUD and FP both have high affinity for the GR, high first-pass inactivation in the liver, and show prolonged binding to target tissue; properties that favour the reduction of systemic side effects.[36]

Effects of ICS Treatment

Ek et al. showed that BUD and FP inhibited the production of IL-6 and IL-8 by the A549 lung epithelial cell line as compared to untreated cells. Additionally, the production of IL-6, IL-8, and TNF-α by AMs induced by LPS was inhibited after exposure to BUD and FP in a dose-dependent manner. Pre-incubation with either steroid was unnecessary to achieve maximal inhibitory effects, suggesting that the onset of action is quick. Moreover, it was found that FP was circa 10 times more potent than BUD in inhibiting the release of IL-6, IL-8, and TNF-α.[11] Patterson et al. were not capable of demonstrating defective *in vivo* capacity of AMs to ingest bacteria following FP treatment, as was shown during previous *in vitro* studies. This study did, however, show that infiltration of the lung parenchyma by neutrophils was elevated after treatment with FP, suggesting that ICS treatment does not impair neutrophil recruitment.[37]

Long-Acting β_2-Agonists

Even though ICS effectively reduce AECOPD and may positively influence decline in lung function in patients, they are relatively ineffective in suppressing the inflammatory response that occurs in COPD patients. Therefore, current guidelines recommend prescribing symptomatic COPD patients a short-acting bronchodilator (SABA) as needed. A LABA is added in case symptoms are inadequately controlled by SABA treatment, especially in patients with more severe disease. The LABAs currently approved for use in COPD are salmeterol and formoterol. The intrinsic activity of salmeterol is lower than that of the SABA salbutamol and its onset of action is delayed. However, its bronchodilatory effects persist for 12 hours. Formoterol has high intrinsic activity and a rapid onset of action; circa 70% of maximal bronchodilatation is observed within the first 5 minutes after inhalation. Whilst salmeterol's bronchodilatory effects are dose-independent, formoterol's duration of action is dose-dependent. The duration of action of both compounds lies around 12 hours and both are thus prescribed as a twice-daily dosing regimen.[38]

Mechanism of Action

LABAs exert their bronchodilatory effects by activating β_2-AR on airway smooth muscle cells (ASMCs), forming an agonist/receptor complex.[38] This complex binds the stimulatory G-protein (G_s) that activates adenylate cyclase, resulting in increased intracellular cyclic adenosine monophosphate (cAMP) levels and the activation of protein kinase A (PKA). PKA, in turn, phosphorylates several intracellular target proteins, which results in the activation of myosin light chain phosphatase and the inhibition of myosin light chain kinase, leading to relaxation of smooth muscle.[33] Relaxation of ASMCs may also occur independent of an increase in cAMP, as β_2-AR are also directly coupled to conductance calcium-activated potassium channels (BK_{ca}) via G_s. Additionally, β_2-agonists are capable of opening BK_{Ca}, repolarizing ASMCs and inducing

sequestration of calcium into intracellular stores. Moreover, β_2-agonists may induce indirect bronchodilatation *in vivo* by acting on inflammatory cells and airway nerves, inhibiting the release bronchoconstriction mediators and neurotransmitters, respectively.[33]
It is hypothesized that the persistent bronchodilatory effects of LABAs are induced by partitioning between the cell membrane and the airway surfactant liquid (ASL), a process that is dependent on the lipophilicity of the compound. This way, the plasma membrane acts as a depot for LABAs; formoterol is continuously released from the lipid bilayer into the ASL, enabling it to interact with the β_2-AR. The initial size of the depot is determined by the concentration of formoterol, also determining its duration of action. Salmeterol is thought to reach the receptor through lateral diffusion, as its partitioning in synthetic plasma membranes was found to be very high and its release therefrom was found to be slow. An alternative to this theory is that dissociated salmeterol is capable of re-associating with either the same receptor or other receptor molecules. Continuous shuffling between β_2-AR would therefore delay salmeterol's escape from the membrane and thereby prolong the effects of this compound.[38]

