Macrolide maintenance treatment for bronchiectasis

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Inhoudsopgave

CHAPTER 1

Introduction and outline of the thesis

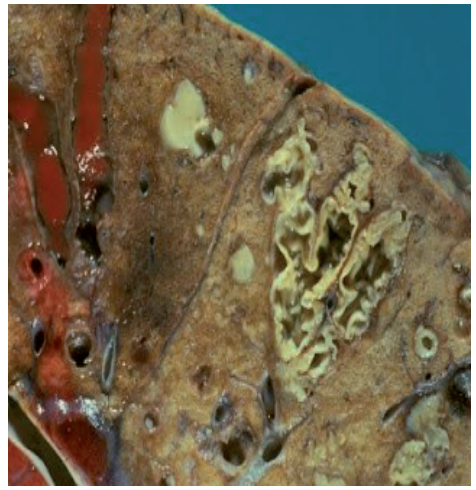
Already in the early 19th century physicians have been fascinated by the main object of this thesis; bronchiectasis. Dr René de Laennec (1781- 1826), a French doctor, pathologist and inventor of the stethoscope wrote down his post mortem observations of this disease in a very lively and illustrative way, able to captivate even 21st century readers:

"the organic lesion which I am now about to notice seems to have been hitherto entirely overlooked (…). It can only be detected by tracing the individual bronchial tubes to their ultimate ramification, a thing which is rarely done in our examination of the lungs".

"ramifications which in their natural state would scarcely admit a fine probe, acquire a diameter equal to that of a crow-quill, or goose-quill or even of the finger"

Dr René Laennec using his stethoscope on a boy. Painting by Robert Thom (1915-1979), reprinted with permission.

The permanent pathological dilatation of bronchi which he observed in his dissecting room was later on named bronchiectasis, derived from the Greek words *bronchos* and *ektasis* (dilatation). And little did he know that up to recently, bronchiectasis would remain a neglected condition, both by the clinical and research community. The cardinal symptom of bronchiectasis; chronic cough with production of substantial amounts of phlegm, is often



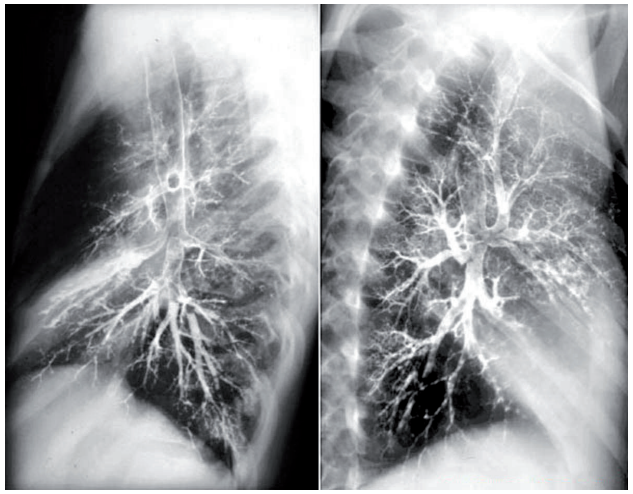
A pathology specimen of a lung segment of a child with severe varicose bronchiectasis (courtesy of J. Haas, Clinical Professor of pathology, University of Washington School of Medicine).

mistakenly attributed to smoking habits or labelled as 'chronic bronchitis'. In addition, affected patients are often reluctant to seek medical advice for their condition, which may be cause for embarrassment when being out in public. Because bronchiectasis is not usually life threatening, many patients probably go undetected.

Doctors frequently believe bronchiectasis to be a near-extinct or 'orphan' disease, due to the evident decline in incidence since antibiotic treatment for tuberculosis and pneumonia – in earlier days the primary causes of bronchiectasis- became widely available. In addition, researchers are frustrated by the vast array of underlying conditions and causes.

In the last two decades, there has been a renewed interest in bronchiectasis, for various different reasons. First, the detection of bronchiectasis was importantly facilitated by the ubiquitous availability of CT scanning. Bronchography, bronchial examination via X-ray following the coating of the inside of the bronchial tree with a radiopaque substance, has been the method of choice for diagnosing bronchiectasis for a long period of time. This rather patient-unfriendly and time consuming procedure, is now considered obsolete. Due to the increase in the number of chest-CT scans, which were obtained for various reasons, one came to realise that the true incidence of bronchiectasis was much higher than expected, particularly in the rapidly expanding elderly population.

Further, developments in cystic fibrosis (CF) research, inspired scientists to look again at "non-CF" bronchiectasis as a field of interest. At first, trials in bronchiectasis were mainly small, non-randomized and involved medication that was first tested in CF. But in the last ten years, larger and better designed trials were executed in bronchiectasis, and the last ten



Bronchography of both sides with deformed, ectatic bronchi of the lingula (chestradiology.net).

years, larger and better designed trials were executed in bronchiectasis, and the research pipeline contains a growing number of trials with agents specifically developed for bronchiectasis treatment.

The research described in this thesis focuses on macrolide treatment in bronchiectasis by combining clinical, pharmacological and immunological data. Our aim is to add to the understanding of the mechanism of action of long term azithromycin treatment and to take a step forward in defining patients who are expected to benefit from this treatment modality, while at the same time trying to balance advantages and disadvantages.

Chapter 2 reviews the available literature on epidemiology, clinical presentation, diagnostic work up and evidence-based treatment options in bronchiectasis. Bronchiectasis is depicted as the result of a final common pathway of bacterial colonization, infection and an exaggerated inflammatory response, which may be present in a variety of disorders, mainly infection (pneumonia, tuberculosis) and immunodeficiency. In order to facilitate the finding of the underlying cause, a protocol-driven workup is proposed. We also propose a protocol-driven stepwise treatment schedule.

An overview of the available evidence on macrolide maintenance treatment in chronic inflammatory respiratory tract disorders and its mode of action is presented in **Chapters 3 and 4**.

The clinical efficacy and safety of long term azithromycin treatment in bronchiectasis was studied in the BAT trial, a multi-center, randomised clinical trial, described in **Chapter 5**.

The effect of long term macrolide treatment on radiological abnormalities and the correlation between CT findings and clinical parameters in bronchiectasis are addressed in **chapter 6**.

The relationship between azithromycin sputum and serum levels and its clinical efficacy during long-term treatment in bronchiectasis is explored in **chapter 7**. We correlate sputum concentrations of azithromycin with respiratory symptoms and inflammatory markers.

Chapter 8 reports on the validation of a newly developed tool for convenient symptom measurement in bronchiectasis patients. Validity, responsiveness and reliability of the *'Lower respiratory tract infections – visual analogue scale'* (LRTI-VAS) are assessed.

CHAPTER 2

Non-cystic fibrosis bronchiectasis: clinical presentation, diagnosis and treatment, illustrated by data from a Dutch Teaching Hospital.

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Abstract

This review article describes epidemiology, clinical presentation, diagnostic workup and treatment options in adult non-cystic fibrosis (non-CF) bronchiectasis (widening of mainly small and medium sized bronchi as seen on chest-CT scan). We illustrate evidence from the literature with our own data retrieved from chart review, involving 236 adult patients with recurrent lower respiratory tract infections and HRCT-proven bronchiectasis, who visited the outpatient clinic of respiratory diseases of a large Dutch teaching hospital between 2000 and 2010.

Bronchiectasis (BE) can be described as a final common pathway of a vicious cycle of exaggerated bronchial inflammation, bacterial colonization and infection. BE may arise from several causes, headed by infection and immunodeficiency and is clinically characterized by a chronic, productive cough and infectious exacerbations. Once BE is diagnosed using high resolution CT scanning, a protocol-driven work-up to identify the underlying cause is recommended.

Non-medicinal treatment options are primarily directed at clearance of bronchial secretions, which can further be improved by inhalation of hyperosmolar agents. Antibiotic treatment of exacerbations is a cornerstone medicinal treatment in bronchiectasis management. Patients with frequent exacerbations can be considered for long-term low dose macrolide treatment, supported by robust evidence. Inhaled antibiotics might be beneficial in selected patients colonized with Pseudomonas aeruginosa. Important developments in the last decade include the introduction of international guidelines and the proposition of a validated scoring system for disease severity.

Bronchiectasis patients are encountered by physicians in diverse medical professions and the disease itself is still underdiagnosed. The authors aim to increase awareness of the condition and provide practical tools for diagnosis and treatment.

Introduction

Bronchiectasis – characterized by irreversible, pathologic dilatation of the small and medium-sized bronchi- is not a disease in its own right, but rather a final common pathway of a vicious cycle of inflammation, bacterial colonization and infection. A variety of respiratory and systemic diseases may be complicated by pathological bronchial dilatation, and therefore various medical specialists will be dealing with the condition in one way or the other. Although general availability of CT-scans has importantly contributed to higher case-finding rates, bronchiectasis is still considered an underdiagnosed condition. In this article, we address different signs and symptoms which can be clues to the diagnosis in order to facilitate recognition of the disease among non-pulmonary physicians. We further discuss our preferred diagnostic approach and give an overview of evidence- based treatment options.

Cystic Fibrosis (CF), an inherited multi-system disorder, is usually discussed separately and here we focus on non-CF bronchiectasis - hereafter referred to as 'bronchiectasis'. The gold-standard for diagnosis has long been bronchography, until the introduction of high resolution CT scanning, the current standard diagnostic test. Due to the abundant amounts of purulent phlegm produced by affected individuals, bronchiectasis was considered offensive and also untreatable before the introduction of antimicrobial agents (1).

Around World War I bronchiectasis was common in the western world and it carried a poor prognosis: over 40% of all patients died of respiratory causes before the age of 40 (2-4). Improved socio-economic status, successful nationwide vaccination programmes for whooping cough and measles, and – most importantly – the availability of antibiotics reduced both incidence and mortality, in developed countries at least. Indeed, bronchiectasis became an 'orphan disease', as a result of which the focus of clinicians and researchers diverted away from this condition which was now considered rare with a relatively benign course. In spite of adequate antibiotic treatment, however, bronchiectasis still has the potential to cause substantial morbidity, including repeated lower respiratory infections complicated by hemoptysis, a disabling productive cough and shortness of breath, all of which importantly affect quality of life (2). Patients with bronchiectasis were found to spend more days in hospital and have higher annual medical care expenditure as compared to matched controls (3).

Recent epidemiological studies show a high incidence of bronchiectasis among New Zealand's and Australia's indigenous population and inhabitants of remote areas in Alaska (4). In the developed world estimated prevalence ranges from 0.42 per 100.000 in 18-34 year olds to 272 per 100.000 in those over 75 (5). Important developments in the last decade include the introduction of international guidelines, the proposition of a validated

scoring system for disease severity and the first large randomized trials on antibiotic maintenance treatment for those with frequent exacerbations, all of which will be discussed in this article (6-9).

We illustrate the evidence from the literature on the diagnosis and treatment of bronchiectasis using the experience gained in a large Dutch teaching hospital. Demographic, epidemiological and clinical data were collected from the entire, unselected, non-CF bronchiectasis cohort of the Alkmaar Medical Centre in 2010, for research purposes. Data were retrieved from chart review of all adult patients with recurrent lower respiratory tract infections and HRCT-proven non-CF bronchiectasis who visited the outpatient clinic of respiratory diseases of the Medical Centre Alkmaar at the time.

