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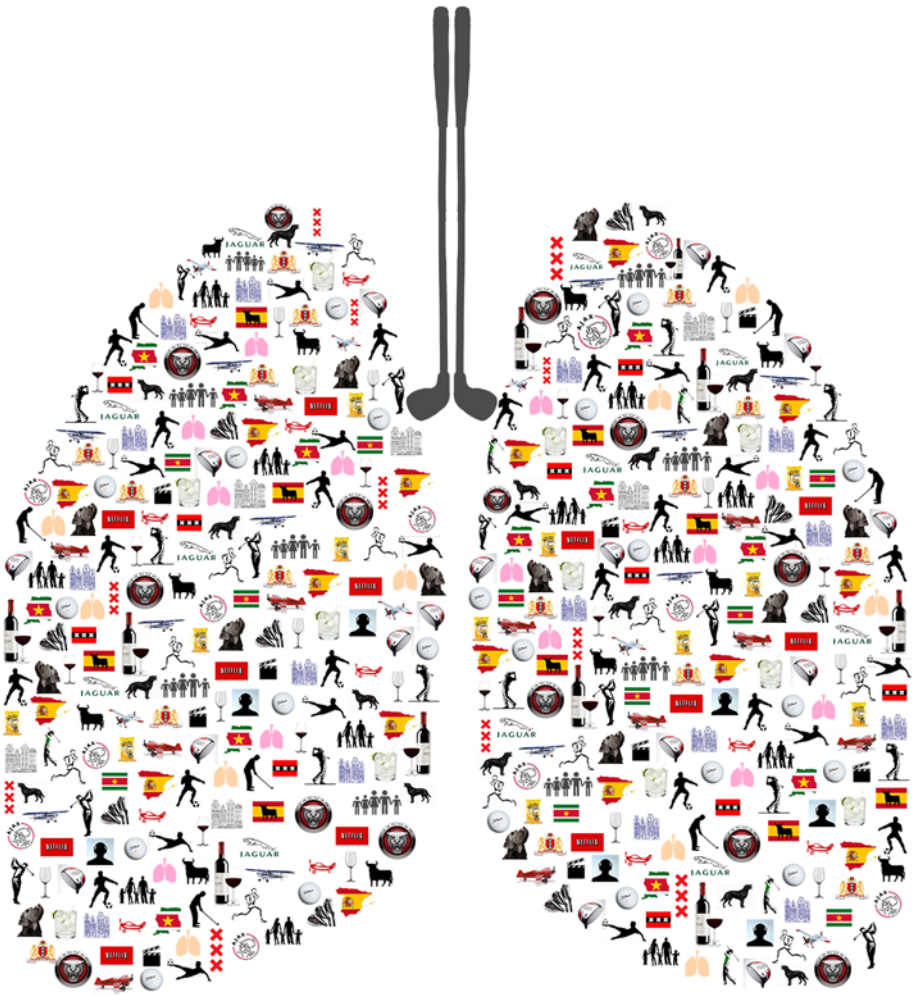

The Effects of Long-term Macrolide Therapy in COPD Patients with Frequent Exacerbations

De effecten van onderhoudsbehandeling met macroliden bij COPD patiënten met frequente exacerbaties

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Voor Rolinde, Yoeri, Daan en Eline

"*Amor tussisque non celantur*"

(Liefde en hoest blijven niet verborgen)

Ovidius

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Chapter 1

General introduction and aim of the thesis: Acute Exacerbations of Chronic Obstructive Pulmonary Disease

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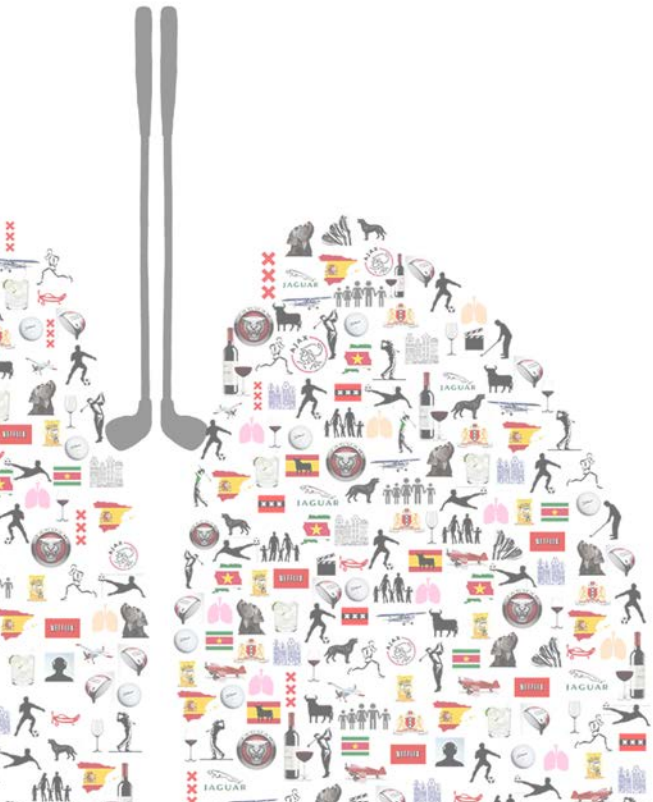

1. Epidemiology

Definition

An exacerbation of COPD is defined as an acute event characterized by a worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations and leads to a change in medication [1].

Exacerbations of COPD have been defined as symptom-based or symptoms plus event based [2]. Anthonisen et al graded exacerbations, based on the three cardinal symptoms during an exacerbation: increased dyspnea, sputum purulence and sputum volume [3]. The Anthonisen classification consists of three types: type 1 (all three cardinal symptoms), type 2 (two cardinal symptoms) and type 3 (one cardinal symptom plus one of the following: an upper respiratory tract infection in the past 5 days, fever without other cause, increased wheezing or cough, or an increase in heart rate or respiratory rate by 20% compared with baseline readings). However, a standardized symptom-based definition is likely to be complicated by the highly varied nature of COPD symptomatology during exacerbations.

Classification of exacerbations based on events offers a simple approach and is therefore widely used in clinical trials. A distinction is made between mild (increased use of rescue bronchodilators), moderate (use of either oral antibiotics and/or corticosteroids) and severe exacerbations (hospitalizations) [4].

A disadvantage of the event-based classification is the underreporting of exacerbations, since only 50% of exacerbations according to Anthonisen criteria are reported to the physician [5, 6].

Prevalence

COPD is a leading cause of morbidity and mortality and the prevalence is expected to increase in the coming decades [7, 8]. The latter is caused by an aging population [9] in combination with continued exposure to COPD risk factors, such as tobacco smoke [8] and air pollution from indoor cooking [10]. There is a large variation in exacerbation-rate between patients [11].

Worsening airflow limitation is associated with an increasing prevalence of exacerbations and risk of death [12-14]. The average exacerbation rate climbs from 0.7-0.9 per year in GOLD 2 (moderate airflow obstruction) to 1.2-2.0 per year in GOLD 4 (very severe) [12-14].

However, the best predictor of frequent exacerbations is a history of previous frequent events (two or more exacerbations per year) [14].

2. Pathophysiology

Increased airway inflammation is probably the primary event of COPD exacerbations and may be caused by bacterial and viral infections, environmental pollutants and cigarette smoke [15]. The inflammation leads to bronchospasm, mucosal edema and sputum impaction, which results in increased airways resistance [15].

The main pathophysiological mechanism during a severe exacerbation of COPD is critical airflow limitation with lung hyperinflation, which leads to serious mechanical consequences [16, 17]. Worsening of VA/Q abnormalities can result in hypoxemia [18].

3. Aetiology

Exacerbations of COPD are triggered by infection with bacteria or viruses, environmental pollutants, or unknown factors [19].

More than 50% of COPD exacerbations are caused by viral and bacterial infections [19-22].

In case of bacterial infections *Haemophilus influenzae* is the most frequently isolated microorganism (20-30%), followed by *Streptococcus pneumoniae* (10-15%), *Moraxella catarrhalis* (10-15%) and *Pseudomonas aeruginosa* (5-10%) [23].

Viral infections also play an important role, both alone and in combination with bacteria and account for approximately 30% of exacerbations [24, 25]. Recent polymerase chain reaction (PCR) or reverse transcription (RT)-PCR based studies suggest an even higher prevalence of viral infections during exacerbations (22-64%) [26, 27]. Rhinovirus, influenza virus and respiratory syncitial virus are most frequently isolated [25].

By inducing impairment of antibacterial host defence, viral infections frequently precipitate bacterial infections in COPD patients [28, 29].

Sethi et al developed the concept of the acquisition of a new bacterial strain as the main cause of a bacterial infection [30]. It was found that the frequency of exacerbations increased more than two-fold when a new strain of a pathogen was isolated from sputum [30].

In a recent study by Bafadhel et al four distinct biologic exacerbation clusters were identified that were clinically indistinguishable [31]. These clusters, bacterial-, viral-, eosinophilic predominant and pauciinflammatory, may identify patients that appropriately require corticosteroids and antibiotics at the onset of an exacerbation. It appeared that the phenotype of the exacerbation remained constant in a given patient [31].

4. Characteristics of COPD exacerbations

Exacerbation frequency

It has been shown that exacerbation frequency increases with disease severity, using a symptom-based definition [32, 33].
In the Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE) study, a large 3-year observational multi-centre international study aimed at defining COPD phenotypes, it was observed that exacerbations became more frequent as the level of airflow obstruction increased [14]. In this longitudinal study exacerbation rates in the first year of follow-up varied from 0.85 per person for patients with GOLD stage 2, 1.34 for patients with stage 3 and 2.00 for patients with stage 4.
However, the single best predictor of exacerbations, across all GOLD stages, was a history of frequent exacerbations. A distinct frequent-exacerbation phenotype could be identified, that appeared to be relatively stable over a period of 3 years. It has been previously established that patients in this subgroup with frequent exacerbations have a poorer quality of life [34], and a faster decline in lung function [35, 36].

Time course of exacerbations

Exacerbations of COPD have a sharp increase of symptoms after a short prodromal stage with respiratory symptoms, followed by a slow recovery of weeks to months [5, 37]. The lasting impact of exacerbations was shown in the study by Spencer et al [38]. In this study duration of worsening health status up to several months after a COPD exacerbation has been demonstrated. Depressive symptoms at admission have a negative impact on recovery and are related to worse survival and increased risk for subsequent exacerbations [39]. The COPD assessment test (CAT) and Clinical COPD Questionnaire (CCQ) are useful tools to evaluate recovery after an exacerbation [40].

Seasonality

Exacerbations occur more frequently during winter months [6]. It has been reported that exacerbations were associated with colder outdoor temperature [41]. The seasonal pattern has a marked impact on exacerbation outcomes, antibiotic treatment and all-cause mortality [42]. We showed that viral infections are an important cause of this seasonality [43].

5. Impact of COPD exacerbations

Mortality

Between 1990 and 2010, chronic obstructive pulmonary disease (COPD) moved from the fourth to third most common cause of death worldwide [44].
It has been shown that acute exacerbations of COPD have been associated with an increased risk of death [45, 46]. Mortality increases with the frequency of severe exacerbations, which require admission to hospital [45]. Mortality following hospitalization for an acute exacerbation of COPD varies between 23% [47] and 80% [48].

Hospital admissions

Acute exacerbations of COPD are a significant cause of hospital admission worldwide [49].
Information about the appropriateness of hospitalization among patients experiencing exacerbations of COPD is scarce [50].
Several guidelines describe the management of patients with COPD and exacerbations [51-53].
Appropriate criteria to identify patients who are more likely to benefit from admission in terms of mortality and COPD progression have been validated [54].

Health care costs

Exacerbations of COPD account for a substantial portion of COPD related costs [55]. Increasing exacerbation frequency is associated with a multiplicative increase in all cause and COPD related costs [56]. Exacerbation prevention and treatment strategies may be a key strategy in COPD disease management in order to reduce costs [56].The optimal duration of hospitalization in individual patients with an exacerbation of COPD has not been established [57].

Quality of life

One of the main objectives in the management of patients with COPD is to preserve or improve their health status [58]. It has been shown that even in patients with mild COPD the health related quality of life (HRQL) can be substantially compromised[59]. Exacerbations lead to a short term deterioration in HRQL [60]. However, long term negative effects have also been established [34], especially in patients

with frequent exacerbations [6]. In order to optimize HRQL it is crucial to develop strategies to prevent COPD exacerbations.

Lung function

Several studies have shown that exacerbations play a role in the decline in FEV1 [35, 36, 61].
Data from the Lung Health Study showed that lower respiratory illnesses promote FEV1 decline in current smokers but not ex-smokers with mild chronic obstructive pulmonary disease [35]. Donaldson et al showed a relationship between the frequency of exacerbations and long term decline in lung function of patients with moderate to severe COPD [36].

6. Management

Bronchodilators

Bronchodilators are useful in mild, moderate and severe exacerbations[62]. Mild exacerbations are defined as an acute worsening of respiratory symptoms, that result in treatment with short acting bronchodilators [63]. It is also recommended to start long-acting bronchodilators during an exacerbation, although there are no clinical studies to support this [62].

Systemic corticosteroids

The use of systemic glucocorticoids in COPD exacerbations improves lung function and leads to a shorter recovery time [62]. Treatment leads also to less treatment failure [64] and a shorter duration of hospitalization [65, 66].

Antibiotics

It is still under debate if antibiotics should be used during exacerbations [62]. It appears that outcome is improved in moderate to severe COPD in patients who have symptoms of an airway infection [67-69]. The clinical efficacy has also been shown in patients requiring mechanical ventilation [70].

Oxygen

It is recommended to start titrated oxygen treatment with a target saturation of 88-92%, in acute exacerbations of COPD in order to reduce mortality, hypercapnia, and respiratory acidosis [71].

Noninvasive ventilation

Non invasive ventilation (NIV) is superior to invasive ventilation as the initial mode of ventilation in COPD patients with acute respiratory failure, with a success rate of 80-85% [72-74]. NIV reduces mortality and intubation rates in these patients [75, 76].

7. Prevention

The prevention of exacerbations is a major goal in the management of COPD patients [62]. In order to reduce exacerbation frequency, pharmacological and non-pharmacological interventions are available [77].

Smoking cessation

Smoking cessation is associated with a significantly reduced risk of COPD exacerbations even after adjusting for age, comorbidity, markers of COPD severity and socio-economic status and the described reduction is dependent upon the duration of abstinence [78].

Vaccinations

Vaccination against influenza has proven to be protective in the prevention of influenza related airway diseases and reduced the total number of exacerbations per patient [79]. The evidence of pneumococcal vaccination is conflicting [80-82]. It has been shown that 23-valent pneumococcal polysaccharide vaccine is effective in preventing community acquired pneumonia in patients with COPD aged less than 65 years and in those with severe airflow obstruction. However, no differences were found among the other groups of patients with COPD [80]. In a Cochrane review it was concluded that injectable polyvalent pneumococcal vaccines may provide some protection against morbidity in persons with COPD, but no significant effect on any of the outcomes could be shown [81].

Lung volume reduction surgery

In patients with severe COPD lung volume reduction surgery reduces the frequency of COPD exacerbations and increases the time to first exacerbation [83]. This effect has not yet been shown by the use of bronchoscopic lung volume reduction techniques [84].

Physical activity/pulmonary rehabilitation

It has been proven that patients with COPD who perform some level of regular physical activity have a lower risk of both COPD admissions and mortality [85]. Also, evidence suggests that pulmonary rehabilitation is a highly effective intervention to reduce hospital admissions and mortality in COPD patients who have recently suffered an exacerbation of COPD [86]. However, one study showed that early rehabilitation during hospital admission for chronic respiratory disease did not reduce the risk of subsequent readmission following the event over 12 months. Moreover, mortality at 12 months was higher in the intervention group [87].

Long-acting bronchodilators

Bronchodilators clearly have a role in the prevention of COPD exacerbations. Long acting anti-muscarinic agents (LAMAs) and long acting β agonists (LABAs) not only improve expiratory airflow, but have also shown to reduce the risk of moderate to severe exacerbations by 22-34% [12, 88-91]. Treatment with LAMA is superior to LABA in reducing exacerbations [92, 93]. There are results that indicate that a LABA/LAMA combination is superior in preventing moderate to severe COPD exacerbations compared with a single long-acting bronchodilator [94].

Inhalation corticosteroids (ICS)

Inhalation corticosteroids (ICS) are effective in reducing the number and severity of COPD exacerbations [95, 96]. In the TOwards a Revolution in COPD Health (TORCH) trial, investigating the combination of salmeterol/fluticasone propionate for 3 years in COPD on all-cause mortality, treatment with fluticasone resulted in an 18% reduction in exacerbation rate compared with placebo [97]. However, a meta-analysis showed only a modest benefit of ICS in preventing COPD exacerbations [98]. Therefore, in guidelines monotherapy with inhaled corticosteroids is not recommended [62].

Combinations of ICS and long-acting bronchodilators

In the TORCH study it was found that an ICS combined with LABA is more effective in reducing COPD exacerbations when compared with placebo, but also when compared with LABA alone [97]**.**
There is conflicting evidence about the benefits of triple therapy (inhaled corticosteroid-LABA plus LAMA) compared to LABA/LAMA on reducing the number of exacerbations [99, 100]**.**
Triple therapy can be considered in patients on LABA/LAMA therapy who develop further exacerbations [62].

Phosphodiesterase-4 inhibitors

It has been shown that the selective phosphodiesterase 4-inhibitor roflumilast reduced COPD exacerbations in a subpopulation of patients with symptoms of chronic bronchitis (chronic cough and sputum production) and a history of exacerbations compared with placebo [101].

It can be concluded that these preventive measures have a limited effect on exacerbation frequency. There is still a need for interventions, which further reduce exacerbation frequency, especially in patients with frequent exacerbations.