ICS/LABA Combination Therapy

Despite the fact that ICS monotherapy has minimal to no effects on lung function and mortality in patients, Wouters et al. showed that withdrawal of FP in patients that use SFC results in 'acute and persistent deterioration in lung function and dyspnoea and in an increase in mild exacerbations and percentage of disturbed nights.'[39] Moreover, several clinical trails support the notion that LABA/ICS combination treatment improves several outcome measures, such as better health status, fewer patient withdrawals, and lower mortality, as compared to placebo and monotherapy with either component.[27,28,38]
Combining LABA-treatment with an ICS results in additive effects of one drug over the other. ICS are known to improve β_2-AR-signaling by a number of mechanisms.[38] For instance, down-regulation of β_2-AR in the lungs has been observed upon long-term use of β-agonists. Mak et al. showed that ICS-treatment provided a protective effect against this down-regulation at the transcriptional level.[40] Corticosteroids are known to induce the transcription of the β_2-AR, increasing the number of receptors expressed on the cell membrane. Additionally, corticosteroids may enhance the effects of β_2-agonists by enhancing coupling of β_2-AR to G_s and by reversing uncoupling of β_2-AR in response to inflammatory mediators.[33] In turn, the effects of ICS can be enhanced by the addition of a LABA to treatment; β_2-agonists may enhance the anti-inflammatory effects of ICS by affecting GR function. As mentioned before, corticosteroids activate the GR, inducing translocation of cytoplasmic GR to the nucleus, and LABAs were shown to increase this translocation by facilitating the entry of the GR/corticosteroid complex into the nucleus.[33,41,42]
It has been shown that combined administration of salmeterol and FP does not result in systemic pharmacokinetic interaction between these compounds, as no significant difference in blood pressure, potassium, and glucose levels were detected. However, it has been shown that salmeterol and FP form particle agglomerations within their aerosol propellant system, and this interaction likely also occurs in DPI. Haghi et al. investigated the effect of salmeterol/FP co-deposition compared to single drug deposition on diffusion through the human Calu-3 epithelial cell layer. It was shown that the addition of salmeterol to the FP formulation significantly decreased the rate of FP transport across these epithelial cells. The trans-epithelial electrical resistance was higher after exposure to salmeterol particles, suggesting a stabilizing role for salmeterol that results in hindered diffusion of FP through the monolayer and, thus, in prolonged anti-inflammatory effects of FP. Heijink and Van den Berge propose that, in addition to suppressing the production of pro-inflammatory mediators, improving epithelial barrier function exerts direct anti-inflammatory effects by dampening the release of pro-inflammatory mediators by the epithelium. Therefore, the addition of salmeterol to the FP formulation may also have a beneficial influence on airway remodelling.[43]

Steroid Resistance

As mentioned before, the inflammation seen in COPD patients is relatively resistant to corticosteroid treatment; ICS do not affect inflammatory cell- and cytokine profiles and are incapable of reversing the protease-antiprotease imbalance. In addition, the response of AMs to corticosteroids is reduced in normal smokers as compared to non-smokers, and is absent in COPD patients. Increased oxidative stress due to chronic exposure to cigarette smoke may result in reduced HDAC activity or even in damaging of the HDAC2 enzyme and subsequent failure of down-regulating pro-inflammatory gene transcription. Another mechanism that has been proposed to explain corticosteroid-resistance in COPD is failure of GR to translocate to the nucleus. In this case, the addition of a LABA, which are known to increase nuclear localization of the GR, may improve the anti-inflammatory effects of ICS.[44]

Recently, it has been proposed that several subsets of COPD patients, also called phenotypes, exist that respond to treatment differently; a subset of patients with eosinophilic COPD has been identified on which ICS treatment has a larger beneficial effect than on other phenotypes. This subset of patients may be identified by sputum eosinophilia and systemic eosinophil counts.[45] Recently, Pascoe et al. showed that blood eosinophil counts were predictive of rates of exacerbation increasing eosinophil counts were positively correlated with exacerbation rates.[29] Therefore, blood eosinophil counts could represent a new biomarker for the response to ICS during acute exacerbations of COPD. Moreover, combination therapy including fluticasone furoate resulted in a decreased exacerbation rate in 29% of patients with blood eosinophil counts ≥2% as compared to monotherapy, but only in 10% of patients with blood eosinophil counts <2%,[26] suggesting that combination therapy is more effective in reducing exacerbation rates that monotherapy. However, eosinophilic COPD remains a controversial subject, as distinguishing it from asthma is difficult. Patients that experience both increased variability of airflow and reversible airflow obstruction are now diagnosed with asthma-COPD overlap syndrome (ACOS), which typically includes patients with early-onset asthma that fulfil the criteria for COPD with age and COPD patients with increased reversibility. This subset of patients comprises 13-19% of patients with obstructive lung disease, a number that increases with age. Many trials on the effects of ICS on COPD exclude patients with a diagnosis of asthma, resulting in low generalizability of the results for this subset of patients. During exacerbations of ACOS, the number of eosinophils in the airway mucus increases more than that of neutrophils, explaining the fact that these patients show improvement of symptoms upon ICS treatment.[45]

Risk of Pneumonia

In spite of the positive effects combination therapy was found to have on patients' health status, the TORCH trial was the first trial to identify an elevated risk of pneumonia in patients treated with medications containing FP.[27] It is not yet fully clear whether this risk is similar for all ICS and whether it is dose-related, although the risk appears to be particularly increased using high doses and shorter durations.[4] Suissa et al. conducted a population-based cohort study to investigate the risk of different ICS on the occurrence of pneumonia and to evaluate possible dose-response relationships. It was found that case subjects had more severe respiratory disease, more prescriptions for respiratory drugs, and a higher prevalence of comorbidities. It was found that the use of ICS was associated with a 69% increase in the risk of serious pneumonia. Interestingly, this effect waned gradually upon ceasing treatment and vanished after 6 months. Moreover, patients using FP showed a doubling of the rate of serious pneumonia that was dose-dependent; a dose of 1000µg per day was associated with a 122% increased risk. Use of BUD resulted in an increase of 17% in the risk of serious pneumonia and was not dose-related.[4] Ernst et al. confirmed these results, showing a 70% increase in the risk of serious pneumonia in patients currently using ICS and that this risk increased with higher doses. Since

this study did not make a distinction between different ICS, no conclusions were drawn on the influence of different ICS on the risk of serious pneumonia.[2]