Pathophysiology

The mechanism of disease that eventually causes bronchiectasis is traditionally depicted as a vicious circle of exaggerated inflammation and bacterial colonization. Diverse stimuli, which can be either endogenic (such as ciliary defects) or exogenic (e.g. foreign body aspiration), may result in structural damage to the airways. This in turn allows for persistent bacterial colonization of the larger and medium-sized bronchi. The host inflammatory responses together with secreted bacterial toxins cause additional damage (hypersecretion, ciliary dysfunction and airway remodeling) which further weakens local resistance (10;11).

The immune response in bronchiectasis is mainly neutrophil driven and increased levels of chemokines and pro-inflammatory cytokines are found in the airways of affected individuals (12;13). High levels of proteases - toxic neutrophil products excreted on neutrophil activation - are present at the site of inflammation, causing release of pro-inflammatory cytokines and exerting proteolytic activity, thus causing even more damage to cells constituting the structure of the airways (14). T-cell infiltration, impaired macrophage phagocytosis, altered epithelial cell function and, more recently, deficiency of mannose binding lectine (MBL) have all been proposed as additional mechanisms responsible for an enhanced inflammatory response (11;15-18). A cycle of oxidative stress is also present, in which (mainly neutrophil derived-) reactive oxygen species cause damage to cells and the surrounding tissues and induce additional oxidative stress through activation of the inflammatory transcription factors nuclear factor-kappa B and activator protein-1 (19).

Causes

Bronchiectasis may arise from several different causes, headed by infection and immunodeficiency, mostly primary antibody deficiency syndromes (table 1). Due to successful prevention programmes for tuberculosis and childhood infections such as whooping cough and measles, post-infectious bronchiectasis tends to become less common in developed countries. In about half of patients, no underlying cause of permanent airway damage is found. Shoemark et al (20) found no causative factor in one third of their patients despite thorough systematic investigations in a tertiary referral centre. Other centres with multidisciplinary specialized bronchiectasis outpatient clinics with diagnostic protocols in place, report 40-50% idiopathic bronchiectasis in spite of an extensive workup (21-24).

Bronchiectasis is seen in 7-25% of patients with asthma or COPD, coinciding with more severe disease (25;26). While asthma has recently been considered a cause of bronchiectasis in the absence of other factors, the link between COPD and bronchiectasis has yet to be established (6).

The underlying cause for our cohort of patients is shown in table 1.

Table 1: *Aetiology of bronchiectasis in 236 patients visiting the out-patient department of the Alkmaar Medical Centre as compared to possible causes for bronchiectasis as found in non-CF bronchiectasis phenotyping studies and clinical trials, n (total) = 1535 (20-22;24;55;60)*

	Literature (n= 1535)	Alkmaar cohort (n=236)
Post infectious Non-tuberculous mycobacteria (NTM) Tuberculosis (TB) Pneumonia Childhood infections (e.g. pertussis, measles, adenovirus)	20- 38%	17.4%
Immunodeficiency *Primary* Hypogammaglobulinemia (CVID) X-linked agammaglobulinemia (XLA) *Secondary* Leukaemia HIV / AIDS Following chemotherapy or immunosuppressive therapy	3% - 24%	7.1%
Asthma Allergic bronchopulmonary aspergillosis (ABPA)	3-11 % 3-8%	11.4% 3.0%
Mechanical obstruction Tumor Corpus alienum Lymfadenopathy	0-1 %	0.4%
Sequelae of inhalation or aspiration Gastro-oesophageal reflux (GERD) Inhalation of toxic fumes	1% - 4%	2.5%

Table 1: *continued*

Auto-inflammatory conditions Rheumatoid Arthritis (RA) Sjögren's syndrome Systemic Lupus Erythematosus (SLE) Ulcerative Colitis or Crohn's disease	2% -3%	4.7%
Congenital conditions Cystic fibrosis (CF) α1 anti-trypsin deficiency Primary ciliary dyskinesia (PCD) Kartagener syndrome (situs inversus, chronic sinusitis, bronchiectasis) Mounier-Kuhn syndrome (tracheobronchomalacia) Williams–Campbell syndrome (cartilage deficiency)	1% - 18%	4.2%
Other uncommon aetiologies Yellow nail syndrome (yellow nails and lymphedema) Young's syndrome (sinusitis-infertility syndrome) Diffuse panbronchiolitis	1% - 3%	0.4%
Idiopathic	26% - 56%	47.9%

Clinical presentation and symptoms

The 'typical' patient with bronchiectasis is supposedly a middle-aged woman, who is a lifelong non-smoker – or at least, this is the profile of the majority of patients in bronchiectasis phenotyping studies (20-23;27;28). Our own data do not completely reflect this picture, as our patients were slightly older and,more frequently, smokers (table 2).This incongruence illustrates the varied clinical presentation of bronchiectasis patients in clinical practice. Bronchiectasis can just as well occur in the 80-yr-old male with frequent and virulent exacerbations of obstructive lung disease as in the 40-yr-old lady with rheumatoid arthritis visiting your practice with complaints of persisting cough. Severity of symptoms is different for each patient, but in general the course of the disease is highly variable, including nearly symptom-free periods interspersed with infectious exacerbations. The most persistent and often presenting symptom is a chronic productive cough, present in 96% of 103 patients referred to a pulmonary out-patient clinic, with the amount of sputum being among the main determinants of quality of life (2). Dyspnoea, fatigue and upper respiratory tract symptoms are encountered in 60-70% of patients. About half of the patients describe having specks of blood in their sputum at any time, but haemoptysis resulting in immediate medical consultation is present in a quarter of patients. Pleuritic or musculoskeletal chest pain is present in 25-50 of patients and chest pain is often the reason for repetitive investigations at emergency departments. Exacerbations are characterized by an increase in symptoms and signs suggesting lower respiratory tract infection. Physical examination is often unremarkable except for the presence of crackles, mostly bilateral at the lower lobes. (27;28).

Table 2: *Patient characteristics (n=236) from patients with recurrent lower respiratory tract infections and non-CF bronchiectasis in a large Dutch teaching hospital.*

Female sex - No (%)	154 (65.3)
Age - yr	65.7 (57.4 – 75.1)
Never smoker - No (%)	134 (56.8)
Current smoker - No (%)	15 (6.4)
FEV-1 - % of predicted	87 (66.0 – 103.0)
FVC - % of predicted	97 (79.0 – 110.0)
Age at first presentation – yr	58.3 (47.0 – 65.5)

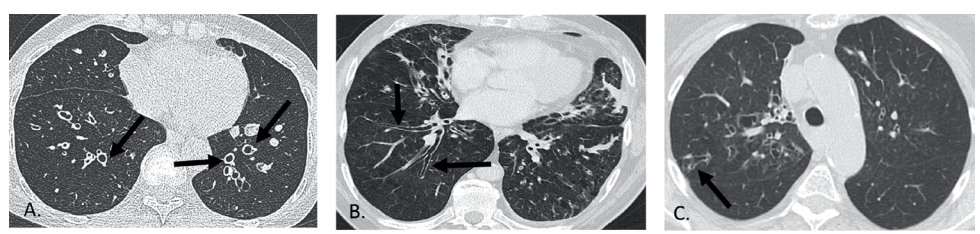
Continuous variables are presented as median (IQR). FEV-1: forced expiratory volume in the first second. FVC: forced vital capacity.

Diagnostic workup

Bronchiectasis ought to be considered in patients with a chronic productive cough and/or recurrent lower airway infections, especially when these symptoms are present in younger, non-smoking individuals. Hemoptysis, recurrent para-nasal sinus infections or successive sputum cultures positive for S aureus or P aeruginosa may also be clues leading to the diagnosis. In patients with asthma or COPD, bronchiectasis should be considered in case of frequent, slow resolving exacerbations, unstable or medication resistant asthma or severe symptoms despite limited exposure to smoking in patients diagnosed with COPD (6).

Key to the diagnosis are imaging studies using high resolution-CT. The chest CT protocol should be a spiral CT with 1 mm slices, able to detect pathology of larger and smaller airways, preferably with software allowing for reconstruction in different planes. In patients with bronchiectasis, the HRCT typically shows a distorted ratio (> 1,0) of the inner bronchial diameter as compared to the accompanying artery, and signs of bronchial dilatation: lack of tapering and increased visibility of small airways in the sub-pleural region (29) (fig 1). Plain chest X-rays show abnormalities in a large proportion of bronchiectasis patients (66% of our cohort), but changes are non-specific and a irremarkable chest X-ray does not rule out bronchiectasis.

Figure 1. *CT scans of bronchiectasis three patients showing typical radiological features of bronchiectasis: A. Increased bronchial diameter (signet ring sign) in a patient with allergic bronchopulmonary aspergillosis. B. Lack of tapering in a patient with COPD complicated by bronchiectasis. C. Increased visibility of small airways in the subpleural region in a patient with rheumatoid arthritis-associated bronchiectasis.*



In symptomatic patients, the radiological finding of bronchiectasis should be followed by investigations to reveal the underlying cause. If a standardized protocol is used, the diagnostic yield may be enhanced, resulting not only in reduction of the proportion of patients diagnosed with 'idiopathic' bronchiectasis, but even in changing the treatment and the prognosis in up to 50% of patients (30;31). We use a diagnostic algorithm based on national and international guidelines (6;32;33) (fig 2). A standardized workup has been shown to reduce diagnostic delay which could last up to several years, especially in patients with underlying immune deficiency (34).

Localized bronchiectasis is usually indicative of a local mechanical cause (e.g middle lobe syndrome) or post-infectious damage. The latter is even more plausible when a clear temporal relationship exists between an infectious episode and development of bronchiectasis-related symptoms. In other subjects, bronchiectasis can occur as a symptom of an already identified disease, such as rheumatoid arthritis or inflammatory bowel disease. In such cases we suggest to refrain from extensive investigations - or to only resort to additional testing if unexplained deteriorations occur. The same holds true for patients with asymptomatic bronchiectasis, as for instance can be seen in stable fibrosis (traction bronchiectasis).

Treatment options

When a specific disorder is found to cause bronchiectasis, disease management should primarily be directed at the underlying cause. This, for instance, applies to bronchiectasis due to allergic bronchopulmonary aspergillosis (ABPA) or common variable immune deficiency (CVID) both requiring their own treatment regimens.

Bronchiectasis management is aimed at preventing disease progression and improving quality of life by reducing symptoms and exacerbations. This includes treatment of exacerbations and optimal airway clearance, complemented with long-term antibiotic therapy (oral or nebulized) or surgery in selected cases. Many treatment options for non-CF bronchiectasis are derived from the treatment regimens developed for cystic fibrosis. At first, treatment modalities were simply extrapolated to non-CF patients, but in the last decade, treatment modalities have been studied for this specific group of patients, resulting in evidence-guided treatment recommendations. Sometimes these recommendations contradict those for CF, as is true for mucolytic treatment with recombinant human DNAse (rhDNAse). Routinely used in CF-treatment, rhDNAse has been found of no benefit in one trial of non-CF bronchiectasis and harmful in another (35). No sufficient evidence is available to support the use of other mucolytics, such as acetylcysteine, in non-CF patients. Inhaled corticosteroids – although widely used by non-specialists in non-CF bronchiectasis patients

– were only found effective in patients with underlying asthma. Current guidelines advise against routine use in non-CF bronchiectasis (6).

Nevertheless, it is worth mentioning that the pharmacological options described below - such as macrolides or inhaled hyperosmolar agents - have been approved by neither the US Food and Drug Authorization nor the European Drug regulators. Use is solely based on outcomes of clinical trials and international guidelines.