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Chapter 2

Macrolides for reducing acute exacerbations of COPD: New evidence. A review

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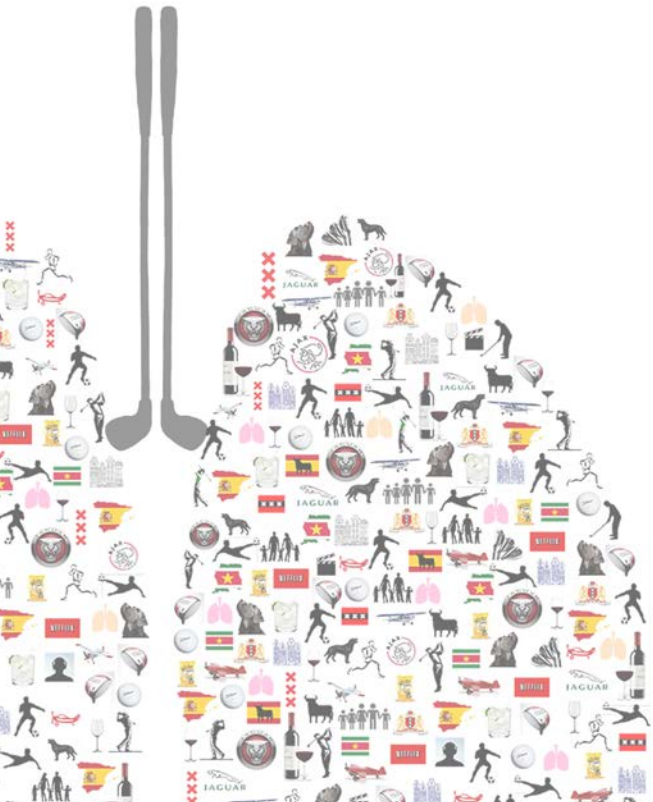


Abstract

In the natural course of chronic obstructive pulmonary disease (COPD), exacerbations are important events influencing the outcome. Pharmacological and nonpharmacological interventions are available to reduce the exacerbation frequency. Despite these strategies to reduce exacerbations of COPD, there is still a subgroup of COPD patients who are experiencing exacerbations of COPD. Macrolide antibiotics may be an alternative therapy in this distinct subgroup of patients. This review describes the current understanding of the value of maintenance therapy with macrolides to reduce COPD exacerbations, according to recent studies. The positioning of macrolides in addition to the usual care, patient selection, and treatment regimen are discussed.

Introduction

In the natural course of COPD, exacerbations are important events influencing outcome. They cause a more rapid decline in lung function [37], worsening of the quality of life [35] and increased mortality [45]. Therefore, prevention of exacerbations is a major goal in the management of COPD patients [53]. Pharmacological and non-pharmacological interventions are available in order to reduce exacerbation frequency [64].

Bronchodilators clearly have a role in the prevention of COPD exacerbations. Long acting antimuscarinic agents (LAMAs) and long acting β agonists (LABAs) not only improve expiratory airflow, but have also shown to reduce the risk of moderate to severe exacerbations by 22-34% [74-78]. In the TORCH study it was found that inhaled corticosteroid-LABA combination inhalers significantly reduced COPD exacerbations when compared with placebo, but also when compared with LABA alone [76]. These findings were confirmed in other studies [91].

The use of triple therapy (inhaled corticosteroid-LABA plus LAMA) may have an additional effect by reducing the number of severe exacerbations [92].

Clinical trials have shown that the selective phosphodiesterase 4-inhibitor roflumilast reduced COPD exacerbations in a subpopulation of patients with symptoms of chronic bronchitis (chronic cough and sputum production) that have had at least one exacerbation within the past year. In this subgroup, it was shown that roflumilast reduced exacerbations by 17% compared with placebo [95].

Prospective studies on the long-term effects of systemic corticosteroids in COPD are limited and show no reduction in exacerbation frequency [53].

Non-pharmacological strategies include influenza vaccination, self-management programs, pulmonary rehabilitation and lung volume reduction surgery (LVRS). Vaccination against influenza has proven to be protective in the prevention of influenza related airway diseases and reduced the

total number of exacerbations per patient [66]. In some patients with severe COPD LVRS reduces the frequency of COPD exacerbations and increases the time to first exacerbation [70].
Despite above strategies to reduce exacerbations of COPD, there is still a subgroup of COPD patients who are experiencing exacerbations of COPD. Therefore, there is a need to generate alternatives to prevent exacerbations in this distinct subgroup of patients. Macrolide antibiotics may be an alternative therapy in this type of patients. This review describes the current understanding of the value of maintenance therapy with macrolides to reduce COPD exacerbations, according to recent studies.

Trials with macrolides in COPD

The effect of macrolides in the treatment of COPD has been evaluated in several studies. We described the results of these studies in an earlier overview [96]. The most recent prospective randomized trials are summarized in Table 1. These recent studies have been set up as double blind randomized controlled trials, in contrast to the majority of the older studies [97-99].

New evidence

Seemungal et al performed a randomized double-blind placebo-controlled trial in patients with COPD to determine whether regular therapy with macrolides reduces exacerbation frequency [97]. Patients with moderate to severe COPD (FEV 1 between 30% and 70% of predicted) were included. Exclusion criteria were a history of asthma, bronchiectasis, neoplasia, or other significant respiratory disease. A total of 109 outpatients were randomized to receive 250 mg erythromycin or placebo twice daily over 12 months. The main result was a significant difference in median exacerbation frequency in one year: 2 in the placebo group versus 1 in the erythromycin group (p=0.006). The time to the first exacerbation was significantly lower in the macrolide arm compared with the placebo arm (271 vs. 89 days). Furthermore, patients in the macrolide arm had shorter duration of exacerbations compared with placebo.
Albert et al performed the largest prospective study concerning long-term macrolide therapy in COPD [98].
They performed a randomized placebo-controlled double-blind trial in COPD patients who had an increased risk of exacerbations to evaluate whether azithromycin decreased the frequency of exacerbations. Inclusion criteria were treatment with systemic glucocorticoids for an AECOPD in the previous year or the use of continuous supplemental oxygen. Asthma was one of the exclusion criteria. The primary outcome was the time to the first AECOPD. A total of 1142 patients were

randomly assigned to receive azithromycin at a dose of 250 mg daily or placebo for 12 months in addition to usual care. The median time to first exacerbation was 266 days (95% CI, 227-313) among participants receiving azithromycin, as compared with 174 days (95% CI, 143-215) among participants receiving placebo (p<0,001). The scores on the St. George's Respiratory questionnaire improved more in the azithromycin group than in the placebo group (p=0,004), although the mean change did not exceed the minimal clinically important difference for the group. No differences were seen between groups in the score on the Medical Outcomes Study 36-Item Short-Form Health Survey. This study showed that azithromycin decreased exacerbation rate in selected patients with COPD.

Uzun et al have published the most recent prospective study concerning long-term macrolide therapy [99].

In contrary to the two previous studies we included COPD patients with the frequent exacerbator phenotype. The aim of our study was to investigate whether COPD patients who had received steroids or antibiotic 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.

The study was a randomised, double-blind, placebo-controlled, single-centre trial. The primary endpoint was the rate of exacerbations of COPD in the year of treatment. Main exclusion criteria were a history of other clinically significant respiratory diseases (eg, asthma, cystic fibrosis) and presence of bronchiectasis. To exclude patients with bronchiectasis a CT scan was performed in all included patients. After randomization patients received either azithromycin 500 mg or placebo, three times a week (Monday, Wednesday, and Friday) for 12 months. Analysis was by intention to treat. 92 patients were randomly assigned to the azithromycin group (n=47) or the placebo group (n=45).

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). 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). 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).

We concluded that maintenance treatment with azithromycin significantly decreased exacerbation rate and increased time to first exacerbation 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.

Table 1 Overview of Recent Trials

	Seemungal et al [97]	**Albert et al [98]**	**Uzun et al [99]**
Design of study	RCT	RCT	RCT
Double blind	Yes	Yes	Yes
No. Patients (treatment-control)	109 (53-56)	1142 (570-572)	92 (47-45)
No. Males (%)	69 (60)	651 (57)	40 (43)
Mean age, treatment-control	67-68	65-66	65-65
Inclusion criteria of COPD severity	FEV_1 between 30% and 70% of predicted	FEV_1 < 80% of predicted FEV_1/FVC ratio <70%	FEV_1/FVC ratio <70%
Mean FEV_1 in % of predicted, treatment-control	49-51	39-40	44-45
Macrolide dose	Erythromycin 250 mg twice daily	Azithromycin 250 mg daily	Azithromycin 500 mg three times a week
Duration of therapy (mo)	12	12	12

RCT = randomized controlled trial

Working mechanism of macrolides

Besides antimicrobial effects, macrolides have anti-inflammatory and anti-viral effects [100-106]
Since COPD exacerbations are mainly caused by bacterial and viral infections, leading to airway inflammation, macrolides can be useful in the treatment and prevention of exacerbations [107].

Antibacterial effects

The main antimicrobial effect of macrolides is inhibition of bacterial protein synthesis, by binding to the 50S subunit of the bacterial ribosome [108]. Macrolides mainly affect gram-positive bacteria and intracellular pathogens such as Mycoplasma, Chlamydia and Legionella [108]. Azithromycin is also effective against gram-negative organisms, such as *Haemophilus influenzae* [108].
A decrease in airway bacterial colonisation in patients receiving maintenance treatment with azithromycin may lead to a reduction in systemic inflammation [109].
Besides a direct antimicrobial effect, macrolides also modulate the virulence and the inflammation caused by the bacteria that induce COPD exacerbations. Studies have shown that macrolides influence the virulence of microorganisms, like *Proteus mirabilis* [110], *S. pneumonia* [111] and *H. influenzae* [112]. Clarithromycin reduces the production of pneumolysin, a key virulence factor in the infection of *S. pneumonia* [111]. Furthermore, the production of pro-inflammatory cytokines, soluble intercellular adhesion molecule (ICAM)-1 and mucin in airway epithelial cells in response to endotoxin and extract of *H. influenzae* is reduced by macrolides [101,113]. In addition, macrolides may reduce the production of exoenzymes [114].
Many studies have been performed investigating the mechanisms of action of macrolides in *P. aeruginosa* infections. Azithromycin maintains the integrity of airway epithelial cells during *P. aeruginosa* infection [115]. Macrolides alter the biofilm around bacteria [112,116]. In *P. aeruginosa* this may facilitate phagocytosis by polymorphonuclear neutrophils [117]. Furthermore, It has been suggested that macrolides block quorum sensing [118] in *P. aeruginosa*, which relates to virulence factor production [119]. In *P. aeruginosa*, macrolides reduce flagellin synthesis and expression [120,121], thereby inhibiting the twitching motility [116].

Antiviral effects

During COPD exacerbations, viruses (most commonly rhinovirus, influenza virus and respiratory syncytial virus) cause injury to epithelial cells with subsequent pulmonary edema, alveolar

destruction and airflow limitation [107]. Macrolides exert their antiviral function through the inhibitory action against virus-induced inflammatory responses in the lung. Most of these effects have been studied in murine models and in in vitro studies.

It was also demonstrated that clarithromycin suppressed the growth of the influenza virus and its release in mouse airways and epithelial cells [104]. Furthermore, Yamaya et al. showed that clarithromycin decreased the release of viruses and cytokines into supernatant fluids in human tracheal epithelial cells that were infected with type A seasonal influenza (H3N2) [106]. This effect was achieved by reducing the expression of the viral receptor and by inhibiting viral RNA entry.

Gielen reported that interferons were induced in human bronchial epithelial cells when the cells were pretreated with azithromycin and infected with rhinovirus [122]. In this study azithromycin reduced rhinovirus replication and release. This effect may relate to the shorter duration of exacerbations in COPD patients who are treated with macrolides. Another study showed that azithromycin could reduce rhinovirus replication in CF bronchial epithelial cells compared to cells from control children [123]. Asada et al. also reported that macrolides have inhibitory effects on RSV virus infection in human airway epithelial cells [105].

To date, the only study investigating the antiviral effect of macrolides in humans was performed by Suzuki et al [124]. In this study the efficacy of erythromycin therapy for the prevention of the common cold and subsequent exacerbations in COPD was evaluated in a prospective, randomized controlled, but not blinded, trial. The relative risk of developing two or more common colds in the control group compared with that in the erythromycin group was 9.26 (95% confidence interval, 3.92 to 31.74; p = 0.0001). The relative risk of experiencing a COPD exacerbation in the control group compared with that in the erythromycin group was 4.71 (95% CI, 1.53 to 14.5; p = 0.007).

Immune modulatory effects

The immune modulatory effects of macrolides have been extensively investigated. Since COPD exacerbations seem to be related to an underlying pro-inflammatory state with an over-activated immune system macrolides may play a role in the treatment of COPD exacerbations [125].

Takizawa et al. [126] and Desaki et al. [127] demonstrated that macrolides had effects on bronchial epithelial cells. The results indicated that erythromycin had inhibitory effects not only on the mRNA expression and release of IL-8, but also on the activation of transcription factors nuclear factor-κB and activator protein-1.

Furthermore, the function of dendritic cells in T -cell regulation can be modulated by macrolides [128].

Airway infiltration by neutrophils in response to activation of alveolar macrophages in bacterial and viral infections is an important feature of lung inflammatory reactions and is involved in most lung

pathologies [129]. Erythromycin was able to inhibit neutrophil chemotaxis by decreasing the expression of adhesion molecules and chemoattractants [101]. Azithromycin was able to reduce the concentrations of G-CSF within the airways [130]. This activity may result in a decrease in epithelial cells-dependent neutrophil survival within the airways.

In vitro studies have demonstrated that macrolide antibiotics such as erythromycin [131], clarithromycin [131] and azithromycin [113] have inhibitory effects on mucin or MUC5AC production or secretion in airway epithelial cells, thereby inhibiting mucus hypersecretion. Shimizu et al. [131] reported that clarithromycin inhibited ovalbumin (OVA)- and lipopolysaccharide (LPS)-induced mucus production in rats.

Macrolide resistance

Mechanisms of bacterial resistance to macrolides

There are two common mechanisms of macrolide resistance in streptococci (Figure 1). The first mechanism is the M phenotype. In this mechanism activation of the efflux pump removes macrolide antibiotics from the cell. The M phenotype is mediated by the MEF (A) gene. The M phenotype usually results in moderate macrolide resistance [132]. The second mechanism is the MLS_B (macrolide, lincosamide, and streptogramin) resistance phenotype. This is the result of erm (erythromycin ribosome methylase) gene mutations. These mutations, lead to ribosomal target modification for these antibiotics, preventing binding and activity. Erm B gene mutation is the most prevalent pneumococcal macrolide resistance genotype [133]. Erm (B) gene mutation is also commonly found in bacteria other than pneumococcus [132].

Macrolide resistance in response to macrolide antibiotic exposure

The development of macrolide resistance related to treatment with macrolide antibiotics is a major concern [134].

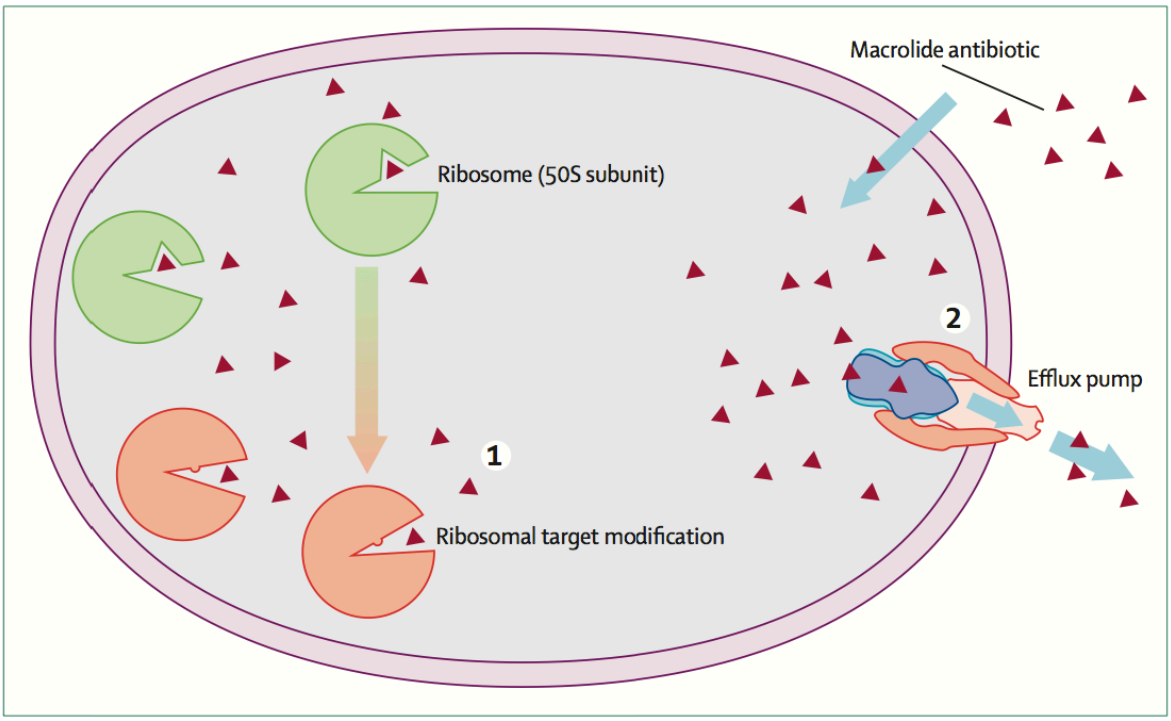
It has been shown that macrolide resistance develops in response to macrolide antibiotic exposure [134]. Since the introduction of long-acting macrolides, such as azithromycin, macrolide resistance rates are increasing in the United States and Europe [135,136]. It has been demonstrated that on a population-level, an increase in macrolide exposure results in higher macrolide resistance rates [135]. However, exposure to macrolides can lead to macrolide resistance on an individual level as well. This effect has been observed after short-term [137] and long-term use of macrolide antibiotics [138].

