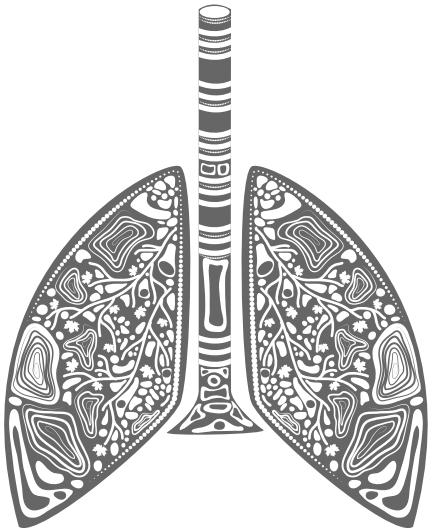
Characterisation and prevention of exacerbations in frequently exacerbating patients with COPD



Sevim Uzun

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Characterisation and Prevention of Exacerbations in Frequently Exacerbating Patients with COPD

Karakterisatie en preventie van exacerbaties in frequent exacerberende patiënten met COPD

Proefschrift

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"*Vincit qui patitur*"
Persius

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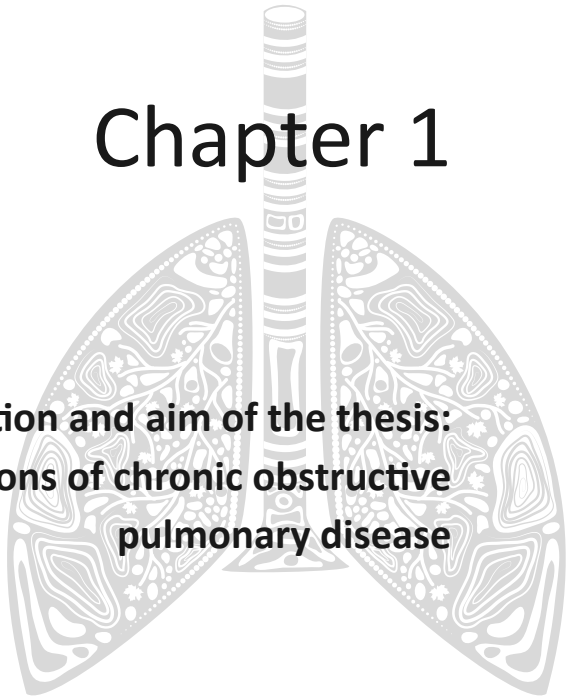
Chapter 1



General introduction and aim of the thesis: Acute exacerbations of chronic obstructive pulmonary disease

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Chapter 4: ***Acute Exacerbations of Chronic Obstructive Pulmonary Disease***
Oncogenesis, Inflammatory and Parasitic Tropical Diseases of the Lung

Introduction

Chronic obstructive pulmonary disease (COPD) is a disease which is characterised by airway inflammation and progressive airflow limitation with poor reversibility. Periods of acute deterioration lie in the natural course of the disease and are called exacerbations. In literature, there are different definitions for an acute exacerbation of COPD (AECOPD). A symptom reported AECOPD is defined solely based on a patient's symptoms.[1] This is regardless of whether the patient seeks medical attention or receives treatment for the exacerbation. An event defined AECOPD requires a therapeutic intervention such as a change in COPD medications or a change in healthcare utilization.[1] Generally accepted is the definition as in the guidelines of the World Health Organization, US National Heart Lung and Blood Institute and Global Initiative for Chronic Obstructive Lung Disease (GOLD), which define an exacerbation as "an event in the natural course of the disease characterised by a change in the patient's baseline dyspnoea, cough, and/or sputum that is beyond normal day-to-day variations, is acute in onset and may warrant a change in regular medication in a patient with COPD".[2]

Frequent exacerbations result in a decreased health related quality of life,[3] a decline in lung function,[4] an increased risk of hospitalization[5], and an increase in mortality.[6] COPD and acute exacerbations of COPD impose a burden on health care and society. It is estimated that COPD is the 4th leading cause of death worldwide and will be the 3rd leading cause of death in 2030.[7] Along with increasing mortality rates the loss in disability-adjusted life years (DALYs) will also rise. By 2030 COPD is predicted to be the 5th leading cause of loss in DALYs globally, where it was only number 13 in 2004. Increasing health care costs will be the consequence of this trend. In the European Union COPD accounts for just over 3% of the total health care budget. In the USA, the direct and indirect costs for COPD are almost 50 billion USD. The majority of these costs are attributed to exacerbations.[8] The importance of exacerbations is reflected in the latest update of the GOLD report, in which the number of exacerbations in the preceding year is incorporated in the new classification of a patient with COPD.[8] In order to try reduce mortality rates, loss in DALYs, and related costs to lower the burden on society and health care, it is a goal to prevent and treat COPD and exacerbations of COPD.

This chapter will give a concise overview of the background of AECOPD and the available tools for its treatment and prevention.

Epidemiology

The prevalence of COPD varies greatly per country and also within countries.[9] This heterogeneity can be contributed to not only differences in diagnostic methods and classification but also to smoking habits, population age, in- and outdoor air pollution, occupational exposure, prevalence of pulmonary tuberculosis, chronic asthma, and socioeconomic status.[10] Prevalences of COPD have been reported varying from 0.2–37%.[11,12]

The prevalence of AECOPD is very difficult to determine since there is no generally agreed definition for an AECOPD. Studies show that only 32–50% of symptom defined AECOPD are reported by patients to health care professionals.[13,14] Although there is no reliable estimate of the prevalence of AECOPD, data are present on the occurrence of exacerbations. Research shows that exacerbations are more frequent in the winter season[15] and may occur clustered in time.[16] Exacerbations are also more frequent and severe as COPD severity increases.[17] Besides COPD severity, the history of exacerbations is also a good predictor of future exacerbations.[17] Furthermore, a strong correlation exists between depressive symptoms and recurrent exacerbations.[18,19]

Pathophysiology of COPD and AECOPD

COPD is the result of a chronic inflammation in the airways. The inflammation is believed to be initiated by chronic exposure to exogenic toxins (e.g. cigarette smoke), which cause damage to the airway epithelium, activate the innate immune system and induce a rapid, nonspecific inflammatory response.[20,21] Of the innate immune response, the neutrophilic inflammation is most prominent in COPD. The cells of the innate immune system activate the adaptive immune system, of which CD8+-cells, CD4+ $T_{helper}1$ cells and B-cells play a role in COPD. This activation of the adaptive immune response is the beginning of a cascade that causes extensive chronic inflammation, oxidative stress and remodelling, and eventually results in destruction of alveolar space and deposition of connective tissue in the subepithelium and adventitium of the airway wall.[22] The degree of chronic inflammation in COPD correlates with the severity of airflow limitation. This is supported by a correlation between the severity of obstruction and presence of inflammatory cells in the small conducting airways[22] and the presence of neutrophils in sputum.[23] Also, bacterial colonisation is more frequently observed in patients with severe to very severe COPD, suggesting that bacterial colonisation induces inflammation and contributes to the progression of COPD.[24,25]

The existence of chronic inflammation and oxidative stress in the lungs is supported by the presence of oxidants and numerous pro-inflammatory cytokines in the airways and serum. Compared to healthy controls, sputum specimens of patients with stable COPD and patients with AECOPD show increased numbers of neutrophils and increased levels of pro-inflammatory cytokines like interleukin-6 (IL-6) and interleukin-8 (IL-8).[21,23,26-29] During an AECOPD neutrophils, IL-6, and IL-8 are also increased in serum.[27,30,31] Interleukin-6 is a cytokine released during initial immune response by different cell types of the native immune system, like macrophages. It induces hepatic acute phase response during inflammation[32] which in turn increases production of C-reactive protein (CRP). Furthermore, IL-6 is a growth factor for T- and B-cells.[33] Interleuking-8 is released by a variety of cell types involved in inflammation, like endothelial cells, fibroblasts and monocytes.[34] It is a potent neutrophil chemotactic and activating factor.[34] The higher inflammatory markers in serum in patients with COPD might be explained by the “overspill theory”, in which the local inflammatory processes in the lung “spill over” to the systemic

circulation.[35] Therefore it is believed that disease activity of COPD can be measured in serum by biomarkers.

Exhaled breath condensate (EBC) components are thought to reflect the physiological state of lining fluid of the airways. Measuring EBC components is a non-invasive mean of obtaining information on oxidative stress and inflammation in the airways. Hydrogen peroxide (H_2O_2, a precursor of potent oxidants OH and HOCl) and 8-isoprostane (formed by the free radical peroxidation of arachidonic acid) are oxidative stress biomarkers in EBC that are proved to be elevated in patients with COPD during stable state and during exacerbations.[31,36-38] The EBC component heme-oxygenase-1 (HO-1) is an inducible catalyser of the degradation of haeme to biliverdin, and is believed to provide protection from oxidative stress. In histopathological studies, HO-1 positive macrophages were decreased in ex-smokers with COPD compared to ex-smokers without COPD and non-smokers.[39,40] Furthermore, HO-1 positive cells were increased during severe exacerbations compared to stable state, indicating increased oxidative stress during exacerbations.[29]

Aetiology of acute exacerbations of COPD

Microbiology

There is a great variety in reported infectious causes of COPD exacerbations. It is of importance to determine, both for bacteria and viruses, whether the presence of the microorganism is actually the cause of the exacerbation. Estimated is that about 50–78% of acute exacerbations of COPD are caused by respiratory infections,[24,27,41,42] in which the clinical presentation ranges from pneumonia to coryzal symptoms with dyspnoea. Patients with AECOPD of proven infectious aetiology have a longer hospital stay and a greater decrease in FEV_1 during the exacerbation than patients with non-infective exacerbations.[27]

Viral causes

In the past, viruses have been an underestimated cause of AECOPD and the causative role of viruses in AECOPD is still not fully established. The observation that as well viral infections and exacerbations are seasonal does suggest that viruses have a role in AECOPD.[15,43] Recently, researchers deliberately exposed COPD patients and healthy smokers to rhinoviruses and observed that this virus was able to cause an exacerbation.[44]

Detection of viruses by serology and by culture is less sensitive and more time consuming than PCR techniques. Because of the advanced PCR techniques in detecting viruses, the percentage of exacerbations they account for can also be overestimated. The presence of viral DNA or RNA does not implicate that the virus is the cause of an exacerbation as several studies have reported that 12–19% of stable COPD patients can carry viruses.[45,46] In exacerbations, several studies have reported that viruses were detected in 20–56% of cases.[24,27,41,42,46,47] In these studies rhinovirus, influenza virus and respiratory syncytial virus and were the most common isolated viruses. A more extensive overview can be found in table 1.

Table 1: Causes of exacerbations of COPD.

COPD exacerbations: divided by cause	
Bacteria	**Atypical microorganisms**
– *Streptococcus pneumoniae*	– *Mycoplasma pneumoniae*
– *Haemophilus influenzae*	– *Chlamydophila pneumoniae*
– *Moraxella catarrhalis*	– *Legionella pneumophila*
– *Haemophilus parainfluenzae*	– *Coxiella burnetii*
– *Pseudomonas aeruginosa*	
– *Staphylococcus aureus*	**Other**
	– Sulphur dioxide (SO_2)
Viruses	– Ozone (O_3)
– Human rhinovirus	– Nitrogen dioxide (NO_2)
– Respiratory syncytial virus	– Particulate matter ($PM_{2,5}$, PM_{10})
– Influenza virus	
– Parainfluenza virus	
– Human metapneumovirus	
– Coronavirus	
– Adenovirus	

Bacterial causes

Bacteria as cause of AECOPD are reported from 30%[48] up till 55%.[27,49] The most common bacterial pathogens as cause of AECOPD are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and in patients with more severe COPD also *Pseudomonas aeruginosa*.[42,48] It is difficult to determine the role of bacteria in AECOPD, as 34–48% of patients with COPD are reported to be colonised with bacteria.[26,27,50,51] Molecular typing of bacteria during exacerbations showed that the acquisition of new strains may cause exacerbations,[52] but not every acquisition of a new strain is linked to an exacerbation.

Non-microbial causes

One tenth of AECOPD are induced by environmental pollution of which ozone, sulphur dioxide, and nitrogen dioxide are known and researched causes.[53,54] Particulate matter (PM) is also related to increased admissions for COPD and other respiratory diseases.[53,55] Particulate matter consists of a mixture of solid particles and liquid aerosols suspended in the air from natural sources and industrial activities, but can also be traffic related.[56] Other possible, non-infectious causes may be left sided heart failure, change in environmental temperature, but about 30% of exacerbations are of unknown origin.[6]

Clinical presentation and diagnosis

History

Patients with an AECOPD usually present with dyspnoea, which may be acute but can also be a history of slowly progressive dyspnoea. Coughing or sputum production may or may not be present. Increase in sputum volume and its purulence are important to assess. Purulent sputum

is usually a sign of infection.[57] Fever or other signs of infection should be looked for. Haemoptysis may be present in case of a severe infection. Risk factors for atypical infections should be thought of.

Laboratory tests

Laboratory test can be performed if necessary. C-reactive protein as marker for inflammation can be performed. Additional laboratory tests can be performed depending on the differential diagnosis. If available, an arterial blood gas can be performed. Hypoxemia may be present and in more severe cases a patient can also retain CO_2. When a hypercapnia is present, it is important to distinguish if the hypercapnia is longer existing or an acute development.

Radiology

A chest X-ray is mainly useful for excluding other pathology like pneumothorax, pleural fluid, congestive heart failure or otherwise. It may reveal consolidations or other pathology.

In the acute phase a chest CT-scan has no additive value in the tract of diagnosing an exacerbation of COPD. In a patient with recurrent airway infections a CT-thorax can be performed to investigate whether bronchiectasis are present.

Biomarkers

Biomarkers can be used as indicators of a physiological state in which a patient is or may become, it can help in diagnosis, aetiology and prognosis. In theory, biomarkers could be used to predict exacerbations, to determine if a patient has increased inflammation, to distinguish types of inflammation, or to predict clinical outcomes.

Many biomarkers have been researched and many of them are of limited clinical use. At this moment the most important biomarkers in AECOPD are CRP, serum IL-6, 8-isoprostane, H_2O_2 and procalcitonin (ProCT). These biomarkers are closely related to oxidative stress and inflammation. C-reactive protein is momentarily the most widely used marker of inflammation in clinical practice.

In patients with frequent exacerbations, both CRP and serum IL-6 levels are increased during a stable phase but also during the recovery period of an AECOPD[58,59] compared to patients with infrequent exacerbations. Interleukin-6 is widely expressed and produced in the body, and is not specific to the lung. Serum IL-6 has no additional value above CRP in clinical decision making. Interleukin-6 levels in sputum may be of use to predict therapy response,[58] although more research is needed before clinical decisions can be made based on this biomarker. Similarly, there is a lack of studies which investigate the use of exhaled biomarkers 8-isoporstane and H_2O_2 for clinical purposes.

Procalcitonin may be a biomarker which can discriminate in aetiology of an exacerbation but may also be used as therapeutic response parameter. Procalcitonin is the precursor of calcitonin and is released in response to a bacterial infection by many tissues under stimulation of several

cytokines. Procalcitonin levels are minimally raised in viral infections,[60] making it a relative specific diagnostic tool for bacterial infections. Most research has been performed in patients with community acquired pneumonia (CAP).[61] It is suggested that ProCT could become a useful tool in clinical decision making regarding antibiotic therapy. There have been several trials to assess the utility of ProCT in AECOPD. In general ProCT-guided antibiotic therapy compared to standard management in AECOPD showed no differences in death from any cause, rates of intensive care unit (ICU) admission for any reason, duration of ICU stay, improvement of symptoms, difference in the quality-of-life score, re-exacerbation and readmission.[62] Procalcitonin-guided antibiotic therapy showed reduction in antibiotic prescription[62] and in one study[63] also reduction in antibiotic therapy duration, which in turn decreases the patient's exposure to antibiotics and related side effects, lowers the burden of antibiotic use and might lower the risk of antimicrobial resistance. Procalcitonin is not yet being implemented in standard care though, as it is relatively expensive and there has been little to no research performed outside Europe.

Management

The treatment of an AECOPD consists of supportive therapy, maximal bronchodilation, steroids to reduce the inflammation and treatment of the cause.

Supportive therapy

Oxygen delivery is one of the first supportive therapies that can be provided for a patient. In some patients, too much oxygen may cause hypercapnia as the drive to breathe in those patients relies on arterial O_2 pressure. Symptoms of acute hypercapnia, like somnolence, headache, drowsiness, confusion, flushed skin or agitation, may be observed then.

Physiotherapy during an admission for an AECOPD can prevent deterioration in skeletal muscle function and improve exercise capacity.[64,65] Because an AECOPD is accompanied by an impaired energy balance due to a decreased dietary intake and an increased resting energy expenditure, nutritional support may also benefit the patient in terms of general well-being and prevention of muscle wasting.[66-68]

Pharmacotherapy

An exacerbation is the result of increased inflammation causing increased flow limitation. Treatment should be directed towards controlling this exacerbated inflammation and maximizing bronchodilation. Short acting agents like salbutamol and ipratropium are mostly used for maximal bronchodilation.

Corticosteroids have been proven to reduce time to recovery and treatment failure, increase FEV_1 and improve arterial hypoxemia.[8] Treatment schemes have been reported varying from 30 mg prednisolone orally to 60 mg intravenous, ranging from 5 days to two weeks. Studies comparing oral and parenteral steroids have shown no differences in clinical outcomes.[69]

Antibiotic treatment can be initiated when a bacterial infection is suspected. The Anthonisen criteria[70] are generally used to decide whether antibiotic treatment is necessary or not. These criteria are derived from a randomized placebo-controlled crossover trial which has been performed in the '80s where patients with COPD exacerbations were treated with antibiotics or placebo. The cardinal symptoms of infection in this study were increased sputum volume and purulence in combination with increased dyspnoea. An exacerbation with all the previous 3 symptoms is called a type 1 exacerbation; two out of three symptoms have to be present for a type 2 exacerbation; one out of three and at least one other "minor symptom" (table 2) have to be present for it to be a type 3 exacerbation. Patients with type 1 and type 2 exacerbations are most likely to benefit from antibiotic therapy. In Spain a pilot study was performed with hospitalized patients with AECOPD, where antibiotic therapy was given to patients with self-reported purulent sputum and withheld in patients with non-purulent sputum.[71] There was no difference between the two groups in treatment failure on day 3, suggesting patient reported non-purulent sputum may be a valid criterion to withhold antibiotics.[71]

Table 2: Classification of acute exacerbations of COPD according to Anthonisen criteria.[70]

Classification of AECOPD according to Anthonisen criteria	Presence of symptoms and findings
Type 1	Increased dyspnoea Increased sputum volume Increased sputum purulence
Type 2	Two symptoms of type 1
Type 3	One of three symptoms of type 1, plus at least one of the following findings: – Upper respiratory infection (sore throat, nasal discharge) within the past 5 days – Fever without other cause – Increased wheezing – Increased cough – Increase in respiratory rate or heart rate by 20% as compared with baseline

A Dutch study showed that addition of doxycycline to the treatment regimen with glucocorticoids of a patient with an exacerbation was superior on day 10 but equivalent on day 30 in terms of clinical success and clinical cure compared to glucocorticoids alone, even in patients not showing signs of infection.[72] Most recently Spanish investigators performed a multicentre trial where they suggested that treatment of a mild to moderate exacerbation with amoxicillin/clavulanate, independent of glucocorticoids treatment, might give better clinical cure after 10 days compared to placebo.[73] In this study the median time to next exacerbation was also increased in patients receiving antibiotics compared to placebo. Unfortunately, because of recruitment problems this study did not reach the calculated amount of patients needed, so that definite conclusions cannot be made from the results of this study.

Prevention

Preventing exacerbations is an important treatment goal in COPD. There is a wide range of preventive measures which have proven to reduce exacerbation frequency or hospitalization in patients with AECOPD.

Supportive measures

Influenza vaccination and pneumococcal vaccination have both been researched as preventive measures for infection associated exacerbations. Current GOLD guidelines[2] advise influenza vaccination for patients with COPD. Pneumococcal vaccination is mainly advised for elderly patients with COPD. Investigation on this subject is ongoing.

Of the non-pharmacologic interventions, pulmonary rehabilitation is the most effective in reducing hospital admissions and mortality and improving health-related quality of life in COPD patients who have recently suffered an exacerbation of COPD.[74]

Long-acting bronchodilators

Long-acting bronchodilators can be divided in two groups: long acting muscarinic receptor antagonists (LAMAs) and long acting β-agonists (LABAs). Many of both have proven to show a positive effect on exacerbation reduction and improvement in quality of life.[75-79] An overview of the long-acting bronchodilators is given in table 3.

Table 3: An overview of available long-acting bronchodilators.

LABA	LAMA
– Formoterol	– Tiotropium
– Arfomoterol	– Glycopyrronium
– Salmeterol	– Aclidinium
– Indacaterol	
– Olodaterol	

Inhalation corticosteroids

Inhalation corticosteroids (ICS) can be given to patients with high risk of exacerbations. In several studies ICS provided a reduction of symptoms (dyspnoea, cough) and reduced the frequency of exacerbations.[86-88]

Phosphodiesterase inhibitors

Currently, two phosphodiesterase inhibitors are available for the treatment of COPD: theophylline and roflumilast. Theophylline is a xanthine derivative which acts as a non-selective phosphodiesterase inhibitor. It has bronchodilator effects, improves symptoms and there is evidence that it can reduce exacerbations.[89-91] It is a drug which needs therapeutic window

monitoring. It can interact with many drugs and can have toxic side effects which may be potentially dangerous, like cardiac arrhythmia. Therapy with theophylline is not recommended if long acting bronchodilators are available but can be used as add-on therapy.[2]

Roflumilast is a selective phosphodiesterase-4 inhibitor. It increases prebronchodilator FEV_1 and can reduce exacerbations in a selected group of patients with COPD.[92,93] In all trials, patients in the roflumilast group experienced more side effects in comparison to patients in the placebo groups. The side effects were mostly gastro-intestinal related (nausea, diarrhoea, weight loss) and headache. These adverse events were associated with increased patient withdrawal in the roflumilast groups. The design of the trials limits the generalizability of these results. The included COPD patients were required to have symptoms of chronic bronchitis and AECOPD in the past. More investigation is needed to determine the exact place of this medication in the treatment of AECOPD.

Maintenance antibiotics

Antibiotic prevention of exacerbations is a highly researched topic in COPD.[88] Studies have been performed using amongst others macrolides, tetracyclines and quinolones. The most promising class of antibiotics appear to be macrolides. In various chronic lung diseases like diffuse panbronchiolitis, cystic fibrosis (CF) and non-CF bronchiectasis macrolides seem to have, besides their antimicrobial activity, an immunomodulatory function.[89]

Aim of the thesis

Exacerbations of COPD are the core of this thesis. Prevention of exacerbations with macrolides, prediction of the occurrence of exacerbations, characterisation of a frequent exacerbating population and the ability to correlate clinical and laboratory parameters in this population will be researched and discussed.