Management of infectious exacerbations

One of the cornerstones of bronchiectasis management is antibiotic treatment of infectious exacerbations. There are no randomized trials evaluating the effect or the duration of antibiotic treatment in bronchiectasis, but antibiotics are generally thought to reduce the time to recovery and to reduce symptoms. By convention, a 14-day-course of antimicrobials is prescribed, either intravenously or orally for exacerbations that last several days at least and are accompanied by increased sputum purulence, volume or reduced viscosity and increased cough, dyspnea and systemic upset such as fatigue or fever (6).

Preceding antibiotic treatment, sputum samples should be submitted for microbiological investigation and therapy should be directed at previously or newly isolated pathogens.

Physiotherapy

Most patients with bronchiectasis, especially those with excessive secretions, are offered physiotherapy. A customary physiotherapy program in the Netherlands would include one or more techniques directed at improved clearance of broncho-pulmonary secretions, combined with a pulmonary rehabilitation program to improve exercise tolerance. Forced expiratory manoeuvres as well as hand-held devices generating positive expiratory pressure ('pep'-devices) such as Flutter™ or Acapella™ are used for optimal sputum clearance. A recent randomized trial, evaluating a similar approach , demonstrated a beneficial effect on exercise capacity, dyspnoea and fatigue in 85 patients (36).

A Cochrane review, evaluating the effect of physiotherapy-taught airway clearance techniques (ACT) as compared to no therapy or active coughing, demonstrated small improvements in sputum expectoration, lung function and health-related quality of life in 5 small and diverse studies, involving 51 patients (37). The choice of an ACT might as well be guided by patient preference, since there is no clear evidence in favor of any of the ACT's available. A small randomized study in 30 patients showed improved exercise tolerance and

health related QOL with pulmonary rehabilitation in addition to ACT as compared to ACT alone (38).

Inhalation of hyperosmolar agents

Due to impaired mucociliairy clearance, many patients with bronchiectasis suffer from mucus hypersecretion and retention, leading to dyspnea, chronic cough and increased susceptibility to infections. We frequently use inhalation of isotonic (0.9%) or hypertonic saline (6-7%) bid in addition to airway clearance techniques for optimal sputum evacuation. No evident benefit of nebulized hypertonic saline over isotonic saline has yet been demonstrated in the small studies available (39) and in our experience, patients report less discomfort in terms of wheezing or dyspnea when using the isotonic solution. Nevertheless, the inhalation process itself is often experienced as time consuming and inconvenient.

The hyperosmolar agent mannitol reduces exacerbations and improves lung function in cystic fibrosis (40). When administered as dry powder through a purpose-designed inhaler device, it is proposed as a less cumbersome alternative to saline inhalation. Several smaller or short-term studies on mannitol inhalations in bronchiectasis yield conflicting results in terms of sputum expectoration and quality of life (39). The sole large - yet slightly underpowered - long-term trial of 400 mg mannitol bd vs a non-therapeutic dose of 50 mg demonstrated that inhaled mannitol increases the time until first exacerbation in patients with bronchiectasis, without improving respiratory quality of life or reducing actual exacerbation rates (41). Mannitol is known for inducing bronchospasm. It is worth noting that ll participants in two large clinical trials were screened for mannitol tolerance at baseline and excluded when mannitol-induced bronchospasm was present (in 16% of all screened subjects). In the other participants mannitol inhalations were safe and well-tolerated (41;42). In the Netherlands, dry powder mannitol (Bronchitol™) is primarily used for optimizing sputum expectoration in cystic fibrosis patients and is not registered for use in other patient groups.

Long-term antibiotic treatment

Treatment with maintenance antibiotics in bronchiectasis can bedirected at simply reducing the increased bacterial load, since chronic colonization has been found to coincide with enhanced inflammation and worse clinical outcome. In case of macrolides it is thought to dampen the exaggerated inflammatory response through multiple pathways (43).

Macrolides

Macrolides, because of their anti-bacterial and anti-inflammatory properties, have long been thought ideal to intervene in the vicious circle of infection and inflammation that underlies bronchiectasis. In three different clinical trials evaluating long-term oral macrolide treatment, exacerbation frequency was significantly reduced. All trials used different dosing régimes and there is ongoing debate on which schedule should be used. Traditionally, many physicians use a dosing schedule equivalent to the CF treatment schedules consisting of azithromycin either 500 mg thrice weekly or 250 mg daily. Similar schedules were used in the BAT- and EMBRACE trials, as opposed to the Australian BLESS-trial which used erythromycin 400 mg twice daily (7-9). In CF patients macrolide antibiotics, and in particular azithromycin, tend to cumulate inside alveolar macrophages and as such have an extended half-life. Based on the pharmacokinetic properties of azithromycin in CF-patients - whose kinetics may differ considerably from those with non-CF - dose levels of 22-30 mg/kg/week divided by 1-7 dosing moments, are proposed (44). Lung function improvement and enhanced quality of life were most distinct in patients with frequent exacerbations. Recent COPD trials show a tendency to a higher yield of macrolide treatment in patients with more exacerbations (45). Although bronchiectasis guidelines consider patients with three or more exacerbations yearly and suffering from chronic symptoms to be candidates for this treatment type, no robust evidence is yet available to justify abstention from macrolide treatment in less frequent exacerbators (7-9).

Benefits of macrolide treatment come with a considerable increase in macrolide resistant pathogens, which demands judicious use of long-term macrolide therapy.

Inhaled antibiotics

Since the late nineties nebulized antibiotics for reducing airway bacterial load have been considered as a treatment option in bronchiectasis. Higher bacterial load is found to coincide with augmented systemic inflammation and an increased morbidity (46). Due to the favorable pharmacokinetic profile of inhaled substances, with minimal systemic drug delivery, systemic adverse effects are mild (47;48). Local, non-severe side effects are frequently encountered in clinical trials with inhaled antibiotics (49). Iinhalation-induced bronchospasm could pose an extra challenge in clinical practice, but is usually overcome through inhalation of a short-acting beta-2 agonist prior to inhalation of antibiotics. Most randomized clinical trials evaluating inhaled antimicrobial agents included bronchiectasis patients colonized with Pseudomonas aeruginosa (PA) and used different types of antibiotics (colomycin, tobramycin), amikacin, or ciprofloxacin) (50-54). In addition, the three distinct trials (using aztreonam, gentimicin and ciprofloxacin), which did not specifically require PA

colonization for inclusion, in fact included many patients with PA-colonization at baseline (48-85%) (49;55;56). Alltrials demonstrated bacterial load reduction in the airways of actively treated patients, but this effect does not correspond consistently with improvement in clinical endpoints (57). The largest trial (n=500) of inhaled aztreonam in bronchiectasis patients - 85% of which PA colonized - failed to demonstrate reduced exacerbation rates or improved quality of life (49). Other authors report prolonged time to exacerbation and improved health related quality of life as secondary findings. The attractive safety profile and encouraging results in some studies have stimulated further research in this field and momentarily no less than seven trials are recruiting patients, most of which studying inhaled ciprofloxacin (58). Awaiting further evidence we think that nebulized antibiotics offer a reasonable alternative to oral treatment in selected patients colonized with P aeruginosa.

Other non-pharmacological options, such as surgery for localized disease and bronchial artery embolization in case of massive hemoptysis will not be discussed here in detail.

Prognosis

Although bronchiectasis can cause considerable morbidity, prognosis in terms of survival is favourable. The largest prospective study up until now found 62 deaths in 608 patients (10.2%) within 4 years, but the majority of deaths (81%) occurred above the age of 70 (59). Independent predictors of mortality were older age, low FEV-1, prior hospitalization and 3 or more exacerbations in the year prior to the study. The authors used these data to compile and validate a clinical prediction tool, the Bronchiectasis Severity Index, which divides patients in three risk groups (low/ intermediate/ high) in order to predict mortality, hospital admissions and exacerbations.
This tool could be very useful in research settings in order to increase homogeneity of study populations. Its value for directing therapy in a clinical setting needs yet to be proven.

In conclusion, the broad range of diseases that cause or coincide with bronchiectasis, make it a frequently encountered entity in various medical specialisations. The authors hope that this article will renew awareness of this still underdiagnosed condition. Exciting new developments are the publication of high quality, randomised studies and new tools for patient selection which are important steps towards improving bronchiectasis management.

Figure 2. Diagnostic workup in adult bronchiectasis patients

1. Symptomatic patients with HRCT-confirmed bronchiectasis:

Medical history:

- ☐ Childhood respiratory symptoms
- ☐ Recurrent or severe upper- or lower respiratory tract infections
- ☐ Other serious or persistent infections (e.g. skin)
- ☐ Symptoms of gastro-oesophageal reflux disease or aspiration
- ☐ Inhalation of noxious gases
- ☐ Infertility, features of malabsorption
- ☐ Symptoms of connective tissue disorder or inflammatory bowel disease.
- ☐ Family history of immune deficiency

Clinical assessment:

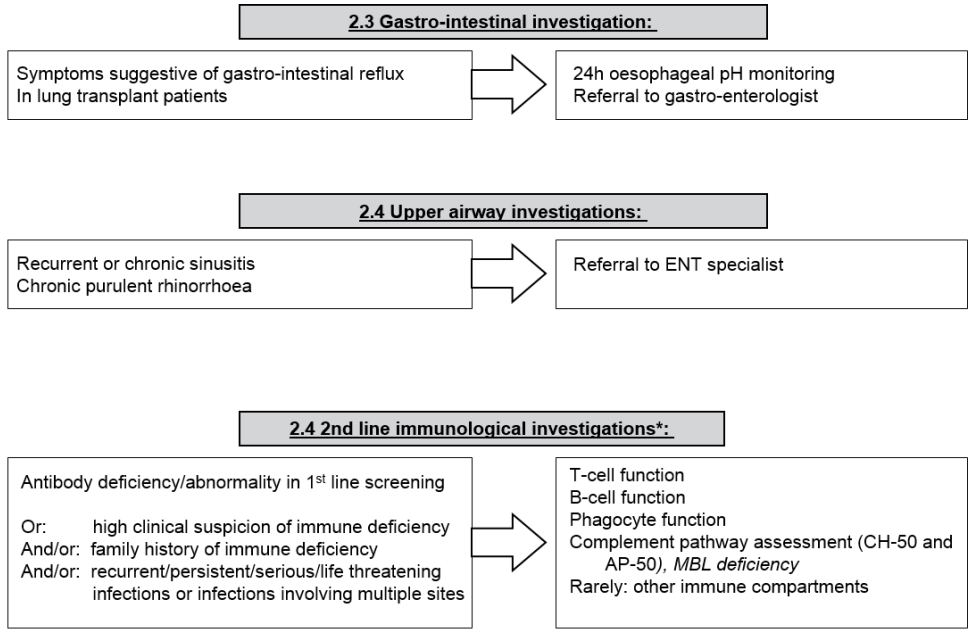
- ☐ Auscultation of heart and lungs
- ☐ Check specifically for: discolouration of nails, arthritis, skin abnormalities.