Clinical Trials on ICS Monotherapy versus ICS/LABA Combination Treatment

Two well-known examples of trials conducted to investigate the use of ICS in COPD are the TORCH and INSPIRE trials.[1,46] The TORCH trial, conducted by Calverley et al., aimed to investigate mortality rates among COPD patients treated with SFC as compared to usual care, i.e. treatment with either component alone. The TORCH trial was conducted in a double-blinded, placebo-controlled, randomized, parallel-group fashion over a period of three years. The primary endpoint was death due to any cause within the three-year trial period, secondary endpoints were frequency of exacerbation and health status according to score on the St. George's Respiratory Questionnaire (SGRQ). The original trial concluded that treatment with combination therapy did not reduce the rate of mortality due to any cause significantly as compared to placebo. However, combination treatment did significantly reduce the number of exacerbations and resulted in improved health status and lung function as compared to placebo. Another important observation was the unexpectedly large number of patients treated with prescriptions containing FP that were diagnosed with pneumonia. This result was unforeseen and was likely found because this study was the first trial conducted on a number of patients that was large enough to detect infrequent events.

The aim of the INSPIRE trial, conducted by Wedzicha et al., was to study the effects of tiotropium bromide monotherapy compared to SFC on the rate of moderate and/or severe exacerbations during the 2-year treatment period and on outcomes that might be related to exacerbations. These secondary endpoints included health status according to SGRQ, post-dose FEV_1, and withdrawal rate. It was concluded that the exacerbation rates between SFC and tiotropium bromide did not differ significantly, although more patients receiving tiotropium failed to complete the study. As in the TORCH trial, a small but significant increase in the number of cases of pneumonia in the SFC group was reported. Interestingly, a mortality reduction benefit in favour of SFC was still found.

The TORCH trial was the first study to find an association between the use of ICS and the development of pneumonia.[47] These findings were supported by the INSPIRE trial, which found an increased incidence of pneumonia in the SFC-treated group.[28] The validity of early trials on the risk of pneumonia upon the use of ICS in COPD is often criticized for reliance on unadjusted adverse event reports of pneumonia, often lacking radiographic confirmation.[48] Additionally, many of these trials did not take into account that the duration of follow-up differed between patients, and therefore did not weigh the rate of exacerbations according to different durations of follow-up. Especially trials with longer durations cope with relatively high dropout rates, i.e. between 20-50%, which may threaten the validity of the results. In addition, during these trials it was often assumed that patients were homogeneous with respect to their rates of acute exacerbations of COPD (AECOPD), disregarding the fact that some patients have many exacerbations and many patients did not experience AECOPD during the trial period.[46] However, Festic et al. recently showed that adjustment for demographic characteristics comorbidities, and concurrent medication regimens slightly attenuated but not eliminated the risk of pneumonia associated with the use of ICS found in earlier studies. Other risk factors that were found to influence the risk of pneumonia were higher potency of ICS compounds, administration of a higher dose, and a longer duration of use. This suggests that there is a dose-effect relationship between the use of ICS and the occurrence of pneumonia.[48]

Development of Pneumonia

It is not yet fully understood by which mechanism FP elevates the risk of developing pneumonia in COPD patients while reducing the number and severity of exacerbations. However, it is known that pneumonia often occurs within weeks after an unresolved exacerbation. The lower airways of COPD patients are often colonized by a spectrum of pathogens, including community-acquired pathogens *Streptococcus (S.) pneumoniae,* and *Haemophilus (H.) influenzae*, and gram-negative enteric bacilli (GNEB) *Pseudomonas* and *Stenotrophomonas.*[49] Soler et al. found that bacterial

colonization of the lower airways in outpatients with stable COPD occurred in around 25% of patients and mainly consisted of *H. influenzae* and *S. pneumoniae*. It was also found that exacerbations of COPD were related to bacterial infections, as both the prevalence of presence (51,7%) and bacterial concentrations were elevated in the lower airways. [50]

The incidence of *de novo* pneumonia without preceding exacerbation in patients is not increased significantly by the use of ICS. However, the risk of pneumonia is increased by 70% in patients who experience an unresolved infective exacerbation. Therefore, it is hypothesized that the innate immune system and ICS treatment fail to adequately control airway infections that are responsible for exacerbation and the associated airway inflammation, enabling the infection and inflammation to enter the alveoli and pleural space. It has been demonstrated that FP is capable of suppressing the immune response against pneumonia pathogens. This may be due to its high lipophilicity, resulting in a high mucosal retention time and thus higher concentrations in the airways than is the case for other ICS, like BUD. [18]

Host generation of reactive oxygen and nitrogen species (ROS and NOS, respectively) play an important role in eliminating bacteria. Patterson et al. showed *in vivo* that the production of H_2O_2 by AMs was unaffected by FP treatment, suggesting that this pathway is not affected by corticosteroids. However, the expression of nitric oxide was inhibited by low doses of FP, confirming the decreased induction of nitric oxide synthase (NOS)2 by dexamethasone found in a prior study. Moreover, it was shown that the amount of inducible nitric oxide synthase (iNOS) mRNA was decreased upon bacterial challenge in the lungs of mice treated with FP. As mentioned before, the number of bacteria found within AMs was found to be increased in FP-treated mice, and Patterson et al. therefore speculate that the expression of NO is impaired as a consequence of ICS-treatment. [37]