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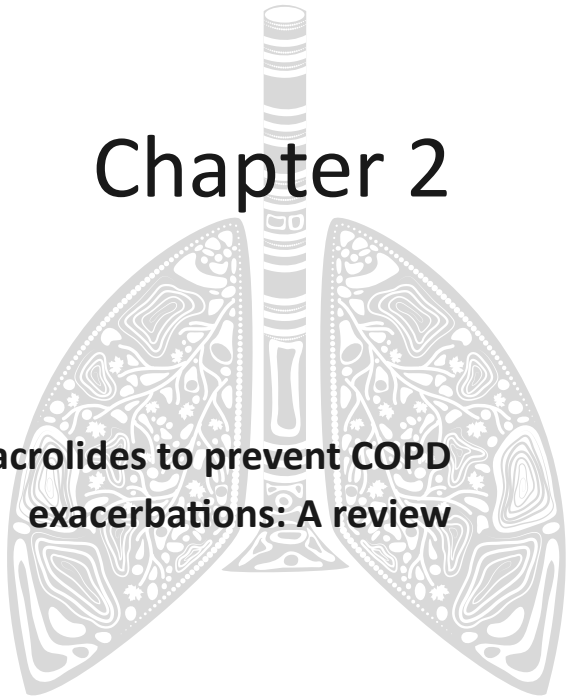
Chapter 2



Macrolides to prevent COPD exacerbations: A review

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Abstract

Chronic obstructive pulmonary disease (COPD) is one of the major health problems in the world. Long-term treatment with macrolide antibiotics is a recent development as it has been reported to have beneficial effects on exacerbation frequency. These effects are not only attributed to the anti-microbial effect but also to the immune modulatory effect. Six randomized trials and one retrospective study have been performed to investigate the efficacy of macrolides in the prevention of acute exacerbations of COPD. Besides the beneficial effects on the occurrence of exacerbations of COPD, this treatment also seems to improve quality of life and is well tolerated. Antimicrobial resistance is one of the future issues to consider before implementing this therapy.

Introduction

Chronic obstructive pulmonary disease is one of the major health problems in the world; it is the third leading cause of death in 2010.[1] The natural history of COPD is characterised by progressive airflow obstruction and the occurrence of exacerbations. Frequent acute exacerbations of COPD (AECOPD) can lead to a decreased health related quality of life,[2] a decline in lung function,[3] an increased risk of hospitalisation,[4] and an increase in mortality.[5] Furthermore, the occurrence of an exacerbation is a risk factor of developing recurrent exacerbations.[6]

Prevention of AECOPD with long-term macrolide antibiotic treatment is a recent development as this therapy does not only have an anti-microbial effect but also seems to have an immune modulatory effect.[7] Several studies investigated the efficacy of macrolides in the prevention of AECOPD.[8-14] In this paper these studies and the role of macrolides in the treatment of COPD will be reviewed.

Macrolide antibiotics

Macrolide antibiotics are derived from the *Streptomyces* species. Erythromycin was the first antibiotic to be widely used for the treatment of infectious conditions. Over decades many other macrolides, like clarithromycin and azithromycin, have been developed in an attempt to create antimicrobial agents with broader antibacterial activity, less side effects, and superior chemical and pharmacodynamic properties (table 1).

All macrolides have a macrocyclic lactone ring consisting of 12 or more atoms. Erythromycin and clarithromycin are 14-membered-ring compounds, while the lactone ring of azithromycin contains 15 atoms. Macrolides bind to the 50S subunit of the bacterial ribosome and inhibit bacterial protein synthesis.[15] Most macrolides have a uniform degree of activity; their antimicrobial spectrum extends from Gram-positive bacteria to a limited activity against Gram-negative bacteria.[16] Of the macrolides, azithromycin displays superior activity against Gram-negative organisms, such as *Haemophilus influenzae*.[15] Compared with other macrolides like erythromycin and clarithromycin, azithromycin has better uptake in peripheral blood polymorphonuclear neutrophils (PMN) with slower release[17,18] and also a better tissue uptake. Furthermore, compared with erythromycin, tissue concentrations of azithromycin are increased for a certain period after the last administered dose.[19,20]

Table 1: Overview of macrolide antibiotics.

14-membered-ring compound	15-membered-ring compound	16-membered-ring compound
	Natural macrolides	
– Erythromycin		– Josamycin
– Oleandomycin		– Spiramycin
	Semisynthetic macrolides	
– Clarithromycin	– Azithromycin	– Rokitamycin
– Flurithromycin		– Miocamycin
– Roxithromycin		
– Dirithromycin		
– Telithromycin		
– Troleandomycin		

Results of the effect of macrolide antibiotics in chronic lung diseases other than COPD

Diffuse panbronchiolitis

The most striking effect of macrolides was observed in survival rates in diffuse panbronchiolitis (DPB). This is a progressive inflammatory disorder of the airways found almost exclusively in Japan. The disease is characterised by chronic cough, excessive sputum production, exertional breathlessness, chronic sinusitis and eventually *Pseudomonas aeruginosa* colonisation.[21] Untreated, the prognosis of diffuse panbronchiolitis is poor, with progressive deterioration of lung function, the development of diffuse bronchiectasis and death caused by respiratory failure. The introduction of long-term macrolide therapy resulted in dramatic improvements in survival with 5-year survival rates increasing from 63% to 92%.[21,22] Significant symptom reduction and improved pulmonary function were also achieved.[23-26] The success of maintenance macrolide therapy in DPB was attributed to immune modulation as serum levels of the macrolides were below the minimum inhibitory concentrations (MIC) of the common microorganisms, and some of the microorganisms were intrinsically not sensitive to macrolides. These results suggested that other effects than the antimicrobial effect were more important.

Cystic fibrosis

In patients with cystic fibrosis (CF) who were colonized with *P. aeruginosa*, macrolide therapy led to improvement in forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC), a reduction in exacerbation rate, and a reduction in hospital days and days of intravenous antibiotic use. Furthermore time until the first exacerbation was delayed and the number of additional courses of antibiotics was reduced.[27-32] A Cochrane review of macrolide therapy also concluded that treatment with azithromycin had a small but significant effect on pulmonary function in patients with cystic fibrosis.[33]

Non-CF bronchiectasis

There are recent advances in the treatment of non-CF bronchiectasis with macrolides. In New Zealand a randomised double-blind placebo controlled trial was performed where three times a week 500 mg azithromycin or placebo was given for six months to patients with non-CF bronchiectasis.[34] The aims of the study were to investigate whether long-term treatment with azithromycin decreased the frequency of exacerbations, increased lung function and improved health-related quality of life in these patients. The study included 141 patients, two patients discontinued the study drug because of gastrointestinal symptoms. An intention to treat analysis showed a statistically significant reduction in exacerbation rate in the azithromycin group compared with the placebo group. Although there were no differences in the overall score of the St. George's Respiratory Questionnaire (SGRQ), a significant improvement was observed in the symptom component of the SGRQ at six months in the azithromycin group compared with the placebo group. This difference was considered clinically important. Furthermore, there were no differences in FEV_1 but there was a small statistically significant change in prebronchodilator FVC after 12 months and in postbronchodilator FVC after 6 and 12 months. In a second randomised placebo-controlled trial from the Netherlands, Altenburg *et al*. treated 83 patients with non-CF bronchiectasis with daily 250 mg azithromycin or placebo during 12 months.[35] Two patients discontinued the study drug because of adverse effects. In an intention to treat analysis a significant reduction in exacerbations in the azithromycin group was observed. This was accompanied by a statistically and clinically significant improved quality of life measured by SGRQ and a statistically significant increase in FEV_1 and FVC compared to placebo.

Trials in COPD

Several studies have been performed to evaluate the effect of macrolides in the treatment of COPD. Until so far there are six prospective randomised trials and one retrospective study of which three have been performed with erythromycin, three with azithromycin and one with clarithromycin (table 2).

The first prospective study to be performed with macrolides in patients with COPD was a Japanese study conducted from 1997 to 1999.[12] Suzuki *et al*. investigated the effect of erythromycin therapy on common colds and exacerbations in patients with COPD. It was a randomised controlled but not blinded study. The included patients fulfilled the criteria for COPD of the American Thoracic Society. Patients with bronchiectasis and diffuse panbronchiolitis were excluded. Patients were randomised to receive erythromycin 200–400 mg/day or riboflavin 10 mg/day for 12 months. One hundred and eight of the recruited 109 patients completed the study, one patient was excluded during the study due to side effects. Analysis showed that patients who received erythromycin therapy had significantly less common colds (1.24 ± 0.07 per person in treatment group versus 4.54 ± 0.02 per person in riboflavin group; $p = 0.0002$) and significant less subsequent AECOPD compared with patients in the control group. The severity of exacerbations was also significantly lower in the treatment group compared with the placebo

group as there was a statistic significant reduction in hospital admissions for an exacerbation in the erythromycin group. This study had a few limitations. The study was not blinded; patients, researchers and clinicians were aware of the treatment received. Furthermore, a sample size calculation was not provided in the paper. Also, the authors did not discuss what the screening methods were for bronchiectasis. The achieved effect of the erythromycin may therefore also be due to the presence of bronchiectasis in some patients. Another point of discussion is the lack of information regarding the severity of the COPD in the studied population. The mean FEV_1 is mentioned but not the percentage of predicted. Nowadays it is recognised that patients with more severe COPD are at higher risk for developing an AECOPD.[6]

Seemungal *et al*. performed a randomised double-blind placebo-controlled trial in patients with COPD to test the hypothesis that maintenance treatment with macrolides reduces exacerbation frequency.[9] Patients were required to have moderate to severe COPD with an FEV_1 between 30% and 70% of predicted. Exclusion criteria were a history of asthma, bronchiectasis, neoplasia or other significant respiratory disease. Of the pre-calculated 136 patients, 115 were recruited of which 109 eventually were randomised to receive 250 mg erythromycin or placebo twice daily during 12 months. The intention to treat analysis showed a significant difference in exacerbations in favour of COPD patients who received erythromycin. Furthermore, there was a significant difference in time to the first exacerbation in the macrolide arm compared with the placebo arm (271 days versus 89 days). Also, the median duration of exacerbations was significantly less in the macrolide group compared with the placebo group. The main limitation of this study was that the total number of patients predefined for the study was not reached. Therefore their conclusions should be cautiously interpreted.

In a randomised placebo-controlled double blind trial He *et al*.[11] investigated whether long-term treatment with erythromycin influenced airway inflammation and health outcome in patients with COPD. They included 36 patients with COPD with an $FEV_1/FVC < 0.7$ and an FEV_1 between 30–70%. Patients with significant other pulmonary diseases were excluded. After six months of three times daily 125 mg erythromycin, patients in the erythromycin group experienced significant less exacerbations compared with patients in the placebo group. Erythromycin also significantly delayed the time to the first COPD exacerbation compared with placebo. Furthermore, a significant decrease of neutrophils in sputum was observed in the erythromycin group compared with baseline. There were no significant differences in health related quality of life between the two groups. The limitations of this study were the small sample size and the six month treatment duration which may be too short to observe a larger effect. In this study no sample size calculation was provided.

Table 2: Overview of trials.

	Suzuki[12]	Seemungal[9]	He[11]	Banerjee[10]	Blasi[13]	Pomares[14]	Albert[8]
Design of study	RT	RCT	RCT	RCT	RT	Retrospective, no control group	RCT
Double blind	No (open-label)	Yes	Yes	Yes	No (open-label)	No	Yes
Number of patients (treatment-control)	109 (55–54)	109 (53–56)	36 (18–18)	67 (31–36)	22 (11–11)	24 (only treatment)	1142 (570–572)
Number of males (%)	91 (83%)	69 (60%)	31 (86%)	46 (69%)	19 (86%)	24 (100%)	651 (57%)
Mean age, treatment-control	69–72	67–68	69–69	65–68	72–73	71	65–66
Inclusion criteria of COPD severity	COPD according to ATS criteria	FEV_1 between 30% and 70% of predicted	FEV_1 between 30% and 70% of predicted. FEV_1/FVC ratio <70%	FEV_1 < 60% of predicted. FEV_1/VC ratio <70%	Severe COPD	FEV_1 <50%	FEV_1 < 80% of predicted. FEV_1/FVC ratio <70%
Mean FEV_1 in % of predicted, treatment-control	NA	49–51	44–42	43–44	NA	32	39–40
Macrolide	Erythromycin	Erythromycin	Erythromycin	Clarithromycin	Azithromycin	Azithromycin	Azithromycin
Dose	200–400 mg once daily	250 mg twice daily	125 mg three times daily	500 mg once daily	500 mg three times a week	500 mg three times a week	250 mg once daily
Duration of therapy	12 months	12 months	6 months	3 months	6 months	12 months	12 months

RT=indicates randomized trial; RCT=randomized controlled trial; NA=not addressed.

Banerjee *et al*. examined whether clarithromycin treatment in patients with COPD improved health status, diminished sputum bacterial numbers and reduced exacerbation rates compared to placebo.[10] This study was set up as a prospective double-blind randomised controlled trial. The researchers included 67 patients with moderate to severe COPD. Patients were excluded when they had a clinical history of asthma, bronchiectasis or lung cancer. The subjects received three months of clarithromycin 500 mg daily or placebo. Overall, no significant benefit was seen in health status, exacerbation rate or sputum bacterial numbers. However, significant improvements in both the SGRQ symptom score and 36-item short-form health survey (SF-36) physical function score were seen. One of the major limitations of this study was the short treatment time of three months. A longer follow-up might have led to the detection of a significant change in clinical parameters as exacerbation frequency.

A study published in 2010 by Blasi *et al*.[13] investigated the efficacy and safety of long-term azithromycin use compared to standard care in outpatients with severe COPD and tracheostomy. The study was a phase II multicentre open-label non-placebo control-arm pilot trial. Inclusion criteria were patients with severe COPD (assessed by pulmonary function test previously) who had a tracheostomy. Patients were excluded if their life expectancy was less than one year. The primary objective was to determine if azithromycin use was associated with a reduction in the number of exacerbations and hospitalisations during the study period. Secondary outcomes were bacteriological and immunological effects and impact on quality of life. Subjects were randomized to receive standard care or standard care plus oral azithromycin three times weekly 500 mg for six months. In total 22 patients were included in the study. Analysis showed that the cumulative number of exacerbations after three months of treatment was significantly lower in the azithromycin group. Also the cumulative number of antibiotic and steroid treatments was significantly lower in the azithromycin group after three months of treatment. The time to first exacerbation was also significantly longer in the azithromycin group compared to the standard care group. The patients in the standard care group had a five-fold significantly higher hazard of having a first exacerbation. After discontinuation of treatment in the azithromycin group the subjects experienced significantly more exacerbations in the six months after drug discontinuation. There was no significant impact on airways bacterial colonisation. A significant improvement in quality of life, measured by the Maugeri Respiratory Failure questionnaire (MRF26), was observed in the azithromycin group after three, six and eight months which was not seen in the standard care group. This study showed that azithromycin had beneficial effects on a well-defined population of patients with COPD who have a high microbial colonisation rate and a subsequent higher chance of exacerbations.[36,37] The small sample size and the short treatment time were the major limitations of this study. Also, the authors did not provide any information on the criteria for the diagnosis of COPD nor the severity of the previously assessed pulmonary function in both groups.

Pomares *et al*.[14] investigated the usefulness of long-term intermittent azithromycin therapy in reducing exacerbation frequency in severe COPD patients at a high risk of AECOPD despite

conventional maximum treatment. This study was a retrospective study. Inclusion criteria were a FEV_1 of <50% of predicted and patients were required to have at least four AECOPD in the previous year. Exclusion criteria were asthma, malignancy and significant bronchiectasis assessed by high-resolution computed tomography (HR-CT). Twenty-four patients were eligible for study participation of which 20 completed the 1-year period. The subjects received 500 mg azithromycin three times a week during 12 months. Compared to the year before treatment, long-term azithromycin therapy resulted in a statistically significant reduction of 58.9% in AECOPD, a 61.2% decrease in hospitalizations, and a reduction of 18.7 days in yearly mean hospital stay due to respiratory disease. The limitations of this study were the retrospective character, the lack of matched controls and the small sample size.

The most recent and largest prospective study concerning long-term macrolide therapy in COPD was performed by Albert *et al*.[8] They investigated whether long-term treatment with azithromycin decreased the frequency of AECOPD when added to the usual care of patients with COPD. The study was conducted as a randomised placebo-controlled double blind trial. Patients were included when they had received treatment with systemic glucocorticoids for an AECOPD in the previous year or were using continuous supplemental oxygen. Asthma was one of the exclusion criteria. The primary outcome was the time to the first AECOPD. In total 1142 subjects were included in the study. The subjects received 250 mg azithromycin or placebo daily for 12 months. An intention to treat analysis showed a statistical significant reduction in time to first exacerbation and a reduced risk for exacerbations in patients receiving daily azithromycin during one year. In the total SGRQ score, one of the secondary outcomes, there was a significant difference between the treatment and the placebo group. However, this mean difference did not exceed the minimal clinically important difference. Significantly more patients in the azithromycin group had an improvement in the SGRQ score compared with the placebo group. No differences were seen between groups in the score on the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36). This study shows that azithromycin lowers exacerbation rate in patients with COPD. One of the limitations of this study is that other obstructive lung diseases such as non-CF bronchiectasis were not described as exclusion criteria.

Effects on antimicrobial resistance

One of the main risks of long-term treatment with macrolide antibiotics is the development of antimicrobial resistance. Four of the seven previously described studies addressed the development of macrolide resistance (table 3).

Seemungal *et al*.[9] tested sputum samples for sensitivity. Baseline sputum samples in which a bacterial pathogen was found showed no antimicrobial resistance to macrolides. After 12 months one erythromycin resistant strain of *S. pneumoniae* was found in the erythromycin group.

Blasi *et al*.[13] cultured tracheal aspirates and tested for azithromycin resistance. At baseline eight of eleven subjects and ten of eleven subjects were colonised with potential pathogenic microorganisms in the azithromycin group and standard care group respectively. After six months

of azithromycin there was no significant change in colonisation rates between the two groups. One strain of *S. pneumonia* was isolated in the azithromycin group which was resistant for macrolides.

Pomares *et al*.[14] retrospectively evaluated sputum cultures for macrolide resistance. In the 12 months before treatment with azithromycin there were three isolates of *S. pneumoniae* which were resistant for macrolides. These strains were also resistant for clindamycin representing a highly resistant phenotype. During therapy with azithromycin four strains of *S. pneumoniae* and one strain of *H. influenzae* were isolated which were resistant for azithromycin. This difference was not statistically significant.

Albert *et al*.[8] collected nasopharyngeal swabs and tested selected respiratory pathogens (i.e., *Staphylococcus aureus*, *S. pneumoniae, Haemophilus* species, and *Moraxella* species) for macrolide resistance. At baseline 79 of 558 (14.1%) patients and 85 of 559 (14,8%) patients were colonised with selected respiratory pathogens in the azithromycin group and placebo group respectively. There was no statistically significant difference in macrolide resistance in these isolates (52% in azithromycin group versus 57% in placebo group). During the course of the study, participants in the azithromycin group became statistically significant less colonised compared with the placebo group (12% versus 31%). However, the isolated pathogens in the azithromycin group were significantly more often resistant compared with the pathogens in the placebo group (34/47 (81%) versus 44/108 (41%)). In a comment on the published paper, Hahn[38] pointed out that the rate of colonisation with macrolide resistant bacteria was actually 24% lower in the azithromycin group than in the placebo group when calculating rates per 100 subjects per year. This was confirmed by the authors by stating that indeed fewer patients receiving azithromycin had macrolide resistant organisms cultured than those receiving placebo. Despite the differences in macrolide resistance in relative and absolute numbers in this study population, antimicrobial resistance will be a future issue to consider when implementing this treatment in large groups of patients.

Besides studies with macrolides in COPD, other studies have also addressed the development of antimicrobial resistance in treatment with macrolides. A Dutch double-blind randomised placebo-controlled trial which studied the effect of two weeks 500 mg daily clarithromycin on flora in nasal and throat swabs in 296 patients showed a statistically significant increase in macrolide resistant microorganisms (*Haemophilus parainfluenzae* and oral flora) compared with the placebo group. This effect persisted for at least eight weeks after cessation of therapy.[39] Malhotra-Kumar *et al*.[40] also performed a randomised placebo-controlled double-blind trial comparing the development of macrolide resistance in throat swabs in volunteers taking three days azithromycin, seven days clarithromycin or three to seven days placebo. After treatment with macrolide antibiotics a significant rise in macrolide-resistant streptococci was seen in both antibiotic groups but not in the placebo group. After 180 days these differences remained significantly higher in the macrolide groups compared with the placebo group. In the previously described study of Altenburg *et al*.[35] also an increase in macrolide resistance was found after 12

months of daily azithromycin in patients with non-CF bronchiectasis. A statistically significant macrolide resistance rate of 88% was noted in azithromycin-treated subjects compared with 26% in the placebo group after 12 months treatment. In another Dutch study where patients with CF were treated with long-term azithromycin, a retrospective analysis showed a significant increase in macrolide resistant *S. aureus* and *Haemophilus* species compared with CF patients who did not receive macrolide treatment.[41]

Adverse effects

The profile of adverse effects of long-term macrolide therapy is important to consider before implementing this treatment. Side effects were not very common in the previously described studies. The main side effects were of gastrointestinal nature. Of all seven studies, in total 27 of the 738 (3.7%) patients in the macrolide groups and 26 of the 747 (3.5%) patients in the placebo groups experienced side effects related to the gastrointestinal tract. There were no statistically significant differences between treatment groups in adverse effects. Other reported adverse events in these studies were rashes, cardiovascular events and other (not specified) causes all of which were also not statistically significant different between treatment and placebo groups.

Of the macrolides, both erythromycin and clarithromycin are known to have proarrhythmic effects and increase the risk of cardiac events. Ray *et al*.[42] performed a retrospective cohort study to assess whether patients who used azithromycin would have an increased risk of cardiovascular death, particularly sudden cardiac death, compared with patients who did not take antibiotics or patients who took other selected antibiotics. Analysis of almost two million prescriptions and controls showed that the risk of cardiovascular death during five days of azithromycin therapy was significantly greater than with either amoxicillin or ciprofloxacin but did not differ significantly from the risk with levofloxacin. Because this population consisted of Medicaid beneficiaries in the United States, a population characterised by a high prevalence of coexisting conditions and high mortality rates, Svanström *et al*.[43] performed a retrospective cohort study in young to middle-aged adults to investigate whether azithromycin was associated with an increased risk of death from cardiovascular causes. Analysis of over one million azithromycin prescriptions and controls showed that the current use of azithromycin significantly increased the risk of death from cardiovascular causes compared with no use. This increased risk was not seen when azithromycin use was compared with penicillin V. Furthermore the authors found that the mortality rates in the study of Ray *et al*. were markedly higher than the mortality rates in their study, indicating that the study population of Ray *et al*. had a higher baseline risk, as compared with their study population. The authors therefore suggested that their findings indicate that the risk of cardiac toxic effects associated with azithromycin may not be generalizable but may rather be limited to high-risk populations, such as patients with coexisting cardiovascular conditions.