Investigations:

- ☐ Full blood count, white cell differential, ESR, CRP, routine biochemistry.
- ☐ Reumatoid factor, anti-CCP
- ☐ Serum immunoglobulins (IgA, IgG, IgM)
- ☐ Serum total IgE, Serum IgE and IgG to *A fumigatus* .
- ☐ Lung function measurement (spirometry)
- ☐ Chest X-ray
- ☐ 3 sputum cultures for microbiology, including fungal and mycobacterial cultures.
- ☐ **In idiopathic bronchiectasis or high clinical suspicion of immune deficiency** :
  Adequacy of antibody responses to specific antigen challenge

2. Additional investigations

Patient characteristics	Test of choice
2.1 Additional lung function tests:	
Airflow obstruction And/or: History of smoking	Measurement of lung volumes and gas transfer coefficient Reversibility testing
2.2 Bronchoscopy:	
Localised disease And/or: Suggestion of mycobacterial infection on HRCT and negative sputum cultures. And/ or: Treatment resistant infections and no growth of pathogens in serial sputum cultures.	Bronchoscopy + cultures for microbiology



Reference List

1. Nicotra MB. Bronchiectasis. Semin Respir Infect 1994 Mar;9(1):31-40.
2. Martinez-Garcia MA, Perpina-Tordera M, Roman-Sanchez P, Soler-Cataluna JJ. Quality-of-life determinants in patients with clinically stable bronchiectasis. Chest 2005 Aug;128(2):739-45.
3. Derek Weycker, John Edelsberg, Gerry Oster, Gregory Tino. Prevalence and Economie Burden of Bronchiectasis. Clin Pulm Med 2005 Jan 7;12(4).
4. Chang AB, Bell SC, Byrnes CA, Grimwood K, Holmes PW, King PT, et al. Chronic suppurative lung disease and bronchiectasis in children and adults in Australia and New Zealand. Med J Aust 2010 Sep 20;193(6):356-65.
5. Seitz AE, Olivier KN, Adjemian J, Holland SM, Prevots R. Trends in bronchiectasis among medicare beneficiaries in the United States, 2000 to 2007. Chest 2012 Aug;142(2):432-9.
6. Pasteur MC, Bilton D, Hill AT. British Thoracic Society guideline for non-CF bronchiectasis. Thorax 2010 Jul;65 Suppl 1:i1-58.
7. Altenburg J, de Graaff CS, Stienstra Y, Sloos JH, van Haren EH, Koppers RJ, et al. Effect of azithromycin maintenance treatment on infectious exacerbations among patients with non-cystic fibrosis bronchiectasis: the BAT randomized controlled trial. JAMA 2013 Mar 27;309(12):1251-9.
8. Wong C, Jayaram L, Karalus N, Eaton T, Tong C, Hockey H, et al. Azithromycin for prevention of exacerbations in non-cystic fibrosis bronchiectasis (EMBRACE): a randomised, double-blind, placebo-controlled trial. Lancet 2012 Aug 18;380(9842):660-7.
9. Serisier DJ, Martin ML, McGuckin MA, Lourie R, Chen AC, Brain B, et al. Effect of long-term, low-dose erythromycin on pulmonary exacerbations among patients with non-cystic fibrosis bronchiectasis: the BLESS randomized controlled trial. JAMA 2013 Mar 27;309(12):1260-7.
10. Cole PJ. Inflammation: a two-edged sword--the model of bronchiectasis. Eur J Respir Dis Suppl 1986;147:6-15.
11. Chalmers JD, Hill AT. Mechanisms of immune dysfunction and bacterial persistence in non-cystic fibrosis bronchiectasis. Mol Immunol 2013 Aug;55(1):27-34.
12. Bergin DA, Hurley K, Mehta A, Cox S, Ryan D, O'Neill SJ, et al. Airway inflammatory markers in individuals with cystic fibrosis and non-cystic fibrosis bronchiectasis. J Inflamm Res 2013;6:1-11.
13. Hsieh MH, Fang YF, Chen GY, Chung FT, Liu YC, Wu CH, et al. The role of the high-sensitivity C-reactive protein in patients with stable non-cystic fibrosis bronchiectasis. Pulm Med 2013;2013:795140.
14. Fuschillo S, De FA, Balzano G. Mucosal inflammation in idiopathic bronchiectasis: cellular and molecular mechanisms. Eur Respir J 2008 Feb;31(2):396-406.
15. Tan HL, Regamey N, Brown S, Bush A, Lloyd CM, Davies JC. The Th17 pathway in cystic

fibrosis lung disease. Am J Respir Crit Care Med 2011 Jul 15;184(2):252-8.
16. Watt AP, Brown V, Courtney J, Kelly M, Garske L, Elborn JS, et al. Neutrophil apoptosis, proinflammatory mediators and cell counts in bronchiectasis. Thorax 2004 Mar;59(3):231-6.
17. Chalmers JD, McHugh BJ, Doherty C, Smith MP, Govan JR, Kilpatrick DC, et al. Mannose-binding lectin deficiency and disease severity in non-cystic fibrosis bronchiectasis: a prospective study. Lancet Respir Med 2013 May;1(3):224-32.
18. Chan SC, Shum DK, Tipoe GL, Mak JC, Leung ET, Ip MS. Upregulation of ICAM-1 expression in bronchial epithelial cells by airway secretions in bronchiectasis. Respir Med 2008 Feb;102(2):287-98.
19. Wong C, Jones S. Oxidative stress and macrolides in bronchiectasis--exhaling few clues. Respirology 2013 Oct;18(7):1037-8.
20. Shoemark A, Ozerovitch L, Wilson R. Aetiology in adult patients with bronchiectasis. Respir Med 2007 Jun;101(6):1163-70.
21. Pasteur MC, Helliwell SM, Houghton SJ, Webb SC, Foweraker JE, Coulden RA, et al. An investigation into causative factors in patients with bronchiectasis. Am J Respir Crit Care Med 2000 Oct;162(4 Pt 1):1277-84.
22. Loebinger MR, Wells AU, Hansell DM, Chinyanganya N, Devaraj A, Meister M, et al. Mortality in bronchiectasis: a long-term study assessing the factors influencing survival. Eur Respir J 2009 Oct;34(4):843-9.
23. Anwar GA, McDonnell MJ, Worthy SA, Bourke SC, Afolabi G, Lordan J, et al. Phenotyping adults with non-cystic fibrosis bronchiectasis: a prospective observational cohort study. Respir Med 2013 Jul;107(7):1001-7.
24. Martinez-Garcia MA, de GJ, Vendrell RM, Giron R, Maiz CL, de la Rosa CD, et al. Multidimensional approach to non-cystic fibrosis bronchiectasis. The FACED score. Eur Respir J 2013 Nov 14.
25. Patel IS, Vlahos I, Wilkinson TM, Lloyd-Owen SJ, Donaldson GC, Wilks M, et al. Bronchiectasis, exacerbation indices, and inflammation in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2004 Aug 15;170(4):400-7.
26. Gono H, Fujimoto K, Kawakami S, Kubo K. Evaluation of airway wall thickness and air trapping by HRCT in asymptomatic asthma. Eur Respir J 2003 Dec;22(6):965-71.
27. King PT, Holdsworth SR, Freezer NJ, Villanueva E, Holmes PW. Characterisation of the onset and presenting clinical features of adult bronchiectasis. Respir Med 2006 Dec;100(12):2183-9.
28. Nicotra MB, Rivera M, Dale AM, Shepherd R, Carter R. Clinical, pathophysiologic, and microbiologic characterization of bronchiectasis in an aging cohort. Chest 1995 Oct;108(4):955-61.
29. van der Bruggen-Bogaarts BA, van der Bruggen HM, van Waes PF, Lammers JW. Assessment of bronchiectasis: comparison of HRCT and spiral volumetric CT. J Comput Assist Tomogr 1996 Jan;20(1):15-9.
30. Pasteur MC, Helliwell SM, Houghton SJ, Webb SC, Foweraker JE, Coulden RA, et al. An investigation into causative factors in patients with bronchiectasis. Am J Respir Crit Care Med 2000 Oct;162(4 Pt 1):1277-84.
31. Li AM, Sonnappa S, Lex C, Wong E, Zacharasiewicz A, Bush A, et al. Non-CF bronchiectasis: does knowing the aetiology lead to changes in management? Eur Respir J 2005 Jul;26(1):8-14.
32. Nederlandse Vereniging van Artsen voor Longziekten en Tuberculose. Diagnostiek en antimicrobiële behandeling van recidiverende lagere luchtweginfecties. Alphen aan den Rijn: Van Zuiden Communications B.V.; 2005.
33. de VE. Patient-centred screening for primary immunodeficiency: a multi-stage diagnostic protocol designed for non-immunologists. Clin Exp Immunol 2006 Aug;145(2):204-14.
34. Gathmann B, Mahlaoui N, Gerard L, Oksenhendler E, Warnatz K, Schulze I, et al. Clinical picture and treatment of 2212 patients with common variable immunodeficiency. J Allergy Clin Immunol 2014 Jul;134(1):116-26.
35. Wilkinson M, Sugumar K, Milan SJ, Hart A, Crockett A, Crossingham I. Mucolytics for bronchiectasis. Cochrane Database Syst Rev 2014;5:CD001289.
36. Lee AL, Hill CJ, Cecins N, Jenkins S, McDonald CF, Burge AT, et al. The short and long term effects of exercise training in non-cystic fibrosis bronchiectasis--a randomised controlled trial. Respir Res 2014;15:44.
37. Lee AL, Burge A, Holland AE. Airway clearance techniques for bronchiectasis. Cochrane Database Syst Rev 2013;5:CD008351.
38. Mandal P, Sidhu MK, Kope L, Pollock W, Stevenson LM, Pentland JL, et al. A pilot study of pulmonary rehabilitation and chest physiotherapy versus chest physiotherapy alone in bronchiectasis. Respir Med 2012 Dec;106(12):1647-54.
39. Hart A, Sugumar K, Milan SJ, Fowler SJ, Crossingham I. Inhaled hyperosmolar agents for bronchiectasis. Cochrane Database Syst Rev 2014;5:CD002996.
40. Bilton D, Bellon G, Charlton B, Cooper P, De BK, Flume PA, et al. Pooled analysis of two large randomised phase III inhaled mannitol studies in cystic fibrosis. J Cyst Fibros 2013 Jul;12(4):367-76.
41. Bilton D, Tino G, Barker AF, Chambers DC, De SA, Dupont LJ, et al. Inhaled mannitol for non-cystic fibrosis bronchiectasis: a randomised, controlled trial. Thorax 2014 Dec;69(12):1073-9.
42. Bilton D, Daviskas E, Anderson SD, Kolbe J, King G, Stirling RG, et al. Phase 3 randomized study of the efficacy and safety of inhaled dry powder mannitol for the symptomatic treatment of non-cystic fibrosis bronchiectasis. Chest 2013 Jul;144(1):215-25.
43. Altenburg J, de Graaff CS, van der Werf TS, Boersma WG. Immunomodulatory effects of macrolide antibiotics - part 1: biological mechanisms. Respiration 2011;81(1):67-74.