Several studies have shown that azithromycin use is also related to increased resistance to other antibiotic classes, especially to penicillin [136].
However, it is difficult to judge the independent effect of macrolides from these studies.
In the study by Uzun et al the acquisition of macrolide resistant bacteria in sputum has been identified [99]. The number of positive sputum cultures was low. In line with Albert and colleagues' findings [98], patients in the azithromycin group were less likely to become colonized with respiratory pathogens than were those in the placebo group. Furthermore, azithromycin significantly reduced acquisition of macrolide-resistant bacteria in sputum compared with placebo. In this study, the increase in acquisition of macrolide-resistant bacteria in the placebo group could not be explained by additional use of macrolides during follow-up for any indication. A limitation of this study was the fact that macrolide resistance has not been assessed in oral commensal flora. Therefore, the results might underestimate macrolide resistance in vivo.

New techniques to detect microbial resistance

The human body serves as a host for a wide range of microorganisms, which has been termed the microbiota [139]. The micobiome is the aggregate collection of genes within the microbiota. The portion which encodes resistance to antibiotics is called the resistome [140]. There is evidence that the use of antibiotics has an impact upon the composition of the resistome [141]. Recently new PCR-based techniques have been developed that provide insight into antibiotic resistance [142]. With these so-called metagenomic approaches, the presence of antimicrobial resistance (AMR) genes can be detected. These non-culture based techniques are used to avoid potential bias associated with difficult to culture bacteria.
Consequently, the composition of the resistome and the changes in response to antibiotic therapy can be monitored more accurately [143]. At present these data are lacking in patients on macrolide treatment.

Figure 1: The two most common mechanisms of macrolide resistance in pneumococcus



From: Serisier DJ. Risks of population antimicrobial resistance associated with chronic macrolide use for inflammatory airway diseases. The Lancet Respiratory medicine. 2013; 1(3):262-74

Relevant aspects of treatment with macrolides

The use of azithromycin for the prevention of COPD exacerbations has not been endorsed by current expert guidelines.

Although there is emerging evidence about the efficacy of maintenance therapy with macrolides in the prevention of AECOPD [97-99], uncertainty remains about several aspects of this treatment. Positioning of macrolides in addition to usual care, patient selection and treatment regimen, must be considered as part of the risk-benefit ratio of this treatment. The different aspects and recommendations are summarized in table 2.

Positioning of macrolides in addition to usual care

As already mentioned, the use of inhaled corticosteroids (ICS) [76], LAMAs [74], LABAs [77] and selective phosphodiesterase 4-inhibitors [95], may reduce the exacerbation frequency in selected sub-groups of patients with COPD with 12-34%. Non-pharmacological strategies, including influenza vaccination,

pulmonary rehabilitation and lung volume reduction surgery (LVRS) also have proven to be effective in reducing the number of exacerbations per patient [66,70].

It can be hypothesized that with macrolide maintenance therapy an additional reduction in exacerbation frequency can be achieved. In the study of Seemungal et al there was no statistically significant difference between the macrolide and placebo arms in ICS use or dosage [97]. A significant reduction in exacerbation rate was found in the macrolide arm compared with the placebo arm. It was suggested that the effect of the macrolide (erythromycin) occurred on top of any effect of ICS on reducing exacerbation frequency.

In the study by Albert et al there were also no significant between-group differences with regard to the use of ICS, LAMAs and LABAs [98]. Subgroup analyses showed that the response to azithromycin seemed to vary according to age (≤65 vs. >65 years), smoking status (former smoker vs. current smoker), use or nonuse of oxygen, GOLD stage, and use of ICS. The use of ICS at enrollment was associated with a decreased effect of azithromycin on the time to first AECOPD.

Uzun et al examined a COPD population that was refractory to usual care [99]. A large part of the population received treatment with ICS (92%), LABAs (93%) or LAMAs (80%). Hence, it can be assumed that macrolide treatment reduced the exacerbation frequency in this highly selected group of patients on top of the effects of inhalation medication. Furthermore, in this study it was demonstrated that there was no statistically significant difference 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)

Patient selection

COPD is a common disease with a reported prevalence of approximately 10% [144]. Widespread use of macrolide treatment has the potential to greatly affect population macrolide resistance rates [134].

The large size of this patient population makes it necessary to choose the right criteria on the basis of which patients will be treated with azithromycin maintenance therapy.

Treatment of contributing factors

It is suggested that only COPD patients in whom other contributing factors, such as smoking, have been optimally managed should be considered for macrolide treatment [134].

Exacerbation frequency

The greatest benefit of treatment with macrolides can be achieved in COPD patients with frequent exacerbations in the past year. This also provides a baseline against which a clinical response can be assessed. Therefore it has been recommended by Wenzel to consider long-term azithromycin

treatment only in selected COPD patients with at least two exacerbations in the previous year [145]. Several studies have shown that an exacerbation frequency greater than three per year is associated with a greater rate of FEV1 decline [37]. These findings were the reason to choose 3 or more exacerbations in the previous year as inclusion criterion in the study by Uzun et al [99]. In this study a higher relative reduction in exacerbation rate (42%) was recorded compared to the trials of Seemungal and colleagues (35%) [97] and Albert and colleagues (27%) [98]. In the study by Albert et al 12% of patients did not have any exacerbation in the year before inclusion [98]. Moreover, this was also seen in 32% of patients in the control group during the study, compared with 7% in the control group of the study by Uzun et al [99]. Therefore, the use of a criterion of three or more exacerbations exposes fewer patients to redundant macrolide treatment and results in a substantial clinical benefit.

Side effects/drug interactions

Ototoxicity, cardiac toxicity, and drug interactions are the three major categories of adverse effects that may be anticipated with long-term treatment with azithromycin [145]. Albert and colleagues found a 5% differential in hearing loss between the azithromycin group (25%) and the placebo group (20%). In the study by Uzun et al patients were actively asked about hearing loss, but no standard audiometry was performed [99]. At the end of the study, one patient in the placebo group reported hearing loss. Seemungal and colleagues did not perform routine screening of hearing as this was not thought to be a significant side effect at this dose of macrolide [97].

Macrolide antibiotics are known to cause ventricular arrhythmias [145]. In recent studies with long-term macrolides, patients with an unstable cardiac status (e.g., cardiac failure, prolonged QTc interval, cardiac arrhythmia or tachycardia) were excluded [97-99]. In a recent review it was concluded that this risk was overestimated and chronic macrolides can be used safely in the majority of COPD patients [146].

Macrolides inhibit the cytochrome P-450 CYP3A4 isoenzyme and cause increased serum levels of other drugs metabolized by this enzyme [145]. Patients taking any drug that are metabolized by this enzyme are recommended to avoid long-term macrolides [147].

Treatment regimen

Dose

In two recent studies COPD patients received long-term treatment with macrolides on a daily basis [97,98].

In the study by Seemungal and colleagues long-term erythromycin 250 mg was administered twice daily to COPD patients [97]. Albert et al also treated COPD patients with daily macrolides (azithromycin

250 mg) [98].

However, with a three times weekly regimen, tissue levels of azithromycin are sufficient for a bactericidal effect [148]. Furthermore, studies involving patients with cystic fibrosis and bronchiectasis showed that the side effects of azithromycin were fewer when a reduced regimen (three times per week instead of daily) was used [149]. This regimen (azithromycin 500 mg three times a week) was used in COPD patients in the recent study by Uzun et al and resulted in a significant reduction of the exacerbation rate compared with placebo [99].

Which macrolide

Azithromycin is the most studied macrolide in long-term treatment in patients with cystic fibrosis, bronchiectasis and COPD and has fewer adverse effects compared with other macrolides [145]. In two out of three recent studies with COPD-patients azithromycin was the drug of choice [98,99].

Duration of long-term treatment

In the three recent clinical trials of Seemungal et al, Albert et al and Uzun et al, patients received treatment with macrolides during a one-year period [97-99]. It is unclear whether maintenance therapy with macrolides should be continued after this first year in case of a relevant reduction of the number of exacerbations. Further studies with different treatment regimens and longer treatment duration should provide an answer to this question.

Table 2 Treatment guidelines for long-term macrolide treatment in COPD patients	
Subject	**Recommendation**
Positioning in addition to usual care	
Pharmacological treatment	ICS, LABAs, LAMAs
Non-pharmacological treatment	Influenza vaccination
	Pulmonary rehabilitation program
Patient selection	
Treatment of contributing factors	Smoking cessation
Exacerbation frequency	≥ 3 exacerbations in the previous year
Side effects/drug interactions	
Ototoxicity	No hearing impairment
Cardiac toxicity	Normal QTc interval
	No use of drugs known to cause QT prolongation
Cytochrome P-450 CYP3A4 isoenzyme inhibition	No use of drugs that are metabolized by this enzyme
Treatment regimen	
Dose	Azithromycin 500 mg three times a week
Duration of treatment	1 year

Conclusions

On the basis of recent studies, macrolides can be used as maintenance therapy in COPD patients with 3 or more exacerbations in the previous year. Treatment should only be considered in patients in whom other contributing factors have been optimally managed and maximum therapy (pharmacological and non-pharmacological) has been used.
Side effects and interactions with other medication must be taken into account.
Azithromycin should be the drug of choice with a dose of 500 mg 3 times a week and duration of treatment of 1 year, pending the results of future studies.

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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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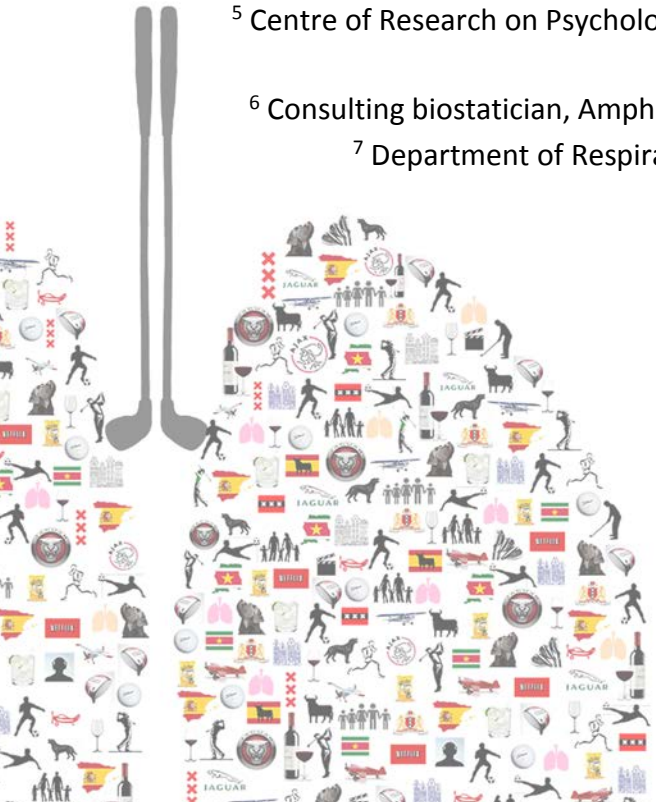

Trials 2012; 13: 82

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 (FEV1/FVC < 70%), classification into GOLD I (FEV1 8-100% predicted), GOLD II (FEV1 50-80% predicted), GOLD III (FEV1 30-50% predicted) or GOLD IV (FEV1 ≤ 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 echocardiography 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.

	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

Table 1: Overview of outpatient department visits and tests

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 $\alpha = 0.88/0.86$), 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 outcome**s***

- Measurement of lung function parameters and 6-min walking distance.
- Assessment of presence of type D personality by DS14 questionnaire.
- Disease-specific health-related quality of life measured by St. George's Respiratory Questionnaire (SGRQ).
- Generic health status measured by the 12-Item Short Form Health Survey (SF-12).
- Indication of anxiety and depression by Hospital Anxiety Depression Scale (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), pro-adrenomedullin, (pro-ADM), 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 FEV1% 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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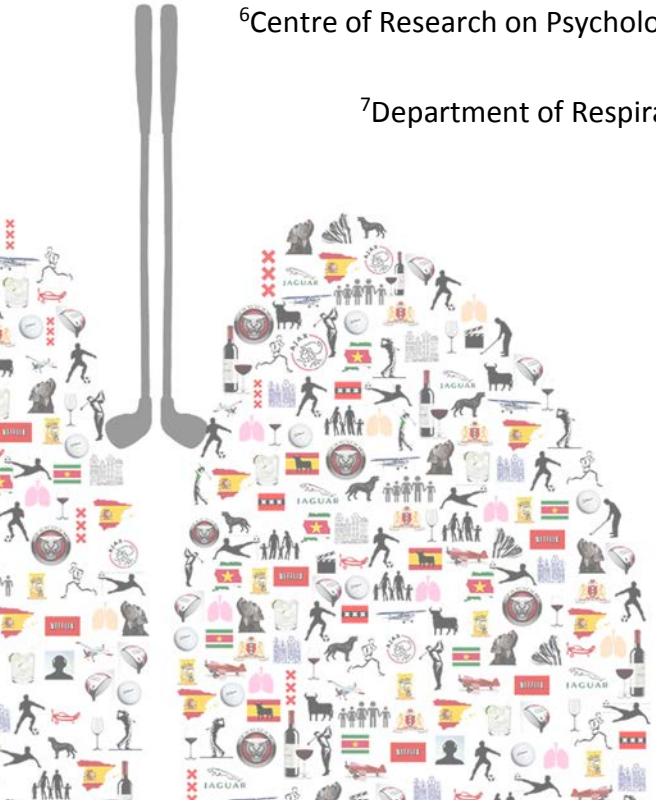

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Summary

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 vs 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

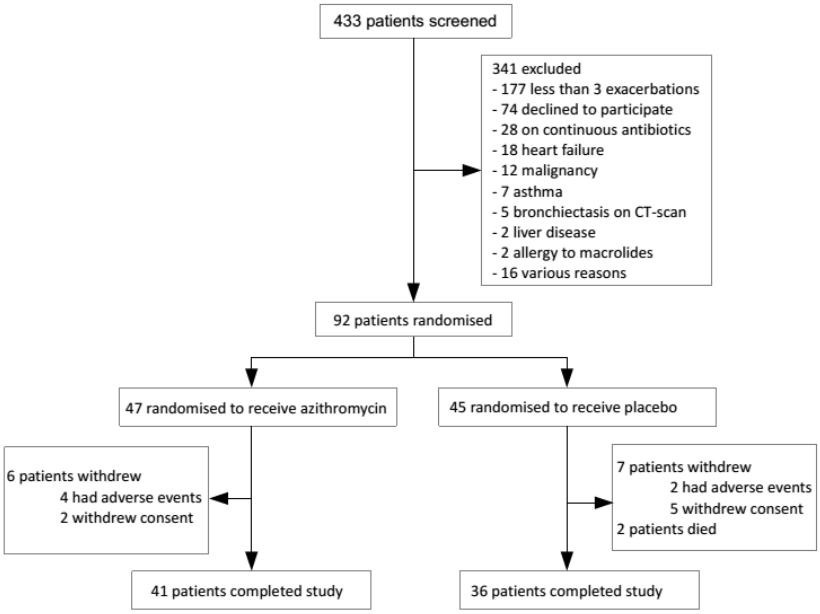

Figure 1: Trial profile

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.

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.

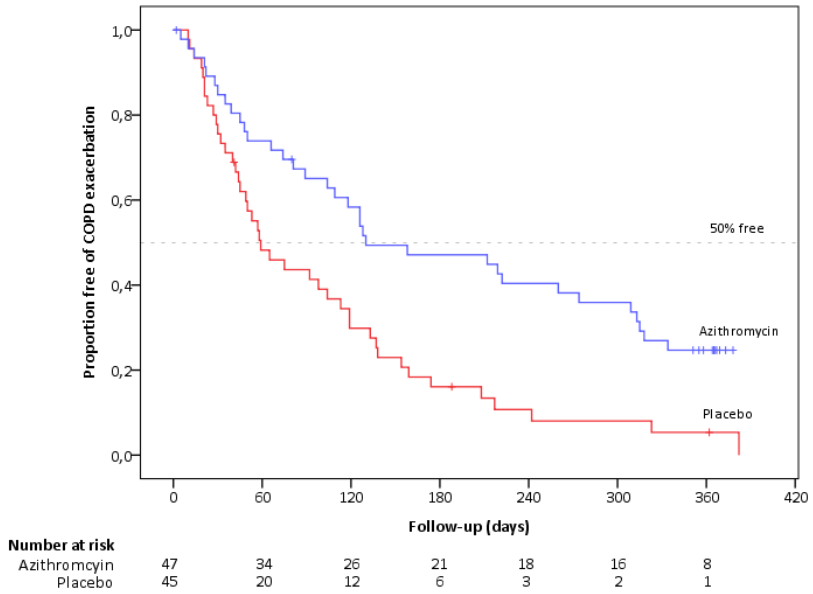

Figure 2: Proportion of patients free from acute exacerbations of COPD according to study group

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

	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/m^2)	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)

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

	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

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

(7%) participants 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. 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).

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 vs 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).

	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)

Table 3: Overview of exacerbations and given treatments during the study
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 vs 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 vs n=2), *Streptococcus pneumoniae* (n=2 vs n=3), and *Pseudomonas aeruginosa* (n=2 vs n=0). During follow-up, fewer patients in the azithromycin group had positive sputum cultures with new respiratory pathogens compared with those in the placebo group (n=4 vs 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 (table4). 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

	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 4: Secondary outcome variables at 12 months

(figure 1). More patients had diarrhoea in the azithromycin group than in the placebo group (p=0.015; table 6).