In the seven previously described studies there were no reports of significant differences in cardiovascular events or deaths between the treatment and placebo groups.

Table 3: Overview of antimicrobial resistance.

	Suzuki[12]	**Seemungal**[9]	**He**[11]	**Banerjee**[10]	**Blasi**[13]	**Pomares**[14]	**Albert**[8]
Antimicrobial resistance described	NA	Yes	NA	NA	Yes	Yes	Yes
Type of sample		Sputum			Tracheal aspirate	Sputum	Nasopharyngeal swabs
Number of samples (Placebo, macrolide)		43 (P=20, M=23)			52 (P=24, M=28)	Not described	248 (P=157, M=91)
Antimicrobial resistance at end of treatment		One macrolide resistant *S. pneumoniae* strain in treatment group			After 6 months one ceftazidim resistant *P. aeruginosa* and one erythromycin resistant *S. pneumoniae,* both in azithromycin group	One azithromycin resistant *H. influenzae* and 4 azithromycin resistant *S. pneumoniae* in the azithromycin group	Macrolide resistant pathogens in 81% in macrolide group versus 41% in placebo group*
P-value		Not calculated			Not calculated	NS	<0.001

NA indicates not addressed; NS=not significant.

*These numbers refer to patients (n=155) who became newly colonized during the course of the study. For a more accurate description of the scenario we refer to the text where the effect of macrolides on antimicrobial resistance is discussed.

Proposed working mechanism

Much *in vivo* and *in vitro* research has been performed with macrolide antibiotics to investigate the antimicrobial, anti-inflammatory and immune modulatory effects.

Pseudomonas aeruginosa is a Gram-negative rod which has an intrinsic resistance for macrolides but has nonetheless been extensively studied in combination with macrolides. Studies have shown that macrolides influence the virulence of not only *P. aeruginosa*[44-46] but also other microorganisms, like *Proteus mirabilis,*[47] *Salmonella enterica,*[48] *Staphylococcus epidermidis*[49] and *H. influenzae*.[50] Macrolides alter the biofilm around bacteria,[49-51] in *P. aeruginosa* this may facilitate phagocytosis by PMN.[45] It is also suggested that macrolides block quorum sensing in *P. aeruginosa*,[52,53] reduce flagellin synthesis and expression[47,48] and reduce production of bacterial exoenzymes.[44]

Macrolides support the airway innate immune system by maintaining airway epithelial integrity.[54,55] In several studies it is shown that macrolides can inhibit mucus hypersecretion and improve the transport of secretions.[7] Also macrolides decrease the hypersecretion of pro-inflammatory cytokines and chemokines in cell cultures, in animal models of disease, in persons with chronic inflammatory pulmonary diseases and in healthy individuals.[7,56,57] Macrolide antibiotics have also shown to be able to inhibit neutrophil chemotaxis by decreasing the expression of adhesion molecules and chemoattractants.[7] *In vitro*[58] and *in vivo*[59] studies show that alveolar macrophage phagocytosis function improves under influence of macrolides. Furthermore the function of dendritic cells in T-cell regulation can be modulated by macrolides.[7]

In murine models and in *in vitro* studies macrolides have shown to influence respiratory viral infections. In one study therapy with erythromycin increased survival rates in mice infected with lethal doses of influenza virus.[60] This effect might be exerted through the inhibitory action of erythromycin against virus-induced inflammatory responses in the lung. The production of interferon-gamma (IFN-γ) in the lungs was significantly decreased by the administration of erythromycin to the infected mice. Two *in vitro* studies which researched the effect of erythromycin and clarithromycin in human tracheal cells infected with rhinovirus and influenza A virus, also showed that macrolides decrease the production of pro-inflammatory cytokines and inhibited activation of nuclear factor-κB (a regulating factor in transcription of DNA in response to cellular stress).[61,62] These effects of macrolides on the immune response to viruses have not yet been proven in patients, although the study of Suzuki *et al*. provides evidence that macrolides may prevent common colds which are mostly of viral aetiology.[12]

Conclusion

Treatment with macrolides has shown many beneficial effects for several chronic lung diseases. Also the results from the previous described studies have shown that maintenance treatment with macrolides resulted in a decrease in exacerbations of COPD. In some studies there was

also an improvement in quality of life in patients with COPD. However, caution should be taken by implementing these results in daily practice. Many studies, except Pomares *et al*., did not perform a HR-CT scan to exclude patients with bronchiectasis or had a small sample size. Also more information needs to be obtained about the occurrence of antimicrobial resistance during long-term treatment with macrolides. This is in accordance with the opinion of Siafakis[63] who stated that the risk of microbial resistance associated with the long-term use of azithromycin in patients with COPD must be considered as part of the risk-benefit ratio of this treatment. Considering this, there is much debate whether each patient with an AECOPD should be treated with maintenance macrolide treatment according to results of the Albert *et al*. study. In theory this could cause a major problem of increasing macrolide resistance. Therefore, there is clearly a need to investigate whether subpopulations of COPD patients can benefit from this treatment. At the moment we are investigating in a prospective randomized double-blind trial, whether COPD patients with three or more exacerbations in the previous year will have a decrease in exacerbation rate using maintenance macrolide treatment and what will be the effect on microbial resistance.[64]

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Chapter 3

Influence of macrolide maintenance therapy and bacterial colonisation on exacerbation frequency and progression of COPD (COLUMBUS): Study protocol for a randomised controlled trial

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Trials 2012; 13: 82

Abstract

Background

Chronic obstructive pulmonary disease (COPD) is characterised by progressive development of airflow limitation that is poorly reversible. Because of a poor understanding of COPD pathogenesis, treatment is mostly symptomatic and new therapeutic strategies are limited. There is a direct relationship between the severity of the disease and the intensity of the inflammatory response. Besides smoking, one of the hypotheses for the persistent airway inflammation is the presence of recurrent infections. Macrolide antibiotics have bacteriostatic as well as anti-inflammatory properties in patients with cystic fibrosis and other inflammatory pulmonary diseases. There is consistent evidence that macrolide therapy reduces infectious exacerbations, decreases the requirement for additional antibiotics and improves nutritional measures. Because of these positive effects we hypothesised that maintenance macrolide therapy may also have beneficial effects in patients with COPD who have recurrent exacerbations. The effects on development of bacterial resistance in patients with COPD to macrolides due to this long-term treatment are unknown.

Until now, studies investigating macrolide therapy in COPD are limited. The objective of this study is to assess whether maintenance treatment with macrolide antibiotics in COPD patients with three or more exacerbations in the previous year decreases the exacerbation rate in the year of treatment and to establish microbial resistance due to the long-term treatment.

Methods/design

The study is set up as a prospective randomised double-blind placebo-controlled single-centre trial. A total of 92 patients with COPD who have had at least three exacerbations of COPD in the previous year will be included. Subjects will be randomised to receive either azithromycin 500 mg three times a week or placebo. Our primary endpoint is the reduction in the number of exacerbations of COPD in the year of treatment.

Discussion

We investigate whether long-term therapy with macrolide antibiotics can prevent exacerbations in patients with COPD. Additionally, our study aims to assess the effect of long-term use of macrolides on the development of antimicrobial resistance and on inflammatory parameters related to COPD. We believe this study will provide more data on the effects of macrolide treatment in patients in COPD and will add more knowledge on its working mechanisms.

Background

Chronic obstructive pulmonary disease (COPD) is generally accepted to become one of the major health problems in the western worlds in the following years. The main issue is the progressive character of the disease, which is characterised by an ongoing development of non-reversible airflow limitation.

COPD imposes a substantial burden on health-care systems worldwide, as the disease is a major cause of morbidity, mortality, reduced health status and a common cause of medical hospital admission.[1] Because of a poor understanding of COPD's pathogenesis, treatment is mostly symptomatic and new therapeutic strategies are limited. One of the known causes of COPD is long-term exposure to noxious particles or gasses. Particularly cigarette smoking is one of the main causes of development of COPD.[2] All smokers show evidence of lung inflammation, but smoking-induced lung injury is variable and appears to be amplified only in a minority of long-term tobacco smokers, suggesting that superimposed processes are the final determinants of COPD development.[3,4] There is a direct relationship between the severity of the disease and the intensity of the inflammatory response.[3,4] Thus, excessive inflammation is likely the key to susceptibility. Inflammation persists long after patients have stopped smoking. The cause of this persistent airway inflammation is unknown although recurrent airway infections seem to play a role in this process.

Macrolide antibiotics have bacteriostatic as well as anti-inflammatory properties.[5-7] The anti-inflammatory capacities of macrolides were firstly established in pulmonary diseases as diffuse panbronchiolitis, a progressive inflammatory disorder of the airways found almost exclusively in Japan.[7] Also in patients with cystic fibrosis macrolide therapy had led to improvement of several clinical parameters.[8-13]

Although currently the use of maintenance antibiotic treatment in COPD, other than for treating infectious exacerbations COPD, is not recommended by the GOLD report.[14] Several studies have been conducted to assess the effect of long-term therapy with macrolide antibiotics in patients with COPD.[15-17] The results of these studies are conflicting; however some suggest that macrolide antibiotics may become a valuable therapeutic option for COPD patients in preventing exacerbations.

In this randomised placebo controlled trial our main aim is to assess whether maintenance treatment with three times weekly azithromycin in COPD patients with three or more exacerbations in the previous year can decrease the exacerbation rate in the year of treatment and to study the effect of this treatment on microbial resistance.

Methods

The study is designed as a prospective randomised double-blind placebo-controlled single-centre trial in the department of respiratory medicine in the Amphia Hospital Breda, The Netherlands. Length of study is determined at a period of 3 years, of which 2 years will be spent on patient inclusion and 1 year on treatment. The end of the study is defined by the last visit of the last included subject.

Patient selection

All patients with COPD who have had three or more exacerbations in the previous year will be asked to participate in the study. An exacerbation of COPD is defined by a (sub)acute increase of pulmonary symptoms like dyspnoea, coughing, increased sputum volume with or without purulence, for which the patient has consulted a general practitioner (GP) or a respiratory physician, or for which the patient has been admitted to the hospital. The health care professional has judged the symptoms to be in such a degree that treatment was given with systemic steroids and/or a course of antibiotics.

The patients will be recruited from the outpatient department. To assess whether the patient fulfils the criteria for study participation the study subjects' GP will be contacted to review the patient chart and medication use. The hospital charts will be reviewed as well by the investigator.

Inclusion criteria

- Diagnosis of COPD according to GOLD criteria (FEV_1/FVC <70%), classification into GOLD I (FEV_1 80–100% predicted), GOLD II (FEV_1 50–80% predicted), GOLD III (FEV_1 30–50% predicted) or GOLD IV (FEV_1 ≤30% predicted).
- Age ≥18 years.
- Three or more exacerbations of COPD in the preceding year of inclusion for which a course of systemic steroids and/or antibiotics therapy was started.
- Clinically stable during 1 month. Patients have to be free of COPD exacerbation or respiratory tract infection within a month prior to involvement in the study, and in this period they should not have received antibiotics or a course of high doses of systemic steroids defined as more than 10 mg of prednisone a day.
- Informed consent.

Exclusion criteria

- Use of antibiotics or a course of high doses of systemic steroids defined as more than 10 mg of prednisone a day within a month prior to involvement in the study.
- Addition of inhalation steroids to the patient's therapy regimen within 1 year prior to study inclusion. Adding inhalation steroids 1 year before trial inclusion can influence the outcome of exacerbation frequencies.

- Pregnant or lactating women.
- Allergy to macrolides.
- Liver disease (alanine transaminase and/or aspartate transaminase levels two or more times the upper limit of normal).
- Asthma, defined as episodic symptoms of airflow obstruction which is reversible with bronchodilators, assessed with lung function testing.
- Bronchiectasis. A CT scan (1-mm slices) was performed in all patients to exclude bronchiectasis. Criteria of the BTS guideline Bronchiectasis (non-CF) are used for radiologic definition of bronchiectasis.[30]
- Malignancy of any kind for which the subject is under treatment or is being monitored as part of follow-up after treatment.
- Heart failure. A patient is excluded when having clinical signs of heart failure and a cardiac function defined as a left ventricular ejection fraction of less than 45% confirmed by echo-cardiography or single photon emission computed tomography (SPECT) scan.
- Use of drugs that can adversely interact with macrolides and for which therapeutic monitoring cannot be undertaken, e.g. ergotamine derivatives.

Intervention

Subjects will be randomised to receive either azithromycin 500 mg three times a week or placebo during a 1-year period. During this year subjects will be followed at the outpatient department at 3, 6, 9 and 12 months after initiating the study. During these visits the following tests will be performed according to the flowchart (table 1):

- Lung function testing.
- Sputum sample collection.
- Peripheral blood collection.
- Throat swab.
- Rectal swab.
- DS14 questionnaire for assessment of type D personality (only on day 1 and month 12).
- Hospital Anxiety Depression Scale (HADS).
- 12-Item Short Form Health Survey (SF-12).
- St. George's Respiratory Questionnaire (SGRQ).

In case of exacerbation subjects have the choice to get treatment from their general practitioner or to visit the hospital to be seen by the investigator. Either way sputum and peripheral blood will be collected for immunological and microbiological investigations. Also the subjects with an exacerbation will be asked to complete the SF-12, HADS and SGRQ.

Table 1: Overview of outpatient department visits and tests.

	Day 1	Month 3	Month 6	Month 9	Month 12	Other*
Informed consent	X					
Blood work	X	X	X	X	X	X
Microbiology	X	X	X	X	X	X
Lung function testing	X	X	X	X	X	
Rectal swab	X		X		X	
Questionnaires	X	X	X	X	X	X

Type D personality

The Type D Scale (DS14) will be administered to assess Type D personality.[31] This 14-item questionnaire comprises two subscales, Negative Affectivity and Social Inhibition, each consisting of seven items. Items are answered on a 5-point Likert scale, ranging from 0 (false) to 4 (true). A standardised cut-off score ≥10 on both subscales is used to classify individuals with a Type D personality.[32] A previous study confirmed that it is the interaction of both traits, rather than the single traits, that incurs an increased risk of adverse health outcomes.[32] Both of the DS14 subscales of Negative Affectivity and Social Inhibition have good internal validity (Cronbach's α=0.88/0.86), are stable over a 3-month period (r=0.82/0.72), and are independent of mood and health status.[31]

Depressive and anxious symptomatology

The Dutch version of the Hospital Anxiety and Depression Scale (HADS) will be used to assess depressive and anxious symptomatology.[33,34] Both subscales consist of seven items that are answered on a 4-point Likert Scale, ranging from 0 to 3. A cut-off score of ≥8 for each subscale represents probable clinical levels of anxiety and depression.[35] Test-retest reliabilities over a 3-week period for the subscales and the total scale are good ($0.86 < r < 0.91$).[34] The dimensional structure and reliability of the HADS has been shown to be stable across medical settings and age groups.[34]

Health status

The Dutch version of the Short-Form Health Survey12 (SF-12) will be administered to assess generic health status.[36,37] This generic instrument measures overall physical and mental health status, as indicated by the Physical Component Scale Summary (PCS) and the Mental Component Summary (MCS) scores.[38] According to standard scoring procedures, all scale scores will be standardised to the general US population (range 0–100, mean=50, SD=10), with higher scores indicating better functioning. The SF-12 has been demonstrated to be a reliable and valid instrument.[37]

Health-related quality of life

Disease-specific health-related quality of life will be measured by the total score on St. George's Respiratory Questionnaire (SGRQ).[39] Three component scores are calculated: symptoms, activity and impacts (on daily life), and a total score. Total scores range from 0 to 100, with lower scores indicating improvement.

Study endpoints

Primary study outcome

Reduction in the number of exacerbations of COPD in the year of treatment.

Secondary study outcomes

- Measurement of lung function parameters and 6-min walk test.
- Assessment of presence of type D personality by DS14 questionnaire.
- Disease-specific health-related quality of life measured by SGRQ.
- Generic health status measured by the SF-12.
- Indication of anxiety and depression by HADS.
- Microbiology: Sputum specimens will be cultured. Polymerase chain reaction (PCR) in sputum and serology in serum for viral and atypical microorganisms will be performed. The rectal swabs will be tested for change in rectal flora as a result of maintenance azithromycin.
- Measurement of inflammatory markers in serum (erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), midregional pro-adrenomedullin (MR-proADM), interleukin-6 and cytokine profiles of T-helper 1, T-helper 2 and T-helper 17 cells).
- Decrease in percentage of clinical versus outpatient department exacerbations.
- Difference in treatment effect between subjects with and without steroid maintenance therapy as hypothesis-generating secondary analysis.
- Adverse events of treatment. Symptoms that are believed to be (possibly) related to therapy will be reviewed at the outpatient department. If a patient has an adverse event that is thought to be drug related and that does not resolve, then the patient will be withdrawn from the study. There will be no routine ECG screening since azithromycin (in contrast to erythromycin) is much less likely to be a cause of a prolonged QTc interval.
- Length of hospital stay.
- Time till first exacerbation.

Sample size and statistical analysis

Power calculation and number of study subjects

This calculation starts with the assumption that the number of exacerbations follows a pure Poisson distribution with a mean rate of 3 per subject per year in the placebo group. A 50% reduction in this rate is considered to be clinically relevant. Hence, in the active treatment group the mean exacerbation rate is set at 1.5 per subject per year. With 33 subjects per treatment

group followed up for 1 year, this reduction is detectable with 90% power, given a test size alpha of 0.05 (2-sided). However, the assumption of a pure Poisson distribution may be too strong. In fact, it is plausible that in this case a zero-inflated Poisson process may be present (the outcome of zero exacerbations has a higher probability than that following from a pure Poisson distribution). In addition there is a risk of overdispersion (the variance is larger than the mean). These phenomena may be expected to have a negative (not exactly quantifiable though) effect on the power of the study. In order to reasonably compensate for the risk of a too low power and for subjects dropping out within 1 year of follow-up, the number of subjects will be augmented by 40%, so that the sample size is set at 46 subjects per treatment group.

Statistical analysis

The exacerbation rate (primary efficacy outcome) will be analysed using Poisson regression, with a log link function and the log of the time-under-treatment as offset. The exacerbation rate ratio of active relatively to placebo treatment will be the efficacy parameter of interest that will be tested for significance at the 5% level (2-sided). In addition a 95% confidence interval of this parameter will be calculated. The following baseline covariates will be entered along with treatment group: steroid maintenance therapy, the number of exacerbations in the year preceding randomisation, age, sex, smoking, and the GOLD criteria (Tiffeneau index and FEV_1% of predicted). In order to generate hypotheses concerning the modification of the treatment effect by the steroid therapy, the treatment-by-steroid interaction term will be added to the model and its effect will be explored. When necessary, the scale parameter will be used to correct the SEs for overdispersion. Additionally, a zero-inflated Poisson distribution will be fitted to the data in order to test if a better fit is obtained.

Other continuous (secondary) outcome variables with measurements at baseline, 3, 6, 9 and 12 months will be analysed using mixed model ANOVA. The following covariates will be entered in the model along with treatment group: the baseline measurement of the outcome variable, age, sex, smoking and the GOLD criteria. When appropriate, the outcome variables will be suitably transformed in order to obtain normally distributed residuals.

Time to first exacerbation will be analysed using Cox proportional hazards regression with the following covariates entered in the model along with treatment group: age, sex, smoking and the GOLD criteria. Also a Kaplan-Meier curve for time to first exacerbation per treatment group will be presented for illustrative purposes.

Concerning safety, the number and type of adverse event will be compared between the two treatment groups using the chi-square (or Fisher's exact) test.

Randomisation

All eligible subjects will be randomised using block randomisation sequences generated by computer. Treatment allocation numbers will be entered into individually sealed opaque envelopes. The envelope contains a number that is concealed to the treatment allocation. The

allocation list will be kept in a safe in the hospital pharmacy and access is possible by a non-investigator independently. In the event of an emergency medical situation the individual's randomisation code and group allocation could be identified.

Ethical aspects

The study has been approved by the ethics committee Toetsingscommissie Wetenschappelijk Onderzoek Rotterdam (TWOR), the ethics committee of the Amphia Hospital Breda and the Centrale Commissie Mensgebonden Onderzoek (CCMO).

The research will be explained in detail (verbally and in writing) to the patient prior to enrolment in the study. The explanation will include the type and method of the research, the tests to be performed and any potential hazards. An informed consent in writing will be obtained from each patient. The patient can withdraw from the study at any time, without any repercussion for the ongoing care.

The study will be conducted according to the International Conference for Harmonization (ICH) principles of Good Clinical Practice (GCP) and the Declaration of Tokyo (2004). The investigator will conduct all aspects of this study in accordance with all national and regional laws of the pertinent regulatory authorities.

Discussion

Despite the clinical efficacy of long-term macrolide treatment in a number of respiratory diseases, until recently, only smaller studies had reported on this subject in patients with COPD. These studies showed conflicting results. Recently Albert et al. showed in a large randomised controlled trial that adding daily 250 mg azithromycin to standard therapy reduced the number of exacerbations in patients with COPD.[23] The major concern with this study, raised in a number of comments and editorials, was the question about the development of antimicrobial resistance, which had almost doubled in that trial.[40] Also the question about the working mechanism of azithromycin, whether it has an antimicrobial or immunomodulatory effect, was not answered in that trial.

With the current study we investigate whether long-term therapy with macrolide antibiotics can prevent exacerbations in patients with an instable COPD. Additionally, our study aims to assess the effect of long-term use of macrolides on the development of antimicrobial resistance and on inflammatory parameters related to COPD. We believe this study will provide more data on the effects of macrolide treatment in patients in COPD and will add more knowledge on its working mechanisms.

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Chapter 4

Effect of azithromycin maintenance treatment in patients with frequent exacerbations of COPD (COLUMBUS): A randomised, double-blind, placebo-controlled trial

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Summary

Background

Macrolide resistance is an increasing problem; there is therefore debate about when to implement maintenance treatment with macrolides in patients with chronic obstructive pulmonary disease (COPD). We aimed to investigate whether patients with COPD who had received treatment for three or more exacerbations in the previous year would have a decrease in exacerbation rate when maintenance treatment with azithromycin was added to standard care.

Methods

We did a randomised, double-blind, placebo-controlled, single-centre trial in the Netherlands between May 19, 2010, and June 18, 2013. Patients (≥18 years) with a diagnosis of COPD who had received treatment for three or more exacerbations in the previous year were randomly assigned, via a computer-generated randomisation sequence with permuted block sizes of ten, to receive 500 mg azithromycin or placebo three times a week for 12 months. Randomisation was stratified by use of long-term, low-dose prednisolone (≤10 mg daily). Patients and investigators were masked to group allocation. The primary endpoint was rate of exacerbations of COPD in the year of treatment. Analysis was by intention to treat. This study is registered withClinicalTrials.gov, number NCT00985244.