Discussion & Conclusions

ICS are widely used in the treatment of COPD due to their ability to improve quality of life and to reduce the frequency of exacerbations in a subset of patients.[16] In other subsets of stable COPD patients, however, the use of ICS monotherapy does not appear to impede the progressive decline in lung function and has small and inconsistent effects on symptoms, quality of life, and the severity of exacerbations. Moreover, long-term use of ICS may cause systemic adverse effects, including bruising of the skin, diabetes, cataracts, and osteoporosis. The GOLD guidelines therefore state that regular treatment with ICS, with or without LABA, should only be prescribed to patients with severe COPD.[10] Moreover, several clinical trials have suggested a link between the use of ICS and an elevated risk of developing pneumonia, and although it remains uncertain whether this risk is similar for all ICS and whether it is dose-related, the risk appears to be particularly increased using high doses and shorter durations.[4] The aim of this thesis was to provide an answer to the questions of whether ICS monotherapy and/or ICS/LABA combination treatment provide a beneficial addition to standard care in COPD, whether differences with regard to the effectiveness of monotherapy versus combination treatment exist and if differences between specific drugs and/or drug combinations exist, and whether this is the case for all patients or if subsets of patients should be distinguished. As several clinical trials found a relation between the use of FP and an increased risk of pneumonia, it was hypothesized that differences between different ICS exist and that the use of some ICS is preferable over the use of others. As it was also shown that the addition of a LABA resulted in fewer exacerbations than monotherapy, it was hypothesized that combination treatment may be more effective in the treatment of COPD than monotherapy. Moreover, since the eosinophilic subset of patients is known to respond well to corticosteroid treatment, it was hypothesized that more subsets can be distinguished that would benefit from personalised treatment.

The use of ICS monotherapy for the treatment of COPD raises some debate due to its inability to suppress inflammatory responses in COPD patients, in spite of being capable of effectively reducing AECOPD. Additionally, corticosteroid resistance as a result of chronic oxidative stress induced by smoking may occur through the inactivation of HDAC2.[31] Instead, ICS/LABA combination treatment is recommended, as combining these types of drugs results in additive effects of one drug over the other, for instance by prolonging anti-inflammatory effects of ICS and/or improving epithelial barrier function by addition of a LABA.[38,43,51] Due to these additive effects, the possibility exists that combination treatment requires lower doses of ICS than are needed for ICS monotherapy, possibly resulting in a lower occurrence of systemic side effects. Moreover, as the risk of developing pneumonia is particularly high for higher doses of ICS,[48] it is likely more beneficial to administer lower doses of ICS/LABA combination therapy for the treatment of patients with severe COPD. This, however, needs to be investigated, as clinical trials on the effects of different doses of ICS combined with a LABA have not yet been performed. As the efficacy of ICS monotherapy in COPD is unclear and long-term use of ICS is associated with systemic adverse effects, including the risk of developing pneumonia, monotherapy should not be prescribed as a treatment for COPD.
Due to differences in pharmacodynamic properties of different ICS, the use of some types of ICS may be preferable over the use of others. FP is retained in the lumen of the airways due to its high lipophilicity and thus has high local activity and a long duration of action. However, the onset of action of salmeterol/fluticasone (SFC; salmeterol/fluticasone combination treatment) is slower than that of budesonide/formoterol (FBC; formoterol/budesonide combination treatment), the latter of which provides a more rapid relief of acute symptoms.[52] Due to its high lipophilicity, FP dissolves in the mucus and thus remains outside the epithelial cells for a longer period, making it more susceptible to mucociliary clearance. BUD is less lipophilic than FP and is retained within airway epithelial cells through fatty acid conjugation, making it less susceptible to mucociliary clearance and thus less affected by lung function. Few studies have been performed that make a direct comparison between the use of SFC versus that of FBC, making

this an interesting topic for further investigation. The PATHOS study, a population-based observational registry study comparing the effects of SFC versus FBC treatment on the prevention of AECOPD, showed that long-term treatment with FBC resulted in fewer moderate and severe exacerbations than long-term treatment with SFC. [52] In addition to affecting exacerbation frequency, bacterial colonization of the lower airways often found in COPD patients is implicated in the onset of pneumonia; as mentioned before, the risk of pneumonia is increased by 70% in patients who experience an unresolved infective exacerbation. Since FP is a more potent immunosuppressant [11] and persists in the airways longer than BUD, it has been hypothesized that FP treatment results in prolonged suppression of local immune responses, facilitating the occurrence of exacerbations and suppressing the immune response against pneumonia pathogens, resulting in pneumonia. [18,52] Therefore, prescribing FBC to patients with severe COPD appears favourable over the prescription of SFC, as was shown in the TORCH, INSPIRE, and PATHOS trials. As mentioned before, currently circa 80% of COPD patients are prescribed long-term ICS treatment, whilst only 10% of COPD patients meet the criteria for severe COPD. As some side effects of long-term treatment with ICS are severe, only patients with severe COPD should be prescribed high doses of ICS.