	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

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

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 (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 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%)

Table 6: Adverse events

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-glutamyltransferase. LDH=lactate dehydrogenase.

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 immunomodulatory 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 [150]. It has been reported that AECOPD are predominantly caused by both bacterial and viral respiratory infections [21,22,151]. Recent polymerase chain reaction (PCR) or reverse transcription (RT)-PCR based studies consistently showed a high prevalence of viral infections during exacerbations (22-64%) [21,26-28,152-154]. Exacerbations caused by respiratory viruses were associated with more severe exacerbations, reflected by increased length of stay and decrease in lung function [25]. 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 [155].

Epidemiological data reported a greater frequency of exacerbations in the winter months [43,156-158]. 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 [156]. In this and in other studies, it was suggested that this increase in exacerbations could be caused by increased exposure to viral infections [159-161]. 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 [99,162].

Inclusion criteria were age ≥ 18 years, a COPD diagnosis according to the guidelines of the Global initiative for chronic Obstructive Lung Disease, [53] 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 [3].We obtained data for the number of exacerbations, the date of onset of exacerbation and the number of preceding influenza vaccinations.

Collection of viral isolates

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 I.

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 [163]. 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.

Table I. Viruses, primers and primer sequences.

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
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

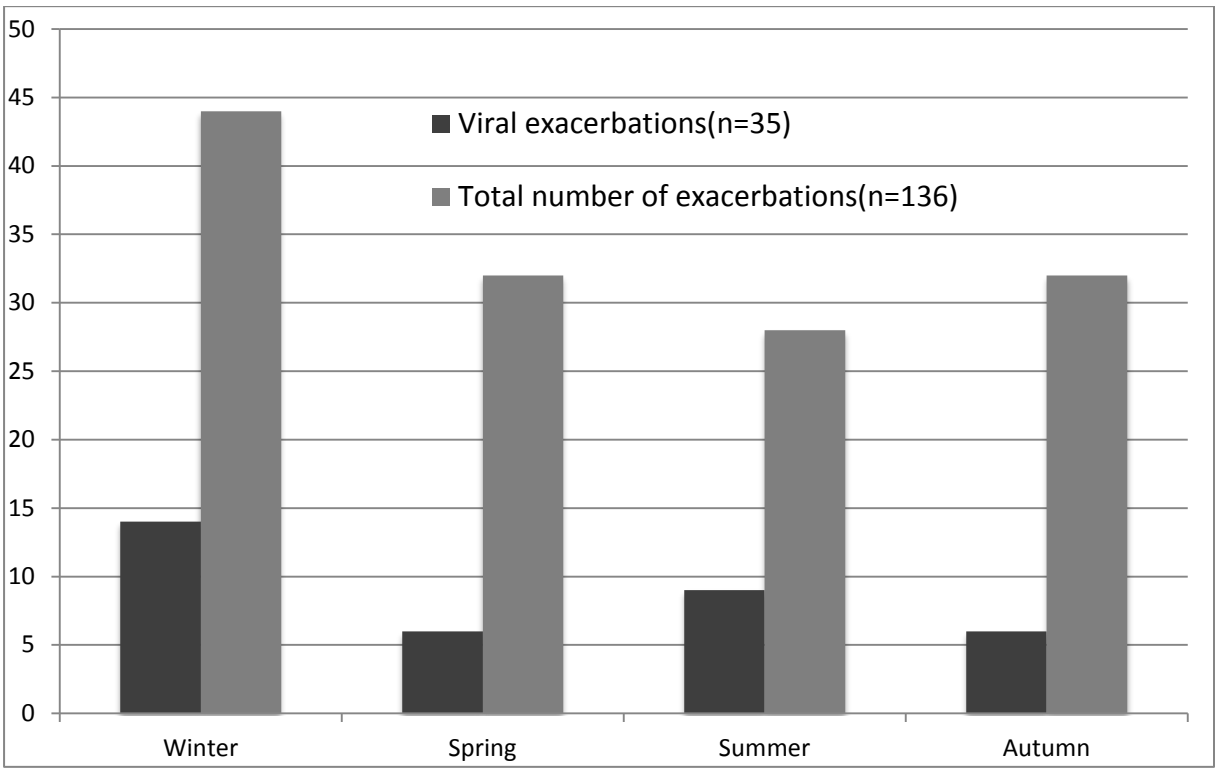
Results

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 I.

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 I. 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 [156].

During all seasons viral infections were responsible for 25,7% of the AECOPD, which is in accordance with other studies [26-28,152,154].

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 [25,26]. 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 [164,165].

It is known that hMPV and RSV infections have a temporal distribution, with a variable activity from year to year[166,167]. The samples in this study were collected during 3 consecutive years, including 3 winter periods. During the first winter period of this study no hMPV infections (n=5) were found. RSV infections (n=6) were equally distributed over these 3 winter periods.

Influenza infection can cause exacerbations in COPD [66]. Since vaccination against influenza is proven to be protective in the prevention of influenza related airway diseases, many COPD patients are now receiving an influenza vaccination on yearly basis [168]. 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 [26].

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 [169]. Third, human coronavirus has been acknowledged as an important cause of COPD exacerbations [170]. However, because we only determined coronavirus in the first 35 COPD exacerbations of the study, in which 2 patients (5.7%) turned out to be positive, we decided not implement these results in this

study.

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

Blood eosinophil count and GOLD stage predict response to maintenance azithromycin treatment in COPD patients with frequent exacerbations

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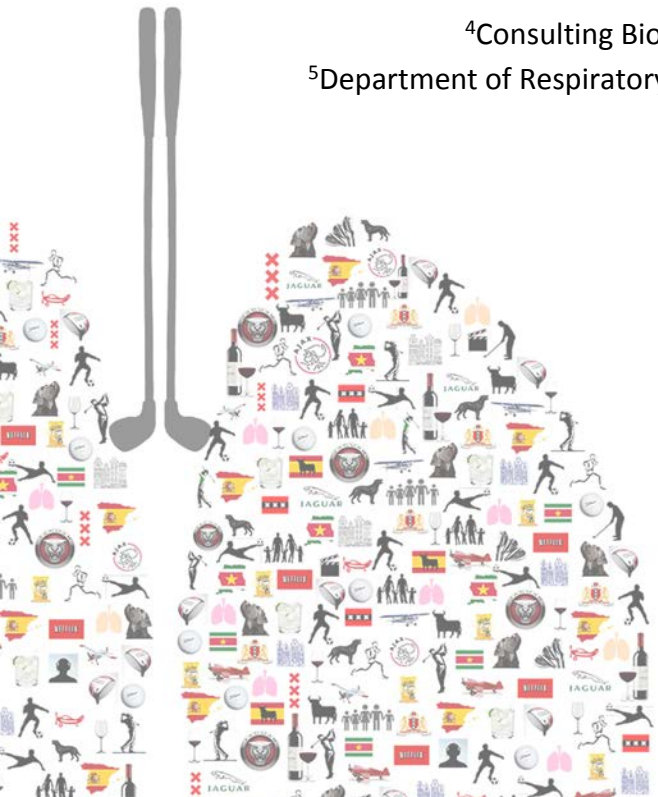


Submitted

Abstract

Macrolides are useful in preventing COPD exacerbations. We investigated which characteristics of COPD patients with frequent exacerbations predicted the best response to maintenance treatment with azithromycin.

This study was part of the COLUMBUS trial, a prospective randomized, double-blind, placebo-controlled study in 92 COPD patients with frequent exacerbations. During the 1-year treatment period, follow-up data were collected for spirometry, mMRC scores, sputum cultures and blood inflammatory markers.

In the azithromycin group a significant lower number of exacerbations per patient was observed in patients with the following characteristics: baseline blood eosinophil count ≥ 2.0% ($\bar{x}$=1.26), compared to an eosinophil count < 2.0% ($\bar{x}$=2.50; p=0.02), GOLD stage 1-2 ($\bar{x}$=1.06), versus GOLD stage 4 ($\bar{x}$=2.62; p=0.02) and GOLD group C ($\bar{x}$=0.45) compared to group D ($\bar{x}$=2.18; p<0.01). Moreover, the number of hospitalizations was significantly lower in patients, with a blood eosinophil count ≥ 2.0% ($\bar{x}$=0.26) compared to an eosinophil count < 2.0% ($\bar{x}$=0.90; p=0.01) and in GOLD stages 1-2 ($\bar{x}$=1.06) compared to stage 4 ($\bar{x}$=2.62; p=0.04).

In conclusion, azithromycin maintenance treatment is most effective in COPD patients with frequent exacerbations, who are either classified in GOLD stage 1-2 or GOLD C and those with a blood eosinophil count of ≥ 2.0%.

Introduction

Chronic obstructive pulmonary disease (COPD) is an important cause of morbidity and mortality [1]. Exacerbations of COPD are associated with worsening health status and lung function and therefore impose a large burden on health care systems, which result in high cost [2,3].

In patients experiencing COPD exacerbations macrolides have demonstrated to be successful in preventing exacerbations [4-6]. However, long-term administration of macrolides can be accompanied by side-effects, such as hearing impairment [5], resting tachycardia or QTc time prolongation [7].

Moreover, of greater concern is the development of bacterial resistance patterns as a result of chronic treatment with macrolide antibiotics [8]. Goossens et al showed that antibiotic resistance was correlated with outpatient antibiotic use in Europe [9]. This observation was further confirmed by Malhotra-Kumar et al, who showed that a higher consumption of

macrolide antibiotics resulted in higher rates of macrolide resistance [10]. In order to limit these harmful effects, selection of patients who are most likely to benefit from long-term treatment with macrolides would be an advantage. Han et al showed that older patients and those with a milder Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) stage had a better treatment response to azithromycin [11]. Others recommended to start macrolides only in patients with frequent exacerbations [8,12]. Previously we showed that a strategy of limiting macrolide treatment to patients with three or more exacerbations a year resulted in a reduction of exacerbations [6].

However, whether the population with frequent exacerbations can be stratified further to identify a patient population with a better response to macrolide treatment remains unclear.

We therefore investigated in this study which characteristics of COPD patients with the frequent exacerbator phenotype showed the best treatment response to maintenance therapy with azithromycin.

Methods

This study was part of the COLUMBUS trial (Clinicaltrials.gov, NCT00985244), a prospective study in which 92 COPD patients who were frequent exacerbators, were followed during 12 months at the Amphia Hospital (Breda, the Netherlands). The study protocol and the primary results have been previously published [13,14]. Inclusion criteria were age ≥ 18 years, a COPD diagnosis according to the guidelines of the Global initiative for chronic Obstructive Lung Disease [15] and ≥ 3 exacerbations of COPD in the previous year that were treated with steroids and/or antibiotics. Patients were required to be clinically stable for one month prior to enrolment. Exclusion criteria were a history of other significant respiratory diseases (e.g. asthma, cystic fibrosis), the presence of bronchiectasis as assessed by computed tomography, heart failure, and an active malignancy. An exacerbation was defined according to the Anthonisen criteria, requiring treatment with steroids and/or antibiotics [16]. An exacerbation was considered severe when hospital admission was necessary, and moderate when it was treated at the outpatient department by the study investigators or the patient's general practitioner. All participants provided written informed consent. Independent and local ethics committees approved the study.

Procedures

Participants were followed up at the outpatient department with scheduled visits at 3, 6, 9, and 12 months. During these visits, we obtained data for spirometry, mMRC-scores, white-blood-cell count, concentrations of C-reactive protein and mid-regional pro-adrenomedullin. Additionally, sputum samples were obtained for culture at baseline and at every scheduled visit. Sputum samples were processed according to American Society of Microbiology guidelines [17]. 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. Sputum was considered positive at baseline if a pathogenic microorganism had been cultured. We established antibiotic susceptibility with breakpoints from the European Committee on Antimicrobial Susceptibility Testing [18]. In case of an exacerbation, patients were seen and treated by the study investigators unless the patient chose to visit their family doctor.

We used the same cut-off values in order to dichotomize, for CRP [19] (3 mg/L) and blood eosinophils [20,21] (2.0% of total white blood cell count) that were used as predictors of COPD outcome in previous studies [22,23].

For blood neutrophils and serum proADM we used as cut-off value the median values at baseline (63.3% of total white blood cell count and 0.69 nmol/L, respectively), a method that has been used in previous studies [24,25].

Outcomes

The total number of COPD exacerbations and the number of hospitalizations due to COPD exacerbations in the year of treatment.

Statistical analysis

Statistical analyses were performed using Statistical Analysis System (SAS) version 9.4 (SAS Institute, Cary, NC, USA).

Subgroups were created for sex, age (<65, ≥ 65), smoking status, long acting bronchodilators (LABA), long acting muscarinic antagonists (LAMA), inhalationcorticosteroids (ICS) use during the study, prednisolone use during the study (yes, no), sputum positive at baseline, C-reactive protein (CRP) at baseline (<3 mg/L, ≥3 mg/L), blood eosinophils at baseline (<2%, ≥2%), blood neutrophils at baseline (<63.3%, ≥63.3%), serum proADM (<0.69 nmol/L, ≥ 0.69

nmol/L), GOLD stage (1-2, 3 and 4), GOLD group (C and D) and home oxygen use during the study.

For all subgroups explored, descriptive statistics were calculated for the number of exacerbations and number of exacerbations leading to hospitalization. Within each treatment arm, subgroups based on phenotypes were compared using Wilcoxon's rank sum test.

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

The baseline characteristics of the 92 patients are described in table 1.

Two subjects in the azithromycin group and one subject in the placebo arm were missing baseline data for COPD group (C or D) classification. There was no significant difference in sex, age, smoking status, use of concomitant medication, exacerbation frequency, lung function, GOLD stage and GOLD group classification across treatment groups at baseline. In 22 (46.8%) patients in the azithromycin group a sputum culture on day 1 could be obtained, versus 20 (44.4%) patients in the placebo arm.

Total number of exacerbations

The total number of exacerbations in the year of treatment was 213, of which 84 (39%) exacerbations occurred in the azithromycin group versus 129 (61%) in the patients receiving placebo ($p<0.01$) (table 2). Patients treated with azithromycin experienced a significant lower number of exacerbations per patient when the eosinophil count at baseline was ≥ 2.0% ($\bar{x}=1.26$), compared to those with a blood eosinophil count < 2.0% ($\bar{x}=2.50$; odds ratio 0.24 [CI] 0.06-1.02; $p=0.02$). The eosinophil count did not differentiate in exacerbation numbers in the placebo group (figure 1a). However, a blood eosinophil count of ≥ 2.0% predicted a significant better response after treatment with azithromycin compared to placebo ($p<0.01$), while a blood eosinophil count of < 2.0% showed no significant difference in response between both groups ($p=0.97$) (figure 1b).

Another significant difference in number of exacerbations in the azithromycin group was observed between patients classified in GOLD stage 1-2 ($\bar{x}$=1.06 ± 1.29) and patients in GOLD stage 4 ($\bar{x}$=2.62 ± 2.22; p=0.02) (figure 2). In the placebo group patients with GOLD stage 1-2 had a significant lower number of COPD exacerbations ($\bar{x}$=2.27 ± 1.28) compared to those in GOLD stage 3 ($\bar{x}$=3.55 ± 1.99; p=0.03) (figure 2).

Furthermore, it appeared that patients in GOLD group C had fewer exacerbations ($\bar{x}$=0.45 ± 0.69) compared to patients in GOLD group D ($\bar{x}$=2.18 ± 1.85) when treated with azithromycin. This difference appeared to be highly significant (odds ratio 8.17 [CI] 1.80-37.05; p<0.01). This difference in outcome was not observed in the placebo group (figure 3).

In the placebo group the only other significant difference in the number of exacerbations could be observed between subjects using LAMA ($\bar{x}$=3.16) versus those not using LAMA ($\bar{x}$=2.15; odds ratio 5.64 [CI] 0.46-68.46; p<0.05) (table 2).

Number of exacerbations requiring hospitalization

We conducted subgroup analyses for the number of exacerbations with hospitalizations. The total number of hospitalizations during the year of treatment was 56, of which 25 (45%) admissions occurred in patients receiving azithromycin and 31 (55%) hospitalizations in the placebo group. Patients that were treated with azithromycin, who had a blood eosinophil count ≥ 2.0% had a significant lower number of hospitalizations ($\bar{x}$=0.26 ± 0.66) than those with a blood eosinophil count of < 2.0% ($\bar{x}$=0.90 ± 1.07; p=0.01).

In the placebo group an eosinophil count of ≥ 2.0% ($\bar{x}$=0.79 ± 1.10) showed no difference in number of hospitalizations compared with a blood eosinophil count < 2.0% ($\bar{x}$=0.57 ± 0.87; p=0.47) (figure 4).

In the azithromycin group, there was a significant difference in hospitalizations between stages 1-2 and 4 (p=0.04), but not between stages 1-2 and 3 (p=0.80) or between stage 3 and 4 (p=0.06) (figure 5). In the placebo group pairwise comparison showed a significant difference in number of hospitalizations between GOLD stages 1-2 ($\bar{x}$=0.00) and 3 ($\bar{x}$=0.90 ± 1.07; p<0.01) and also between stages 1-2 and 4 ($\bar{x}$=1.30 ± 1.06; p<0.001), but not between stage 3 and 4 (p=0.24) (figure 5).

For both treatment groups there was no significant difference in number of hospitalizations between patients classified in GOLD group C compared to patients stratified in group D (p=0.07) (data not shown).

Inflammatory markers

The median value of pro-ADM was 0.69 nmol/L. A higher pro-ADM value was not predictive for the number of exacerbations during one-year follow-up. Also there was no difference seen in number of exacerbations when patients were treated with azithromycin or were receiving placebo. The same results were seen for the median value of CRP (3 mg/l) and median neutrophil count (63.3% of total white blood cell count).