Findings

We randomly assigned 92 patients to the azithromycin group (n=47) or the placebo group (n=45), of whom 41 (87%) versus 36 (80%) completed the study. We recorded 84 exacerbations in patients in the azithromycin group compared with 129 in those in the placebo group. The unadjusted exacerbation rate per patient per year was 1.94 (95% CI: 1.50–2.52) for the azithromycin group and 3.22 (2.62–3.97) for the placebo group. After adjustment, azithromycin resulted in a significant reduction in exacerbation rate versus placebo (0.58, 95% CI: 0.42–0.79; p=0.001). Three (6%) patients in the azithromycin group reported serious adverse events compared with five (11%) in the placebo group. During follow-up, the most common adverse event was diarrhoea in the azithromycin group (nine [19%] patients versus one [2%] in the placebo group; p=0.015).

Interpretation

Maintenance treatment with azithromycin significantly decreased exacerbation rate compared with placebo and should therefore be considered for use in patients with COPD who have the frequent exacerbator phenotype and are refractory to standard care.

Introduction

Acute exacerbations of chronic obstructive pulmonary disease (COPD) have important implications for the natural course of COPD and cause high mortality rates in patients with COPD.[1] Patients with three or more exacerbations for which hospital admission is needed have a risk of mortality that is four times higher than those with no exacerbations.[2] Prevention of exacerbations is therefore an essential strategy, not only for improvement of mortality rates, but also for improvement of health-related quality of life[3] and deceleration of further decline of lung function in patients with COPD.[4] Prevention of acute exacerbations of COPD with long-term macrolide treatment is a recent development and the beneficial effect of this treatment has been postulated to result from both an antimicrobial and an immunomodulatory effect.[5] The largest study to date of this approach showed that long-term treatment with daily azithromycin significantly decreased the frequency of acute exacerbations of COPD.[6] However, this study included patients with at least one exacerbation within the previous year and those who were receiving continuous supplemental oxygen without having had any exacerbation. Implementation of this strategy in clinical practice might result in an excessive use of macrolides in patients with COPD. However, the main risk of the increasing consumption of azithromycin is the induction of macrolide resistance in a large group of patients, with the additional risk of induction of resistance to the general population.[7] To benefit maximally from macrolide treatment and to reduce the risk of resistance simultaneously, restrictive use of azithromycin is presently warranted.[7] Proposals have been made to reserve long-term macrolide treatment for patients with two or more COPD exacerbations;[7,8] however, this recommendation was not supported by findings from clinical studies.

We did the COpd: infLUence of Macrolides on exacerBation freqUency in patientS (COLUMBUS) trial to investigate whether patients with COPD who had three or more exacerbations in the previous year would have a decreased rate of exacerbation when maintenance macrolide treatment was added to standard care.

Methods

Study design and participants

The study protocol has been published elsewhere.[9] We undertook this prospective, randomised, double-blind, placebo-controlled, single-centre trial at the Amphia Hospital (Breda, the Netherlands) between May 19, 2010, and June 18, 2013. Eligible patients were 18 years or older, had been diagnosed with COPD according to the guidelines of the Global initiative for chronic Obstructive Lung Disease,[10] and had received treatment for three or more exacerbations of COPD in the previous year for which they received steroids or antibiotic treatment. Patients had to be clinically stable and could not have had a COPD exacerbation or respiratory-tract infection in

the month before involvement in the study. Exclusion criteria were a history of other clinically significant respiratory diseases (e.g., asthma, cystic fibrosis); presence of bronchiectasis, as assessed by CT-scan; maintenance antibiotic treatment; use of more than 10 mg prednisolone a day; allergy to macrolides; pregnancy or lactation in women; liver disease (alanine transaminase or aspartate transaminase concentrations that were two or more times the upper limit of normal); malignant disease of any kind for which the patient received treatment or was being monitored as part of follow-up after treatment; heart failure; and the use of drugs that could adversely interact with macrolides and for which therapeutic monitoring could not be undertaken. All participants provided written informed consent.

The study was approved by independent and local ethics committees.

Randomisation and masking

An independent pharmacy randomly assigned patients (1:1), via a computer-generated randomisation sequence with permuted blocks of ten (five per treatment group), to receive either azithromycin dihydrate 500 mg (Teva Pharmachemie, Haarlem, the Netherlands) or placebo, three times a week (Monday, Wednesday, and Friday) for 12 months. Randomisation was stratified by use of long-term, low-dose prednisolone (≤10 mg daily). The randomisation list was retained by the clinical trials pharmacist of the Amphia Hospital. Patients were enrolled by SU, RSD, and JGJVA, and were automatically given the next allocated treatment by clinical trials staff at the hospital pharmacy. Participants and investigators were masked to treatment allocation throughout the study. After data collection and data cleaning were completed, and after final database lock, investigators were unmasked and could assess outcomes and do data analysis.

Procedures

Participants were followed up at the outpatient department at scheduled visits at months 3, 6, 9, and 12. During these visits, we obtained data for spirometry, the 6 min walk test, white-blood-cell count, concentrations of C-reactive protein, mid-regional pro-adrenomedullin, erythrocyte sedimentation rate, interleukin-6, and cytokine profiles of T-cell subsets. Additionally, patients completed the 12-Item Short-Form Health Survey (SF-12), the Hospital Anxiety and Depression Scale, and the St George's Respiratory Questionnaire at baseline and every 3 months. The type-D scale – a 14-item questionnaire to assess type D personality – was completed at baseline and at 12 months. Sputum samples were obtained for culture at baseline and at every scheduled visit. Sputum samples were processed according to American Society of Microbiology guidelines.[11] Sputum samples were additionally washed in sterile saline to avoid possible contamination from the oropharynx. We regarded a sputum sample as representative when more than 25 polymorphonuclear leucocytes and less than ten squamous cells per low-power field were identified by Gram stain. We established antibiotic susceptibility with breakpoints from the European Committee on Antimicrobial Susceptibility Testing.[12] In case of an exacerbation,

patients were seen and treated by the study investigators unless the patient chose to visit their family doctor. All exacerbations were defined according to Anthonisen criteria, and whether the patient needed treatment with steroids or antibiotics, or both.[13] An exacerbation was regarded as severe when hospital admission was necessary, and mild when it was treated at the outpatient department by the study investigators or the patient's family doctor.

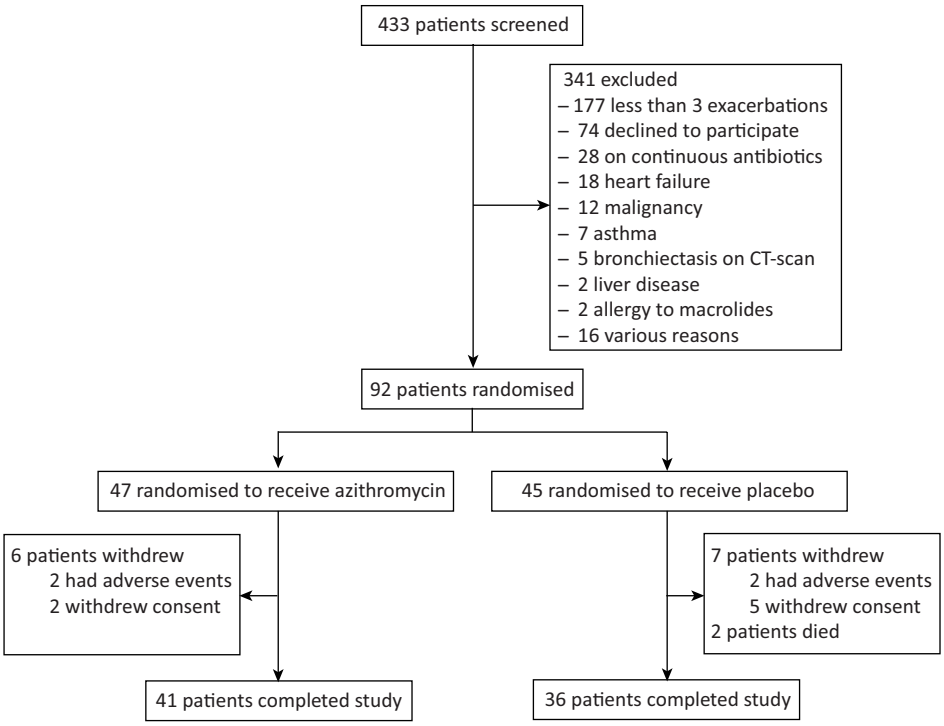
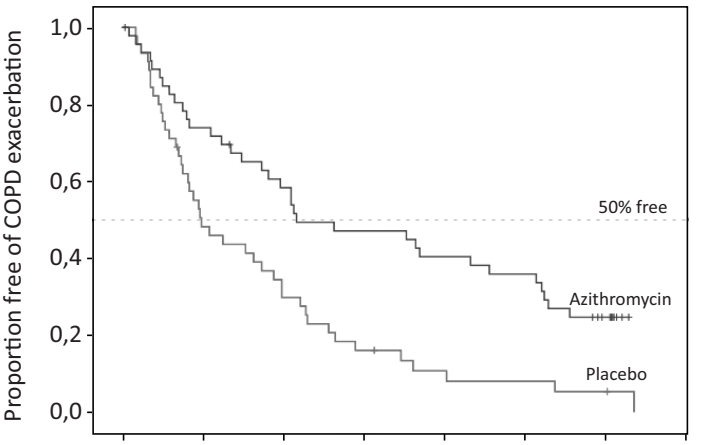


Figure 1: Trial profile.

Outcomes

The primary endpoint was rate of exacerbations of COPD in the year of treatment. Secondary outcomes were time to first exacerbation; hospital admission for acute exacerbations; change in proportion of exacerbations needing admission to hospital versus treatment in an outpatient department compared with the previous year; treatment for an acute exacerbation of COPD; forced expiratory volume in 1 s (FEV_1) after bronchodilation; forced vital capacity after bronchodilation; 6 min walking test; quality of life, as assessed by the SF-12 and the St. George's Respiratory Questionnaire; acquisition of macrolide resistant microorganisms in sputum; and adverse events.



Number at risk							
Azithromycin	47	34	26	21	18	16	8
Placebo	45	20	12	6	3	2	1

Figure 2: Proportion of patients free from acute exacerbations of COPD according to study group.

Results of mid-regional pro-adrenomedullin, erythrocyte sedimentation rate, interleukin-6, cytokine profiles of T-cell subsets, the Hospital Anxiety and Depression Scale, and the type-D scale will be presented elsewhere.

Statistical analysis

The sample size was calculated with the assumption that the number of exacerbations followed a Poisson distribution with a mean rate of three exacerbations per patient per year in the placebo group. With 33 participants per treatment group followed up for 1 year, a 50% reduction was detectable with 90% power (two-sided α 0.05). The calculation was based on an exact conditional binomial test, allowing exact inference on the rate ratio. To account for possible zero inflation, overdispersion, and participants dropping out earlier than 1 year after start of the study, the sample size was augmented by 40%, to 46 individuals per treatment group.

We analysed exacerbation rate with Poisson regression, with a log-link function and the log of time-in-study as an offset variable, and with covariates of long-term, low-dose prednisolone use, number of exacerbations in the preceding year, age, sex, smoking, and FEV_1. We corrected for overdispersion by multiplying the standard errors by the square root of the ratio of the Pearson χ^2 value to its number of degrees of freedom. We included all randomly assigned patients in the intention-to-treat analysis; for the per-protocol analysis we included only those who completed follow-up. The exacerbation rate ratio of azithromycin versus placebo treatment was tested for significance at the 5% level (two sided). Interaction between treatment and long-term, low-dose prednisolone use was also examined in an exploratory analysis. Time to first exacerbation

was analysed with Kaplan Meier survival analysis and log-rank test. To investigate the effect of treatment, with discrimination between occurrences of mild and severe exacerbations, we used generalised linear modelling with a logit-link function and a robust variance estimator to analyse the probability of hospital admission due to a given acute exacerbation of COPD; treatment was the only variable entered in this model. We did a similar analysis for the proportion of patients' exacerbations treated with antibiotics. Furthermore, the effect of treatment on the difference in the proportion of exacerbations requiring hospital admission versus outpatient treatment between the treatment year and the previous year was analysed with similar generalised linear modelling, whereby the correlation between the hospital proportions of the previous and treatment year was accounted for through the generalised estimation equations method. Secondary continuous outcome variables measured at baseline and at months 3, 6, 9, and 12 were analysed with linear mixed modelling. In addition to treatment, the baseline measurement of the outcome variable of interest was included as a covariate. Missing values over time for lung function parameters, 6 min walking test, C-reactive protein, and white-blood-cell count, caused by patients who withdrew before the end of the study, were appropriately imputed by the maximum likelihood estimation procedure used in linear mixed modelling, on the basis of the multivariate structure of the available measurements in time. Treatment effects were estimated by visit and overall across visits if the treatment-by-visit interaction was not significant ($p>0.01$). For adverse events and baseline characteristics, comparisons of parameters between treatment groups were calculated with a t-test if normally distributed and with a Mann-Whitney U test if not. We compared categorical data between the treatment groups with the exact χ^2 trend or Fisher's test, as appropriate. Statistical analysis was done with SPSS (version 21).
This study is registered with ClinicalTrials.gov, number NCT00985244.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Figure 1 shows the trial profile. We randomly assigned 92 patients to the azithromycin group (n=47) or the placebo group (n=45), of whom 41 (87%) versus 36 (80%) completed the study. All 92 patients received at least one dose of the assigned treatment (figure 1). In 91 (99%) patients bronchiectasis was excluded by chest CT scan. Table 1 shows baseline characteristics. We recorded 84 exacerbations in patients in the azithromycin group compared with 129 in those in the placebo group (table 2). 13 (28%) participants in the azithromycin group did not have any exacerbation compared with three (7%) participants in the placebo group. The unadjusted exacerbation rate

Table 1: Baseline characteristics determined on day 0 of study treatment.

	Azithromycin group (n=47)	Placebo group (n=45)
Male	22 (46·8%)	18 (40%)
Age	64·7 (10·2)	64·9 (10·2)
Current smoker	20 (42·6%)	9 (20%)
Body-mass index (kg/m²)	25·9 (4·6)	26·3 (5·7)
AECOPD in past year	4·0 (1·2)	4·0 (1·1)
Hospitalisation due to AECOPD	1·0 (1·1)	0·7 (0·8)
Symptoms		
– Cough	28 (59·6%)	34 (75·6%)
– Sputum production	29 (61·7%)	32 (71·1%)
Spirometry after bronchodilation		
– FEV_1 (L)	1·1 (0·47)	1·1 (0·43)
– FEV_1 (% of predicted)	44·2 (19·3)	45·0 (19·5)
– FVC (L)	2·9 (0·8)	2·7 (0·92)
– FVC (% of predicted)	92·5 (22·2)	88·9 (20·3)
– FEV_1/FVC (%)	38·0 (11·7)	40·3 (12·4)
GOLD stages		
– I	2 (4·3%)	3 (6·7%)
– II	14 (29·8%)	12 (26·7%)
– III	18 (38·3%)	20 (44·4%)
– IV	13 (27·7%)	10 (22·2%)
6-min walk test (m)	402 (101)	365 (136)
6-min walk test (% of predicted)	79 (20)	74 (27)
Medications		
– LABA	45 (95·7%)	41 (91·1%)
– LAMA	42 (89·4%)	32 (71·1%)
– ICS	42 (89·4%)	43 (95·6%)
– SABA	32 (68·1%)	33 (73·3%)
– Prednisolone	11 (23·4%)	9 (20·0%)
Influenza vaccination in past year		
– Yes	34 (72·3%)	41 (91·1%)
– No	5 (10·6%)	1 (2·2%)
– Not registered	8 (17·0%)	3 (6·7%)
SGRQ total score	57·4 (14·7)	57·6 (14·7)
– Symptoms	61·4 (19·1)	61·9 (16·4)
– Activity	77·7 (20·6)	75·0 (19·5)
– Impacts	43·3 (15·2)	45·8 (17·2)
SF-12		
– Physical component score	33·9 (10·0)	33·5 (9·0)
– Mental component score	37·4 (12·7)	39·9 (11·4)
CRP (mg/L)*	2 (1–180)	4 (1–42)
Leukocytes (x10^9/L)*	8·1 (5·8–17·1)	8·4 (5·1–17·4)

Data are in n (%) or mean (SD), unless otherwise stated. AECOPD=acute exacerbations of COPD. FEV_1=forced expiratory volume in 1 second; FVC=forced vital capacity; LABA=long-acting beta agonist; LAMA=long-acting muscarinic antagonist; ICS=inhalation corticosteroid; SABA=short-acting beta agonist; SGRQ=St. George's Respiratory Questionnaire; SF-12=12-Item Short Form Health Survey. *Median (range).

per patient per year was 1.94 (95% CI: 1.50–2.52) for the azithromycin group and 3.22 (2.62–3.97) for the placebo group. The rate ratio (RR) of azithromycin to placebo was 0.60 (95% CI: 0.43–0.84; p=0.003). After adjustment for covariates, the analysis remained significant (azithromycin versus placebo 0.58, 0.42–0.79; p=0.001). Results from the unadjusted (RR 0.60, 95% CI: 0.42–0.85; p=0.004) and adjusted (0.58, 0.42–0.79; p=0.001) per-protocol analyses were almost identical to those from the intention-to-treat analysis. No statistically significant difference was shown in the exacerbation rate ratio of azithromycin treatment to placebo between patients who did and did not already receive long-term, low-dose prednisolone treatment (p=0.12).

Table 2: Overview of exacerbations and hospitalisations in the year prior to the study and during follow-up.

	Azithromycin group (n=47)	**Placebo group (n=45)**
AECOPD in previous year	190	179
– Hospitalisation, n (%)	48 (25·3%)	32 (17·9%)
Odds hospitalisation/outpatient department AECOPD	0·34	0·22
AECOPD during follow-up	84	129
– Hospitalisation, n (%)	25 (29·8%)	31 (24·0%)
Odds hospitalisation/outpatient department AECOPD	0·42	0·32
Odds ratio of change (treatment year compared to previous year)*	1.24	1.46

AECOPD=acute exacerbation of COPD.
*Azithromycin to placebo ratio of the OR of changes 0·86, 95% CI: 0·35–2·07; p=0·73.

The median time to first exacerbation was 59 days (95% CI: 31–87) in the placebo group and 130 days (28–232) in the azithromycin group (p=0.001; figure 2). A post-hoc analysis showed that the probability of remaining free of exacerbations of COPD at 6 months was 0.14 (95% CI: 0.04–0.24) in the placebo group and 0.47 (0.32–0.62) in the azithromycin group (p=0.0005). In the year of treatment the odds for hospital admission due to acute exacerbations of COPD did not differ between groups (OR 1.34, 95% CI: 0.67–2.70; p=0.41). To assess whether this result was affected by only a few patients needing frequent admission, we did a post-hoc analysis in which no difference in mean time-to-first admission was noted between patients in the azithromycin group and those in the placebo group (282 days versus 258 days; p=0.48). Furthermore, no difference was shown between groups in change of rate of hospital admission for acute exacerbations versus exacerbations treated in the outpatient department (table 2). We noted no difference between groups in treatment of severe exacerbations with additional antibiotics (OR 0.34, 95% CI: 0.10–1.14; p=0.08). Mild exacerbations in the azithromycin group were treated significantly less often with additional antibiotics than were those in the placebo group (OR 0.20, 0.08–0.49; p=0.0001; table 3). During the study, macrolides were prescribed to four (9%) patients in the placebo group and to none in the azithromycin group.

No significant changes took place between groups in post-bronchodilator forced vital capacity, FEV_1, and 6 min walking test from baseline to 12 months (table 4). The mean change in total score on the St. George's Respiratory Questionnaire differed significantly between groups at 3 months in favour of azithromycin (-4.2, 95% CI: -8.3 to -0.1; p=0.043), but this change did not persist at 12 months (table 4). No differences between groups were noted in mean change from baseline in the component scores at 12 months (table 4). However, after undertaking an estimation of the overall treatment effect across all visits, we recorded a significant difference in symptom score on the St George's Respiratory Questionnaire between patients in the azithromycin group and those in the placebo group, but not in the total score or component scores of activities and impacts (table 4). The SF-12 showed a significant difference in mean change in the mental component score at 3 months in favour of azithromycin (6.6, 95% CI: 1.4–11.8; p=0.013), but not at 12 months (table 4). No differences were shown between groups in mean change in the physical component score at 3 months (data not shown) or 12 months (table 4).

Table 3: Overview of exacerbations and given treatments during the study.

	AECOPD in azithromycin group (n=84)	**AECOPD in placebo group (n=129)**
Severe exacerbation, n (%)	**25 (29·8)**	**31 (24·0)**
– Prednisolone	9 (10·7)	5 (3·9)
– Antibiotics	0 (0)	0 (0)
– Prednisolone & antibiotics	16 (19·0)	26 (20·2)
Mild exacerbation, n (%)	**59 (70·2)**	**98 (76·0)**
– Prednisolone	36 (42·9)	25 (19·4)
– Antibiotics	0 (0)	16 (12·4)
– Prednisolone & antibiotics	23 (27·4)	57 (44·2)

A severe exacerbation was defined as an exacerbation for which hospitalisation was necessary. A mild exacerbation was defined as an exacerbation treated at the outpatient department by the study investigators or by the general practitioner. Provided are percentages (%) of the number of exacerbations per treatment arm.
AECOPD=acute exacerbations of COPD.

No significant changes were recorded between groups in concentrations of C-reactive protein and white-blood-cell counts at 12 months compared with baseline (table 4). However, across all visits, significantly lower concentrations were noted in patients in the azithromycin group for both C-reactive protein and white-blood-cell counts than in those in the placebo group (table 4).

One or more sputum samples were obtained in 32 (68%) of the 47 patients in the azithromycin group, and in 32 (71%) of the 45 patients in the placebo group. At baseline, 42 sputum samples were obtained (22 in the azithromycin group and 20 in the placebo group), and 108 samples (51 versus 57) were obtained during 1 year of follow-up (table 5). The most commonly cultured bacteria in the azithromycin and placebo groups at baseline were *Haemophilus influenzae* (n=3 versus n=2), *Streptococcus pneumoniae* (n=2 versus n=3), and *Pseudomonas aeruginosa* (n=2 versus n=0). During follow-up, fewer patients in the azithromycin group had positive

Table 4: Secondary outcome variables at 12 months.