As mentioned, several subsets of COPD patients can be distinguished, including an α_1-antitrypsin subset that has a genetic predisposition to develop COPD irrespective of smoking habits and an eosinophilic subset that is characterized by high numbers of eosinophils in induced sputum and serum. Therefore, it is not surprising that patients with different phenotypes of the disease respond to treatment differently. From this logically follows that, in order to optimize treatment, treatment is ought to be adjusted to the patient. An example is the subset on which the focus of this thesis lies; frequent exacerbators who respond best to LABA, LAMA, and/or LABA/ICS treatment, which results in reduced exacerbations and improved lung function and quality of life, and possibly in reduced FEV_1 decline and mortality. In order to provide optimal treatment for COPD patients and reducing health care costs, it is advisable that phenotypes are distinguished and treatments are adjusted to these phenotypes, as types of treatments that work well in one subset of patients do not necessarily provide relief for other subsets.

Several clinical trials, including the PATHOS, INSPIRE, and TORCH trials, have shown an increased risk of the development of pneumonia associated with the use of ICS, in particular for treatments including FP. [27,28,52] In the case of cohort studies for which computerized databases are consulted, the availability of the drugs or drug combinations used will determine the number of patients that receive these treatments. In the case of ICS/LABA combinations prescribed to COPD patients, the availability of the SFC and FBC drug combinations may have been an influencing factor on the number of patients in the groups, for example in the study conducted by Suissa et al. [4] . At the time this study was conducted, SFC was approved for use in COPD, whilst the FBC combination was not. [4] Therefore, the group receiving FBC, which consisted of 9542 subjects versus 24 198 subjects in the SFC group, may not have been large enough to detect irregular effects, potentially resulting in an underestimation of the risk of pneumonia in this group. [4] Since then, FBC has been approved for use in COPD treatment, [18] which likely results in a larger number of COPD patients that use FBC becoming available for cohort studies.
Another aspect that should be taken into account when comparing results from different trials is the fact that the inclusion criteria that are used for the selection of subjects differ per trial. Based on the FEV_1 inclusion criteria used, the INSPIRE trial selected patients with severe COPD, i.e. with a post-bronchodilator FEV_1 of <50% of predicted and reversibility, [28] whilst for the TORCH trial patients with moderate COPD were selected, i.e. with a pre-bronchodilator FEV_1 of <60% of the predicted value. [27] It should thus be taken into account that differences between results found in different trials may result from this difference in the inclusion criteria that were used.
The GOLD Criteria also state that in older adults, especially individuals over the age of 70, a FEV_1/FVC ratio between 0.65-0.7 may be normal, which may lead to over-diagnosing of COPD when this is not taken into account. Similarly, in individuals under the age of 45, the use of a

ratio of 0.7 may lead to under-diagnosis of COPD.[53] This is especially relevant for the TORCH trial, in which patients aged 40-80 with a ratio of ≤0.7 were included. However, as patients also had to meet other criteria characteristic for COPD, this does not necessarily pose a threat to the validity of this trial. Moreover, since COPD is a heterogeneous disease and test performance is affected by several factors including day of testing and the number of drugs given to test, Calverley et al. suggest that FEV_1 reversibility may not be the ideal parameter for the assessment of COPD severity.[54] Classification based on FEV_1 reversibility is therefore said to be 'an illusion', as results can be easily misinterpreted due to lack of understanding of both between and within test variability. Instead, it is suggested that FEV_1 reversibility is ought to be used as a robust way of identifying the disease, supporting a clinical diagnosis of COPD.[54]
In addition to the different inclusion or exclusion criteria that are used during trials, the definitions used for AECOPD also differ per trial. For instance, the INSPIRE and TORCH trials defined an exacerbation as a symptomatic deterioration that requires treatment with antibiotic agents, systemic corticosteroids, hospitalization, or a combination of these.[27] In a trial conducted by Bhowmik et al., the definition used for diagnosing AECOPD were based on patients' experience of two of three major symptoms, being dyspnoea, sputum purulence, and/or increased sputum volume, or at least one of these major symptoms in combination with at least one minor symptom, including a cold, wheeze, cough, or fever. In addition to experiencing these symptoms, this study required a diagnosis of the exacerbation confirmed by a doctor in the study team.[55] These are only some examples of the different diagnoses used for AECOPD, and even though both diagnoses are both in accordance with the GOLD criteria, the generalizability of the results would, as is the case for the inclusion criteria used to select subjects, benefit from setting a standard that is used in every trial. This especially holds true for clinical trials that investigate the effects of one certain compound instead of comparing two or more compounds within the same trial. In the first case, a lack of standardised criteria and diagnoses may lead to unjustified attribution of the found effect to the design of the study, instead of acknowledging that the found effects are due to properties specific for the tested drug. Interestingly, some trials on the use of ICS in COPD only use exclusion criteria that patients need to meet in obtaining a group of subjects. For instance, in a study by Suissa et al.[4] on the influence of ICS use on the development of serious pneumonia, subjects were selected based on several exclusion criteria, including the prescription of an ICS or any form of β-agonist, age prior to and year of entering the cohort, and hospital admission for asthma. Strikingly, no diagnosis of COPD was required for entering the cohort. This raises the question of whether all patients included in the cohort were, in fact, COPD patients, as a lacking diagnosis of asthma does not guarantee a diagnosis COPD in these patients. Therefore, it cannot be guaranteed that the cohort contained subjects suffering from airway disease other than COPD. In addition, the diagnosis of pneumonia in this study did not require chest radiography as a confirmation, since the database that was used did not contain this information.[4] In this study, cases of pneumonia were defined as hospitalization or death from pneumonia. However, this raises the question of whether it is possible to make a distinction between patients who died as a result of having pneumonia and patients who died with pneumonia in whom pneumonia was not the cause of death. As mentioned before, lack of a radiographic diagnosis of pneumonia is an issue in more trials, especially the earlier trials that were conducted on the use of ICS in COPD that did not include pneumonia as a primary or secondary endpoint. Therefore, the possibility exists that not all cases of pneumonia included in the results of these trials were, in fact, cases of pneumonia, resulting in the possibility of an under- or, more likely, overestimation of the effects of ICS use on the risk of pneumonia. Therefore, the effect of the use of ICS, and specifically FP, on the risk of developing pneumonia may not be as dramatic as was initially thought.