Discussion

In this study we analysed which characteristics of COPD patients with frequent exacerbations might be predictive of a positive outcome, with regards to exacerbation, from maintenance treatment with azithromycin. We showed that an eosinophil count of ≥2.0% and classification in a lower GOLD stage (1 and 2) and in GOLD C [compared to D]) predicted the best treatment response to azithromycin. Additionally, we demonstrated that in patients in GOLD stage 1 and 2, azithromycin had a positive effect on the number of hospital admissions. In the placebo group there were less exacerbations in the patients with GOLD stage 1 or 2 when compared with those in GOLD stage 3 or 4. Eosinophil count did not have any effect in the placebo group. We did not observe a difference in response to azithromycin by sex, age, smoking, positive sputum culture, CRP, proADM, neutrophilia, other concomitant COPD therapy or use of home oxygen.

Long-term use of macrolides has shown benefits in a broad range of respiratory diseases with airway inflammation [26-30], including COPD [4-6]. However, long-acting macrolides, especially azithromycin, are particularly associated with increased rates of population bacterial macrolide resistance [31,32]. The development of macrolide resistance after maintenance treatment has also been demonstrated on individual level [29,33]. Recent studies in COPD patients that were treated with long-term azithromycin addressed this issue as well [4-6]. In order to prevent the widespread use of macrolides in the large population of COPD patients, the selection of patients who are likely to benefit the most from long-term macrolide treatment is desirable.

The results from the current study suggest that the level of blood eosinophils can also be used as a prognostic marker as they predict the response to azithromycin therapy.

This is a finding that has not been observed before. The role of eosinophilic inflammation has been described in stable COPD patients [34]. In this study higher blood eosinophil counts were associated with an increased risk of exacerbations. Furthermore, in recent studies it

was found that COPD patients with higher levels of blood eosinophils had greater reduction in exacerbation rate during treatment with inhaled corticosteroids [21,35] or with a combination of ICS/LABA [36]. However, a similar positive effect during treatment with macrolides had not been established in earlier studies. In contrast, macrolides have shown to be effective in chronic respiratory disorders, in which neutrophilic inflammation played a central role [28,37,38].

Han et al demonstrated that treatment benefit of azithromycin is greatest in milder GOLD stages [11]. This finding was confirmed in the present study. However, this was also been seen in the placebo arm. In contrast with the previous study, we didn't observe a greater efficacy of azithromycin in older patients or non-smokers [11]. It has to be stressed though, that both analyses were performed on different COPD (phenotypically distinct) populations.

Another finding in our study was the efficacy of azithromycin observed in patients with GOLD group C compared to group D. It appeared that the effect of azithromycin was most pronounced in patients with a lower level of respiratory symptoms. The distinction between the categories C and D is made on the basis of symptoms, by using the modified British Medical Research Council dyspnea scale (mMRC), with a mMRC grade ≥ 2 indicating a high level of symptoms and thus inclusion in GOLD D [39]. All patients in our study were categorised in GOLD group C and D, since only having three or more exacerbations was an a priori inclusion criteria.

The main limitation of this study was that, compared to the study performed by Han et al [11], data were analysed from a relatively small number of patients. Therefore, we recommend that this analysis be repeated in larger trials. Furthermore, the current study had not specifically been powered for subgroup analyses.

The great importance of this study is that these results contribute to a better selection of COPD patients that will benefit the most from long-term treatment with macrolides. We previously already showed that selection of patients with three or more exacerbations reduced the exacerbation rate with less patients being exposed to macrolide treatment unnecessarily [6].

In conclusion, in this study we showed that patients with a blood eosinophil count of ≥ 2.0% have a better response to azithromycin maintenance therapy resulting in less exacerbations and less hospital admissions. Also patients classified in GOLD C and in GOLD stage 1 and 2 showed a reduction in number of exacerbations after treatment with long-term azithromycin.

Table 1 Baseline characteristics of 92 patients determined on day 0 of study treatment.

	Azithromycin group (n=47)	Placebo group (n=45)
Male	22 (46·8%)	18 (40%)
Age (years)	64·7 (10·2)	64·9 (10·2)
Current smoker	20 (43%)	9 (20%)
Medication		
- **LABA**	45 (96%)	41 (91%)
- **LAMA**	42 (89%)	32 (71%)
- **Inhaled corticosteroids**	42 (89%)	43 (96%)
- **SABA**	32 (68%)	33 (73%)
- **Prednisolone**	11 (23%)	9 (20%)
Number of AECOPD in past year (mean, SD)	4·0 (1·2)	4·0 (1·1)
Number of hospitalisations due to AECOPD (mean, SD)	1·0 (1·1)	0·7 (0·8)
Spirometry after bronchodilation (mean, SD)		
- **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%)
GOLD groups		
- **A**	0	0
- **B**	0	0
- **C**	11 (23.4%)	16 (35.6%)
- **D**	34 (72.3%)	28 (62.2%)
- **Unknown**	2 (4.3%)	1 (2.2%)

Definition of abbreviations: LABA=long-acting β-agonists; LAMA=long-acting muscarinic agents; SABA=short-acting β-agonists; AECOPD=acute exacerbation of chronic obstructive pulmonary disease; FEV1=forced expiratory volume in 1 second; FVC=forced (expiratory volume) vital capacity; GOLD=global initiative for chronic obstructive pulmonary disease. Data are ± standard deviation unless otherwise indicated.

Table 2 Number of exacerbations in the year of treatment

	Azithromycin (n exacerbations=84)				**Placebo** (n exacerbations=129)			
	n	**Mean number of exacerbations**	**p**	**Odds ratio (CI)**	**n**	**Mean number of exacerbations**	**P**	**Odds ratio (CI)**
Male	22	1.50	0.55	1.04 (0.29-3.74)	18	3.39	0.11	
Female	25	2.04			27	2.52		
Age <65 (yrs)	24	1.71	0.59	1.17 (0.32-4.20)	20	2.80	0.80	2.67 (0.22-31.75)
Age >=65 (yrs)	23	1.87			25	2.92		
Current smoker	20	1.95	0.93	0.82 (0.23-2.96)	9	3.33	0.55	
Ex-smoker	27	1.67			36	2.75		
No LABA	2	0.50	0.26	2.75 (0.16-47.52)	4	3.00	0.90	6.50 (0.45-94.08)
LABA	45	1.84			41	2.85		
No LAMA	5	2.20	0.40		13	2.15	<0.05	5.64 (0.46-68.46)
LAMA	42	1.74			32	3.16		
No ICS	**5**	0.60	0.08	4.80 (0.70-32.90)	2	1.50	0.31	20.50 (0.91-461.50)
ICS	42	1.93			43	2.93		
No Prednisolone	37	1.70	0.23	4.32 (0.49-38.13)	36	2.89	0.78	0.47 (0.04-5.85)
Prednisolone	10	2.10			9	2.78		
Sputum positive	7	1.43	0.16	0.38 (0.04-3.52)	6	3.50	0.17	0.38 (0.02-7.40)
Sputum negative	15	2.07			14	2.50		
CRP < 3 (mg/L)	26	1.62	0.27	2.25 (0.58-8.73)	19	2.47	0.27	2.94 (0.25-35.06)
CRP ≥ 3 (mg/L)	21	2.00			26	3.15		
Eosinophils<2,0%	22	2.50	0.02	0.24 (0.06-1.02)	21	2.52	0.23	0.41 (0.03-4.91)
Eosinophils ≥ 2,0%	25	1.26			24	3.17		
Neutrophils<63,3%	30	1.73	0.53	2.33 (0.54-10.05)	16	3.00	0.79	4.00 (0.33-47.99)
Neutrophils ≥ 63,3%	17	1.88			29	2.79		
ProADM<0,69 (nmol/L)	28	2.14	0.11	0.72 (0.20-2.63)	21	3.00	0.65	2.30 (0.19-27.59)
ProADM ≥ 0,69 (nmol/L)	19	1.26			23	2.83		
GOLD 1-2	16	1.06	0.19 (vs 3)		15	2.27	0.03 (vs 3)	
GOLD 3	18	1.83	0.21 (vs 4)		20	3.55	0.09 (vs 4)	
GOLD 4	13	2.62	0.02 (vs 1-2)		10	2.40	0.85 (vs 1-2)	
GOLD C	11	0.45	<0.01	8.17 (1.80-37.05)	16	2.63	0.38	
GOLD D	34	2.18			28	3.11		
No Home Oxygen	38	1.68	0.12		38	2.63	0.12	0.33 (0.03-4.27)
Home Oxygen	9	2.22			7	4.14		

Definition of abbreviations: LABA=long-acting β-agonists; LAMA=long-acting muscarinic agents; SABA=short-acting β-agonists; ICS=inhalation corticosteroids; CRP=C reacting protein; ProADM=midregional pro adrenomedullin; nmol=nanomol/Liter; GOLD=global initiative for chronic obstructive pulmonary disease. Data are ± standard deviation unless otherwise indicated

Figure 1a: Average number of exacerbations during treatment period per treatment group and levels of eosinophils in blood.

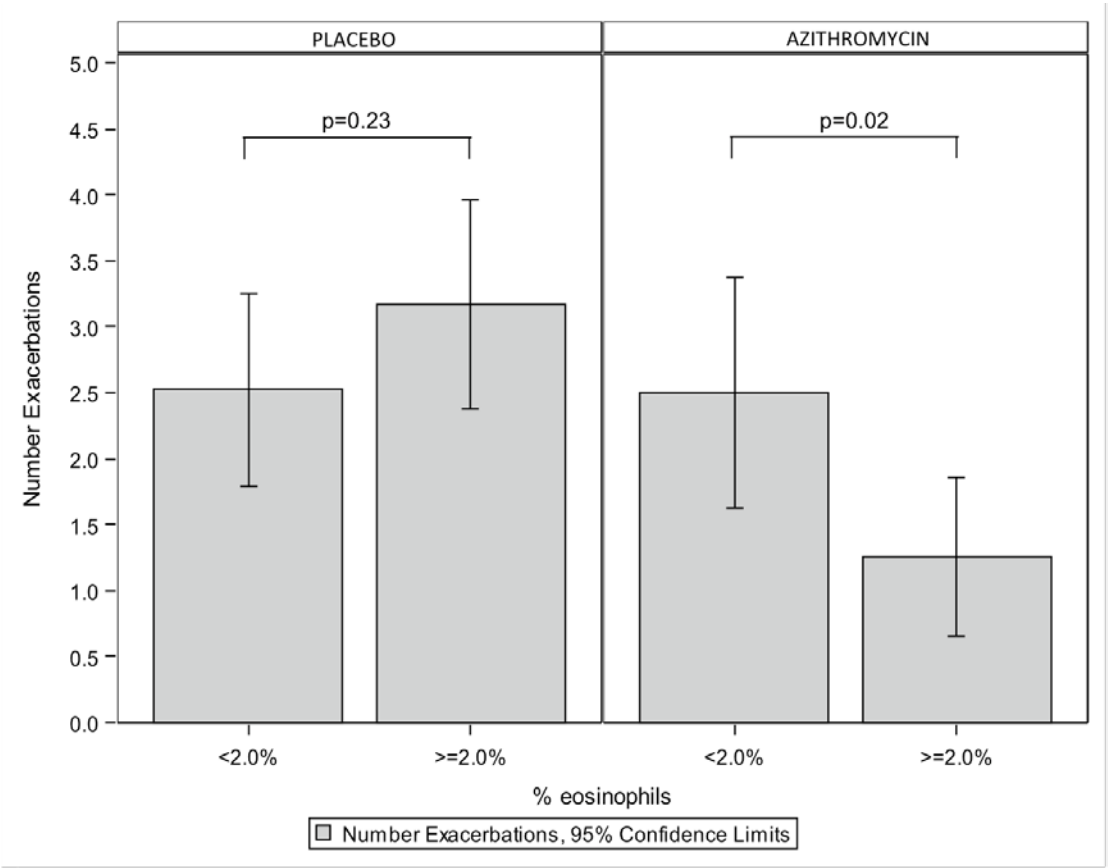


Figure 1b: Average number of exacerbations during treatment period per level of eosinophils in blood and treatment group.

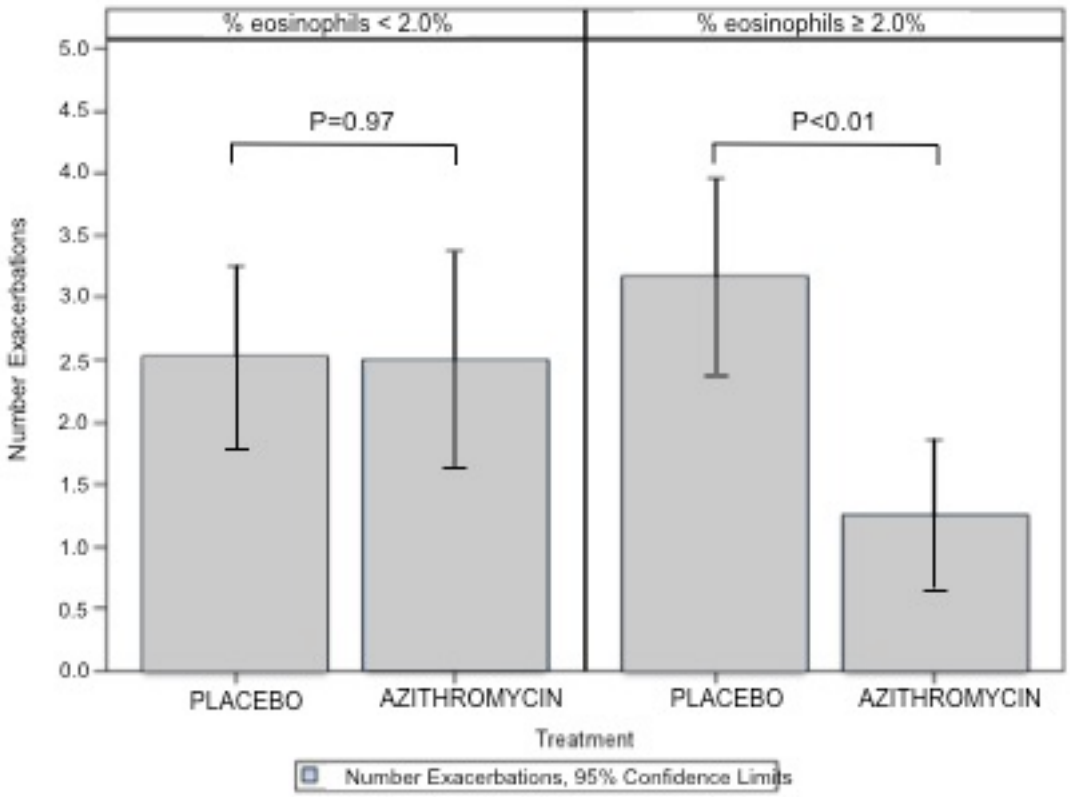


Figure 2: Average number of exacerbations during treatment period per treatment group and GOLD stage.

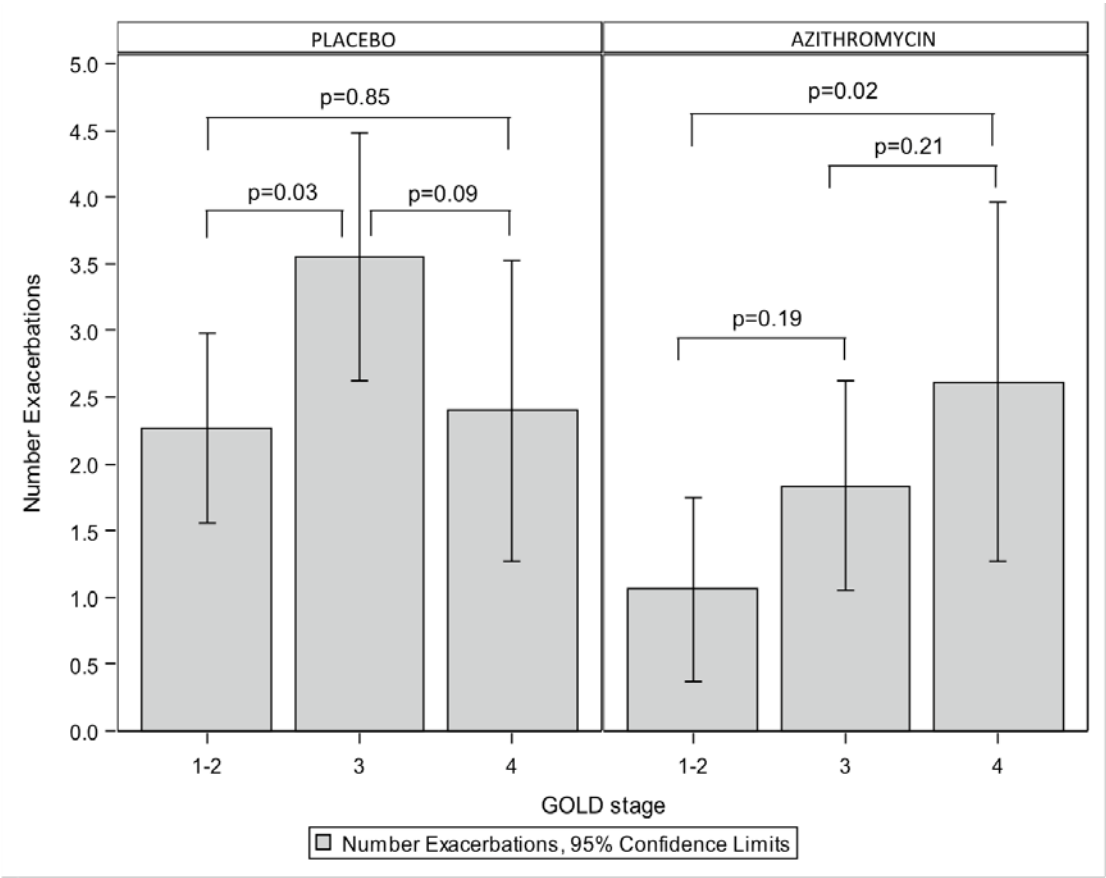


Figure 3: Average number of exacerbations during treatment period per treatment group and ABCD GOLD group.