44. Wilms EB, Touw DJ, Heijerman HG. Pharmacokinetics of azithromycin in plasma, blood, polymorphonuclear neutrophils and sputum during long-term therapy in patients with cystic fibrosis. Ther Drug Monit 2006 Apr;28(2):219-25.
45. Uzun S, Djamin RS, Kluytmans JA, Mulder PG, van't Veer NE, Ermens AA, et al. Azithromycin maintenance treatment in patients with frequent exacerbations of chronic obstructive pulmonary disease (COLUMBUS): a randomised, double-blind, placebo-controlled trial. Lancet Respir Med 2014 May;2(5):361-8.
46. Chalmers JD, Smith MP, McHugh BJ, Doherty C, Govan JR, Hill AT. Short- and long-term antibiotic treatment reduces airway and systemic inflammation in non-cystic fibrosis bronchiectasis. Am J Respir Crit Care Med 2012 Oct 1;186(7):657-65.
47. Stass H, Weimann B, Nagelschmitz J, Rolinck-Werninghaus C, Staab D. Tolerability and pharmacokinetic properties of ciprofloxacin dry powder for inhalation in patients with cystic fibrosis: a phase I, randomized, dose-escalation study. Clin Ther 2013 Oct;35(10):1571-81.
48. Barker AF, Couch L, Fiel SB, Gotfried MH, Ilowite J, Meyer KC, et al. Tobramycin solution for inhalation reduces sputum Pseudomonas aeruginosa density in bronchiectasis. Am J Respir Crit Care Med 2000 Aug;162(2 Pt 1):481-5.
49. Barker AF, O'Donnell AE, Flume P, Thompson PJ, Ruzi JD, de GJ, et al. Aztreonam for inhalation solution in patients with non-cystic fibrosis bronchiectasis (AIR-BX1 and AIR-BX2): two randomised double-blind, placebo-controlled phase 3 trials. Lancet Respir Med 2014 Sep;2(9):738-49.
50. Couch LA. Treatment With tobramycin solution for inhalation in bronchiectasis patients with Pseudomonas aeruginosa. Chest 2001 Sep;120(3 Suppl):114S-7S.
51. Drobnic ME, Sune P, Montoro JB, Ferrer A, Orriols R. Inhaled tobramycin in non-cystic fibrosis patients with bronchiectasis and chronic bronchial infection with Pseudomonas aeruginosa. Ann Pharmacother 2005 Jan;39(1):39-44.
52. Orriols R, Roig J, Ferrer J, Sampol G, Rosell A, Ferrer A, et al. Inhaled antibiotic therapy in non-cystic fibrosis patients with bronchiectasis and chronic bronchial infection by Pseudomonas aeruginosa. Respir Med 1999 Jul;93(7):476-80.
53. Serisier DJ, Bilton D, De SA, Thompson PJ, Kolbe J, Greville HW, et al. Inhaled, dual release liposomal ciprofloxacin in non-cystic fibrosis bronchiectasis (ORBIT-2): a randomised, double-blind, placebo-controlled trial. Thorax 2013 Sep;68(9):812-7.
54. Haworth CS, Foweraker JE, Wilkinson P, Kenyon RF, Bilton D. Inhaled colistin in patients with bronchiectasis and chronic Pseudomonas aeruginosa infection. Am J Respir Crit Care Med 2014 Apr 15;189(8):975-82.
55. Murray MP, Govan JR, Doherty CJ, Simpson AJ, Wilkinson TS, Chalmers JD, et al. A randomized controlled trial of nebulized gentamicin in non-cystic fibrosis bronchiectasis. Am J Respir Crit Care Med 2011 Feb 15;183(4):491-9.
56. Wilson R, Welte T, Polverino E, De SA, Greville H, O'Donnell A, et al. Ciprofloxacin dry powder for inhalation in non-cystic fibrosis bronchiectasis: a phase II randomised study. Eur Respir J 2013 May;41(5):1107-15.
57. Brodt AM, Stovold E, Zhang L. Inhaled antibiotics for stable non-cystic fibrosis bronchiectasis: a systematic review. Eur Respir J 2014 Aug;44(2):382-93.
58. Rubin BK, Williams RW. Aerosolized antibiotics for non-cystic fibrosis bronchiectasis. Respiration 2014;88(3):177-84.
59. Chalmers JD, Goeminne P, Aliberti S, McDonnell MJ, Lonni S, Davidson J, et al. The bronchiectasis severity index. An international derivation and validation study. Am J Respir Crit Care Med 2014 Mar 1;189(5):576-85.
60. Goeminne PC, Nawrot TS, Ruttens D, Seys S, Dupont LJ. Mortality in non-cystic fibrosis bronchiectasis: a prospective cohort analysis. Respir Med 2014 Feb;108(2):287-96.

CHAPTER 3

Immunomodulatory effects of macrolide antibiotics – part 1: biological mechanisms.

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Respiration.
2011;81(1):67-74

Abstract

Macrolide antibiotics are well known for their antibacterial and anti-inflammatory properties. This article provides an overview of the biological mechanisms through which macrolides exert this 'double effect'. Their anti-bacterial effect consists of inhibition of bacterial protein synthesis, impaired bacterial biofilm synthesis and attenuation of other bacterial virulence factors. Apart from these direct anti-microbial effects, macrolides are known for their modulating effect on many components of the human immune-system. By influencing the production of cytokines, they have a dampening effect on the pro-inflammatory response. Furthermore, the majority of cells, involved in the immune-response, are, one way or the other, influenced when macrolide antibiotics are administered. Having such an obvious effect on the various aspects of the immune system macrolides seem to be exceptionally suited for the treatment of chronic inflammatory diseases.

Introduction

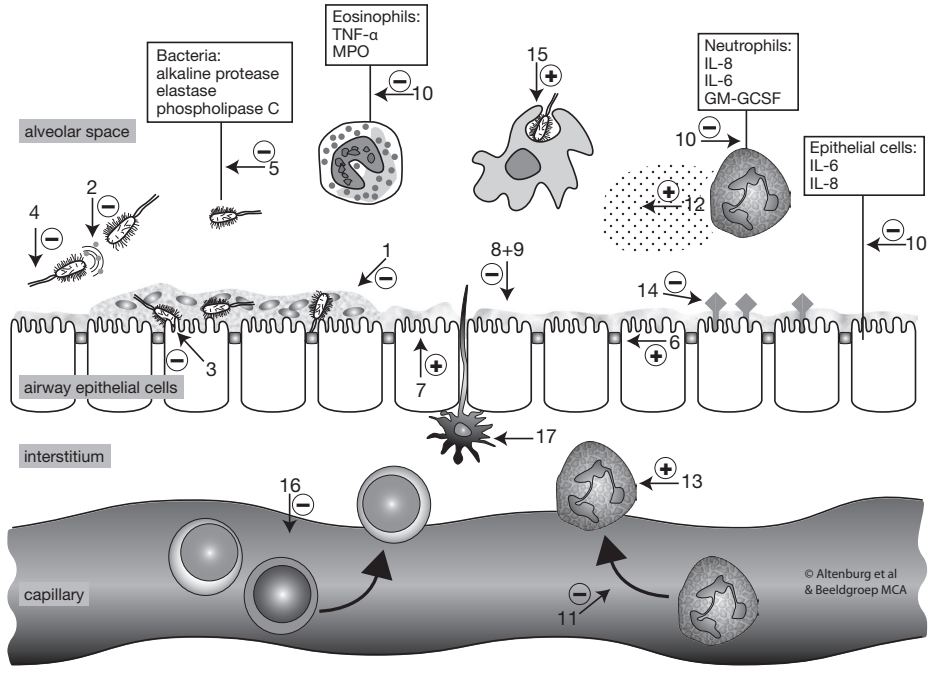
Since their discovery in 1952, many beneficial effects have been attributed to antibiotics belonging to the macrolide family, originally isolated from cultures of *Streptomyces erytraea*. Macrolide antibiotics were named after their main characteristic; a macrocyclic lactone ring which can contain up to 23 atoms [1]. The most commonly used macrolides have 14 (e.g. erythromycin, clarithromycin, roxithromycin) or 15 (e.g azithromycin) atoms attached to their macrocyclic rings and are therefore defined as 14- or 15-membered ring macrolides. Over the last decades macrolide antibiotics have been used as a treatment for common infectious diseases like pneumonia, bronchitis, pharyngitis or skin infections, possessing a moderately broad spectrum of antibacterial activity.

An accumulating body of evidence has emerged, indicating that 14- and 15 membered ring macrolides possess modes of action independent of their antimicrobial activity. This became first known in 1987, when Kudoh and colleagues [2] reported a spectacular decrease in symptoms and increase in life expectancy in patients with diffuse panbronchiolitis (DPB) when they were treated with the macrolide erythromycin. Until then, DPB had been a rapidly progressive and debilitating inflammatory airway disorder carrying a very poor prognosis. After 1987, when erythromycin was introduced as standard therapy for DPB, an impressive increase of 10-year survival was seen; from 10-20% to more than 90% [3-6].

The unexpected success was attributed to a previously unknown anti-inflammatory effect of erythromycin. This theory was supported by the fact that serum levels of erythromycin in these DPB-patients were well below minimal inhibitory concentrations (MIC) for the detected pathogens and the known lack of susceptibility of most gram-negative organisms to erythromycin.

Exhaustive evidence has shown that macrolides indeed have a direct anti-microbial effect, but, more importantly, also modulate many components of the immune-response. Because of this anti-inflammatory or 'immune modulating' effect, macrolide antibiotics have been widely used as maintenance treatment for various chronic inflammatory pulmonary diseases. Chronic inflammatory diseases generally feature a distorted inflammatory response. Instead of protecting the human body against exogenous attacks, the cascade of anti-inflammatory responses fails, damaging cells and making them more vulnerable to new attacks. In this article we aim to clarify the biological mechanisms through which macrolides exert their immune-modulating and anti-bacterial effect. These mechanisms are shown schematically in figure 1.

Figure 1: Cellular and non-cellular effects of macrolides



1. Attenuation of biofilm function
2. Suppression of bacterial quorum sensing
3. Decrease of bacterial adherence
4. Loss of flagellar mobility
5. Reduced production of bacterial toxins
6. Consolidation of epithelial tight junctions
7. Increasing ciliary beat frequency
8. Reduction of sputum quantity
9. Deminished sputum viscosity
10. Inhibition of synthesis of pro-inflammatory agents by bacteria, eosinophlis, neutrophils and epithelial cells.
11. Reduction of neutrophil chemotaxis
12. Stimulation of neutrophil degranulation
13. Acceleration of neutrophil apoptosis
14. Down-regulation of adhesion molecules
15. Stimulation of phagocytosis by alveolar macrophages
16. Reduction of T-cell numbers and T-cell migration
17. Modulation of dendritic cell function

1. Effects on host-pathogen interactions

Most macrolides are active against gram-positive cocci (including anaerobes) and have limited gram-negative activity. They inhibit bacterial protein synthesis by binding to the 50S subunit of the ribosome [1,7,8]

Biofilm

A biofilm is an aggregate of micro-organisms immersed in a polysaccharide matrix, adherent to each other and to the airway mucosa. Biofilm-forming bacteria are protected from phagocytosis, antimicrobial agents and the ciliary action of the airway epithelial cells. Furthermore, micro-organisms gathered in a biofilm develop significantly different genetic properties, compared to planktonic species. Research on biofilm effects of macrolides mainly focuses on *Pseudomonas aeruginosa* (PA), being one of the more virulent biofilm-forming micro-organisms with a natural resistance to macrolides.

Effects of macrolides however, were also demonstrated on biofilm formation in *H influenzae* and *S epidermidis* [9,10]. Macrolides were shown to alter the structure and architecture of the bacterial biofilm [11-13]. Results of Japanese in vitro studies indicate that azithromycin and clarithromycin change the structure of bacterial biofilms via inhibition of polysaccharide synthesis [12,14]. An insufficient biofilm allows for enhanced phagocytosis and clearance of bacteria by alveolar macrophages [11,15].

Quorum sensing

During infection, bacteria employ mutual communication (quorum sensing [QS]) to coordinate the expression of genes, e.g. genes encoding for tissue-damaging factors [16]. Through production of auto-inducer molecules, genes can be switched on or off, depending on local pathogen density. Furthermore, activation of the QS cascade is claimed to promote biofilm formation and to stimulate IL-8 production, causing enhanced neutrophil influx at the site of infection [6]. Several authors suggest that suppression of QS-systems, through reduced transcription of QS-genes is also one of the mechanisms of macrolide action [16-18].