Due to ethical considerations, the INSPIRE trial did not include a placebo group. As this trial was conducted in patients with severe COPD, it was not possible to include a group in which treatment was withheld. However, this means that this trial did not include a group that did not receive any treatment, and it is therefore not possible to determine if the results found in this trial were caused by the ICS used or if they were a result of the severity of the disease, resulting

in greater frequency of hospitalization and number of prescriptions.[48] This should be taken into account when interpreting the results and in drawing conclusions on the risk of pneumonia due to the use of ICS. The TORCH trial was conducted in a group of COPD patients with moderate disease, and therefore this trial did include a placebo group. The results found in the INSPIRE trial were in accordance with those of the TORCH trial,[28] the PATHOS trial[52] and a clinical study conducted by Suissa et al.[4] Therefore, the increased risk of developing pneumonia in COPD patients appears to represent a true potential side effect of the use of ICS, and especially the use of FP. The proposed mechanism behind this increased risk is a failure of the innate immune system to adequately control the airway infections responsible for exacerbation and the associated airway inflammation, resulting in the infection and inflammation to enter the alveoli and pleural space.[18] As mentioned before, ICS and LABA have additive effects over each other. Therefore, it is possible that the addition of a LABA results in a lowering of the dose of ICS that is needed to effectively treat COPD. In turn, this lower dose of ICS may result in fewer side effects, such as pneumonia. If this were, in fact, the case, ICS/LABA combination treatment would be favourable over ICS monotherapy. However, no studies comparing different doses of ICS/LABA combination treatment have yet been performed, making this a suitable topic for future research.

In conclusion, high doses of ICS should only be prescribed to patients with severe COPD, as the long-term use of ICS is associated with systemic side effects, including diabetes, osteoporosis, and cataracts. Since ICS and LABA have additive effects over each other, combining treatment may lower the dose of the ICS needed in order to achieve a beneficial effect and thus reduce these adverse effects. Long-term treatment of COPD patients with FBC resulted in fewer exacerbations than long-term treatment with SFC, and FP is thought to increase the occurrence of exacerbations and the risk of developing pneumonia. Therefore, prescription of FBC appears favourable over the prescription of SFC for the treatment of COPD. In addition, treatment of COPD patients may be optimized by distinguishing several phenotypes of the disease and by adjusting treatment to the specific needs of the different subsets of COPD patients.
When interpreting the results found during trials on the use of ICS in COPD, one should take into account that the outcome of the trial may have been influenced by the availability of the drug on the market. Additionally, when comparing the results from different trials it should be taken into consideration that the inclusion criteria and requirements for diagnosis used may not be the same for every trial, possibly resulting in the selection of a different group of patients. Therefore, the generalizability and comparability of clinical trials may benefit from setting standards with regard to inclusion criteria that are used to select subjects, defining the diagnosis of AECOPD, and ascertaining that radiographic diagnosis of pneumonia is required. If possible, a placebo group should be included to ascertain that possible results found are, in fact, caused by the treatment and not, for instance, by disease severity.

Acknowledgements

I would like to thank dr. H.I. Heijink for giving feedback and supervising this thesis.

References

1. Cave AC, Hurst MM. The use of long acting β 2-agonists, alone or in combination with inhaled corticosteroids, in chronic obstructive pulmonary disease (COPD): A risk–benefit analysis. *Pharmacol Ther*. 2011;130(2):114-143.

2. Ernst P, Gonzalez AV, Brassard P, Suissa S. Inhaled corticosteroid use in chronic obstructive pulmonary disease and the risk of hospitalization for pneumonia. *American journal of respiratory and critical care medicine*. 2007;176(2):162-166.

3. Sin DD, Man SF. Why are patients with chronic obstructive pulmonary disease at increased risk of cardiovascular diseases? the potential role of systemic inflammation in chronic obstructive pulmonary disease. *Circulation*. 2003;107(11):1514-1519.

4. Suissa S, Patenaude V, Lapi F, Ernst P. Inhaled corticosteroids in COPD and the risk of serious pneumonia. *Thorax*. 2013;68(11):1029-1036.

5. Fischer BM, Pavlisko E, Voynow JA. Pathogenic triad in COPD: Oxidative stress, protease-antiprotease imbalance, and inflammation. *Int J Chron Obstruct Pulmon Dis*. 2011;6:413-421.

6. Pauwels RA, Rabe KF. Burden and clinical features of chronic obstructive pulmonary disease (COPD). *Lancet*. 2004;364(9434):613-620.