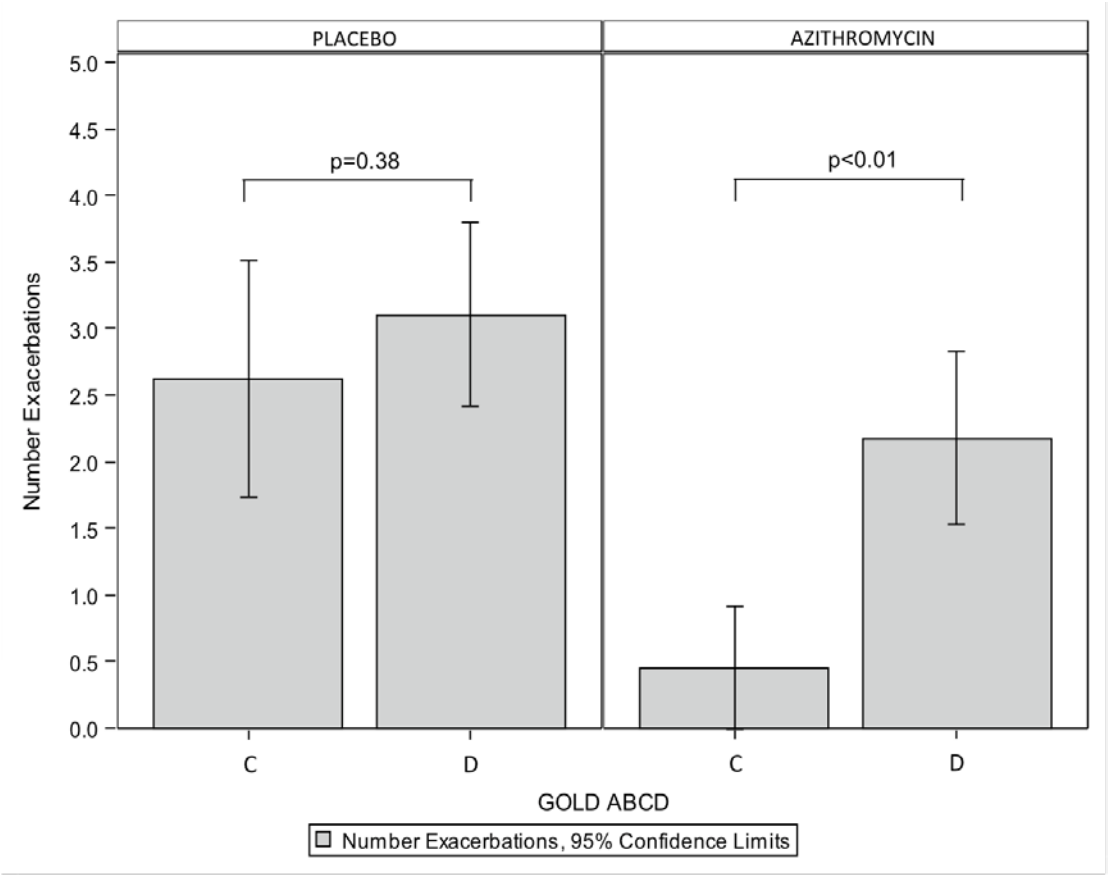


Figure 4: Average number of hospitalizations due to exacerbations during treatment period per treatment group and levels of eosinophils in blood

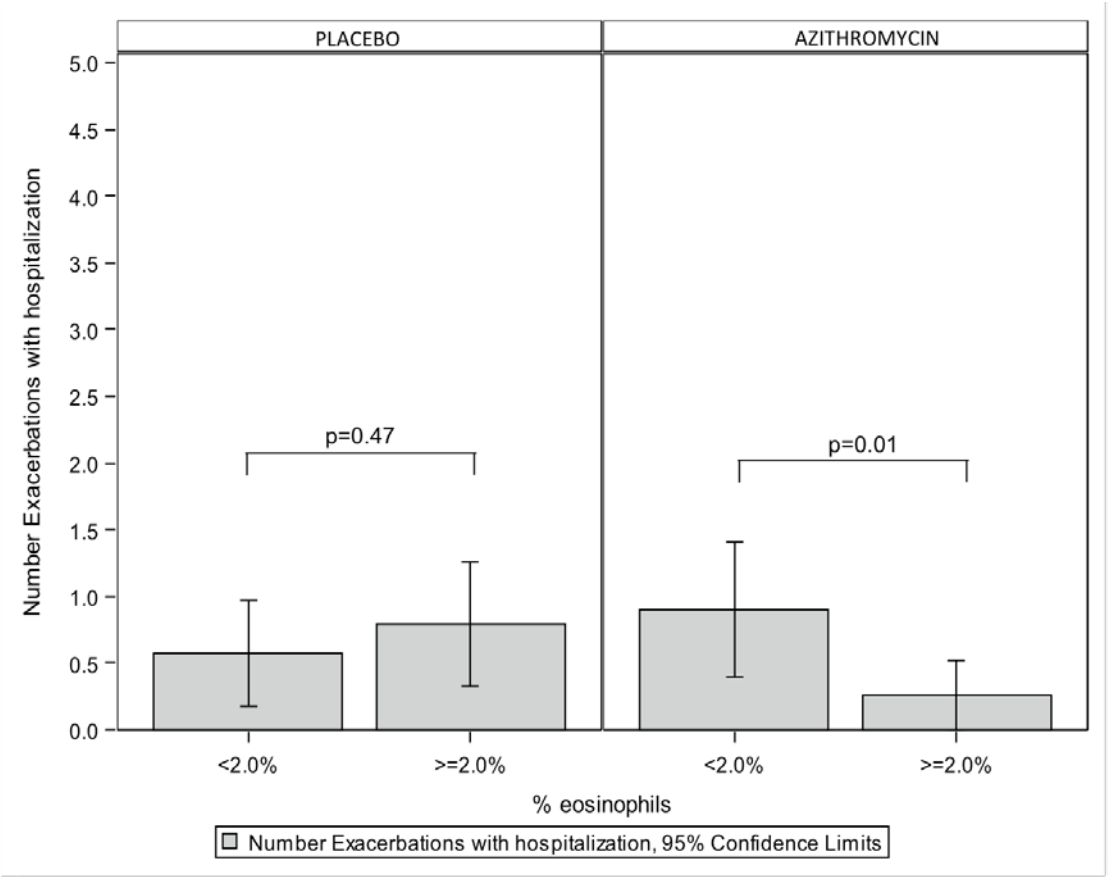
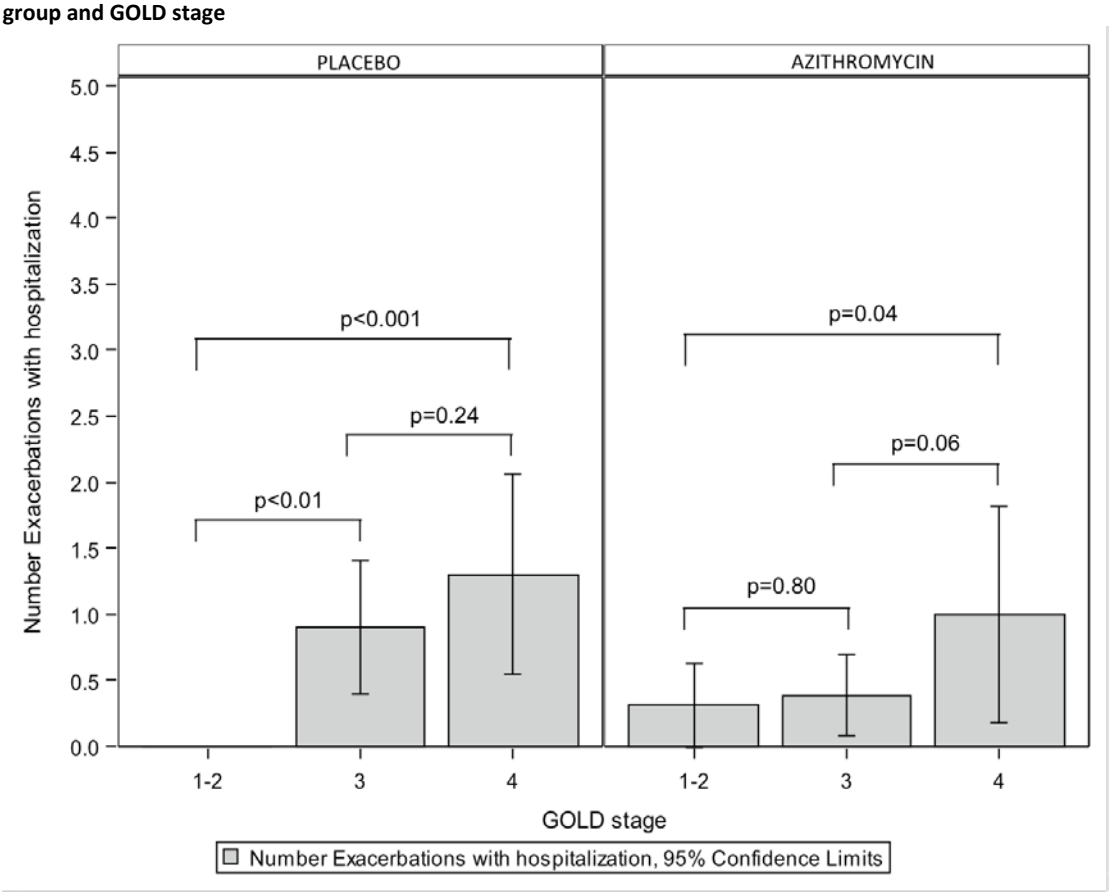


Figure 5: Average number of hospitalizations due to exacerbations during treatment period per treatment group and GOLD stage



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Chapter 7

Molecular mechanisms modulating macrolide resistance in COPD patients during maintenance treatment with azithromycin

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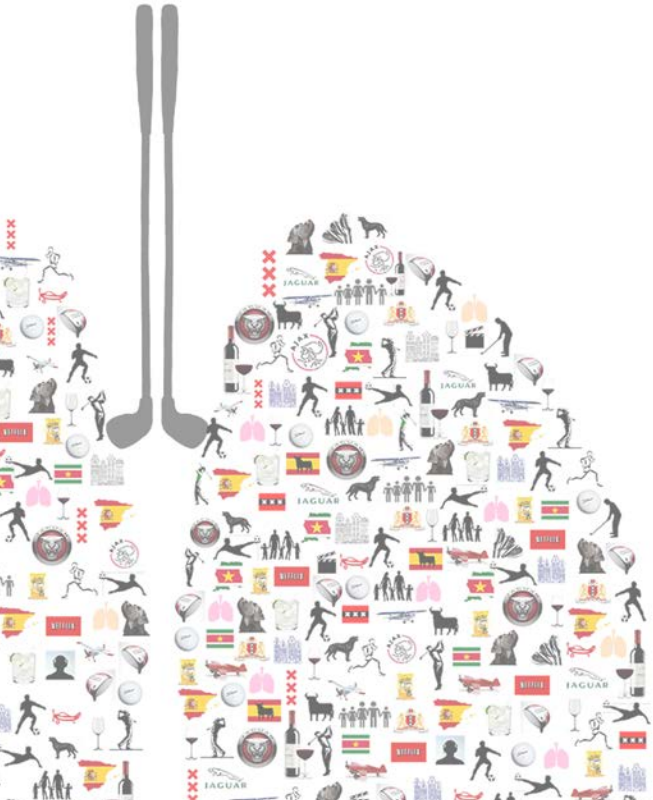


Submitted

Introduction

Chronic obstructive pulmonary disease (COPD) is an important cause of morbidity and mortality [1]. Exacerbations in COPD patients impose a large burden on health care costs and are important events in disease progression [2,3].

COPD exacerbations are mainly caused by bacterial and viral infections, leading to airway inflammation [4,5]. Macrolides have antimicrobial, anti-inflammatory and anti-viral effects, which make them potentially useful in reducing COPD exacerbations [6]. Hence, maintenance treatment with macrolide antibiotics has shown to be effective in reducing exacerbations in COPD patients [7-9].

A major concern with prolonged treatment with antibiotics is the development of bacterial resistance [10,11]. The use of macrolides has been associated with the development of macrolide resistance in oral commensal streptococcal microbiota [12]. However, the effect of maintenance treatment with macrolides on resistance in patients with COPD has given controversial results [7-9,13].

Macrolide resistance can be caused by several mechanisms. Target modification is mediated by one or more rRNA *erm* methylases, which change a site in 23S rRNA [14]. In addition, the *mefA* gene is responsible for a macrolide efflux pump system [15,16]. Some of these genes are known to persist on mobile genetic elements, which easily facilitate the spread of these resistance genes.

In the present study we performed a randomized control trial to determine the effect of azithromycin maintenance therapy on the dynamics of macrolide resistance genes in the pharyngeal microbiota of COPD patients. We used a targeted (PCR-based) metagenomic approach to determine the presence and relative abundance of specific macrolide resistance genes; *ermB*, *ermF* and *mefA*.

Methods

Study design and participants

This study was part of the COLUMBUS trial (Clinicaltrials.gov, NCT00985244), a randomised, double-blind, placebo-controlled trial to measure the effect of maintenance treatment with azithromycin in COPD patients on the exacerbation rates during a 12-month period. The study protocol and the primary results have been published earlier [17,18]. Adult patients (≥18 years) with a diagnosis of COPD who had received treatment for three or more exacerbations in the previous year were randomly assigned to receive 500 mg azithromycin or placebo three times a week for 12 months (total of 92 patients). Additional inclusion criteria were a COPD diagnosis according to the guidelines of the Global initiative for chronic Obstructive Lung Disease [19], and 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 an active malignancy.

Randomisation was stratified by use of long-term, low-dose prednisolone (≤10 mg daily). Patients and investigators were masked to group allocation. The study was approved by the Mater Human Research Ethics Committee and all participants provided written informed consent.

Sample collection

During the treatment period, throat samples (e-swabs) were collected at baseline, 6 months and 12 months, as well as during each exacerbation that required admission to the hospital. E-swabs were stored at –80°C until molecular analysis was performed.

Molecular methods

The extraction of DNA was performed from the collected e-swabs™ (COPAN BV), using the EasyMAG (Biomérieux). Real-time PCR was performed to detect and quantify genes responsible for resistance to macrolides; *ermB*, *ermF* and *mefA*. Amplification of *ermB* was performed as described earlier [20]. Primers to target *mefA* and the forward primer for *ermF* were adapted from earlier described studies [21,22]. A reverse primer for *ermF* was designed by performing an nBLAST in GenBank for the *ermF* gene sequence (NG_047826.1) and aligning all resulting sequences with >75% query coverage (identity: 94-100%) using MAFFT (http://mafft.cbrc.jp/alignment/software/), after which a primer homologous to all sequences was chosen.

The 16S ribosomal DNA was amplified as a reference gene to normalize for the amount of bacterial DNA in the samples, using previously described primers [23]. All targets were amplified by using a MyiQ Single-Color Real-Time PCR Detection System (BioRad, Hercules, CA, USA) in 25-µL reactions containing 12.5 µL iQ SYBR Green Supermix (BioRad), 300 nM of both the respective targets forward and reverse primer and 5-µL template DNA. Primer sequences, amplicon sizes and PCR cycling conditions are displayed in Table 1. For all antibiotic resistance gene targets, specificity of the assay was investigated by melting curve analysis of all samples and amplicon sequencing of 10 random positive samples using the PCR primers and an ABI BigDye Terminator v1.1 Cycle Sequencing Kit. Sequencing data were obtained on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Efficiencies of the assays were determined to be 103.1% (16S rDNA), 99.7% (*ermF*) and 105.1% (*mefA*).

Statistical methods

The prevalence of the macrolide genes between the treatment groups was compared using a χ^2 test. In addition, acquisition and loss of the different resistance genes between the different treatment groups were compared using a χ^2 test.

A comparison of the resistance gene abundances between treatment groups was performed based on the samples of both month 6 and month 12. These comparisons were based on the change from baseline, relative to the amount of 16S DNA present, using real-time PCR. These ratios – or fold changes – were calculated for *ermB*, *ermF* and *mefA* using the ΔΔCT method with a Pfaffl modification to correct for PCR efficiency as described earlier (20). This method is standard to measure the relative change in mRNA expression levels by using real-time PCR. Here, we measure the relative amount of target DNA present rather than measuring mRNA expression. The 16S rDNA was used as the reference gene. In order to perform paired-analysis, multiple throat samples from one patient have to be available in which the presence of the gene of interest was detected. Ratio's log-transformed were compared between treatment arms using the Wilcoxon rank sum test. In addition, descriptive statistics (n, mean, median, SD) and graphical presentations were provided for both time points.

Changes from baseline in relative resistance gene abundances (ratio) were evaluated between samples of month 6 (and month 12) and samples of baseline using the same ΔΔCT method with a Pfaffl modification to correct for PCR efficiency as described earlier (20).

Results

Study population

The COLUMBUS trial was a single centre study that took place at the Amphia Hospital (Breda, the Netherlands) between May 19, 2010 and June 18, 2013.

The placebo group consisted of 47 patients and the azithromycin group of 45 patients. The baseline characteristics of these 92 patients are described in table 2.

Prevalence of macrolide resistance genes present in pharyngeal microbiota

At baseline throat samples were taken in 36 (77%) patients in the placebo group and in 44 (98%) patients in the azithromycin group. At month 6 and 12 the available samples were, 30 and 27 in placebo group and 34 and 32 in azithromycin group, respectively.