Bacterial adherence

In vitro and in vivo evidence suggests that PA bacilli, when cultured in the presence of low levels of macrolides, e.g. erythromycin, are less adherent to cells of the airway epithelium [19-21]. Since adherence of bacteria to mucosal surfaces is an important initial event in the pathogenesis of most bacterial infectious diseases, this could help explain the clinical efficacy of low-dose macrolide therapy in patients colonized with PA.

Mobility

The effect of macrolides on PA is accompanied by impairing the mobility of this micro-organism. *Pseudomonas* spp are mobile thanks to two distinctive modalities; flagella, tail-like structures that project from the cell body and move in a whip-like manner; and type IV pili (fimbriae), that provide twitching motility.

Exposure to sub-MIC concentrations of macrolide antibiotics results in loss of mobility, partly due to the inhibition of flagellin production [22-24] -the principal constituent of bacterial flagella- and partly because some macrolides alter the assembly of type IV pili [13,25]. This loss of mobility facilitates easier phagocytosis and killing of bacteria by alveolar macrophages.

Bacterial toxins

Cytotoxic enzymes, produced by bacteria when causing infection, including exotoxin A, alkaline protease, elastase and and phospholipase C, are important factors in bacterial virulence. Erythromycin, and, more recently, also azithromycin, have been shown to suppress the production of those enzymes and, consequently, to diminish bacterial virulence [23] [26-28].

Intracellular effects

Macrolides accumulate and show a prolonged retention in human cells after oral or IV administration, an effect that is augmented when macrolide treatment is given for a longer period of time [29-32]. In CF-patients treated with azithromycin (500mg daily) for at least 35 consecutive days, the concentration of azithromycin in neutrophils appeared to be up to 3000 times higher as compared to the concentration in plasma (wilms). Macrolides have also been shown to accumulate in alveolar macrophages [30,33].

The above suggests that tissue and intracellular concentrations may be more useful for assessing the antibacterial activity of azitromycin than serum concentrations [34,35]. Because intracellular concentrations of macrolide antibiotic often exceed the minimal inhibitory concentration (MIC) of phagocytized pathogens, macrolides have also been demonstrated to be effective against micro-organisms with *in vitro* macrolide resistance [35,36]. The excellent intracellular penetration of macrolides also appears to explain their effectiveness against intracellular pathogens [34],

2. Effects on airway epithelial cells and mucus properties

Besides inhibiting production of pro-inflammatory cytokines by bronchial epithelial cells [37,38], macrolides distinctly modulate features of bronchial epithelium, making it better armed against exogenous attacks. The bronchial epithelium is critically important in lung defense. In addition to being a mechanical barrier, it regulates electrolyte content of the airway surface liquid, by means of its tight junctions between adjacent cells. In vitro studies demonstrate that azithromycin increases transepithelial electrical resistance of human airway epithelium by changing the processing of tight junction proteins, as such preventing excess leakage of electrolytes and ameliorating mucus properties [39].

Furthermore, when airway epithelial cells are exposed to inflammatory mediators in vitro, macrolides display a protective effect against epithelial damage and ciliary dysfunction [40,41]. This positive effect on ciliary beat frequency, however, was not confirmed in in vivo studies in patients with chronic rhinosinusitis or bronchiectasis [42,43].

Airway mucus hypersecretion and the resulting excess sputum expectoration is an important characteristic of several chronic inflammatory pulmonary diseases. Mucus hypersecretion may lead to more exacerbations and poor health related quality of life (HRQL) [44]. Macrolides have been shown not only to reduce the quantity of expectorated sputum in vivo, for example in bronchiectasis, but also to change the composition of mucus, thereby enhancing mucus clearance [45-51].

3. Effects on the immune system

Innate immunity

<u>Cytokine and chemokine response</u>

Cytokines are hormone-like proteins that enable immune cells to communicate, and play an integral role in the initiation, perpetuation and subsequent down regulation of the immune response. Chemokines are cytokines with a particularly strong chemotactic capacity.Production of cytokines is effectuated by a variety of cell types, including alveolar macrophages, eosinophils, neutrophils and bronchial epithelial cells. Pro-inflammatory cytokines (such as interleukin (IL) 1, 2, 4, and 6, IFN-gamma, TNF-α, GM-GCSF) and –chemokines (such as IL-8, RANTES) amplify the immune response through positive feedback loops. Anti-inflammatory cytokines, e.g. IL-10, prostaglandins and Transforming Growth Factor (TGF) –β, attenuate the immune response through a negative-feedback mechanism. In general, macrolides inhibit synthesis and/or secretion of pro-inflammatory cytokines

while increasing the release of anti-inflammatory cytokines [1]. Some recent research however, promotes a more discerned view in which macrolides can differentially modulate pro-inflammatory cytokine secretion. [37]. Changes in cytokine and chemokine production are probably related to an effect of macrolides on the activation of transcription factors: nuclear factor (NF)- κB and activator protein (AP)-1 [52]. Inhibition of the production of pro-inflammatory cytokines has been described in several *in vivo* studies in healthy subjects and patients with cystic fibrosis (CF), asthma or chronic rhinosinusitis [53-59].

Alveolar macrophages

Macrophages play a critical role in the phagocytosis of apoptotic cells and the removal of exogenous particles, such as bacteria. Recent studies prove that macrolides promote phagocytosis of apoptotic cells by alveolar macrophages, thus avoiding secondary necrosis and the release of cell contents that may induce further inflammation [60-62].
In addition, some authors propose that macrolides promote monocyte-to-macrophage differentiation, increasing the number of active macrophages [63,64]. Results of earlier research suggest that macrolides antibiotics also enhance other macrophage functions, including their cytocidal activity [65].

Neutrophils

Neutrophils are key players of the inflammatory response in patients with chronic airway disease [66]. They accumulate at the site of infection, responding to increased levels of chemokines and cytokines, primarily IL-8 and TNF- α. Macrolide antibiotics exert influence on several domains of neutrophil function.

Reaction to chemokines

Macrolide antibiotics cause a significant reduction in the chemotactic response of neutrophils to chemokines [67,68]. Together with the above described inhibition of chemo-attractant generation, this results in markedly decreased airway neutrophilia in patients with various inflammatory pulmonary diseases [8,51,59,69-72].

Degranulation

Upon activation, neutrophils release granules containing cytotoxic enzymes, such as elastase, a process called neutrophil degranulation or exocytosis. In general, macrolides seem to stimulate exocytosis, which may result in enhanced anti-bactericidal activity [1,73-76].

Adhesion

Leukocyte adhesion is a hallmark of the inflammatory cascade and cell adhesion molecules (CAM) are the mediators of this event [1]. Cultured bronchial epithelial cells treated with erythromycin show reduced levels of intercellular adhesion molecule (ICAM)-1 [38,77]. These findings suggest that reducing release of adhesion molecules in bronchial epithelial cells is another anti-inflammatory effect of macrolide antibiotics.

Oxidative burst

The production and release of reactive oxygen species by neutrophils to enhance their cytotoxic capability, is referred to as the 'oxidative' or 'respiratory' burst, a process mediated by NADPH-dependent oxidase. Contradictory data have been reported with regard to the effect of macrolides on the oxidative burst. Previously, evidence was presented showing an attenuation of oxidative burst capacity, but more recent studies disclosed an opposite effect or no effect at all [74,78-80].

Apoptosis

In the previous decade, it had already been proposed that apoptosis (programmed cell death) limits the ability of neutrophils to damage tissue while being involved in an inflammatory response [81,82]. Since then several in vitro studies demonstrated that macrolides shorten neutrophil survival by accelerating neutrophil apoptosis [74,79,82-84].

Adaptive immunity

The aforementioned research data indisputably show the existence of a direct modulating effect of macrolides on the innate immune system. Studies focusing on effects of macrolide antibiotics on cellular immunity also clearly demonstrate impact on T-cell regulation and antigen presentation.

Long-term use of macrolide antibiotics reduces the elevated number of lymphocytes in BAL-fluid of DPB patients to sub-normal levels [85,86]. In addition, 14- and 15 membered ring macrolides appear to be involved in the augmentation of apoptosis of activated lymphocytes and, as such, reduce inflammation [87]. Dendritic cells are the most important antigen-presenting cells and play a central role in the initiation and regulation of immune responses. Sugiyama et al [88] demonstrated that clarithromycin and azithromycin modulate the function of dendritic cells, each macrolide shows a different, immune-dampening effect. In addition, macrolides appear to have a suppressive effect on pro-inflammatory cytokine production by T-cells [89,90].

An early *in vivo* study in healthy volunteers showed a small but significant positive effect of azithromycin on the proliferative B-cell response of stimulated lymphocytes [91]. A more recent study in patients with bronchiectasis however, failed to confirm this finding [92], while research in vitro demonstrated an opposite effect [93].

Conclusion

Macrolide antibiotics are well known for their antibacterial and anti-inflammatory properties. They clearly possess an anti-bacterial effect, that consists of inhibition of bacterial protein synthesis, impaired bacterial biofilm synthesis, and attenuation of other bacterial virulence factors. Apart from these direct anti-microbial effects, macrolides are known for their modulating effect on many components of the human immune system. By influencing the production of cytokines, they have a dampening effect on the pro-inflammatory response. Furthermore, the majority of cells involved in both the innate and adaptive immune-response, are, one way or the other, influenced when macrolide antibiotics are administered. The most distinct effect of macrolides is found on neutrophils, the key players of the anti-inflammatory response. Among other things, neutrophil accumulation, adhesion and apoptosis are clearly reduced, which results in markedly decreased airway neutrophilia. Studies focusing on effects of macrolide antibiotics on cellular immunity also clearly demonstrate impact on T-cell regulation and antigen presentation.

Future perspectives

In the near future, clinicians might add new immunomodulatory drugs of the macrolide family to their armamentarium Immunomodulatory macrolide antibiotics devoid of anti-infective properties are developed by modifiying the molecular structure of the atoms attached to the macrocyclic ring. These purely immunomodulatory macrolides would offer a way to circumvent bacterial resistance. This concept has been investigated for tetracyclines, an other group of antibiotics which also have anti-inflammatory properties. Chemically modified tetracyclines (CMT), without anti-bacterial capacity, induce an anti-inflammatory response by modulating cytokine and matrix metalloproteinase secretion [94-98]. However, only in vitro and animal studies have been performed investigating the effect of CMT. To our knowledge, no phase 1 studies are yet available describing the efficacy and safety of purely immunomodulatory drugs.