	Values at 12 months		Change from baseline at 12 months				Overall effect	
	Azithromycin group (n=41)	Placebo group (n=36)	Azithromycin group (n=47)	Placebo group (n=45)	Difference (95% CI)	P-value	Difference (95% CI)	P-value
Spirometry after bronchodilation								
– FEV_1 (L)	1·1 (0·47)	1·0 (0·42)	-0·03	-0·07	0·03 (-0·04 to 0·11)	0·37	0·03 (-0·02 to 0·08)	0·19
– FEV_1 (% of predicted)	43·4 (17·9)	44·2 (20·1)	-1·13	-1·80	0·67 (-2·36 to 3·71)	0·66	0·86 (-1·14 to 2·85)	0·40
– FVC (L)	2·9 (0·93)	2·7 (0·79)	-0·04	-0·12	0·08 (-0·09 to 0·25)	0·35	0·05 (-0·06 to 0·17)	0·35
– FVC (% of predicted)	91·0 (23·5)	88·9 (20·9)	-0·73	-1·21	0·48 (-4·86 to 5·82)	0·86	0·22 (-3·33 to 3·78)	0·90
6-minute walk test (m)	415 (108)	379 (121)	-1·5	-20·8	19·3 (-17·8 to 56·5)	0·31	8.4 (-15·2 to 31·9)	0·48
6-minute walk test (% of predicted)	82 (20)	76 (23)	0·42	-3·55	3·97 (-3·66 to 11·60)	0·31	1·40 (-3·32 to 6.13)	0·56
SGRQ total score	56·2 (17·2)	57·3 (15·2)	-1·05	-0·44	-0·61 (-5·75 to 4·53)	0·82	-1·12 (-4·37 to 2·23)	0·49
– Symptoms	57·3 (18·0)	63·0 (14·4)	-4·97	1·80	-6·77 (-14·22 to 0·67)	0·075	-5·06 (-9·64 to -0·49)	0·030
– Activity	75·5 (22·4)	76·1 (19·9)	-1·66	1·37	-3·02 (-8·72 to 2·67)	0·30	-2·91 (-6·32 to 0·49)	0·09
– Impacts	44·6 (17·8)	44·5 (18·3)	1·12	-1·19	2·31 (-4·43 to 9·05)	0·50	0·89 (-3·19 to 4·96)	0·67
SF-12								
– Physical component score	32·3 (10·7)	32·7 (10·3)	-0·76	1·13	-1·89 (-6·13 to 2·36)	0·38	1·30 (-1·26 to 3·86)	0·31
– Mental component score	36·8 (11·7)	35·9 (13·1)	-0·04	-1·80	1·76 (-4·02 to 7·53)	0·55	2·68 (-0·51 to 5·87)	0·10
CRP (mg/L)*	2 (1-30)	3 (1-90)	-20·6%	-2·1%	-18.9 (-50·6 to 33·2)	0·41	-27.1 (-42.3 to -8.0)	0.008
Leukocytes (x10^9/L)*	8·5 (3·1–16·2)	8·9 (4·8–16·3)	2·6%	9·9%	-6·7 (-17·2 to 5·1)	0·25	-8.4 (-14.2 to -2.3)	0.008

Table 5: Overview of sputum samples per treatment group at baseline and during follow-up.

	Azithromycin group (n=47)	Placebo group (n=45)
Baseline		
– Number of sputum samples	22	20
– Number of patients with sputum samples	22	20
– Number of patients with pathogens in sputum	7	6
– Number of patients with macrolide resistant bacteria	5	4
Follow-up		
– Number of sputum samples	51	57
– Number of patients with sputum samples	25	27
– Number of patients with newly acquired pathogens	4	12
– Number of patients with newly acquired macrolide resistant bacteria	3	11

sputum cultures with new respiratory pathogens compared with those in the placebo group (n=4 versus n=12; p= 0.044; table 5). Acquisition of macrolide-resistant bacteria was noted in three (6%) patients in the azithromycin group compared with 11 (24%) patients in the placebo group (p=0.036; table 5).

No significant differences were shown in the frequency of adverse events or serious adverse events between treatment groups (table 4). During treatment, three (6%) patients in the azithromycin group had serious adverse events (table 4): two were diagnosed with lung carcinoma and a third had an acute coronary syndrome. Five (11%) patients in the placebo group had serious adverse events (table 4): two developed respiratory failure due to an acute exacerbation of COPD, both of whom died; the third patient had a transient ischaemic attack, the fourth had an acute coronary syndrome, and the fifth had cholecystitis for which a cholecystectomy was done. Four (9%) patients in the azithromycin group and two (4%) patients in the placebo group discontinued the study because of side-effects (figure 1). More patients had diarrhoea in the azithromycin group than in the placebo group (p=0.015; table 6).

Discussion

This study is the first to investigate macrolide treatment in patients with frequent exacerbations of COPD. Our findings show that treatment with azithromycin for 12 months decreased the rate of exacerbations and increased time to first exacerbation compared with placebo (panel).

We examined a COPD population who were refractory to usual care. The proportions of patients who received treatment with inhaled corticosteroids (92%), long-acting beta agonists

Table 6: Adverse events.

	Azithromycin group (n=47)	Placebo group (n=45)
Any adverse events	68	74
Serious adverse events	3 (6·4%)	5 (11·1%)
Most frequent adverse events*		
Gastrointestinal		
– Diarrhoea	9 (19·1%)	1 (2·2%)
– Nausea or vomiting	3 (6·4%)	2 (4·4%)
– Other	4 (8·5%)	7 (15·6%)
Laboratory investigations		
– Creatinine increase	7 (14·9%)	3 (6·7%)
– Elevated BUN	4 (8·5%)	10 (22·2%)
– Hyperchloremia	6 (12·8%)	5 (11·1%)
– Alkaline phosphatase increase	4 (8·5%)	1 (2·2%)
– ALT increase	5 (10·6%)	4 (8·8%)
– AST increase	3 (6·4%)	3 (6·7%)
– γ-GT increase	6 (12·8%)	1 (2·2%)
– LDH increase	3 (6·4%)	4 (8·8%)
– Other	9 (19·1%)	17 (37·8%)

Data are numbers of adverse events (%). There were no significant differences except for diarrhoea (p=0·015).
*Those with an incidence of 2·5% or higher.
BUN=blood urea nitrogen; ALT=alanine aminotransferase; AST=aspartate aminotransferase; γ-GT=gamma-glutamyl-transferase; LDH=lactate dehydrogenase.

(LABAs) (93%), and long-acting muscarinic antagonists (LAMAs) (80%) were substantially higher in our study than in two prospective randomised trials investigating the effect of long-term macrolide treatment in patients with COPD.[6,14] In Albert and colleagues' study,[6] inhaled corticosteroids were prescribed in 77% of the patients, LABAs in 74%, and LAMAs in 63%, whereas in Seemungal and colleagues' study,[14] 78% of patients received inhaled corticosteroids, 63% received LABAs, and 33% received LAMAs. Our main inclusion criterion was the presence of three or more acute exacerbations of COPD in the preceding 12 months. This criterion is in contrast with that of Albert and colleagues' trial,[6] in which 12% of patients did not have any exacerbations in the year before inclusion, and that of Seemungal and colleagues' trial, in which 65% of patients had fewer than three exacerbations in the year before inclusion.[14] Therefore, our main outcome cannot be directly compared with those from these two studies. We recorded a higher relative reduction (42%) in exacerbation rate than in Albert and colleagues' trial (27%)[6] and Seemungal and colleagues' trial (35%).[14] Furthermore, median time to first exacerbation in the azithromycin (130 days) and placebo groups (59 days) in the COLUMBUS study was substantially shorter than that in the trials by Albert and colleagues[6] (azithromycin 266 days [95% CI: 227–313], placebo 174 days [143–215]) and Seemungal and colleagues (erythromycin 271 days, placebo 89 days).[14] Another important finding is that 7% of patients in our control group did not have any exacerbation, compared with 32% of those in Albert and colleagues' control group.[6] This

result suggests that use of a criterion of three or more exacerbations exposes fewer patients to redundant macrolide treatment, which consequently reduces the possibility of side-effects and the development of macrolide resistance. An additional difference between our study and that by Albert and colleagues was our use of a thrice-weekly regimen compared with their use of daily zithromycin.[6] When designing the study protocol, most data of long-term treatment with azithromycin were for thrice-weekly regimens in studies of patients with cystic fibrosis.[19,20] Until now, no study has been done comparing a daily dosage with a thrice-weekly schedule.

Azithromycin did not improve generic and disease specific health-related quality of life, as assessed by SF-12 and the St. George's Respiratory Questionnaire. However, we noted a clinically and statistically significant average treatment effect in the symptom component score of the St George's questionnaire in patients in the azithromycin group compared with those in the placebo group at 12 months. This improvement in symptom score might be attributable to the reduction in exacerbations. In a 2 year study done to assess exacerbations and their effect on health-related quality of life in patients with COPD, Miravitlles and colleagues showed that the greatest differences between frequent and infrequent exacerbators in the St George's Respiratory Questionnaire were in the symptoms scale.[21]

Macrolide treatment is an important cause of development of macrolide resistance in oral commensal streptococcal flora.[22] We identified acquisition of macrolide-resistant bacteria in sputum; however, the number of positive sputum cultures was low. In line with Albert and colleagues' findings, patients in the azithromycin group were less likely to become colonised with respiratory pathogens than were those in the placebo group.[6] Furthermore, azithromycin significantly reduced acquisition of macrolide-resistant bacteria in sputum compared with placebo. In Albert and colleagues' study, fewer patients (in absolute numbers) given azithromycin were colonised with macrolide-resistant respiratory pathogens compared with those given placebo.[6,23] In our study, we could not explain this difference in acquisition of macrolide-resistant bacteria by additional use of macrolides during follow-up for any indication.

Several randomised trials have proven the effectiveness of maintenance macrolide treatment for prevention of exacerbations of non-cystic-fibrosis bronchiectasis.[24-26] Inclusion of patients with COPD with bronchiectasis in our study could have resulted in substantial bias because the achieved results could have been affected by patients with non-cystic-fibrosis bronchiectasis. Therefore, we chose to exclude these patients. During the screening period, we excluded five of 433 patients because of bronchiectasis. This number is relatively low compared with that in a study by Martinez-Garcia and colleagues in which almost 58% of the patients with COPD had bronchiectasis.[27] However, in that study, patients with COPD with and without previous exacerbations were included. Another notable observation in our study was the presence of a larger number of female than male patients with COPD. Additionally, in the ECLIPSE and POET studies, women had a higher tendency of exacerbating more frequently than did men.[28-30]

Macrolides have been extensively investigated on the basis of their postulated immuno-modulatory effects. Evidence suggests that macrolides decrease the production of pro-

inflammatory cytokines in response to viral infections,[31] decrease the hypersecretion of pro-inflammatory cytokines and chemokines,[32] improve alveolar macrophage phagocytosis function,[33] and maintain integrity of the airway epithelium.[34] In addition to the immunomodulatory effects, the decrease in airway bacterial colonisation in patients receiving azithromycin as shown in our study might also be associated with reduction in systemic inflammation.[35]

Azithromycin was well tolerated in our trial. Adverse events were mostly gastrointestinal, with roughly a fifth of patients in the azithromycin group reporting diarrhoea, a finding similar to that seen in other studies of azithromycin.[16,24,25] However, in Albert and colleagues' study, only 5% of patients in the azithromycin group reported gastrointestinal complaints.[6] Although, by contrast with erythromycin and clarithromycin, azithromycin does not change the concentrations of theophylline, we therapeutically monitored theophylline as described in the study protocol.[9,36] We did not record theophylline concentrations greater than the therapeutic range.

Our study has some limitations. First, we had small numbers of culture-positive sputum samples for assessment of the development of antimicrobial resistance. We did not assess macrolide resistance in oral commensal flora; therefore, our results might underestimate macrolide resistance *in vivo*. Second, although patients were actively asked about hearing loss, no standard audiometry was done. In several studies done with macrolides, no reports of hearing loss were made.[24,25,37] At the end of our study, one patient in the placebo group reported hearing loss. Third, electro cardiographs were not done as standard before and during the study. However, apart from two patients, one in each treatment group, who had an acute coronary syndrome, no other cardiovascular related events or deaths were reported.

In summary, our results show that long-term treatment with azithromycin could be recommended in patients with COPD with the frequent exacerbator phenotype who are refractory to standard care. However, careful monitoring of the emergence of macrolide resistance is warranted.

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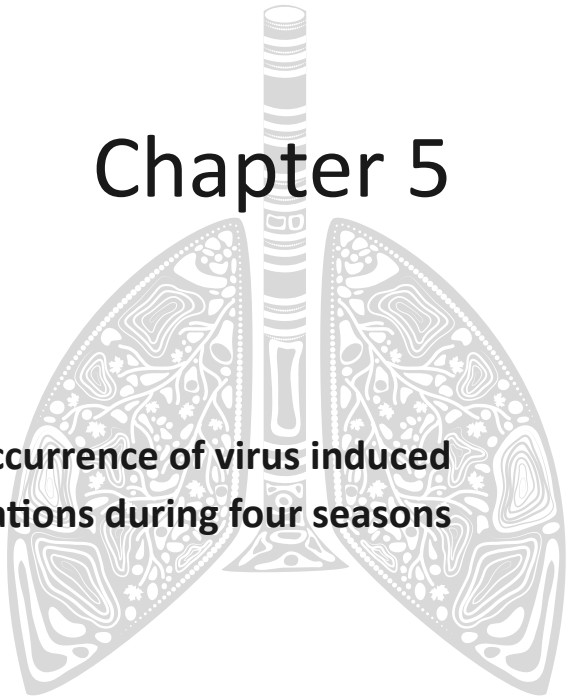
Chapter 5



Occurrence of virus induced COPD exacerbations during four seasons

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Abstract

In this study, we investigated the occurrence of viral infections in acute COPD exacerbations during four seasons.

Viral infections were detected by the use of real-time reverse transcriptase polymerase chain reaction on pharyngeal swabs. During a 12-month period pharyngeal swabs were obtained in 136 exacerbations of 63 patients. In 35 exacerbations (25.7%) a viral infection was detected.

Most viral infections occurred in winter (n=14; 40.0%), followed by summer (n=9; 25.7%), autumn (n=6; 17.1%) and spring (n=6; 17.1%). Rhinovirus was the most frequently isolated virus (n=19; 51.4%), followed by respiratory syncytial virus (n=6; 16.2%), human metapneumovirus (n=5; 13.5%), influenza A (n=4; 10.8%), parainfluenza 4 (n=2; 5.4%) and parainfluenza 3 (n=1; 2.7%).

This study showed that virus induced COPD exacerbations occur in all four seasons with a peak in the winter months. The distribution of rhinovirus infections however, showed a different pattern with most infections occurring in July.

Introduction

Acute exacerbations of COPD (AECOPD) have important effects on the natural course of COPD. AECOPD are associated with morbidity and mortality and with worse health-related quality of life.[1] It has been reported that AECOPD are predominantly caused by both bacterial and viral respiratory infections.[2-5] Recent polymerase chain reaction (PCR) or reverse transcription (RT)-PCR based studies consistently showed a high prevalence of viral infections during exacerbations (22–64%).[2,6-11] Exacerbations caused by respiratory viruses were associated with more severe exacerbations, reflected by increased length of stay and decrease in lung function.[12] In a recent review on the prevalence of respiratory viruses in AECOPD it was found that human rhinovirus (HRV) was most prevalent, followed by respiratory syncytial virus (RSV) and influenza virus.[13]

Epidemiological data reported a greater frequency of exacerbations in the winter months.[14-17] In the TORCH (TOwards a Revolution in COPD Health) study population an almost two-fold increase of exacerbations in the northern and southern regions during the winter months was observed.[14] In this and in other studies, it was suggested that this increase in exacerbations could be caused by increased exposure to viral infections.[18-20] However, data about seasonal variation in the prevalence of AECOPD caused by viral infections are lacking.

The aim of our study was to investigate the occurrence of acute exacerbations of COPD caused by viral infections during four seasons.

Material and methods

This study was part of the COLUMBUS trial (Clinicaltrials.gov, NCT00985244), a 12-month prospective study performed at the Amphia Hospital, Breda, the Netherlands. Patients were included between May 2010 and June 2013. The study investigated azithromycin maintenance therapy compared to placebo in 92 patients with the frequent COPD exacerbator phenotype. After inclusion, patients were followed during a period of one year. The study protocol and the primary results have been published earlier.[21,22]

Inclusion criteria were age ≥18 years, a COPD diagnosis according to the guidelines of the Global initiative for chronic Obstructive Lung Disease,[23] and ≥3 AECOPD in the previous year that were treated with steroids and/or antibiotics. Clinical stability during one month was required prior to enrolment.

Exclusion criteria were a history of other significant respiratory diseases (e.g. asthma, cystic fibrosis), the presence of bronchiectasis assessed by computed tomography, heart failure, liver disease and malignancy of any kind for which the subject received treatment or was being monitored as part of follow up after treatment.

All participants provided written informed consent. Independent and local ethics committees approved the study. In case of an exacerbation patients were seen and treated by the study

investigators unless the patient chose to visit the general practitioner. All exacerbations were defined according to the Anthonisen criteria, requiring treatment with steroids and/or antibiotics.[24] We obtained data for the number of exacerbations, the date of onset of exacerbation and the number of preceding influenza vaccinations.

Table 1: Overview of viruses and primers used for real-time reverse transcriptase polymerase chain reaction.

Virus	Primer	Primer sequence 5'- 3' direction
RSV A	RSVA-F1	AGATCAACTTCTGTCATCCAGCAA
	RSVA-R1	TTCTGCACATCATAATTAGGAGTATCAAT
	RSVA-1-FAM	RSVA-1-FAM
		6FAM-CACCATCCAACGGAGCACAGGAGAT
RSV B	RSVB-F1	AAGATGCAAATCATAAATTCACAGGA
	RSVB-R1	TGATATCCAGCATCTTTAAGTATCTTTATAGTG
	RSVB-2-VIC	RSVB-2-VIC
		VIC-TTCCCTTCCTAACCTGGACATAGCATATAACATACCT
Influenza A	>InfAF2	CTTCTRACCGAGGTCGAAACGTA
	>InfAR2	TCTTGTCTTTAGCCAYTCCATGAG
	>InfA2FAMBhq1	FAMTCAGGCCCCCTCAAAGCCGAGABhq1
	>InfA3FAMBhq1	FAMTCAGGCCCCCTCAAAGCCGAAABhq1
Influenza B	InfB-F2Bhq1	GRA-CAA-CAT-GAC-CAC-AAC-ACA-AAT
	InfB-R2Bhq1	CAC-TCC-ARA-ATT-CCT-GCT-TCA-AA
	InfB-2-YYBhq1	YY-CGG-GAG-CAA-CCA-ATG-CCA-CCA-TAA-ABhq1
Parainfluenza 1	PIV1-F2	AAAAACTTAGGGTTAAAGACAATCCA
	PIV1-R2	GCCAGATGTRTGTCYTTCCTGCTGGT
	PIV1-3-ATTOBhq3 (RG)	ATTO_680-CAAACGATGGCTGAAAAAGGGABhq3
Parainfluenza 2	PIV2-F2	CCATTTACCTAAGTGATGGAA
	PIV2-R2a	CGTGGCATAATCTTCTTTTT
	PIV2-R2b	TGTGGCATAATCTTCTTTCT
	PIV2-2-YYbhq1	YY-AATCGCAAAAGCTGTTCAGTCACBhq1
Parainfluenza 3	PIV3-F2	CAGGAAGCATTGTRTCATCTGT
	PIV3-R2	ATAGTGTGTAATGCAGCTYGT
	PIV3-2-FAMbhq1	FAMACCCAGTCATAACTTACTCAACAGCAACBhq1
Parainfluenza 4	PIV4-F1	CAAAYGATCCACAGCAAAGATTC
	PIV4-R1	ATGTGGCCTGTAAGGAAAGCA
	PIV4-1-Cy5bhq2	Cy5GTATCATCATCTGCCAAATCGGCAATTAAACAbhq2

Virus	Primer	Primer sequence 5'- 3' direction
HRV	HRV-F2a	GACAGGGTGTGAAGAGCC
	HRV-F2b	GACATGGTGTGAAGACCC
	HRV-F2c	GACAAGGTGTGAAGAGCC
	HRV-F2d	GACATGGTGTGAAGACTC
	HRV-F2e	GACATGGTGTGAAGATCT
	HRV-R2	ACACGGACACCCAAAGTAGT
	HRV-2-VIC	HRV-2-VIC
		VIC-TCCTCCGGCCCCTGAATGYGGCTAA
hMPV	hMPV-F2	CATATAAGCATGCTATATTAAAAGAGTCTC
	hMPV-R2	CCTATTTCTGCAGCATATTTGTAATCAG
	hMPV-2-FAM	hMPV-2-FAM
		6FAM-TGYAATGATGAGGGTGTCACTGCGGTTG

RSV=respiratory syncytial virus; HRV=human rhinovirus; hMPV=human metapneumovirus.

Collecting and processing pharyngeal swabs

Pharyngeal swabs were obtained during exacerbations. All pharyngeal samples were screened for the presence of viral respiratory pathogens by real time RT-PCR with primer sequences as shown in table 1. Nucleic acids were extracted from one aliquot of 200 µL swab 'rinse' solution using the Qiagen QIA symphony automated nucleic acid extraction. Samples were tested using real-time PCR specific for respiratory syncytial virus (A and B), human influenza virus A and B, parainfluenza virus 1–4, human rhinoviruses and human metapneumovirus.

Primers, probes and PCR assay conditions used for this study have been previously reported in detail.[25] DNA PCR was performed by using the Qiagen Qantitect Mastermix (Qiagen) and the RNA RT-PCR with Taqman Fast Virus-1 step mastermix (Life technologies) both according to manufacturer's protocol.

The astronomical definition of seasons for the northern hemisphere (22.5–67.5˚N) was used: winter (December 21 – March 20), spring (March 21 – June 20), summer (June 21 – September 20), autumn (September 21 – December 20).

The clinical outcomes of interest were: the number of AECOPD caused by viral infections and the total number of AECOPD per season.

Results

Ninety-two patients were included of which 77 patients completed the study. Overall there were 213 exacerbations. A pharyngeal swab was collected in 136 AECOPD of 63 patients (1–8 AECOPD per patient). In 35 episodes of AECOPD (25.7%) a positive pharyngeal swab was found with a

total of 37 viruses. Multiple viruses were detected in 2 of the 35 AECOPD. The following viruses were isolated: HRV (n=19; 51.4%), RSV (n=6; 16.2%), human metapneumovirus (hMPV) (n=5; 13.5%), influenza A (n=4; 10.8%), parainfluenza 4 (n=2; 5.4) and parainfluenza 3 (n=1; 2.7%).

Most viral infections occurred in winter (n=14; 40.0%), followed by summer (n=9; 25.7%), autumn (n=6; 17.1%) and spring (n=6; 17.1%). The results are shown in figure 1.

In the year preceding the study, 53 patients (84.1%) received influenza vaccination. All four patients who developed influenza A infection had been vaccinated in the year preceding the study.