At baseline, the macrolide resistance gene *mefA* was present in all available throat samples. After both placebo or azithromycin treatment, the *mefA* gene was still present in all available throat samples at 6 and 12 months.

Before treatment, prevalence of the macrolide resistance genes *ermF* and *ermB* were respectively 44.4% and 86.1% in the placebo group (n=36), and respectively 59.1% and 97.7% in the azithromycin group (n=44) (p=0.261 *ermF*, p=0.085 *ermB*) (Table 3). After 6 and 12 months of placebo treatment, the *ermF* and *ermB* genes were detected in 43.3%, 80% (6 months), 48.1% and 74.1% (12 months) of the throat samples tested, correspondingly, with no statistical differences regarding the presence of resistance genes between the treatment groups.

Regarding the azithromycin group, the prevalence of the *ermF* and *ermB* genes at 6 months was 67.7% and 97.1% versus 68.8% and 100% at 12 months (p=n.s.). Comparison of the *ermF* prevalence between the placebo and azithromycin groups showed no significant differences at 6 and 12 months (p=0.05 and p=0.109). The prevalence of *ermB* increased significantly over time in the azithromycin group compared to the placebo treated group (p=0.029 6 months, p=0.002; 12 months).

Loss and acquisition of macrolide-resistance in pharyngeal microbiota during and after treatment with placebo or azithromycin

In the placebo group, 27 patients had throat swabs available from visits at baseline and 6 months while 26-paired samples were available from baseline and 12 months. For the azithromycin group, there were 34 paired samples (from baseline and 6 months) and 30 pairs (from baseline and 12 months).

The loss and acquisition of macrolide resistance genes (*mefA, ermF* and *ermB*) in pharyngeal microbiota before and after treatment of the paired samples is shown in table 3. During the trial, no

differences were detected in the presence of the *mefA* gene in the pharyngeal microbiota, since the gene was always detected in both groups.

For the patients without the macrolide genes *ermF* and *ermB* present in their pharyngeal microbiota at baseline (n_{ermF}=15 and n_{ermB}=4 in placebo, n_{ermF}=16 and n_{ermB}=1 in azithromycin), no statistical differences were observed in the acquisition rates between the placebo and azithromycin treated groups (see table 4).

However, from the patients with the macrolide genes *ermF* and *ermB* present (n_{ermF}=12 and n_{ermB}=23in placebo, n_{ermF}=18 and n_{ermB}=33 in azithromycin) none of the patients treated with azithromycin lost the *ermF* and *ermB* gene over time, while for the placebo group, 1 and 3 patients lost the *ermF* and *ermB* gene after 6 months, respectively. Moreover, in 5 patients in the placebo group, the *ermB* gene was lost after 12 months, therefore, the number of patients that lost the gene was statistically significant higher in the placebo group compared to the azithromycin group (p=0.012).

In other words, this study suggests that there is no or limited increased risk for acquisition of macrolide resistance genes in the azithromycin group compared to the placebo group. However, more patients lost the macrolide resistance gene *ermB* in the placebo treated group compared to the azithromycin group.

Relative gene abundances of the macrolide resistant genes during and after treatment with placebo or azithromycin

A large part of the patients in both groups already had detectable levels of macrolide genes at baseline. This enabled us to compare the relative abundance of the genes in throat samples to determine the effect of the treatment on the abundance of these genes. Abundance ratios which were calculated with the Pfaffl method (24) were converted to log ratios compared to baseline. To determine the overall abundance change of a resistance gene, ratios were log-transformed and are depicted in figure 1.

The relative gene abundance of *mefA* after 6 months of treatment was substantially higher in the azithromycin group compared to the placebo group (p=0.0001) (Figure 1, table 5). Moreover, After 12 months of treatment, this difference was also statistically significant (p=0.002) (Figure 1, table 5). Determining the overall increase or decrease of the abundance of the *ermF* gene showed that this gene increased over time after treatment with azithromycin compared to the placebo group as well, which was only significant at 12 months p=0.0124 (Figure 1, table 5). With regard to the macrolide gene *ermB,* the relative gene abundance was significantly increased over time in the azithromycin group compared to the placebo group after 6 and 12 months of treatment (p=0.01 and p=0.001, respectively) (Figure 1, table 5).

In conclusion, determining the relative change of the abundance of each macrolide gene showed that all the investigated genes increased during treatment with azithromycin. This was significant for all genes with the exception of *ermF* at month 6.

Discussion

Within the COLUMBUS study, maintenance treatment with azithromycin significantly decreased exacerbation rate compared with placebo in COPD patients [9]. In this follow-up study, throat samples were subjected to a molecular analysis to investigate macrolide resistance. Using real-time PCR, quantitative levels of *mefA, ermF* and *ermB* were determined at different time-points in order to measure changes over time.

During the study, only for the *ermB* gene, a significant difference in prevalence between the azithromycin group and the placebo group was measured over time. However, this difference was not based on an increase in prevalence over time within patients of the azithromycin group, but was attributed to a loss of this resistance gene within the placebo group. For the *ermF* and *mefA* gene, no differences were detected in the acquisition rates. However, the high prevalence of all resistance genes at baseline, with *mefA* being present in 100% of cases should be taken into consideration.

Looking at the relative abundance of the macrolide-resistance genes over-time, a statistical increase of all tested genes in the azithromycin group compared to the placebo group was observed.

Long-term treatment with macrolides might influence the microbiological profile and antibiotic resistance in airways. The acquisition of respiratory pathogens and macrolide resistant microorganisms as a result of maintenance treatment with macrolides in COPD patients has been addressed in three recent studies [7,9,25]. It is important to note that these studies did not have the ability to measure quantitative differences over-time. Seemungal and colleagues found no difference in colonization rates with macrolide-resistant organisms between the macrolide and placebo group during one year of treatment [7]. Only one case of erythromycin resistance was detected in the macrolide treated group at 12 months. In contrast with these findings, earlier analysis of our COLUMBUS study found fewer patients in the azithromycin group with macrolide-resistant bacteria in sputum samples compared to those in the placebo group [9]. Albert et al however, observed an increase in the incidence of colonization with macrolide-resistant organisms in the azithromycin group compared to the placebo group [25].

In summary, it can be stated that there is conflicting evidence about the influence of maintenance treatment with macrolides on the acquisition of macrolide resistant respiratory pathogens in COPD patients. In the current study, only a small difference in acquisition rate of macrolide resistance genes between patients treated with azithromycin or placebo could be demonstrated, nevertheless,

a statistical increase in the relative abundance of the tested genes was found. This latter finding suggests that maintenance therapy with azithromycin does influence the presence of macrolide resistance genes, which indicates towards changes in microbiological profile.

To our knowledge this is the first randomised controlled double blind study in a COPD population, in which the effect of long-term treatment with macrolides on the acquisition and relative abundance of macrolide resistance genes using a targeted metagenomic approach has been evaluated. However, this study has some limitations. Unfortunately, throat samples were not obtained from all patients at regular visits. Furthermore, throat samples were not cultured in order to assess the changes in the microbiological profile and resistance patterns. Finally, in this study we focused on three genes, which are involved in macrolide resistance. It is known that more genes are involved in this process [26,27].

The consequences of this study for daily practice are unclear. The clinical benefit of macrolide maintenance therapy in COPD patients with frequent exacerbations has been demonstrated repeatedly [7,9,25]. In the most recent update of the GOLD guidelines it is recommended to consider the addition of a macrolide in COPD patients treated with long-acting beta2 agonists/long-acting muscarinic antagonists/inhalation corticosteroids combination, who still have exacerbations [28]. This recommendation is accompanied by the advice that the possibility of developing resistant organisms should be taken into consideration in the decision making.

As indicated, at the start of the study the prevalence of macrolide-resistance genes were already high in throat samples. This may be the result of historical exposure to (macrolide) antibiotics in this specific study population, since only COPD patients with a minimum of three exacerbations in the previous year, have been included in this study. This could be an argument to consider macrolide maintenance treatment only in this specific category of COPD patients. However, this high prevalence has also been observed in a healthy travel population, as shown in the study of von Wintersdorff et al., with an *ermB* gene presence in 99.2 % in fecal samples [20].

Future research should focus on the changes of the microbiological profile and macrolide resistance patterns in pharyngeal microbiota, sputum and fecal microbiota, during long-term treatment with macrolides.

In conclusion, this study showed that the acquisition rate of macrolide resistance genes in COPD patients treated with azithromycin maintenance therapy was limited, but the relative abundance of macrolide resistance genes increased significantly over time compared to placebo. The clinical implications of these findings are unclear and we consider the benefits for this specific group of patients to outweigh the risks of antimicrobial resistance. Nevertheless, it is recommended to monitor the development of resistance carefully when treating patients for prolonged periods with antibiotics.

Table 1. PCR conditions and primer sequences

Primer	Sequence 5’ - 3’	Amplicon size (bp)	Cycling conditions
16SrDNA_F	CCTACGGGNGGCWGCAG	465	1x 95°C, 3’
16SrDNA_R	GACTACHVGGGTATCTAATCC		35x 95°C, 15”; 55°C, 20”; 72°C, 30”
*ermB*_F	AAGGGCATTTAACGACGAAACTG	438	1x 95°C 3’
*ermB*_R	ATTTATCTGGAACATCTGTGGTATG		40x 95°C 15”, 60°C 20", 72°C 30"
*ermF*_F	CGACACAGCTTTGGTTGAAC	120	1x 95°C 3'
*ermF*_R	TTTGACACCACTTTGAAAGGAAA		40x 95°C 15", 58°C 20", 72°C 30"
mefA	CCTGCAAATGGCGATTATTT	199	1x 95°C 3'
mefA	AATAGCAAGCACTGCACCAG		40x 95°C 15", 58°C 20", 72°C 30"

Table 2. Baseline characteristics.

	Azithromycin group (n=47)	Placebo group (n=45)
Male	22 (46·8%)	18 (40%)
Age (years)	64·7 (10·2)	64·9 (10·2)
Current smoker	20 (43%)	9 (20%)
AECOPD in past year	4·0 (1·2)	4·0 (1·1)
Hospitalisation due to AECOPD	1·0 (1·1)	0·7 (0·8)
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%)

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. GOLD=Global Initiative for Chronic Obstructive Pulmonary Disease.

Table 3. Prevalence of *ermF* and *ermB* macrolide resistance genes over time

	ermF % (pos/all samples)			*ermB* % (pos/all samples)		
Prevalence	Placebo	Azithromycin	P value	Placebo	Azithromycin	P value
Baseline	44,4 (16/36)	59,1 (26/44)	0.261	86,1 (31/36)	97,7 (43/44)	0.085
M6	43,3 (13/30)	67,6 (23/34)	0.050	80,0 (24/30)	97,1 (33/34)	0.029*
M12	48,1 (13/27)	68,8 (22/32)	0.109	74,1 (20/27)	100,0 (32/32)	0.002*

***** Prevalence of *ermB* is statistically significant in the Azithromycin group at M6 and M12 compared to the Placebo group (Chi-square, Pearson corrected)

Table 4. Macrolide-resistant genes presence in pharyngeal microbiota before and after treatment in both groups

		Placebo			Azithromycin		
Baseline*	M6*	*mefA*	*ermF*	*ermB*	*mefA*	*ermF*	*ermB*
neg	neg	-	14	2	-	11	1
	pos	-	1	2	-	5	-
pos	neg	-	1	3	-	-	-
	pos	27	11	20	34	18	33
Baseline*	M12*						
neg	neg	-	12	1	-	8	-
	pos	-	2	2	-	5	-
pos	neg	-	-	5**	-	-	-
	pos	26	12	18	30	17	30

*Throat sample at baseline, 6 and 12 months after placebo or azithromycin treatment

**Loss of *ermB* gene in the placebo group after 12 months is statistically significant compared to the Azithromycin group (p=0.012, Chi-square, Pearson corrected)

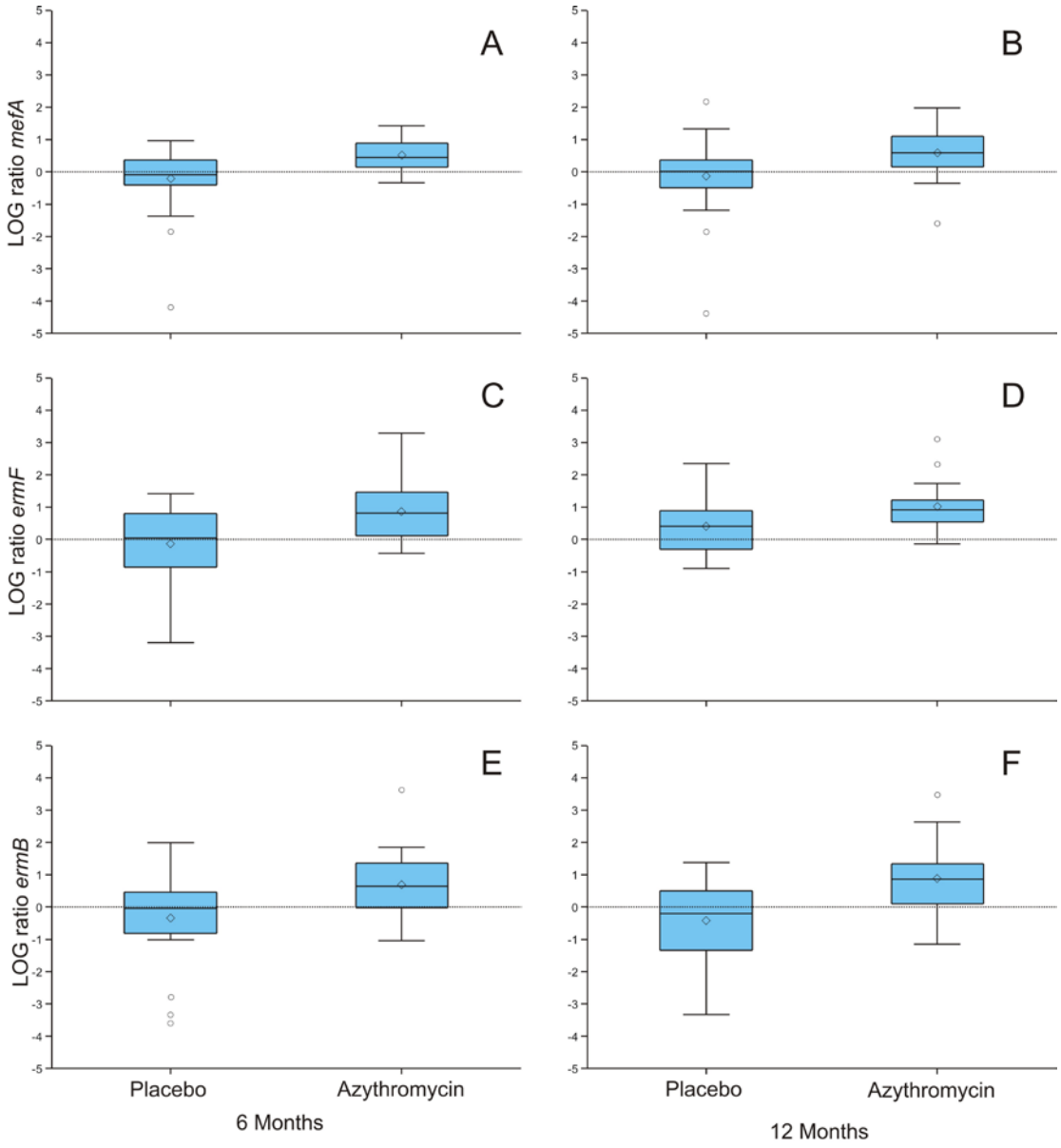
Table 5. Comparison of mean logs ratios of the different macrolide–resistant genes before, during and after treatment

		Placebo		Azithromycin		
		Mean*	SD	Mean	SD	P value**
mefA	M6	-0.22 (n=27)	1.00	0.51 (n=34)	0.47	0.0001
	M12	-0.39 (n=26)	1.21	0.33 (n=30)	0.68	0.002
ermF	M6	-0.14 (n=11)	1.35	0.86 (n=18)	0.99	0.0687
	M12	0.15 (n=12)	0.94	1.04 (n=17)	0.80	0.0124
ermB	M6	-0.32 (n=20)	1.48	0.69 (n=33)	0.93	0.0116
	M12	-0.42 (n=18)	1.33	0.89 (n=30)	1.05	0.0013

*mean of the log gene abundance ratio compared to baseline

**p values for comparison of mean abundance at either 6 or 12 months and baseline, by Wilcoxon ranked sum test.

Figure 1. Relative changes in gene abundance at 6 and 12 months after treatment with placebo or azithromycin.



Changes related to baseline gene abundance of *mefA (*A, B), *ermF* (C, D) and *ermB* (E, F) at 6 and 12 months are shown. Results are visualized in box-plots with median and 10th and 90th percentiles (dots show outliers). The dotted line shows the zero line. Mean logs ratio and statistics are shown in table 5.