Reference list

1. Culic O, Erakovic V, Parnham MJ: Anti-inflammatory effects of macrolide antibiotics. Eur J Pharmacol 2001; 429: 209-29.
2. Kudoh S, Uetake T, Hagiwara K: [Clinical effects of low-dose long-term erythromycin chemotherapy on diffuse panbronchiolitis]. Nihon Kyobu Shikkan Gakkai Zasshi 1987; 25: 632-42.
3. Fujii T, Kadota J, Kawakami K: Long term effect of erythromycin therapy in patients with chronic Pseudomonas aeruginosa infection. Thorax 1995; 50: 1246-52.
4. Kudoh S, Azuma A, Yamamoto M: Improvement of survival in patients with diffuse panbronchiolitis treated with low-dose erythromycin. Am J Respir Crit Care Med 1998; 157: 1829-32.
5. Nagai H, Shishido H, Yoneda R: Long-term low-dose administration of erythromycin to patients with diffuse panbronchiolitis. Respiration 1991; 58: 145-49.
6. Tateda K, Ishii Y, Kimura S: Suppression of Pseudomonas aeruginosa quorum-sensing systems by macrolides: a promising strategy or an oriental mystery? J Infect. Chemother. 2007; 13: 357-67.
7. Healy DP: Macrolide immunomodulation of chronic respiratory diseases. Curr.Infect. Dis Rep 2007; 9: 7-13.
8. Idris SF, Chilvers ER, Haworth C: Azithromycin therapy for neutrophilic airways disease: myth or magic? Thorax 2009; 64: 186-89.
9. Starner TD, Shrout JD, Parsek MR: Subinhibitory concentrations of azithromycin decrease nontypeable Haemophilus influenzae biofilm formation and Diminish established biofilms. Antimicrob.Agents Chemother. 2008; 52: 137-45.
10. Yasuda H, Ajiki Y, Koga T: Interaction between clarithromycin and biofilms formed by Staphylococcus epidermidis. Antimicrob.Agents Chemother. 1994; 38: 138-41.
11. Ichimiya T, Yamasaki T, Nasu M: In-vitro effects of antimicrobial agents on Pseudomonas aeruginosa biofilm formation. J Antimicrob.Chemother. 1994; 34: 331-41.
12. Ichimiya T, Takeoka K, Hiramatsu K: The influence of azithromycin on the biofilm formation of Pseudomonas aeruginosa in vitro. Chemotherapy 1996; 42: 186-91.
13. Wozniak DJ, Keyser R: Effects of subinhibitory concentrations of macrolide antibiotics on Pseudomonas aeruginosa. Chest 2004; 125: 62S-9S.
14. Kondoh K, Hashiba M, Baba S: Inhibitory activity of clarithromycin on biofilm synthesis with Pseudomonas aeruginosa. Acta Otolaryngol.Suppl 1996; 525: 56-60.
15. Takeoka K, Ichimiya T, Yamasaki T: The in vitro effect of macrolides on the interaction of human polymorphonuclear leukocytes with Pseudomonas aeruginosa in biofilm. Chemotherapy 1998; 44: 190-97.
16. Skindersoe ME, Alhede M, Phipps R: Effects of antibiotics on quorum sensing in Pseudomonas aeruginosa. Antimicrob.Agents Chemother. 2008; 52: 3648-63.
17. Nalca Y, Jansch L, Bredenbruch F: Quorum-sensing antagonistic activities of

azithromycin in Pseudomonas aeruginosa PAO1: a global approach. Antimicrob. Agents Chemother. 2006; 50: 1680-88.
18. Tateda K, Comte R, Pechere JC: Azithromycin inhibits quorum sensing in Pseudomonas aeruginosa. Antimicrob.Agents Chemother. 2001; 45: 1930-33.
19. Baumann U, Fischer JJ, Gudowius P: Buccal adherence of Pseudomonas aeruginosa in patients with cystic fibrosis under long-term therapy with azithromycin. Infection 2001; 29: 7-11.
20. Tsang KW, Ng P, Ho PL: Effects of erythromycin on Pseudomonas aeruginosa adherence to collagen and morphology in vitro. Eur Respir J 2003; 21: 401-06.
21. Yamasaki T: [Adherence of Pseudomonas aeruginosa to mouse tracheal epithelium--the effect of antimicrobial agents]. Kansenshogaku Zasshi 1990; 64: 575-83.
22. Molinari G, Paglia P, Schito GC: Inhibition of motility of Pseudomonas aeruginosa and Proteus mirabilis by subinhibitory concentrations of azithromycin. Eur J Clin Microbiol. Infect.Dis 1992; 11: 469-71.
23. Molinari G, Guzman CA, Pesce A: Inhibition of Pseudomonas aeruginosa virulence factors by subinhibitory concentrations of azithromycin and other macrolide antibiotics. J Antimicrob.Chemother. 1993; 31: 681-88.
24. Kawamura-Sato K, Iinuma Y, Hasegawa T: Effect of subinhibitory concentrations of macrolides on expression of flagellin in Pseudomonas aeruginosa and Proteus mirabilis. Antimicrob.Agents Chemother. 2000; 44: 2869-72.
25. Hentzer M, Givskov M: Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. J Clin Invest 2003; 112: 1300-07.
26. Hirakata Y, Kaku M, Mizukane R: Potential effects of erythromycin on host defense systems and virulence of Pseudomonas aeruginosa. Antimicrob.Agents Chemother. 1992; 36: 1922-27.
27. Kita E, Sawaki M, Oku D: Suppression of virulence factors of Pseudomonas aeruginosa by erythromycin. J Antimicrob.Chemother. 1991; 27: 273-84.
28. Mizukane R, Hirakata Y, Kaku M: Comparative in vitro exoenzyme-suppressing activities of azithromycin and other macrolide antibiotics against Pseudomonas aeruginosa. Antimicrob.Agents Chemother. 1994; 38: 528-33.
29. Bosnar M, Kelneric Z, Munic V: Cellular uptake and efflux of azithromycin, erythromycin, clarithromycin, telithromycin, and cethromycin. Antimicrob.Agents Chemother. 2005; 49: 2372-77.
30. Capitano B, Mattoes HM, Shore E: Steady-state intrapulmonary concentrations of moxifloxacin, levofloxacin, and azithromycin in older adults. Chest 2004; 125: 965-73.
31. Wildfeuer A, Laufen H, Zimmermann T: Distribution of orally administered azithromycin in various blood compartments. Int J Clin Pharmacol Ther 1994; 32: 356-60.
32. Wilms EB, Touw DJ, Heijerman HG: Pharmacokinetics of azithromycin in plasma, blood, polymorphonuclear neutrophils and sputum during long-term therapy in patients with cystic fibrosis. Ther.Drug Monit. 2006; 28: 219-25.

33. Lucchi M, Damle B, Fang A: Pharmacokinetics of azithromycin in serum, bronchial washings, alveolar macrophages and lung tissue following a single oral dose of extended or immediate release formulations of azithromycin. J Antimicrob.Chemother. 2008; 61: 884-91.
34. Nakamura S, Yanagihara K, Araki N: Efficacy of clarithromycin against experimentally induced pneumonia caused by clarithromycin-resistant Haemophilus influenzae in mice. Antimicrob.Agents Chemother. 2010; 54: 757-62.
35. Yanagihara K, Izumikawa K, Higa F: Efficacy of azithromycin in the treatment of community-acquired pneumonia, including patients with macrolide-resistant Streptococcus pneumoniae infection. Intern Med 2009; 48: 527-35.
36. Hoffman HL, Klepser ME, Ernst EJ: Influence of macrolide susceptibility on efficacies of clarithromycin and azithromycin against Streptococcus pneumoniae in a murine lung infection model. Antimicrob.Agents Chemother. 2003; 47: 739-46.
37. Shinkai M, Foster GH, Rubin BK: Macrolide antibiotics modulate ERK phosphorylation and IL-8 and GM-CSF production by human bronchial epithelial cells. Am J Physiol Lung Cell Mol.Physiol 2006; 290: L75-L85.
38. Khair OA, Devalia JL, Abdelaziz MM: Effect of erythromycin on Haemophilus influenzae endotoxin-induced release of IL-6, IL-8 and sICAM-1 by cultured human bronchial epithelial cells. Eur Respir J 1995; 8: 1451-57.
39. Asgrimsson V, Gudjonsson T, Gudmundsson GH: Novel effects of azithromycin on tight junction proteins in human airway epithelia. Antimicrob.Agents Chemother. 2006; 50: 1805-12.
40. Feldman C, Anderson R, Theron AJ: Roxithromycin, clarithromycin, and azithromycin attenuate the injurious effects of bioactive phospholipids on human respiratory epithelium in vitro. Inflammation 1997; 21: 655-65.
41. Anderson R, Theron AJ, Feldman C: Membrane-stabilizing, anti-inflammatory interactions of macrolides with human neutrophils. Inflammation 1996; 20: 693-705.
42. Cervin A, Kalm O, Sandkull P: One-year low-dose erythromycin treatment of persistent chronic sinusitis after sinus surgery: clinical outcome and effects on mucociliary parameters and nasal nitric oxide. Otolaryngol.Head Neck Surg 2002; 126: 481-89.
43. Shibuya Y, Wills PJ, Cole PJ: The effect of erythromycin on mucociliary transportability and rheology of cystic fibrosis and bronchiectasis sputum. Respiration 2001; 68: 615-19.
44. Bhowmik A, Chahal K, Austin G: Improving mucociliary clearance in chronic obstructive pulmonary disease. Respir Med 2009; 103: 496-502.
45. Cymbala AA, Edmonds LC, Bauer MA: The disease-modifying effects of twice-weekly oral azithromycin in patients with bronchiectasis. Treat.Respir Med 2005; 4: 117-22.
46. Davies G, Wilson R: Prophylactic antibiotic treatment of bronchiectasis with azithromycin. Thorax 2004; 59: 540-41.
47. Rubin BK, Druce H, Ramirez OE: Effect of clarithromycin on nasal mucus properties

in healthy subjects and in patients with purulent rhinitis. Am J Respir Crit Care Med 1997; 155: 2018-23.
48. Shirai T, Sato A, Chida K: Effect of 14-membered ring macrolide therapy on chronic respiratory tract infections and polymorphonuclear leukocyte activity. Intern Med 1995; 34: 469-74.
49. Tagaya E, Tamaoki J, Kondo M: Effect of a short course of clarithromycin therapy on sputum production in patients with chronic airway hypersecretion. Chest 2002; 122: 213-18.
50. Tsang KW, Ho PI, Chan KN: A pilot study of low-dose erythromycin in bronchiectasis. Eur Respir J 1999; 13: 361-64.
51. Yalcin E, Kiper N, Ozcelik U: Effects of claritromycin on inflammatory parameters and clinical conditions in children with bronchiectasis. J Clin Pharm.Ther. 2006; 31: 49-55.
52. Desaki M, Takizawa H, Ohtoshi T: Erythromycin suppresses nuclear factor-kappaB and activator protein-1 activation in human bronchial epithelial cells. Biochem.Biophys. Res Commun. 2000; 267: 124-28.
53. Kurdowska A, Noble JM, Griffith DE: The effect of azithromycin and clarithromycin on ex vivo interleukin-8 (IL-8) release from whole blood and IL-8 production by human alveolar macrophages. J Antimicrob.Chemother. 2001; 47: 867-70.
54. Equi A, Balfour-Lynn IM, Bush A: Long term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled crossover trial. Lancet 2002; 360: 978-84.
55. Kraft M, Cassell GH, Pak J: Mycoplasma pneumoniae and Chlamydia pneumoniae in asthma: effect of clarithromycin. Chest 2002; 121: 1782-88.
56. Takizawa H, Desaki M, Ohtoshi T: Erythromycin and clarithromycin attenuate cytokine-induced endothelin-1 expression in human bronchial epithelial cells. Eur Respir J 1998; 12: 57-63.
57. Wallwork B, Coman W, Mackay-Sim A: A double-blind, randomized, placebo-controlled trial of macrolide in the treatment of chronic rhinosinusitis. Laryngoscope 2006; 116: 189-93.
58. Yamada T, Fujieda S, Mori S: Macrolide treatment decreased the size of nasal polyps and IL-8 levels in nasal lavage. Am J Rhinol. 2000; 14: 143-48.
59. Simpson JL, Powell H, Boyle MJ: Clarithromycin targets neutrophilic airway inflammation in refractory asthma. Am J Respir Crit Care Med 2008; 177: 148-55.
60. Hodge S, Hodge G, Brozyna S: Azithromycin increases phagocytosis of apoptotic bronchial epithelial cells by alveolar macrophages. Eur Respir J 2006; 28: 486-95.
61. Yamaryo T, Oishi K, Yoshimine H: Fourteen-member macrolides promote the phosphatidylserine receptor-dependent phagocytosis of apoptotic neutrophils by alveolar macrophages. Antimicrob.Agents Chemother. 2003; 47: 48-53.
62. Hodge S, Hodge G, Jersmann H: Azithromycin improves macrophage phagocytic function and expression of mannose receptor in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2008; 178: 139-48.