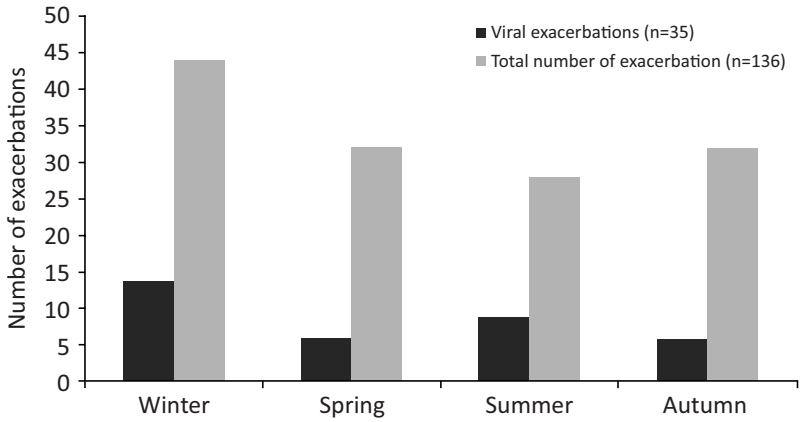

Figure 1: Number of viral (n=35) and total exacerbations (n=136) in each season.

Discussion

Our study showed that virus induced exacerbations occurred in all seasons, but were most frequently seen during winter months. The highest prevalence of AECOPD by all causes was present in the winter season as well. This seasonal pattern of COPD exacerbations has recently been described in the TORCH study.[14]

During all seasons viral infections were responsible for 25.7% of the AECOPD, which is in accordance with other studies.[6-8,10,11]

In our study HRV was the most predominant virus and was found in more than 50% of virus related exacerbations. It is noteworthy that no single case of HRV infection was found from December to February with the highest prevalence in July. Others have also demonstrated that HRV is the most frequently detected virus during COPD exacerbations.[6,12] However, in contrast to our study, several studies have shown that HRV induced respiratory tract infections occurred most often in all seasons but not in summer.[26,27]

Influenza infection can cause exacerbations in COPD.[28] Since vaccination against influenza is proven to be protective in the prevention of influenza related airway diseases, many COPD patients are now annually receiving an influenza vaccination.[29] In the year preceding the study, 53 patients (84.1%) were vaccinated for influenza virus. This could explain the low percentage of influenza infection (10.8%) in our study. In other studies a higher percentage (25%) has been found.[6]

Our study has some limitations. First, we obtained only in 136 of 213 exacerbations (63.8%) a pharyngeal swab for virus PCR. Second, we did not determine the presence of viral infections in sputum, which could have resulted in an underestimation of the presence of viral infections. However, the diagnostic yield in sputum and oropharyngeal samples for the detection of viral pathogens has shown to be equivalent.[30] In summary, our results show that virus induced COPD exacerbations and total COPD exacerbations occur in all four seasons but have a peak in the winter months. The distribution of HRV infections however, shows a different pattern with most infections occurring in July.

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Chapter 6

Comparison of gender in a real life COPD population with the non-exacerbator and the frequent exacerbator phenotype

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Manuscript in preparation

Abstract

Rationale

Despite the increasing number women with COPD, gender-based differences in COPD have been investigated sparsely. The main objective of this observational study was to examine the differences between males and females with the non-exacerbator and frequent exacerbator phenotypes.

Methods

An observational study was performed in a general hospital. Patients were included when they had a diagnosis of COPD according to the Global Initiative for chronic Obstructive Lung Disease and had none or ≥3 exacerbations (treated with antibiotics and/or prednisolone) in the previous year. Exclusion criteria were other significant respiratory diseases. Data were prospectively collected for smoking status, comorbidities, spirometry, 6 minute walk test, COPD assessment test (CAT), medical research council score, and inflammation parameters.

Results

190 (138 non-exacerbators and 52 frequent exacerbators) were included. The frequent exacerbator group consisted of more women (60%) than the non-exacerbator group (42%; p=0.045). In the non-exacerbator group females were younger, had smoked less pack-years, had better 6MWT and had less comorbidity than men. Furthermore, frequent exacerbating females were younger and more often current smokers than frequent exacerbating males.

Conclusion

There are differences between men and women within two distinct phenotypes in COPD that need further research. Special attention should be given to the characterisation of female frequent exacerbators.

Introduction

Despite the increasing number of female patients with (COPD),[1] the role of gender in COPD has not been extensively studied. Till so far studies have shown that smoking female COPD patients have a higher risk of hospitalisation due to COPD,[2] and have more impaired quality of life than male patients.[3,4] However, these studies were performed in a relatively stable COPD population. Furthermore, there is a growing body of evidence that women tend to exacerbate more frequently than men do. This was first reported by De Torres *et al*. in an observational study of 106 COPD patients.[5] In this study it was shown that females had experienced more significantly more exacerbations in the previous year than FEV_1-matched males. This observation was confirmed in the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) study.[6] To explain this difference, it was suggested that women had a heightened awareness of symptoms or a greater tendency to report changes in symptoms to a health care provider.[6] Another explanation might be that certain biologic differences between genders contribute to this tendency of women to exacerbate more frequently. To date, no study evaluated gender-related differences in the frequent exacerbator phenotype.

As COPD is to become one of the major causes of death in future[7] there is need to characterise females with COPD and identify differences in disease manifestation in comparison to males. In particular, investigating females with the frequent exacerbator phenotype is an important objective, but this has not been done before.

The aim of this study was to characterise and compare female and male COPD patients in the non-exacerbator phenotype and the frequent exacerbator phenotype.

Material and methods

Study design and patients

This was a prospective observational study performed in the Amphia Hospital in Breda, the Netherlands. Data were obtained from patients that had attended the Asthma & COPD Centre (ACC) of the department of Respiratory Medicine from April 2012 to April 2014. Patients who attended the ACC were either referred by a pulmonary physician for re-assessment of their disease or by a general practitioner.

Patients with a diagnosis of COPD according to the guidelines of the Global initiative for chronic Obstructive Lung Disease (GOLD)[8] that had none or ≥3 treated exacerbations in the previous year were included in this analysis. Exclusion criteria were a history of other significant respiratory diseases (e.g. asthma, cystic fibrosis), and insufficient data for a diagnosis of COPD.

At baseline, exacerbation frequency was checked with the patient, and cross-checked using a list of medication that were prescribed in the past year. An exacerbation was defined as an increase of pulmonary symptoms like dyspnoea, coughing, increased sputum volume, for which

the patient has consulted a general practitioner or a respiratory physician, and was treated with steroids and/or antibiotics.

Methods

At the ACC, patients were fully screened in 2 consecutive visits (1 week between visit 1 and 2). During these visits data were collected for demographics, body mass index (BMI), smoking status, spirometry, 6 minute walk test (6MWT) in metres with reference values according to Troosters *et al*.,[9] Medical Research Council (MRC) score,[10] COPD assessment test (CAT),[11] C-reactive protein (CRP), capillary blood gas analysis (BG) and white blood cell (WBC) counts. Also, data for the following comorbidities were collected from patient charts: myocardial infarction, heart failure, cerebrovascular disease (e.g. stroke or transient ischemic attack), peripheral vascular disease, diabetes mellitus with or without end organ damage, decreased renal function defined as MDRD-GFR of <60,[12] ulcer disease, liver disease (e.g. chronic hepatitis, cirrhosis), malignancy of any kind, lymphoma, leukaemia, AIDS, obstructive sleep apnoea syndrome, anaemia (defined as <7.5 mmol/L in females and <8.5 mmol/L in males), hypertension, osteoporosis or osteopenia, and depression or anxiety. From these comorbidities the Charlson comorbidity index was calculated for each patient.

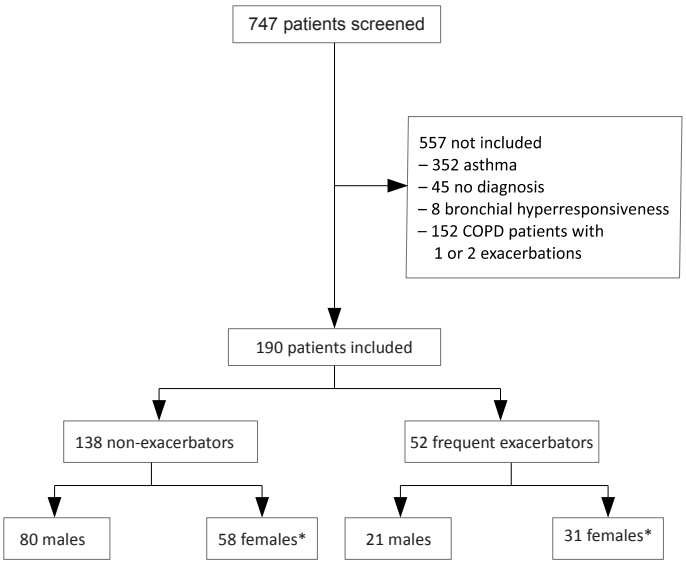

Figure 1: Study profile.

*There were significantly more women in the frequent exacerbator group (60%) than in the non-exacerbator group (42%; p=0.045).

Analysis

Data were presented as mean (standard deviation (SD)) or as median (interquartile range (IQR)) as appropriate. Categorical variables were presented as number (%).

Comparison of gender between the non-exacerbator and frequent-exacerbator groups and comparison of categorical data between males and females in both groups were performed using the exact chi square trend or Fisher's test, as appropriate. For comparisons of continuous variables between males and females in the non-exacerbator and frequent exacerbator groups the *t* test was used if variables were normally distributed and Mann-Whitney U test if not normally distributed. CRP and WBC counts were logarithmically transformed because of the highly skewed distributions. One female patient in the frequent exacerbator group had a B-cell lymphocytic lymphoma and was excluded from the analyses with leucocytes. All results with a $p<0.05$ were considered to be significant. Data were analysed using SPSS version 21 (IBM).

Table 1: Overview of non-exacerbators and differences in gender.

	Non-exacerbators			
	Total (n=138)	**Male (n=80)**	**Female (n=58)**	**P-value***
Age	63.8 (10.3)	65.7 (9.7)	61.1 (10.5)	0.009
BMI^	25.3 (22.3–28.6)	25.8 (22.5–28.7)	24.2 (22.3–28.5)	0.29
Smoking (%)	60 (43.5%)	35 (43.8%)	25 (43.1%)	1.00
Pack-years^	34 (21–45)	38 (25–50)	30 (20–44)	0.028
Partner	96 (69.9%)	61 (77.2%)	35 (62.5%)	0.10
Employment	49 (35.5%)	27 (34.2%)	22 (38.6%)	0.73
Charlson comorbidity index^	1 (1–2)	1 (1–2)	1 (1–1)	0.004
BODE-index^	1 (0–3)	1 (0–3)	1 (0–3)	0.57
FEV_1 in L	1.8 (0.7)	2.0 (0.8)	1.5 (0.6)	<0.001
FEV_1 in % of predicted	64 (21)	63 (20)	65 (22)	0.68
6MWT in m	458 (104)	459 (106)	455 (101)	0.84
6MWT in % of predicted	70 (14)	68 (14)	74 (13)	0.011
Sat <90% after 6MWT (%)	16 (11.6%)	8 (10.1%)	8 (14.3%)	0.64
MRC score	2 (1–3)	2 (1–3)	2 (1–3)	0.72
CAT	16 (7)	16 (7)	16 (8)	0.65
CRP^	2 (1–5)	3 (1–7)	2 (1–4)	0.14
WBC count^	8.9 (7.7–10.6)	8.9 (7.2–10.6)	8.7 (7.9–10.7)	0.42
pCO2 (kPa)	5.3 (0.6)	5.3 (0.6)	5.3 (0.6)	0.61

*P-values calculated for female versus male.

Continuous variables are presented as mean (SD) unless otherwise stated. Categorical variables are presented as number (%).

^Data presented as median (interquartile range).

Results

737 patients were screened of which 190 were included for analysis (figure 1). Frequent exacerbators were more often of the female sex.

Females versus males: non-exacerbators

Analysis showed that females were younger and had smoked less pack-years than non-exacerbating men (table 1). Furthermore, females had a better 6MWT than males (table 1). 88 patients (64%) had comorbidities. Females had less comorbidity than men measured by Charlson comorbidity index (table 1). When examining comorbidities separately, significantly more males had a history of myocardial infarction, peripheral vascular disease and anaemia than females (figure 2).

Females versus males: frequent exacerbators

In the frequent exacerbator group females were younger and more often current smokers than men (table 2). In total, 34 patients (65%) had comorbidities. Unlike in the non-exacerbator group, there were no differences in comorbidities between men and women (table 2 & figure 3).

Discussion

In this study we found that significantly more women were present in the frequent exacerbator group than in the non-exacerbator group. Also we showed that in the non-exacerbator group females were younger, had smoked less pack-years, had better 6MWT and had less comorbidity than men. Furthermore, frequent exacerbating females were younger and more often current smokers than frequent exacerbating males.

With this observational study we have confirmed previous observations that females tend to exacerbate more frequently than males. Apart from the results of the ECLIPSE study[6] and the study performed by De Torres *et al*.,[5] there are also others who reported this difference in exacerbation frequency. Beeh *et al*. reported that frequent exacerbators (defined as ≥2 exacerbations per year) were more likely to be female than in the non- and infrequent exacerbator groups.[13] Celli *et al*. reported that in a 3-year follow-up period in the TORCH trial women had a 25% higher exacerbation rate than men.[14] Furthermore, in the COLUMBUS trial, a prospective study performed in frequent exacerbating (≥3 exacerbations per year) COPD patients, it was observed that 57% of the study population consisted of women.[15] In contrast with the previous findings, two studies did not observe these gender-related differences in exacerbation frequency in patients with COPD. A national cross-sectional study performed in Greece did not show any differences between males and females in median number of exacerbations in the previous year.[16] Also, in a FEV_1- and age-matched study performed in France, women did not have more exacerbations than men.[17]

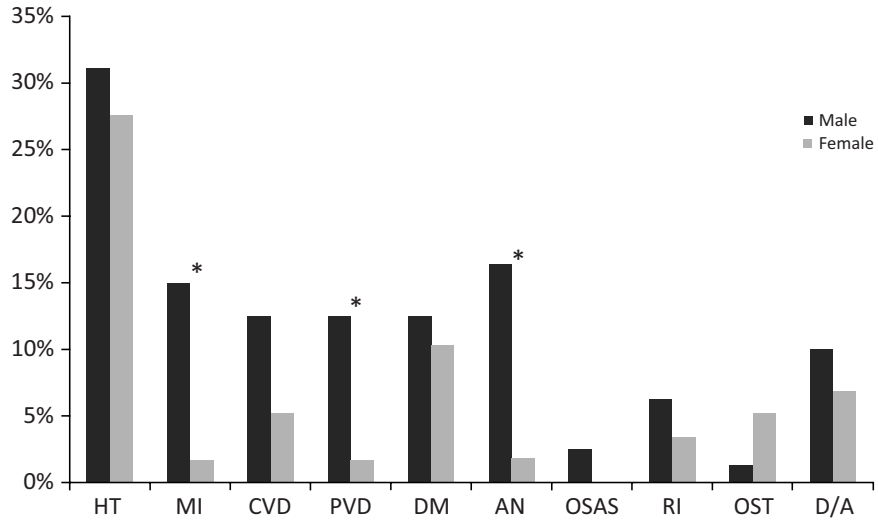

Figure 2: Overview of comorbidities per gender in the non-exacerbator group.

*Indicates statistical significance.
HT=hypertension; MI=myocardial infarction; CVD=cerebrovascular disease; PVD=peripheral vascular disease; DM=diabetes mellitus; AN=anaemia; OSAS=obstructive sleep apnoea syndrome; RI=renal insufficiency; OST=osteoporosis or osteopenia; D/A=depression or anxiety.

In our study we observed that in the non-exacerbator group females had less myocardial infarction, peripheral vascular disease and anaemia than men. Previously, Ferrari *et al*. and De Torres *et al*. also reported less comorbidity in females than in males in stable COPD patients.[5,18] The lower amount of tobacco consumption and less smoking related conditions has been postulated to be the reason for the lower comorbidity in females in comparison with males.[5]

An interesting finding was that there were no differences in comorbidity between males and females in the frequent exacerbating COPD group, an observation that has not been reported before. In contrast to the non-exacerbators, the female frequent exacerbators had smoked as many pack-years as men in this group. The combination of similar tobacco consumption as men at younger age in these women might be an explanation for the absence of differences between men and women in comorbidity. Because the presence of comorbidities in COPD patients is related with an increased risk of mortality,[19,20] the frequent exacerbating females in our study population might have worse prognosis in terms of morbidity and survival due to their younger age than men. However, several studies have reported that women with COPD had better or similar survival rates after adjustment for age and comorbidities than men.[14, 21, 22] Till so far, no studies have been performed investigating gender differences in morbidity and mortality in frequent exacerbators.

Table 2: Overview of frequent exacerbators and differences in gender.

	Frequent exacerbators			
	Total (n=52)	**Male (n=21)**	**Female (n=31)**	**P-value***
Age	61.4 (9.0)	65.4 (7.8)	58.7 (8.9)	0.007
BMI^	24.2 (22.4–30.2)	24.3 (22.7–27.0)	23.7 (22.3–30.5)	0.90
Smoking (%)	32 (61.5%)	7 (33.3%)	25 (80.6%)	0.002
Pack-years	36 (17)	36 (22)	36 (13)	0.95
Partner	36 (69.2%)	15 (71.4%)	21 (67.7%)	1.00
Employment	20 (38.5%)	5 (23.8%)	15 (48.4%)	0.13
Charlson comorbidity index^	1 (1–2)	1 (1–2)	1 (1–1)	0.47
BODE-index^	2 (1–4)	3 (1–5)	2 (1–4)	0.99
FEV_1 in L	1.4 (0.6)	1.6 (0.6)	1.3 (0.5)	0.16
FEV_1 in % of predicted	54 (19)	51 (20)	55 (18)	0.40
6MWT in m^	455 (333–481)	462 (285–512)	453 (368–479)	0.70
6MWT in % of predicted^	68 (51–76)	66 (43–73)	70 (61–77)	0.19
Sat <90% after 6MWT (%)	9 (17.3%)	4 (19.0%)	5 (17.9%)	1.00
MRC score^	3 (1–4)	2 (1–4)	3 (2–4)	0.27
CAT	20 (8)	18 (9)	22 (8)	0.12
CRP^	2 (1–5)	2 (2–7)	2 (1–5)	0.52
WBC count^	9.6 (8.4–12.0)	9.5 (8.1–10.4)	10.0 (8.4–12.8)	0.18
pCO2 (kPa)	5.2 (0.6)	5.4 (0.5)	5.1 (0.7)	0.08

*P-values calculated for female versus male.
Continuous variables are presented as mean ((SD) or as median (IQR). Categorical variables are presented as number (%).
^Data presented as median (interquartile range).

Another observation in our study was that frequent exacerbating females were more often active smokers than frequent exacerbating males. To the best of our knowledge, there are no studies in literature describing differences in smoking status between males and females in a frequent exacerbator phenotype. However, a few studies did report on gender related differences, exacerbations and smoking status in relatively stable COPD populations. Celli *et al*. observed that females, who had 25% higher exacerbation rates than males, were more often active smokers.[14] However, in the study of de Torres *et al*. there were no differences between more frequent exacerbating women and less exacerbating men in current smoking status,[5] and Papaioannou *et al*. reported that women were more often active smokers than men without observing any differences in exacerbation rate.[16] As the number of smoking women is rising,[23] and evidence exists that females might be more susceptible to the hazardous effects of cigarette smoke than men,[24,25] this could result in major health problems in this specific population.

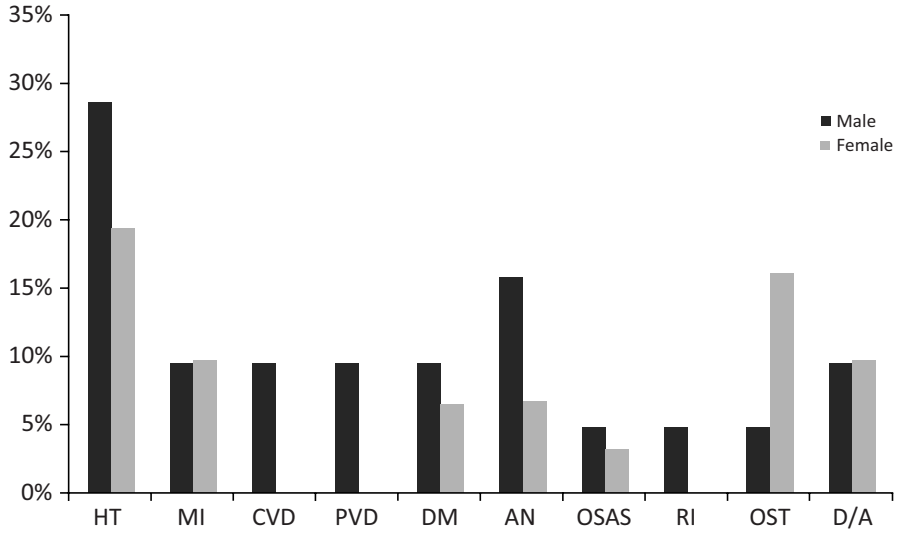

Figure 3: Overview of comorbidities per gender in the frequent exacerbator group.

HT=hypertension; MI=myocardial infarction; CVD=cerebrovascular disease; PVD=peripheral vascular disease; DM=diabetes mellitus; AN=anaemia; OSAS=obstructive sleep apnoea syndrome; RI=renal insufficiency; OST=osteoporosis or osteopenia; D/A=depression or anxiety.

Despite several reports that COPD has a higher impact on health status in women than in men,[4,14,18,26] we could not find this difference in our population measured with the CAT. In one European study performed by Jones *et al*.[27] no differences were found as well between men and women in CAT scores. The majority of the patients in that study had experienced at least one exacerbation in the previous 6 months. To our knowledge there is no data published regarding differences in CAT score between men and women in specifically non-exacerbating and frequent exacerbating phenotypes.

Our study has some limitations. First, we had a limited sample size in the frequent exacerbator group. Second, because patient follow-up was not recorded in this database we could not assess whether the observed differences were also present after some time.

In summary, we have shown that the frequent exacerbator phenotype consisted of more women than the non-exacerbator phenotype. Furthermore, we found that within the two distinct phenotypes in COPD there were differences between men and women. In particular, women in the frequent exacerbator group were younger than males, were more often current smokers, had similar pack-years and similar prevalence of comorbidities. Considering the previous in addition to the poor health outcomes[28-30] and high costs[31] that are related to frequent exacerbations, it is to be expected that female COPD patients might drive a substantial part of the future burden that will come with COPD. Accordingly, strategies should be focussed on identifying young and frequent exacerbating female patients in order to provide adequate interventions. Therefore, in future more longitudinal studies are needed in larger groups to further investigate and uncover the gender based differences in the frequent exacerbator phenotype.