7. O'Donnell R, Breen D, Wilson S, Djukanovic R. Inflammatory cells in the airways in COPD. *Thorax*. 2006;61(5):448-454.

8. Tetley TD. New perspectives on basic mechanisms in lung disease. 6. proteinase imbalance: Its role in lung disease. *Thorax*. 1993;48(5):560-565.

9. Kumagai K, Ohno I, Okada S, et al. Inhibition of matrix metalloproteinases prevents allergen-induced airway inflammation in a murine model of asthma. *J Immunol*. 1999;162(7):4212-4219.

10. Adcock IM, Ito K. Glucocorticoid pathways in chronic obstructive pulmonary disease therapy. *Proceedings of the American Thoracic Society*. 2005;2(4):313-319.

11. Ek A, Larsson K, Siljerud S, Palmberg L. Fluticasone and budesonide inhibit cytokine release in human lung epithelial cells and alveolar macrophages. *Allergy*. 1999;54(7):691-699.

12. Davies RJ, Devalia JL. Asthma. epithelial cells. *Br Med Bull*. 1992;48(1):85-96.

13. Larsen CG, Anderson AO, Appella E, Oppenheim JJ, Matsushima K. The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. *Science*. 1989;243(4897):1464-1466.

14. Warringa RA, Koenderman L, Kok PT, Kreukniet J, Bruijnzeel PL. Modulation and induction of eosinophil chemotaxis by granulocyte-macrophage colony-stimulating factor and interleukin-3. *Blood*. 1991;77(12):2694-2700.

15. Retamales I, Elliott WM, Meshi B, et al. Amplification of inflammation in emphysema and its association with latent adenoviral infection. *American journal of respiratory and critical care medicine*. 2001;164(3):469-473.

16. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. . 2015.

17. Abboud R, Vimalanathan S. Pathogenesis of COPD. part I. the role of protease-antiprotease imbalance in emphysema [state of the art series. chronic obstructive pulmonary disease in high- and low-income countries. edited by G. marks and M. chan-yeung. number 3 in the series]. *The International Journal of Tuberculosis and Lung Disease*. 2008;12(4):361-367.

18. Latorre M, Novelli F, Vagaggini B, et al. Differences in the efficacy and safety among inhaled corticosteroids (ICS)/long-acting beta2-agonists (LABA) combinations in the treatment of chronic obstructive pulmonary disease (COPD): Role of ICS. *Pulm Pharmacol Ther*. 2015;30:44-50.

19. Brightling CE, McKenna S, Hargadon B, et al. Sputum eosinophilia and the short term response to inhaled mometasone in chronic obstructive pulmonary disease. *Thorax*. 2005;60(3):193-198.

20. Halpin DM, Gray J, Edwards SJ, Morais J, Singh D. Budesonide/formoterol vs. salmeterol/fluticasone in COPD: A systematic review and adjusted indirect comparison of pneumonia in randomised controlled trials. *Int J Clin Pract*. 2011;65(7):764-774.

21. Gold PM. The 2007 GOLD guidelines: A comprehensive care framework. *Respir Care*. 2009;54(8):1040-1049.

22. Siva R, Green RH, Brightling CE, et al. Eosinophilic airway inflammation and exacerbations of COPD: A randomised controlled trial. *Eur Respir J*. 2007;29(5):906-913.

23. Burge S, Wedzicha JA. COPD exacerbations: Definitions and classifications. *Eur Respir J Suppl*. 2003;41:46s-53s.

24. Saetta M, Di Stefano A, Maestrelli P, et al. Airway eosinophilia in chronic bronchitis during exacerbations. *Am J Respir Crit Care Med*. 1994;150(6 Pt 1):1646-1652.

25. Singh D, Kolsum U, Brightling CE, et al. Eosinophilic inflammation in COPD: Prevalence and clinical characteristics. *Eur Respir J*. 2014;44(6):1697-1700.

26. Brusselle GG, Bracke K, Lahousse L. Targeted therapy with inhaled corticosteroids in COPD according to blood eosinophil counts. *The Lancet Respiratory Medicine*. 2015.

27. Calverley PM, Anderson JA, Celli B, et al. Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. *N Engl J Med*. 2007;356(8):775-789.

28. Wedzicha JA, Calverley PM, Seemungal TA, Hagan G, Ansari Z, Stockley RA. The prevention of chronic obstructive pulmonary disease exacerbations by salmeterol/fluticasone propionate or tiotropium bromide. *American journal of respiratory and critical care medicine*. 2008;177(1):19-26.

29. Pascoe S, Locantore N, Dransfield MT, Barnes NC, Pavord ID. Blood eosinophil counts, exacerbations, and response to the addition of inhaled fluticasone furoate to vilanterol in patients with chronic obstructive pulmonary disease: A secondary analysis of data from two parallel randomised controlled trials. *The Lancet Respiratory Medicine*. 2015.

30. Alsaeedi A, Sin DD, McAlister FA. The effects of inhaled corticosteroids in chronic obstructive pulmonary disease: A systematic review of randomized placebo-controlled trials. *Am J Med*. 2002;113(1):59-65.