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Chapter 8

Summary and future perspectives

Summary

At the start of the study it was clear that exacerbations of COPD have a major impact on lung function, morbidity and mortality. Therefore it is important to reduce exacerbations, especially in those COPD patients who are prone to recurrent exacerbations.
Despite several interventions, that have proven to be effective in reducing exacerbations, there is still an unmet need to a further reduction.
Several studies have demonstrated the causative role of bacteria in COPD exacerbations. Hence, long-term treatment with antibiotics could be useful. Besides antimicrobial effects macrolide antibiotics have anti-inflammatory effects as well. Macrolides have been successfully used as maintenance treatment in several chronic inflammatory pulmonary diseases.
Chapter 1 provides a comprehensive overview of different aspects of acute exacerbations of COPD and the aim of this study.
In chapter 2 the current understanding of the value of maintenance therapy with macrolides to reduce COPD exacerbations, according to recent studies is described. Several recent studies show that a significant reduction of exacerbation frequency in COPD patients can be achieved with long-term macrolide therapy. Based on the review of the literature we decided, in our COLUMBUS study to evaluate long-term macrolide therapy in the subgroup of COPD patients with at least three exacerbations in the previous year. Azithromycin was chosen based on the fact that it has fewer adverse effects compared with other macrolides. The study protocol is described in chapter 3. We demonstrated that a regimen of azithromycin treatment 500 mg three times a week during one year compared to placebo, leads to a larger reduction in exacerbation frequency in COPD patients with a minimum of three exacerbations in the previous year (chapter 4). Since it is known that besides bacterial infections, viral infections also play an important role in COPD exacerbations we investigated which viruses could be detected during the treatment period (chapter 5). We found a viral infection in a quarter of exacerbations. It was found that human rhinovirus (HRV) was most prevalent, followed by respiratory syncytial virus (RSV) and human metapneumovirus (hMPV). It appeared that most viral infections occurred in winter, followed by summer, autumn and spring. A different pattern was observed for HRV with a peak in July.
In chapter 6 we investigated which characteristics of COPD patients with frequent exacerbations predicted the best response to maintenance treatment with azithromycin. Long-term treatment with azithromycin resulted in a significant lower number of exacerbations in patients with the following characteristics: baseline blood eosinophil count ≥ 2.0% (compared to an eosinophil count < 2.0%), GOLD stage 1-2 (versus GOLD stage 4) and GOLD group C (compared to group D). Also, the number of

hospitalizations was significantly lower in patients with a blood eosinophil count ≥ 2.0% (compared to an eosinophil count < 2.0%) and in GOLD stages 1-2 (compared to stage 4).
Since the development of bacterial resistance during prolonged treatment with macrolide antibiotics is a major concern, we determined the effect of azithromycin maintenance therapy on the dynamics of macrolide resistance genes in the pharyngeal microbiota of the COPD patients in the COLUMBUS trial. In chapter 7 we showed that the acquisition of macrolide resistance genes was limited but the relative abundance of macrolide resistance genes increased significantly over time compared to placebo. However the clinical implications of these results are unclear, it is therefore recommended to monitor the development of resistance carefully when treating patients for prolonged periods with antibiotics.

Future perspectives

It has been shown that long-term treatment with macrolides is effective in reducing exacerbations and the severity of exacerbations in COPD patients. However, in view of the large population of COPD patients and potential spread of macrolide use, patient selection is very important. This intervention should only be considered in optimally treated patients. Non-pharmacological interventions such as influenza vaccination and pulmonary rehabilitation should be deployed. Finally, patients should be treated with maximum pharmacological therapy (ICS, LABA, LAMA) in order to reduce exacerbation frequency. If these measures do not result in reduction of exacerbation frequency, long-term treatment with azithromycin should be used.
In our study we demonstrated that, in comparison with other studies, the greatest benefit of treatment with macrolides could be achieved in COPD patients with frequent exacerbations in the past year. We showed that a criterion of three or more exacerbations in the previous year, results in a higher relative reduction in exacerbation rate.
There is no consensus about the optimal duration of therapy. Todate, in all recent studies patients received one-year treatment with macrolides. It is not clear if a shorter period of treatment leads to equal results or perhaps a prolonged treatment to an even higher reduction in exacerbation frequency. Also, periodic treatment during winter season could be considered. This should be investigated in future research.
It is also unknown if the reduction in exacerbation rate persists in patients after prolonged treatment with long-term therapy. And if not, whether intermittent treatment leads to a more persistent reduction in exacerbation frequency.
Based on recent studies, azithromycin seems to be the drug of choice due to its more favorable side effects profile.

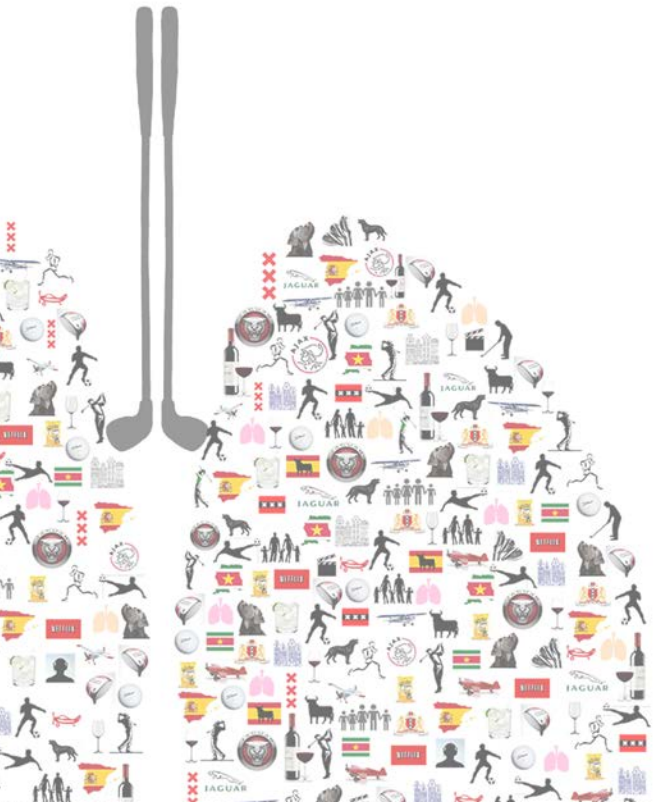
However, the ideal dosage has not been determined. In our study we used the lowest cumulative dosage/year that has been used so far (78 g), with a regimen of 500 mg three times a week. Perhaps similar results can be achieved with a schedule of 250 mg three times a week.

We showed that maintenance therapy is most effective in patients in GOLD stages I-II and C and in patients with blood eosinophils above 2.0%. Prospective randomized controlled studies using these parameters should be performed in order to confirm these findings.

The exact working mechanism of macrolides, which leads to exacerbation reduction in susceptible COPD patients, is still under debate. In order to evaluate the anti-bacterial and anti-viral effects of macrolides in relation to reduction of exacerbations, new long-term studies with extensive assessment of airway colonisation, bronchial cultures of bacteria and PCR based viral detection techniques during exacerbations should be performed. The influence of anti-inflammatory effects of macrolides should be assessed by measuring the change in profile of inflammatory mediators (including cytokines) in sputum and serum during treatment and the relationship with exacerbation frequency. The effects of long-term use of non-antibiotic macrolides in COPD patients with frequent exacerbations could provide a clear answer to this question.

A major concern remains the development of macrolide resistance during long-term treatment. Extensive surveillance cultures with measurement of resistance patterns of bacteria in the nasopharynx and intestines should be deployed in order to monitor the development of resistance. In the current study we assessed the development of macrolide resistance genes in pharyngeal flora. Since the gut microbiota has the largest number of bacteria, the effect of long-term macrolide use can be large. In a future study the development of these genes in intestinal flora should be assessed. Finally the changes in the microbial flora of nasopharynx and intestines during long-term treatment with macrolides should be assessed. New techniques such as IS-PRO or metagenomic sequencing, which determine bacteria without culture, could be used. Consequently, long-term effects of antibiotic therapy can be observed in detail, thereby enabling a better judgement of risks and benefits.

Samenvatting en toekomstige ontwikkelingen



Samenvatting

Bij aanvang van de studie was reeds duidelijk dat COPD exacerbaties een grote impact hebben op longfunctie, morbiditeit en mortaliteit. Het beleid bij COPD patiënten moet er derhalve op gericht zijn om exacerbaties te verminderen, met name bij patiënten die frequent exacerbaties doormaken. Ondanks diverse bewezen effectieve interventies, is er nog steeds een behoefte aan interventies die de exacerbatiefrequentie verder kunnen reduceren.
Diverse studies hebben de rol van bacteriën als oorzaak van COPD exacerbaties aangetoond. Hieruit kan worden opgemaakt dat onderhoudsbehandeling met antibiotica zinvol zou kunnen zijn.
Naast antimicrobiële effecten, hebben macrolide antibiotica ook anti-inflammatoire eigenschappen. Macroliden zijn succesvol gebruikt als onderhoudsbehandeling bij diverse chronische inflammatoire longaandoeningen.
Hoofdstuk 1 geeft een uitvoerig overzicht van verschillende aspecten die bij acute COPD exacerbaties van belang zijn.
In hoofdstuk 2 wordt de huidige stand van zaken van onderhoudsbehandeling met macroliden teneinde COPD exacerbaties te reduceren op basis van recente studies, beschreven. In diverse recente onderzoeken wordt aangetoond dat door middel van onderhoudsbehandeling met macroliden een significante reductie van de exacerbatiefrequentie kan worden bereikt. In onze COLUMBUS studie besloten wij om onderhoudsbehandeling met macroliden te onderzoeken in de specifieke subgroup van COPD patiënten met tenminste drie exacerbaties in het voorafgaande jaar. Er is gekozen voor azithromycine, aangezien dit middel minder bijwerkingen heeft dan andere macroliden. Het studie protocol wordt beschreven in hoofdstuk 3. In dit onderzoek hebben we aangetoond dat een schema met azitromycine 500 mg drie maal per week gedurende een jaar, leidt tot een grotere reductie van de exacerbatiefrequentie in vergelijking met placebo, bij COPD patiënten met tenminste drie exacerbaties in het voorafgaande jaar (hoofdstuk 4). Aangezien bekend is dat, naast bacteriële infecties, virale infecties een belangrijke rol spelen als oorzaak van COPD exacerbaties, is onderzocht welke virussen gedetecteerd konden worden tijdens de behandelperiode (hoofdstuk 5). Tevens werd de verdeling over de seizoenen onderzocht. Een virale infectie werd in een kwart van de exacerbaties vastgesteld. Humaan rhinovirus (HRV) werd het meest frequent gevonden, gevolgd door respiratoir syncytieel virus (RSV) en humaan metapneumovirus (hMPV). Ook werd vastgesteld dat de meeste virale infecties in de winter plaatsvinden, gevolgd door zomer, herfst en voorjaar. Een afwijkend patroon werd gezien bij HRV, met een piek in juli.
In hoofdstuk 6 onderzochten wij welke eigenschappen van COPD patiënten met frequente exacerbaties het best de respons op onderhoudsbehandeling met macroliden konden voorspellen.

Het bleek dat onderhoudsbehandeling met azitromycine tot een significant lager aantal exacerbaties leiden in patiënten met de volgende karakteristieken: uitgangs bloed eosinofielen getal ≥ 2.0% (vergeleken met een eosinofielen getal < 2.0%), GOLD stadium 1-2 (versus GOLD stadium 4) en GOLD groep C (vergeleken met groep D). Ook het aantal opnames was significant lager bij patiënten met een bloed eosinofielen getal ≥ 2.0% (vergeleken met een eosinofielen getal < 2.0%) en bij hen in GOLD stadium 1-2 (vergeleken met GOLD stadium 4).

Aangezien de mogelijke ontwikkeling van bacteriële resistentie tijdens langdurige behandeling met macrolide antibiotica een groot punt van zorg is, onderzochten wij het effect van deze behandeling op het verloop van macrolide resistentie genen in de pharyngeale microbiële flora van de COPD patiënten in de COLUMBUS trial.

In hoofdstuk 7 hebben we laten zien dat de acquisitie van macrolide resistentie genen beperkt was tijdens onderhoudsbehandeling, maar dat het relatieve overschot van deze genen een significante toename liet zien, vergeleken met placebo. Alhoewel de klinische implicaties van deze resultaten niet duidelijk zijn, is het aan te raden om het optreden van resistentie zorgvuldig te monitoren bij patiënten die langdurig met antibiotica worden behandeld.

Toekomstige ontwikkelingen

Het is aangetoond dat onderhoudsbehandeling met macroliden effectief is in het reduceren van het aantal en de ernst van de exacerbaties. Gelet op de grote populatie van COPD patiënten en dientengevolge de effecten van wijdverspreid gebruik van macroliden , is patiënt selectie echter belangrijk. Deze behandeling moet pas overwogen worden bij patiënten, die optimaal worden behandeld. Non-farmacologische maatregelen die de exacerbatiefrequentie gunstig kunnen beïnvloeden, zoals influenza vaccinatie en longrevalidatie moeten eerst zijn toegepast. Tot slot moeten patiënten maximaal farmacologisch zijn behandeld met ICS, LABA en LAMA, teneinde de exacerbatiefrequentie te minimaliseren.

In ons onderzoek hebben we laten zien dat, in vergelijking met andere studies, het grootste behandeleffect bereikt kan worden bij de groep COPD patiënten met frequente exacerbaties (tenminste drie) in het voorafgaande jaar. We hebben aangetoond dat dit criterium resulteert in een hogere relatieve reductie in exacerbatiefrequentie.

Er bestaat geen consensus over de optimale duur van de behandeling. Tot op heden hebben alle patiënten in de meest recente studies een onderhoudsbehandeling van een jaar gekregen. Het is niet duidelijk of een kortere behandelduur tot gelijkwaardige resultaten leidt of een langere duur wellicht tot een nog hogere reductie in exacerbatiefrequentie. Ook periodieke behandeling tijdens de

wintermaanden zou zinvol kunnen zijn. Dit zou in vervolgonderzoek onderzocht dienen te worden. Het is ook niet bekend of het effect van de afname in exacerbaties aanhoudt na de onderhoudsbehandeling met macroliden. En, als dat niet het geval is, of intermitterende behandeling tot een blijvende reductie leidt.

Op basis van recente studies lijkt azitromycine het aangewezen middel te zijn voor onderhoudsbehandeling, op basis van een gunstiger bijwerkingen profiel. De ideale dosis is echter nog niet vastgesteld. In het huidige onderzoek werd in vergelijking met andere onderzoeken de laagste cumulatieve jaardosis (78 g) gebruikt, met een schema van 500 mg driemaal per week. Wellicht kunnen vergelijkbare resultaten worden bereikt met een schema van 250 mg driemaal per week.

We hebben aanwijzingen gevonden dat onderhoudsbehandeling het meest effectief is bij patiënten in GOLD stadia 1-2 en C en bij patiënten met bloed eosinofielen ≥ 2.0%. Deze bevindingen zouden bevestigd moet worden in prospectief gerandomiseerd gecontroleerd onderzoek.

Op basis van welk werkingsmechanisme van macroliden exacerbatiereductie in COPD patiënten wordt bereikt is nog niet duidelijk. Nieuwe studies bij COPD patiënten zijn noodzakelijk waarbij geëvalueerd wordt of anti-bacteriële en anti-virale effecten van macroliden gerelateerd kunnen worden aan de exacerbatiereductie , door middel van uitgebreide assessment van luchtwegkolonisatie, bronchiale kweken van bacteriën en virus detectie technieken op PCR basis gedurende exacerbaties. De eventuele component van de anti-inflammatoire werking van macroliden moet onderzocht worden door de verandering van het inflammatoire profiel (inclusief cytokines) te meten in sputum en serum gedurende behandeling, in relatie tot de afname in exacerbaties.

Studies naar de effecten van langdurige behandeling met non-antibiotische macroliden bij COPD patiënten met frequente exacerbaties zou ook een helder antwoord kunnen geven op de vraag of de exacerbatiereductie met name berust op de anti-inflammatoire werking.

De ontwikkeling van macrolide resistentie tijdens onderhoudsbehandeling blijft een belangrijke reden tot zorg.

Bij onderzoek naar deze vorm van therapie is het van belang om uitgebreide kweken te verrichten met bepaling van resistentie patronen van bacteriën in de nasopharynx en de darmen teneinde de eventuele ontwikkeling van resistentie te kunnen vaststellen.

In het huidige onderzoek is de ontwikkeling van verschillende macrolide resistentie genen in pharyngeale flora onderzocht. Bij toekomstig onderzoek zou ook de ontwikkeling van deze genen in darmflora onderzocht moeten worden.

Tenslotte is van belang om te onderzoeken of onderhoudsbehandeling met macroliden gevolgen heeft voor de samenstelling van microbiële flora in farynx en darm.

Hierbij zou gebruik gemaakt kunnen worden van nieuwe technieken zoals IS-PRO of metagenomische sequencing, waarbij bacteriën kunnen worden vastgesteld zonder kweek. Zodoende kunnen de (lange termijn) effecten van antibiotische therapie nog beter in kaart gebracht worden zodat een goede afweging mogelijk is van de baten en de nadelige effecten.

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'Laten we maar beginnen', waren destijds de woorden van mijn tweede promotor, Prof. Dr. J.A.J.W. Kluytmans; beste Jan, ik wil je bedanken voor de bijdrage die je hebt geleverd om te promoveren in deze fase van mijn carrière. De steun die je hebt gegeven, de wijze waarop je de afdeling Microbiologie hebt opengesteld en het wetenschappelijke klimaat dat je daar hebt gecreëerd, heeft mij zeer geholpen.

Onderzoek doen en artikelen schrijven heb ik moeten leren. The hard way.
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Lieve Ro, love of my life… Amor omnia vincit…

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The author of this thesis was born on May 8th 1964 in Amsterdam, the Netherlands. He completed secondary school (Gymnasium beta) in 1982 at the Thorbecke Scholengemeenschap in Utrecht. In the same year he started his medical education at the University of Amsterdam, where he graduated in 1987. In 1988 he went to Curaçao for a one-year internship of Respiratory Medicine at the Thorax Centrum. After he received his medical degree in 1990, he worked as a resident at the department of Internal Medicine at the Medisch Centrum Alkmaar. In 1991 he started to work as a resident (ANIOS) at the department of Respiratory Medicine of the Onze Lieve Vrouwe Gasthuis (OLVG) in Amsterdam. In 1992 he started his residency in Pulmonary Medicine in the same hospital.

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