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Chapter 7

Midregional pro-adrenomedullin correlates with the severity of exacerbations of COPD in the frequent exacerbator phenotype

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Introduction

Many biomarkers have been analysed for their role in exacerbations of chronic obstructive pulmonary disease (COPD).[1-3] A biomarker of recent interest is adrenomedullin (ADM), a peptide with endocrine, anti-inflammatory, and vasodilatory effects. It can be used as a marker for inflammation but also for cardiopulmonary stress.[4,5] However, a reliable measurement of ADM is difficult as it is rapidly cleared from the circulation. Midregional pro-adrenomedullin (MR-proADM), a stable biologically inactive midregional fragment of the ADM prohormone, is believed to directly reflect levels of ADM.[6]

We evaluated whether MR-proADM levels were different during stable state, and at mild and severe exacerbations of COPD. Furthermore, we investigated whether MR-proADM levels in stable state were related to the occurrence of exacerbations within 3 months and during one year follow-up in patients with the frequent exacerbator phenotype.

Methods

This study was part of the COLUMBUS trial (Clinicaltrials.gov, NCT00985244), which comprised of 92 COPD patients with the frequent exacerbator phenotype who were followed for 1 year. The study protocol and the analysis of the primary results have been published earlier.[7,8] It was a prospective study performed in the Netherlands between May 2010 and June 2013. Inclusion criteria were age ≥18 years, a diagnosis of COPD according to the guidelines of the Global initiative for chronic Obstructive Lung Disease,[9] and ≥3 exacerbations of COPD in the previous year that were treated with steroids and/or antibiotics. Patients were required to be clinically stable during one month prior to enrolment. Exclusion criteria were a history of other significant respiratory diseases (e.g. asthma, cystic fibrosis), the presence of bronchiectasis assessed by computed tomography, heart failure, and malignancy of any kind for which the subject received treatment or was being monitored as part of follow up after treatment. All participants provided written informed consent. The study was approved by independent and local ethics committees.

MR-proADM levels were measured during stable state at enrolment, at scheduled visits at months 3, 6, 9 and 12, and during exacerbations. An exacerbation was defined according to the Anthonisen criteria, requiring treatment with steroids and/or antibiotics.[10] An exacerbation was considered severe when hospital admission was necessary, and mild when it was treated at the outpatient department by the study investigators or the patient's general practitioner.

The MR-proADM samples were frozen at -80°C until later analysis. MR-proADM was detected with an automated immunofluorescent assay (KRYPTOR®, Thermo Scientific, Hennigsdorf, Germany).

Mean MR-proADM levels during stable state, mild and severe exacerbations were outcome parameters. Also, the occurrence of an exacerbation during a follow-up interval of 3 months and

one year between high (>0.75 nmol/L) and low level (≤0.75 nmol/L) MR-proADM groups were outcomes parameters.

Statistical analysis

Mean differences in MR-proADM levels between stable state, mild and severe exacerbations were estimated using mixed model analysis; covariates in this analysis were time since baseline, sex, smoking status, age, renal function, body mass index and use of macrolide maintenance therapy. MR-proADM was logarithmically transformed before analysis because of its positively skewed distribution. Maximally 13 repeated measurements of MR-proADM (5 stable state visits and 8 exacerbations) per patient were involved in this analysis. Estimates were back-transformed to be interpretable as percent differences between geometric means of MR-proADM.

Table 1: Patient characteristics at baseline.

	Patients (n=92)
Age	64.8 (10.1)
Male	40 (43.5%)
BMI	26.1 (5.1)
Smoking	29 (31.5%)
Medication	
– LABA	86 (93.5%)
– LAMA	74 (80.4%)
– ICS	85 (92.4%)
– Azithromycin maintenance therapy	47 (51.1%)
FEV_1 in L	1.1 (0.5)
FEV_1 in % of predicted	44.6 (19.3)
FEV_1/FVC in %	39 (12)

LABA=long-acting beta agonists; LAMA=long-acting muscarinic antagonists; ICS=inhalation corticosteroids; FEV_1=forced expiratory volume in 1 second; FVC=forced vital capacity. Data are in n (%) or in mean (standard deviation).

Logistic regression with a robust variance matrix was used to analyse the occurrence of an exacerbation in a follow-up interval of 3 months (between two successive stable visits) in relation to MR-proADM levels at the beginning of the interval; covariates were sex, age, smoking status, macrolide maintenance therapy, forced expiratory volume in 1 second (FEV_1) in percentage of predicted at the beginning of the interval, and the interval itself as categorical variable. Maximally four 3- months intervals per patient were involved in the analysis (0–3 months, 3–6 months, 6–9 months and 9–12 months after baseline). The follow-up intervals were required to be left and right sided closed intervals (i.e. complete intervals). MR-proADM was dichotomized into high or low MR-proADM based on the cut-off value of 0.75 nmol/L found by Stolz *et al*. who used it for the prediction of mortality.[11] We assessed its value in predicting exacerbations.

Poisson regression analysis was used for evaluating the differences between high and low level MR-proADM groups in exacerbation rate, with covariates age, sex, use of macrolide maintenance therapy, smoking status, exacerbations in previous year, and FEV_1 in % of predicted.

All tests were two-sided, and a p-value of 0.05 was considered statistically significant. The data were analysed using SPSS, version 21 (IBM).

Results

Of the 92 patients, 77 completed the 12 months study period (table 1). Samples for MR-proADM were obtained in 407 of the 417 stable state visits and in 140 (41 severe, 99 mild) of the 213 recorded exacerbations (56 severe, 157 mild). In 91 patients baseline MR-proADM samples were available. Azithromycin maintenance therapy did not influence MR-proADM levels (data not shown).

MR-proADM levels during severe exacerbations were 12.9% higher compared to stable state ($p<0.0005$; figure 1a) and 14.6% higher compared to mild exacerbations ($p<0.0005$; figure 1a). MR-proADM levels during mild exacerbations were not different compared to stable state (-1.5%, $p=0.34$; figure 1a).

For the analysis of occurrence of exacerbations there were 315 closed 3-months intervals and 153 (116 mild, 37 severe) exacerbations in those intervals. An MR-proADM level ≥0.75 nmol/L was not predictive of occurrence of an exacerbation (mild and severe) (odds ratio (OR) 0.73, 95% CI: 0.43–1.23; $p=0.24$) nor for a severe exacerbation alone (OR 0.57, 95% CI: 0.24–1.38; $p=0.21$) within 3 months. Also, after 12 months there were no differences between patients with baseline MR-proADM levels above or below 0.75 nmol/L in exacerbation rate (figure 1b).

Discussion

We are the first to show that MR-proADM levels were significantly higher during severe exacerbations than in mild exacerbations. Additionally, MR-proADM levels were increased on hospital admission compared to stable state. Also, we showed that elevated MR-proADM levels were not related to the occurrence of exacerbations. In a study of Stolz *et al*. MR-proADM levels were also elevated on hospital admission compared to recovery and were independently predictive for mortality.[5] One could suggest that the higher MR-proADM levels measured during severe exacerbations compared to mild exacerbations, were due to increased anti-inflammatory, vasodilatory, and bronchodilating effects of ADM.[4]

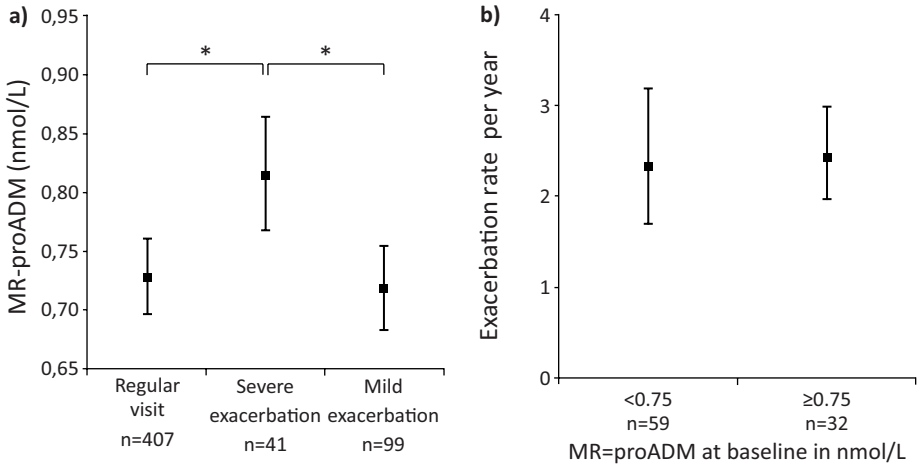

Figure 1: a) The geometric mean of midregional pro-adrenomedullin (MR-proADM) during the regular visits was 0.727 (95% CI: 0.695–0.760), 0.819 (95% CI: 0.771–0.870) at severe exacerbations, and 0.715 (95% CI: 0.680–0.751) at mild exacerbations. *p<0.005 for both comparisons. **b**) The exacerbation rate after 12 months in patients with a baseline MR-proADM level <0.75 nmol/L was 2.32 (95% CI: 1.69–3.19) and 2.41 (95% CI: 1.96–2.98) in patients with a baseline MR-proADM level ≥0.75. The exacerbation rate ratio <0.75 to ≥0.75 was 0.96 (95% CI: 0.64–1.45; p=0.85).

Although there is a relation between MR-proADM levels in stable state and mortality,[11,12] we could not establish a relation with the occurrence of exacerbations of COPD. This might suggest that MR-proADM in stable state in not only a reflection of inflammation, but also of another important physiological conditions, like cardiopulmonary stress, that might be more correlated with mortality than with exacerbations.

In our study we investigated patients of the frequent exacerbator phenotype, therefore these results cannot be generalizable to all patients with COPD.

In summary, we have shown that MR-proADM levels ≥0.75 nmol/L were not predictive of the occurrence of exacerbations in a follow-up period of 3 or 12 months in patients with the frequent exacerbator phenotype. Also, we have shown that MR-proADM levels were elevated during severe exacerbations compared to mild exacerbations and stable state. Further research should be performed to investigate whether MR-proADM could be used as an important biomarker in a clinical decision model, whether to admit a patient for an exacerbation or to treat as an outpatient.

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Chapter 8

Midregional pro-adrenomedullin and clinical outcomes in COPD patients with the frequent exacerbator phenotype

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Abstract

Midregional pro-adrenomedullin (MR-proADM) is able to independently predict mortality in patients with COPD. We investigated whether MR-proADM could predict clinical outcomes other than mortality in COPD patients. We performed a 12-month prospective study in 92 patients with a diagnosis of COPD who had 3 or more treated exacerbations in the previous year. Main exclusion criterion was the presence of another clinically significant respiratory disease. MR-proADM samples were obtained at enrolment in 91 patients and a cut-off level of 0.75 nmol/L was used to dichotomize patients. Linear regression analysis showed that elevated MR-proADM levels at baseline did not predict changes in forced expiratory volume in 1 second, 6-minute walk test, St. George's Respiratory Questionnaire, 12-Item Short Form Health Survey and Hospital Anxiety and Depression Scale at 12 months.

Introduction

To prevent disease progression in chronic obstructive pulmonary disease (COPD) it is important to assess the risk of adverse clinical outcomes and to intervene accordingly.[1] Several prediction models and biomarkers have been developed and examined in order to identify patients with COPD with high risk of poor clinical outcomes.[2,3] Midregional pro-adrenomedullin (MR-proADM) is one of the biomarkers that has shown to independently predict mortality in patients hospitalised with exacerbations and in patients with clinically stable COPD.[4-6] MR-proADM directly reflects circulating levels of adrenomedullin (ADM), a peptide with endocrine, anti-inflammatory, and vasodilatory effects, and is believed to reflect inflammation and cardiopulmonary stress in patients with COPD.[4,7,8]

We evaluated whether MR-proADM levels during stable state could predict clinical outcomes other than mortality in COPD patients with the frequent exacerbator phenotype.

Methods

This study was part of the COLUMBUS trial (Clinicaltrials.gov, NCT00985244), a 12-month prospective study in 92 COPD patients performed in the Netherlands. The study protocol and the primary results have been published earlier.[9,10] Inclusion criteria were age ≥18 years, a COPD diagnosis according to the guidelines of the Global initiative for chronic Obstructive Lung Disease,[11] and ≥3 exacerbations of COPD in the previous year that were treated with steroids and/or antibiotics. Patients were required to be clinically stable during one month prior to enrolment. Exclusion criteria were a history of other significant respiratory diseases (e.g. asthma, cystic fibrosis), the presence of bronchiectasis assessed by computed tomography, heart failure, and malignancy of any kind for which the subject received treatment or was being monitored as part of follow up after treatment. All participants provided written informed consent. The study was approved by independent ethics committees.

We obtained data for spirometry, 6-minute walk test (6MWT), C-reactive protein (CRP), white blood cell count (WBC), renal function (creatinine in mmol/L and blood urea nitrogen (BUN) in mmol/L), St. George's respiratory Questionnaire (SGRQ; higher scores indicating worse health related quality of life), 12-Item Short Form Health Survey (SF-12; higher scores indicating better quality of life), and Hospital Anxiety and Depression Scale (HADS; higher scores indicating more depressive or anxious symptoms) at enrolment and at 12 months. Samples for MR-proADM were obtained at enrolment and were frozen at -80°C until later analysis. MR-proADM was detected with an automated immunofluorescent assay (KRYPTOR®, Thermo Scientific, Hennigsdorf, Germany).

For assessing a relation between MR-proADM levels at baseline and clinical outcomes at 12 months, patients were classified as having low or high MR-proADM levels based on cut-off level of 0.75 nmol/L determined in the study of Stolz *et al*.[5]

The clinical outcomes of interest were: forced expiratory volume in 1 second (FEV_1), 6MWT, SGRQ, SF-12 and HADS scores at 12 months.

We used linear regression to analyse the relation between MR-proADM levels at baseline and each of the outcome variables, adjusted for the variables age, sex, FEV_1 in % of predicted at baseline, smoking status, use of macrolide maintenance therapy, exacerbations in the previous year, and the baseline value of the dependent variable being examined. All tests were two-sided, and a p-value of 0.05 was considered statistically significant. Data were analysed using SPSS, version 21 (IBM).

Results

Of the 92 patients, 77 completed the 12 months study period. Baseline MR-proADM levels were available in 91 patients; 59 and 32 patients had MR-proADM levels <0.75 nmol/L and ≥0.75 nmol/L, respectively. There were no differences between groups in sex, smoking status, CRP, WBC, SGRQ, SF-12, HADS and comorbidities (data not shown). Compared to the high level MR-proADM group, patients in the low level group were younger (61.2, 95% CI: 58.9–63.5 versus 71.6, 95% CI: 68.3–74.9; p<0.001), had lower BMI (25.0, 95% CI: 24.0-26.1 versus 28.2, 95% CI: 26.0–30.4; p=0.003), had a lower FEV_1 in % of predicted (40.7, 95% CI: 36.6–44.7 versus 52.5, 95% CI: 44.4–60.9; p=0.004), had lower serum creatinine (72, 95% CI: 68–76 versus 89, 95% CI: 80–99; p<0.001), and lower BUN levels (4.9, 95% CI: 4.6–5.3 versus 6.8, 95% CI: 5.9–7.6; p<0.001).

At 12-month follow-up, baseline MR-proADM levels above or below 0.75 nmol/L had no significant effects on FEV_1, 6MWT, SGRQ, SF-12 or HADS scores (table 1).

Discussion

This study has shown that baseline MR-proADM levels were not related to FEV_1, 6MWT, SGRQ, SF-12 and HADS at 12 months.

The higher values of FEV_1 in the high level MR-proADM group were a remarkable finding which was in contrast to studies of Stolz *et al*.[5] and Jehn *et al*.[12] In the study of Stolz *et al*. COPD patients that did not survive 2-years had significantly higher MR-proADM levels compared to survivors, however the survivors and non-survivors did not differ in FEV_1.[5] In the study of Jehn *et al*. COPD patients with lower GOLD stages had higher MR-proADM levels,[12] however this correlation was not found in a Spearman's correlation test. Another contrast with the study of

Jehn *et al*. was the significant correlation they found between MR-proADM and steps per day assessed by accelerometer; in our study 6MWT, which is correlated to physical activity, was not related to MR-proADM levels.[12, 13]

Table 1: Results of multiple linear regression analyses of clinical outcome variables at 12 months.

Dependent variable at 12 months	Effect of MR-proADM ≥0.75 nmol/L	95 % CI	P-value
FEV_1 (litres)	0.03	-0.06 to +0.12	0.56
FEV_1 (% of predicted)	0.30	-3.41 to +4.02	0.87
6MWT (meters)	-9.82	-51.3 to +31.6	0.64
6MWT (% of predicted)	-1.45	-10.4 to +7.53	0.75
SGRQ total score (SD=16.3)	-2.16	-8.63 to +4.31	0.51
HADS anxiety (SD=4.66)	-0.22	-2.26 to +1.82	0.83
HADS depression (SD=4.42)	-0.67	-2.64 to +1.31	0.50
SF-12 physical component (SD=10.5)	-1.82	-6.93 to +3.29	0.48
SF-12 mental component (SD=12.4)	3.31	-3.19 to +9.81	0.31

Each model contained the covariates age, sex, smoking status, FEV_1 in % of predicted, exacerbations in the previous year, use of macrolide maintenance therapy, and the baseline value of the dependent variable being examined, along with MR-proADM ≥0.75 nmol/L. None of the covariates was significantly associated with the dependent variable, except the baseline value of the dependent variable itself.
SD's at 12 months for SGRQ, HADS and SF-12 were provided to judge the effect size.

Furthermore, baseline MR-proADM levels did not significantly influence SGRQ, SF-12 and HADS at 12 months. Jehn *et al*. also showed that SGRQ did not correlate with MR-proADM levels in patients with stable COPD.[12] There is no data on a correlation between MR-proADM levels and SF-12 in patients with COPD. In a study of Akpinar *et al*., ADM levels were elevated in patients with major depression diagnosed according to the DSM-IV.[14] In our study we used the HADS questionnaire, which is merely an instrument to assess symptoms of anxiety and depression and is not a diagnostic tool for major depression.[15]

The differences we found between high and low level MR-proADM groups in age, BMI and renal function are in accordance with earlier findings.[16]

Our study has some limitations. First, this study was not powered for the outcome variables that were analysed. Second, follow-up might have been too short to detect potential differences in the clinical outcomes.

In summary, we have shown that elevated MR-proADM levels in stable state in COPD patients with the frequent exacerbator phenotype, were not able to predict FEV_1, 6MWD, SGRQ, HADS or SF-12 after 12 months follow-up.

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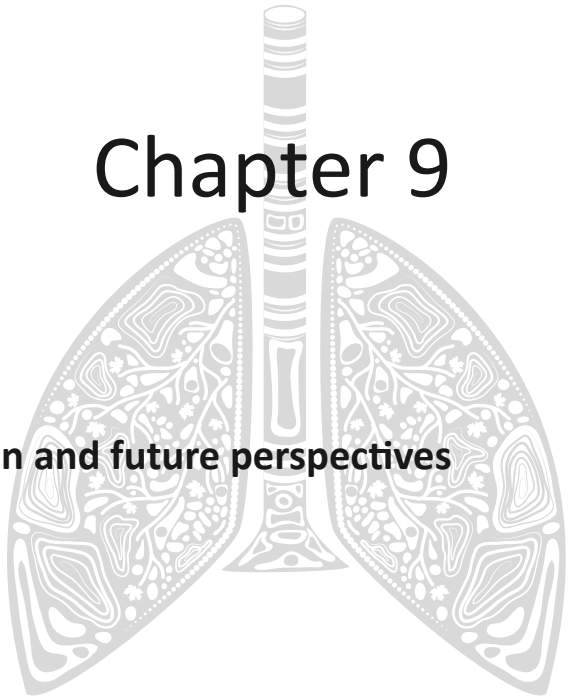
Chapter 9



General discussion and future perspectives

The studies described in this thesis address several matters concerning exacerbations in patients with COPD. The COLUMBUS study provided an answer to the question what role to give macrolide antibiotics in the prevention of COPD exacerbations (chapters 3 and 4). Also, by describing the viruses that caused exacerbations during different seasons in the COLUMBUS study, we provided new information in the seasonal variation of circulating viruses in patients with frequent exacerbations (chapter 5). Furthermore, we investigated non- and frequent exacerbators and compared males and females with each other as we believe the role of gender in COPD has not been sufficiently researched yet (chapter 6). To also identify a biomarker that has correlation with exacerbations and clinical endpoints in COPD, we investigated if MR-proADM might aid in treatment strategies (chapters 7 and 8).

Azithromycin and COPD exacerbations

Although several studies have shown that macrolides prevent exacerbations of COPD, there is much debate when to implement maintenance therapy with macrolide antibiotics as there is concern about antimicrobial resistance and side effects.[1,2] Also, the studies that have been performed did not include standard screening for bronchiectasis by computed tomography (CT), therefore not completely excluding patients with non-CF bronchiectasis, and subsequently risking that the obtained results were influenced by these patients. Furthermore, there was no minimum required number of exacerbations before entry into the study, creating a treatment strategy of prescribing macrolides to many COPD patients. Proposals of reserving long-term macrolide treatment for patients with two or more COPD exacerbations were not supported by clinical studies.[3,4]

In the COLUMBUS trial all patients had 3 or more exacerbations and those with bronchiectasis assessed by CT scan were excluded. We found a relative reduction of 42% in yearly exacerbation rate with azithromycin three times a week 500 mg during one year compared to placebo (chapter 4). Also, with azithromycin there was a doubling in time to first exacerbation compared to placebo. Patients also had fewer symptoms as measured by the symptom score of the St. George's Respiratory Questionnaire and had lower inflammation measured by CRP and circulating leucocytes than patients in the placebo group. We found no increase in adverse effects of azithromycin therapy compared to placebo besides diarrhoea.

A remarkable observation was the increase in the acquisition of macrolide resistant bacteria in the placebo group compared to the azithromycin group. However, as we had a limited number of sputum samples and subsequently limited numbers of positive sputum cultures, we believe these results might underestimate the occurrence of antimicrobial resistance in both groups. As is already known, an abundance of studies have shown that macrolide antibiotics increase antimicrobial resistance.[5,6] In particular, the increasing number of macrolide resistant *S. pneumoniae* and the parallel course of prescription rates of azithromycin in the USA is an

illustrative example.[4] The question remains however, whether individuals receiving macrolide maintenance treatment will face the consequences of the resistant microorganisms. In none of the studies performed with macrolides it was reported that the study patients with COPD had infections with macrolide resistant bacteria.[7-11] Nevertheless, as maximum follow-up in those studies was 12 months caution should be taken with prescribing long-term macrolide treatment. To date, no studies have been performed investigating the long-term effects of antimicrobial resistance on infectious complications, but this is certainly called for. In addition to the previous, it is interesting to note that we saw less mild exacerbations that were treated with additional antibiotics in the azithromycin group compared with the placebo group. It may be hypothesized that less use of antibiotics due to acute exacerbations of COPD might outweigh the long-term use of azithromycin. Future ongoing research by our group may shed light unto this. We are evaluating in more detail the resistance patterns in pharyngeal flora.