31. Barnes PJ. Glucocorticosteroids: Current and future directions. *Br J Pharmacol*. 2011;163(1):29-43.

32. Adcock IM. Glucocorticoids: New mechanisms and future agents. *Curr Allergy Asthma Rep*. 2003;3(3):249-257.

33. Barnes PJ. Biochemical basis of asthma therapy. *J Biol Chem*. 2011;286(38):32899-32905.

34. Malo de Molina R, Mortensen EM, Restrepo MI, Copeland LA, Pugh MJ, Anzueto A. Inhaled corticosteroid use is associated with lower mortality for subjects with COPD and hospitalised with pneumonia. *Eur Respir J*. 2010;36(4):751-757.

35. Dalby C, Polanowski T, Larsson T, Borgstrom L, Edsbacker S, Harrison TW. The bioavailability and airway clearance of the steroid component of budesonide/formoterol and salmeterol/fluticasone after inhaled administration in patients with COPD and healthy subjects: A randomized controlled trial. *Respir Res*. 2009;10:104-9921-10-104.

36. Borchard G, Cassará ML, Roemelé PE, Florea BI, Junginger HE. Transport and local metabolism of budesonide and fluticasone propionate in a human bronchial epithelial cell line (Calu‐3). *J Pharm Sci*. 2002;91(6):1561-1567.

37. Patterson CM, Morrison RL, D'Souza A, Teng XS, Happel KI. Inhaled fluticasone propionate impairs pulmonary clearance of klebsiella pneumoniae in mice. *Respir Res*. 2012;13(1):40.

38. Fuso L, Mores N, Valente S, Malerba M, Montuschi P. Long-acting beta-agonists and their association with inhaled corticosteroids in COPD. *Curr Med Chem*. 2013;20(12):1477-1495.

39. Wouters EF, Postma DS, Fokkens B, et al. Withdrawal of fluticasone propionate from combined salmeterol/fluticasone treatment in patients with COPD causes immediate and sustained disease deterioration: A randomised controlled trial. *Thorax*. 2005;60(6):480-487.

40. Mak JC, Nishikawa M, Shirasaki H, Miyayasu K, Barnes PJ. Protective effects of a glucocorticoid on downregulation of pulmonary beta 2-adrenergic receptors in vivo. *J Clin Invest*. 1995;96(1):99-106.

41. Roth M, Johnson PR, Rudiger JJ, et al. Interaction between glucocorticoids and beta2 agonists on bronchial airway smooth muscle cells through synchronised cellular signalling. *Lancet*. 2002;360(9342):1293-1299.

42. Hanania NA, Darken P, Horstman D, et al. The efficacy and safety of fluticasone propionate (250 μg)/salmeterol (50 μg) combined in the diskus inhaler for the treatment of COPD. *CHEST Journal*. 2003;124(3):834-843.

43. Heijink IH, Berge M. Are salmeterol's beneficial effects on corticosteroid action in the airways executed at the epithelial barrier? *Respirology*. 2013;18(8):1165-1166.

44. Agusti A. COPD, a multicomponent disease: Implications for management. *Respir Med*. 2005;99(6):670-682.

45. Turner AM, Tamasi L, Schleich F, et al. Clinically relevant subgroups in COPD and asthma. *European Respiratory Review*. 2015;24(136):283-298.

46. Ernst P, Saad N, Suissa S. Inhaled corticosteroids in COPD: The clinical evidence. *Eur Respir J*. 2015;45(2):525-537.

47. Sibila O, Soto-Gomez N, Restrepo MI. The risk and outcomes of pneumonia in patients on inhaled corticosteroids. *Pulm Pharmacol Ther*. 2015.

48. Festic E, Scanlon PD. Incident pneumonia and mortality in patients with chronic obstructive pulmonary disease. A double effect of inhaled corticosteroids? *American journal of respiratory and critical care medicine*. 2015;191(2):141-148.

49. Soler N, Torres A, Ewig S, et al. Bronchial microbial patterns in severe exacerbations of chronic obstructive pulmonary disease (COPD) requiring mechanical ventilation. *American journal of respiratory and critical care medicine*. 1998;157(5):1498-1505.

50. Monso E, Ruiz J, Rosell A, et al. Bacterial infection in chronic obstructive pulmonary disease. A study of stable and exacerbated outpatients using the protected specimen brush. *Am J Respir Crit Care Med*. 1995;152(4 Pt 1):1316-1320.

51. Haghi M, Traini D, Postma DS, Bebawy M, Young PM. Fluticasone uptake across Calu‐3 cells is mediated by salmeterol when deposited as a combination powder inhaler. *Respirology*. 2013;18(8):1197-1201.

52. Larsson K, Janson C, Lisspers K, et al. Combination of budesonide/formoterol more effective than fluticasone/salmeterol in preventing exacerbations in chronic obstructive pulmonary disease: The PATHOS study. *J Intern Med*. 2013;273(6):584-594.

53. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Spirometry for health care providers. . Updated 2010.

54. Calverley PM, Albert P, Walker PP. Bronchodilator reversibility in chronic obstructive pulmonary disease: Use and limitations. *Lancet Respir Med*. 2013;1(7):564-573.

55. Bhowmik A, Seemungal TA, Sapsford RJ, Wedzicha JA. Relation of sputum inflammatory markers to symptoms and lung function changes in COPD exacerbations. *Thorax*. 2000;55(2):114-120.