Due to the issues with antimicrobial resistance, there is a need to research alternatives for macrolide maintenance therapy. A recent study reported on a new non-antibiotic azithromycin analogue, CSY0073, which influenced lipopolysaccharide induced inflammation in respiratory tract but did not inhibit growth of *P. aeruginosa, H. influenzae* or *S. aureus*, and also did not have an inhibitory effect on the biofilm of *P. aeruginosa*.[12] Furthermore, CSY0073 attenuated lung inflammation, like azithromycin. Whether COPD patients will have the same clinical benefit from a non-antibiotic macrolide in preventing exacerbations is yet to be established. The achieved results in COPD might partly be influenced by the antimicrobial effects of macrolides, and therefore the effects of CSY0073 might not be as large as antibiotic macrolides. Nonetheless, the findings with CSY0073 merit further research.

Still there remain questions to be answered regarding long-term treatment with macrolides. Issues worth considering are which macrolide to give and which patient to give it to. In the studies performed with macrolides in COPD, the only trial performed with clarithromycin showed no effect in reduction of exacerbations (chapter 2). The other trials used erythromycin or azithromycin. Several important aspects are to be reviewed before making a choice between azithromycin and erythromycin, namely side effects, drug interactions, and dosage.

There were some differences in adverse events between trials conducted with erythromycin and azithromycin. The main category of adverse events in macrolides is known to be of gastro-intestinal (GI) nature like nausea, vomiting or diarrhoea. With erythromycin, adverse events of the GI tract in COPD patients varied from 2–15%.[8,9,11] In the studies conducted with azithromycin GI tract adverse events were reported to be 5–36%.[7,10] In the COLUMBUS trial GI tract related adverse events occurred in 10 patients (22%). However, in a report published earlier, in which thousands of patients were reviewed, it was shown that azithromycin was better tolerated than erythromycin.[13] Furthermore, cardiovascular death has been an issue in macrolides.[14,15] While erythromycin was known for its Qtc prolonging property, azithromycin was considered relatively safe until one study reported that current use of azithromycin increased risk of cardiovascular death in elderly with comorbidity.[14] A second study in younger patients with less comorbidity

also found that cardiovascular death was higher with current use of azithromycin compared with no use, but this effect was probably entirely contributable to the risk of death associated with acute infection.[15] In a recent review Albert *et al*. questioned whether the risk of arrhythmias with macrolides is as great as suggested by several reports.[16] With respect to the previous, we believe no difference should be made between azithromycin and erythromycin. When considering drug interactions however, azithromycin should be chosen over erythromycin. Because azithromycin in contrast to erythromycin does not interfere with the cytochrome P450 system, drug interactions are less common with azithromycin.[17] Additionally, azithromycin can be given in a thrice weekly regimen instead of daily. An advantage of azithromycin is the prolonged presence of the drug in the body, which might make it less sensitive to poor therapy compliance, although this has not been specifically researched. With the advantages of azithromycin described above, and taking into account that more COPD patients have participated in azithromycin trials than in erythromycin trials, it is justifiable to prefer azithromycin over erythromycin in maintenance treatment for preventing COPD exacerbations.

We believe that patients with COPD who might benefit from maintenance treatment with azithromycin should be carefully selected. There are several reasons why the targeted group should consist of patients with three or more exacerbations in one year who are refractory to standard care. First, the poor health[18,19] and prognosis[20,21] of patients with the frequent exacerbator phenotype warrant intervention. Second, considering the need for prudent use of antibiotics to contain antimicrobial resistance,[22,23] a small group of COPD patients that is responsible for a substantial part of the burden of COPD on health-care systems should receive treatment.[24] Third, with the COLUMBUS study we have shown that only few patients with 3 or more exacerbations in the previous year, would receive unnecessary treatment with macrolides as only 7% of patients in placebo group did not have any exacerbation in the 12-month study period. Compared to the largest study performed with azithromycin, 32% of the control patients did not experience any exacerbation.[7] We therefore believe a main criterion of at least 3 exacerbation for implementation of maintenance therapy with macrolides is a reasonable one to maintain, and should considered to be incorporated into treatment guidelines for COPD.

Exacerbations, seasonality and viral causes

The seasonal pattern of exacerbations is a known phenomenon.[25] In the COLUMBUS study this was in accordance with earlier findings from larger studies.[26] This seasonality of exacerbations is partly attributed to several factors, including increased exposure to viruses and greater risk of infection due to changes in meteorological conditions.[27] However, specification and description of the viruses causing exacerbations per season had not been done before. In the COLUMBUS study the viral pathogens causing the exacerbations were in accordance with viruses described in literature.[28] However, as we found rhinoviruses exclusively in exacerbations in summer, while

other viral microorganisms were found mostly in other seasons, new information has been provided in seasonally circulating viruses in patients with frequent exacerbations. It should be considered though that there is a possibility that the incidence of viral induced exacerbations were influenced by maintenance treatment with azithromycin in some of the patients. Several *in vitro* studies have shown that macrolides inhibit rhinovirus and influenza A virus infections in human airway epithelial cells.[29,30] One could hypothesize that due to the inhibition of viral infection by macrolides, an exacerbation was not induced and therefore an underestimation of virus associated exacerbations was presented. Further analysis of the data might provide an answer to this hypothesis.

Future studies could focus on the specification of the seasonal pathogens causing exacerbations, and accordingly treatment and preventive strategies could be developed.

Gender and COPD

A remarkable observation in the COLUMBUS trial was that 57% of the frequent exacerbating patients were female, whereas in general most patients with COPD are male. In several other studies it was suggested that females tend to exacerbate more than men do.[31,32] To further analyse the gender-based differences, we investigated a real life COPD population and specifically aimed at non- and frequent exacerbators (chapter 6). Here, we also found that the frequent exacerbator population consisted of significantly more women than the non-exacerbator population. We showed that women in the non-exacerbating population were younger and had smoked less pack-years than men, but this had been shown earlier. However, a new observation was that in our frequent exacerbator population females were more often current smokers. Also, despite the significant younger age of the women, they had smoked as many pack-years and had similar comorbidities as the males. As comorbidities are related to higher mortality rates in COPD,[33,34] it is therefore possible that this specific subgroup of patients of young females who exacerbate frequently, are current smokers and have considerable comorbidities, might have a more severe prognosis than males.[35] An interesting point of discussion is that despite the previous, most studies investigating sex-related mortality differences show that women have higher survival rates. However, these studies were performed in large COPD populations and did not analyse the frequent exacerbators specifically.

Concerning gender differences, there is need to put our results into context and investigate if women with frequent exacerbations of COPD indeed are a distinct and more high-risk patient category than men. Therefore, a greater number of COPD patients with frequent exacerbations should be researched, with particular attention to females. Subsequently, when certain factors are identified that contribute to frequent exacerbations, treatment strategies can be developed in order to obtain more adequate disease control in these patients. For example, more intensive

counselling for smoking cessation and screening for comorbidities can contribute to a better disease management.

Midregional pro-adrenomedullin and COPD

As stated earlier, a frequent exacerbating population is prone to poor clinical outcomes. A biomarker to predict the occurrence of exacerbations, decline in lung function, exercise capacity, quality of life or mortality would be ideal in these patients in order to provide adequate intervention. In an attempt to predict clinical outcomes in patients with COPD, there has been an abundance of research to biomarkers and COPD and exacerbations. However, none of these biomarkers have proven to be an ideal predictor of exacerbations or poor clinical outcomes. Midregional pro-adrenomedullin (MR-proADM), a surrogate marker for adrenomedullin, is one of the biomarkers that has been researched and has been proven to predict mortality in patients with COPD, with increased levels indicating higher risk of mortality. However, no data were available on elevated MR-proADM levels and other clinical outcomes. We therefore investigated if MR-proADM could predict or was related to events and outcomes in frequent exacerbating COPD patients. Despite the fact that we did not show MR-proADM to predict the occurrence of exacerbations or other clinical parameters (chapters 7 & 8), we did find that MR-proADM levels were elevated in COPD exacerbations for which hospital admission was necessary in comparison to exacerbations that were treated at home. This is a first ever observation, and might become useful in clinical decision models in determining whether to admit a patient for an exacerbation or not.

As MR-proADM is a relatively new biomarker with sparse research performed in COPD patients, there are many studies to be thought of in order to establish its role in the management of COPD patients. The observation that MR-proADM levels are significantly higher in severe exacerbations compared to mild exacerbations warrants further research. First, a study should be set up in a larger group of COPD patients to confirm the observation we made. If this would be the case, a clinical decision model to aid physicians in daily practice could be developed. A study could be set up where patients presenting themselves at an emergency department with COPD exacerbations are admitted to the hospital or treated at home based on several clinical parameters in combination with MR-proADM levels.

Concluding remark

In COPD, preventing exacerbations of COPD is one of the most important treatment goals. Currently, we have an array of pharmacological and non-pharmacological interventions to achieve disease control. However, we are still far from completely understanding, classifying and

predicting these events as are we from understanding the clinical diversity in the current COPD population. With the upcoming worldwide increase in COPD patients, and with other causative factors of COPD like air pollution and burning of biomass fuels, the disease itself as the patient population will become more and more diverse. More research to uncover strategies in the treatment of this disease is therefore crucial.

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Summary

Chronic obstructive pulmonary disease (COPD) is an incapacitating disease characterised by chronic airway inflammation and progressive airflow limitation with poor reversibility. Worldwide, COPD is predicted to be the 3rd cause of mortality by 2030. In **chapter 1** we provide a concise overview of the current knowledge on exacerbations of COPD. The hazardous effects of frequent exacerbations are discussed and also the preventive measures, for which nowadays an array of non-pharmacological and pharmacological interventions exists. One of those interventions is long-term treatment with macrolide antibiotics.

In **chapter 2** the effect of macrolide maintenance treatment on diffuse panbronchiolitis, cystic fibrosis (CF), and non-CF bronchiectasis is discussed. We provide a comprehensive overview of studies performed with macrolides in COPD. These studies have proved that macrolides reduce exacerbations of COPD. Concerns about antimicrobial resistance and possible side effects have arisen though, leading to discussions if and when to implement maintenance therapy with macrolides.

With the COLUMBUS trial described in **chapters 3 and 4** we investigated whether COPD patients with frequent exacerbations would have a decreased rate of exacerbation when maintenance macrolide treatment was added to standard care. The study was a 12-month during randomised, double-blind placebo controlled trial comparing azithromycin with placebo. The study comprised of 92 patients with 3 or more exacerbations in the previous year and was performed in the Amphia Hospital in Breda. We showed that azithromycin resulted in a significant reduction in exacerbation rate versus placebo. Also, azithromycin doubled the median time to first exacerbation compared to placebo. Furthermore, we measured decreased inflammatory parameters and fewer symptoms in the patients taking azithromycin. In addition to this, acquisition of macrolide-resistant bacteria was more commonly seen in the placebo group.

From these results, we concluded that azithromycin should be considered for use in patients with COPD who have the frequent exacerbator phenotype and are refractory to standard care.

In **chapter 5** the seasonality of the virus induced exacerbations during the COLUMBUS trial was described. We observed that between May 2010 and June 2013 most exacerbations, regardless of what cause, occurred in winter. Most virus induced exacerbations also occurred in winter, followed by summer, autumn and spring. The rhinovirus was the most frequently isolated viral pathogen, followed by respiratory syncytial virus, human metapneumovirus, influenza A, parainfluenza 4 and parainfluenza 3. However, the distribution of rhinovirus induced exacerbations showed a different pattern in comparison to the other viruses; most rhinovirus induced exacerbations occurred during summer.

An unusual observation in the COLUMBUS study was that 57% of the study population was female. In literature, several studies had described that female COPD patients tend to exacerbate more frequently than men do. To further investigate this, we examined the differences between

males and females in a real life study population of non-exacerbator and frequent exacerbator phenotypes (**chapter 6**). This study population was selected from a prospectively collected database of patients attending the outpatient department of Respiratory Medicine in a general hospital for (re)assessment of their respiratory disease. In total, 190 COPD patients (138 non-exacerbators and 52 frequent exacerbators) were analysed. We observed differences between males and females in the two distinct phenotypes that are described in further detail in the chapter. Future research to further investigate this is certainly necessary.

In COPD patients a biomarker to reliably predict adverse outcomes has not been uncovered yet. Midregional pro-adrenomedullin (MR-proADM) is a biomarker of more recent interest that has been proved to independently predict mortality in patients with COPD during stable state but also during exacerbations. In **chapter 7** we showed that MR-proADM levels during severe exacerbations were significantly higher compared to stable state and mild exacerbations. MR-proADM levels during mild exacerbations were not different compared to stable state. MR-proADM levels were not predictive of occurrence of exacerbations. In **chapter 8** we reported that MR-proADM levels did not predict changes in other clinical outcomes like forced expiratory volume in 1 second, 6-minute walk test, St. George's Respiratory Questionnaire, 12-Item Short Form Health Survey and Hospital Anxiety and Depression Scale at 12 months. Considering the results in **chapter 7**, further research should be performed to investigate whether MR-proADM could be used as a biomarker in a clinical decision model, whether to admit a patient for an exacerbation or to treat as an outpatient.

In **chapter 9** we discuss and give an interpretation of our results. Some limitations of the described studies are also highlighted. With respect to some results, future perspectives and suggestions are given to further investigate the prevention, prediction and characterisation of exacerbations.

Samenvatting

Chronisch obstructieve longziekte (COPD; in het Engels chronic obstructive pulmonary disease) is een invaliderende zieke welke gekarakteriseerd wordt door een chronische ontsteking van de luchtwegen en een progressieve luchtwegobstructie die niet volledig omkeerbaar is. Voorspeld wordt dat COPD in 2030 de derde grootste doodoorzaak wereldwijd zal zijn. In **hoofdstuk 1** geven we een beknopt overzicht over exacerbaties COPD (tijdelijke verergering van de symptomen waarvoor behandeling noodzakelijk is). De schadelijke effecten van exacerbaties op de gezondheidstoestand van patiënten worden besproken, alsook preventieve maatregelen waarvoor tegenwoordig een reeks aan medicamenteuze en niet-medicamenteuze interventies bestaan. Een van die interventies is langdurige behandeling met macrolide antibiotica.

In **hoofdstuk 2** wordt het effect van onderhoudsbehandeling met macroliden op longziekten zoals diffuse panbronchiolitis, taaislijmziekte (cystische fibrose (CF)) en niet-CF bronchiëctasieën besproken. We geven tevens een uitgebreid overzicht van studies die verricht zijn met macrolide antibiotica bij COPD. Deze studies hebben bewezen dat macroliden exacerbaties COPD voorkomen. Echter, er is bezorgdheid over antimicrobiële resistentie en bijwerkingen. Dit heeft tot discussies geleid óf en wanneer deze therapie te implementeren bij patiënten met COPD.

Met de COLUMBUS studie, welke beschreven is in **hoofdstukken 3 en 4**, onderzochten we of langdurige behandeling met macrolide antibiotica als aanvulling op standaard behandeling exacerbaties kon voorkomen in patiënten met frequente exacerbaties. Het was een gerandomiseerde, dubbelblinde, placebo-gecontroleerde studie welke uitgevoerd is in het Amphia Ziekenhuis in Breda. We hebben 92 patiënten gerandomiseerd om gedurende 12 maanden 500 mg azitromycine of placebo 3x per week in te nemen. We zagen dat azitromycine behandeling resulteerde in een significante afname van het aantal exacerbaties. Ook lieten we zien dat azitromycine de mediane tijd tot de eerste exacerbatie verdubbelde in vergelijking met placebo. Verder zagen we dat patiënten die azitromycine onderhoudsbehandeling kregen lagere ontstekingswaarden in het bloed hadden, alsook minder symptomen. Daarnaast waren er minder patiënten in de azitromycine groep in vergelijking met de placebo groep die acquisitie van macrolide resistente bacteriën in het sputum hadden.

Concluderend lieten we zien dat azitromycine overwogen zou moeten worden in patiënten met COPD die frequent exacerbaties hebben en die refractair zijn onder standaard behandeling.

In **hoofdstuk 5** werden de virus geïnduceerde exacerbaties tijdens de COLUMBUS studie beschreven, die per seizoen wisselend voorkwamen. Tussen mei 2010 en juni 2013 vonden de meeste exacerbaties plaats in de winter, ongeacht de oorzaak van de exacerbaties. De virus geïnduceerde exacerbaties kwamen ook het vaakst voor in de winter, gevolgd door de zomer, herfst en lente. Het rhinovirus was de ziekteverwekker die het vaakst werd geïsoleerd, gevolgd door het respiratoir syncytieel virus, humane metapneumovirus, influenza A virus, parainfluenza 4 virus en parainfluenza 3 virus. Echter, de distributie van de rhinovirus geïnduceerde exacerbaties

toonde een ander patroon in vergelijking met de andere virussen; de meeste exacerbaties veroorzaakt door rhinovirus vonden plaats in de zomer.

Een ongewone bevinding in de COLUMBUS studie was dat 57% van de studie populatie bestond uit vrouwen. De meeste COPD patiënten zijn immers van het mannelijke geslacht. In de literatuur zijn er enkele studies die beschreven dat vrouwelijke COPD patiënten vaker neigen te exacerberen dan mannen. Om dit verder te onderzoeken, hebben we de verschillen tussen mannen en vrouwen in een COPD populatie van stabiele en instabiele patiënten onderzocht (**hoofdstuk 6**). Deze studiepopulatie was geselecteerd uit een prospectief verzamelde database van patiënten die op de polikliniek longgeneeskunde kwamen voor (her)beoordeling van een longziekte. In totaal werden er 190 patiënten geanalyseerd (138 geen exacerbaties, 52 frequente exacerbaties). We zagen dat er verschillen waren tussen mannen en vrouwen in deze twee uitgesproken fenotypes van COPD welke in detail zijn besproken in **hoofdstuk 6**. Toekomstig onderzoek om de beschreven bevindingen verder in kaart te brengen is nodig.

Er is nog geen biomarker gevonden welke betrouwbaar uitkomsten voorspelt in patiënten met COPD. Midregionaal pro-adrenomedulline (MR-proADM) is een biomarker welke mortaliteit in patiënten met COPD kan voorspellen bij patiënten met COPD in stabiel vaarwater, maar ook gedurende exacerbaties. In **hoofdstuk 7** hebben laten zien dat de waardes van MR-proADM hoger waren gedurende ernstige COPD exacerbaties dan in een stabiele fase of gedurende een milde exacerbatie. Ook lieten we zien dat MR-proADM het optreden van exacerbaties niet kon voorspellen. In **hoofdstuk 8** rapporteerden we dat MR-proADM waardes geen andere klinische uitkomsten zoals longfunctie, 6 minuten looptest, St. George's Respiratory Questionnaire, 12-Item Short form Health Survey en de Hospital Anxiety and Depression Scale, 12 maanden na meting kon voorspellen. Gezien de beschreven resultaten in **hoofdstuk 7** zou er verder onderzoek gedaan kunnen worden om te bekijken of MR-proADM als een biomarker gebruikt kan worden in een klinisch beslismodel, om te bepalen of een patiënt opgenomen moet worden voor een exacerbatie of niet.

In **hoofdstuk 9** worden de reeds gepresenteerde resultaten geïnterpreteerd en bediscussieerd. Enige tekortkomingen van de studies worden ook aangestipt. Kijkend naar een aantal resultaten, worden er toekomstige perspectieven gegeven en suggesties gedaan voor om verder onderzoek te doen naar het voorkomen, voorspellen en karakteriseren van exacerbaties.

About the author

Sevim Uzun was born on October 3rd 1984 in Rotterdam, the Netherlands. In 1985 she moved to Breda where she grew up. In 2002 she graduated from Gymnasium at the Newman College in Breda and started medical school at the Erasmus University in Rotterdam. In 2006 she went for 6 months to Pondicherry, India for a clinical study to investigate the prevalence of lymphatic filariasis in the local population. In 2008 she did an internship Respiratory Medicine in the Amphia Hospital in Breda, which was when she decided to become a pulmonologist. She finished her studies with an internship Anaesthesiology at the Austin Hospital in Melbourne, Australia. After obtaining her medical degree, she started the working at the Amphia Hospital in Breda. In collaboration with the Erasmus Medical Centre, a study was set up under supervision of prof. H.C. Hoogsteden, dr. J.G.J.V. Aerts and dr. M.M. van der Eerden from which this thesis has arisen. Currently, she is still working at the department as a resident (ANIOS) of Respiratory Medicine in the Amphia Hospital and will start the training to become a pulmonologist on January 1st 2015.

PhD portfolio

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Research training

2009 Introduction to medical statistics and SPSS, Brabant Medical School, the Netherlands.

2010 Writing in scientific English, Brabant Medical School, the Netherlands.

2010 Evidence Based Medicine, Brabant Medical School, the Netherlands.

2010 Good Clinical Practice in scientific research, Amphia Hospital Breda, the Netherlands.

Poster presentation

2012 American Thoracic Society International Conference, San Francisco, United States of America.
Title: "Frequent exacerbations of COPD result in decline in lung function but not in 6 minutes walking distance and Medical Research Council score"

National and international conferences

2011 Masterclass Infectious Diseases, Hospital Clinic, Barcelona, Spain.

2012 New insights into pulmonary infectious diseases, Rotterdam, the Netherlands.

2012 Respiratory Forum, Manchester, United Kingdom.

2013 Respiration Day, University of Parma, Parma, Italy.

List of publications

S. Uzun, R.S. Djamin, J.A.J.W. Kluytmans, N.E. van 't Veer, A.A.M. Ermens, A.J. Pelle, P.G.H. Mulder, M.M. van der Eerden, J.G.J.V. Aerts
"Influence of macrolide maintenance therapy and bacterial colonisation on exacerbation frequency and progression of COPD (COLUMBUS): Study protocol for a randomised controlled trial."
Trials. 2012 June; 13: 82.

S. Uzun, R.S. Djamin, H.C. Hoogsteden, J.G.J.V. Aerts, M.M. van der Eerden
"Chapter 4: Acute Exacerbations of Chronic Obstructive Pulmonary Disease"
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S. Uzun, R.S. Djamin, N.E. van 't Veer, J.A.J.W. Kluytmans, A.A.M. Ermens, H.C. Hoogsteden, J.G.J.V. Aerts, M.M. van der Eerden
"Macrolides to prevent exacerbations of COPD, a review"
Clinical Pulmonary Medicine. 2014; 21: 61-67.

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"Effect of azithromycin maintenance treatment in patients with frequent exacerbations of COPD (COLUMBUS): a randomised, double-blind, placebo-controlled trial"
The Lancet Respiratory Medicine. 2014; 2: 361-68.

Submitted articles
R.S. Djamin, **S. Uzun**, E. Snelders, J.A.J.W. Kluytmans, H.C. Hoogsteden, J.G.J.V. Aerts, M.M. van der Eerden
"Occurrence of virus induced COPD exacerbations during four seasons"

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