63. Keicho N, Kudoh S, Yotsumoto H: Erythromycin promotes monocyte to macrophage differentiation. J Antibiot.(Tokyo) 1994; 47: 80-89.
64. Yoshida K, Sunazuka T, Nagai K: Macrolides with promotive activity of monocyte to macrophage differentiation. J Antibiot.(Tokyo) 2005; 58: 79-81.
65. Xu G, Fujita J, Negayama K: Effect of macrolide antibiotics on macrophage functions. Microbiol.Immunol. 1996; 40: 473-79.
66. Tamaoki J, Kadota J, Takizawa H: Clinical implications of the immunomodulatory effects of macrolides. Am J Med 2004; 117 Suppl 9A: 5S-11S.
67. Tamaoki J, Nakata J, Tagaya E: Effects of roxithromycin and erythromycin on interleukin 8-induced neutrophil recruitment and goblet cell secretion in guinea pig tracheas. Antimicrob.Agents Chemother. 1996; 40: 1726-28.
68. Tsai WC, Rodriguez ML, Young KS: Azithromycin blocks neutrophil recruitment in Pseudomonas endobronchial infection. Am J Respir Crit Care Med 2004; 170: 1331-39.
69. Piacentini GL, Peroni DG, Bodini A: Azithromycin reduces bronchial hyperresponsiveness and neutrophilic airway inflammation in asthmatic children: a preliminary report. Allergy Asthma Proc 2007; 28: 194-98.
70. Sakito O, Kadota J, Kohno S: Interleukin 1 beta, tumor necrosis factor alpha, and interleukin 8 in bronchoalveolar lavage fluid of patients with diffuse panbronchiolitis: a potential mechanism of macrolide therapy. Respiration 1996; 63: 42-48.
71. Suzuki H, Shimomura A, Ikeda K: Effects of long-term low-dose macrolide administration on neutrophil recruitment and IL-8 in the nasal discharge of chronic sinusitis patients. Tohoku J Exp Med 1997; 182: 115-24.
72. Verleden GM, Vanaudenaerde BM, Dupont LJ: Azithromycin reduces airway neutrophilia and interleukin-8 in patients with bronchiolitis obliterans syndrome. Am J Respir Crit Care Med 2006; 174: 566-70.
73. Abdelghaffar H, Vazifeh D, Labro MT: Comparison of various macrolides on stimulation of human neutrophil degranulation in vitro. J Antimicrob.Chemother. 1996; 38: 81-93.
74. Culic O, Erakovic V, Cepelak I: Azithromycin modulates neutrophil function and circulating inflammatory mediators in healthy human subjects. Eur J Pharmacol 2002; 450: 277-89.
75. Labro MT, el Benna J, Babin-Chevaye C: Comparison of the in-vitro effect of several macrolides on the oxidative burst of human neutrophils. J Antimicrob.Chemother. 1989; 24: 561-72.
76. Vazifeh D, Preira A, Bryskier A: Interactions between HMR 3647, a new ketolide, and human polymorphonuclear neutrophils. Antimicrob.Agents Chemother. 1998; 42: 1944-51.
77. Tamaoki J: The effects of macrolides on inflammatory cells. Chest 2004; 125: 41S-50S.
78. Hand WL, Hand DL, King-Thompson NL: Antibiotic inhibition of the respiratory burst response in human polymorphonuclear leukocytes. Antimicrob.Agents Chemother.

1990; 34: 863-70.
79. Koch CC, Esteban DJ, Chin AC: Apoptosis, oxidative metabolism and interleukin-8 production in human neutrophils exposed to azithromycin: effects of Streptococcus pneumoniae. J Antimicrob.Chemother. 2000; 46: 19-26.
80. Levert H, Gressier B, Brunet C: Time and concentration dependent influence of dirithromycin on neutrophils oxidative burst. J Antibiot.(Tokyo) 1999; 52: 127-33.
81. Whyte MK, Meagher LC, MacDermot J: Impairment of function in aging neutrophils is associated with apoptosis. J Immunol. 1993; 150: 5124-34.
82. Aoshiba K, Nagai A, Konno K: Erythromycin shortens neutrophil survival by accelerating apoptosis. Antimicrob.Agents Chemother. 1995; 39: 872-77.
83. Inamura K, Ohta N, Fukase S: The effects of erythromycin on human peripheral neutrophil apoptosis. Rhinology 2000; 38: 124-29.
84. Yamasawa H, Oshikawa K, Ohno S: Macrolides inhibit epithelial cell-mediated neutrophil survival by modulating granulocyte macrophage colony-stimulating factor release. Am J Respir Cell Mol.Biol. 2004; 30: 569-75.
85. Kawakami K, Kadota J, Iida K: Phenotypic characterization of T cells in bronchoalveolar lavage fluid (BALF) and peripheral blood of patients with diffuse panbronchiolitis; the importance of cytotoxic T cells. Clin Exp Immunol. 1997; 107: 410-16.
86. Mukae H, Kadota J, Kohno S: Increase in activated CD8+ cells in bronchoalveolar lavage fluid in patients with diffuse panbronchiolitis. Am J Respir Crit Care Med 1995; 152: 613-18.
87. Kadota J, Mizunoe S, Kishi K: Antibiotic-induced apoptosis in human activated peripheral lymphocytes. Int J Antimicrob.Agents 2005; 25: 216-20.
88. Sugiyama K, Shirai R, Mukae H: Differing effects of clarithromycin and azithromycin on cytokine production by murine dendritic cells. Clin Exp Immunol. 2007; 147: 540-46.
89. Asano K, Kamakazu K, Hisamitsu T: Modulation of Th2 type cytokine production from human peripheral blood leukocytes by a macrolide antibiotic, roxithromycin, in vitro. Int Immunopharmacol. 2001; 1: 1913-21.
90. Williams AC, Galley HF, Watt AM: Differential effects of three antibiotics on T helper cell cytokine expression. J Antimicrob.Chemother. 2005; 56: 502-06.
91. Tomazic J, Kotnik V, Wraber B: In vivo administration of azithromycin affects lymphocyte activity in vitro. Antimicrob.Agents Chemother. 1993; 37: 1786-89.
92. Harita S, Kuyama S, Okada T: Effect of long-term and low-dose administration of erythromycin on proliferation of T lymphocytes stimulated with mitogens. J Chemother. 2008; 20: 604-08.
93. Keicho N, Kudoh S, Yotsumoto H: Antilymphocytic activity of erythromycin distinct from that of FK506 or cyclosporin A. J Antibiot.(Tokyo) 1993; 46: 1406-13.
94. Cazalis J, Tanabe S, Gagnon G: Tetracyclines and chemically modified tetracycline-3 (CMT-3) modulate cytokine secretion by lipopolysaccharide-stimulated whole blood. Inflammation 2009; 32: 130-37.

95. Golub LM, Suomalainen K, Sorsa T: Host modulation with tetracyclines and their chemically modified analogues. Curr Opin Dent 1992; 2: 80-90.
96. Maisi P, Kiili M, Raulo SM: MMP inhibition by chemically modified tetracycline-3 (CMT-3) in equine pulmonary epithelial lining fluid. Ann N Y Acad Sci. 1999; 878: 675-77.
97. Maitra SR, Bhaduri S, Chen E: Role of chemically modified tetracycline on TNF-alpha and mitogen-activated protein kinases in sepsis. Shock 2004; 22: 478-81.
98. Steinberg J, Halter J, Schiller H: Chemically modified tetracycline prevents the development of septic shock and acute respiratory distress syndrome in a clinically applicable porcine model. Shock 2005; 24: 348-56.

CHAPTER 4

Immunomodulatory effects of macrolide antibiotics – part 2: advantages and disadvantage of macrolide maintenance therapy.

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Abstract

The available evidence for long-term, low-dose treatment with 14- and 15-membered ring macrolides in non-CF bronchiectasis, COPD, chronic sinusitis and asthma is reviewed, with special attention to possible adverse effects and emergence of resistance during long term macrolide treatment.

Macrolide maintenance therapy has been proven to be of benefit in diffuse panbronchiolitis and cystic fibrosis, presumably due to an anti-inflammatory mechanism of action, on top of its direct anti-microbial effect. Solid evidence to justify this treatment regimen non-CF bronchiectasis, asthma or sinusitis is still lacking, although a beneficial effect of long term macrolide therapy has been found in small clinical trials on these subjects. Data from randomised trials of long term macrolide treatment in COPD are conflicting. A sufficiently long duration of treatment and careful selection of patients appears to be crucial. Apart from beneficial effects, possible side effects of macrolide treatment should be taken into account, the most important being gastro-intestinal upset and cardiac arrythmias. Development of macrolide resistance among respiratory pathogens is very common during long-term macrolide treatment. Whether this finding is clinically significant is a matter of debate.

Introduction

In pulmonary practice, prolonged macrolide therapy with reduced dosages has become increasingly popular for the treatment of patients with chronic inflammatory conditions, such as non-CF bronchiectasis and sinusitis, who have recurrent infections or other signs of 'badly-regulated disease'. In this article we aim to provide an overview of the available evidence for macrolide maintenance therapy in these chronic inflammatory pulmonary conditions.

The concept of treating inflammatory lung diseases with macrolide maintenance therapy originates from Japan, where, in 1987, Kudoh and colleagues [1] reported a spectacular decrease in symptoms and increase in life expectancy in patients with diffuse panbronchiolitis (DPB) following treatment with the macrolide erythromycin. Until then, DPB had been a rapidly progressive and debilitating inflammatory airway disorder carrying a very poor prognosis. This condition is almost exclusively found in Japan and is pathologically characterized by chronic recurrent bronchiolitis and peribronchiolitis with infiltration of the small airways. This can eventually lead to complete occlusion of the lumen of the respiratory bronchioles, through the formation of lymph follicles, granulomas and scar tissue. Clinically, pulmonary complaints, such as dyspnoea and productive cough are almost always accompanied by features of chronic rhinosinusitis [2,3]. High numbers of neutrophils and lymfocytes, together with high levels of IL-8 and other pro-inflammatory cytokines and chemokines are found in BAL fluid of DPB-patients [4,5]. This indicates a chronic inflammatory process, which is further deteriorated by the presence of pathogenic micro-organisms. In the early course of the disease, sputum cultures of DPB-patients mainly yield *Haemophilus influenzae*, which is in many cases replaced by *Pseudomonas_aeruginosa* (PA) in a more advanced stage of DPB [4,6].

In addition, there appears to be a genetic component contributing to the disease mechanism. In approximately 60% of DPB patients the human leukocyte antigen (HLA)-B54 haplotype is found, which is only present in 11% of the healthy Japanese population [7].

After the introduction of erythromycin as standard therapy for DPB in 1987, an impressive increase of 10-year survival was reported; 10-20% to more than 90% [2,8-10].

The unexpected success was attributed to a previously unknown anti-inflammatory effect in addition to the anti-microbial potency of erythromycin. This theory is supported by more recent studies, which demonstrated that serum levels of erythromycin in DPB-patients were well below minimal inhibitory concentrations (MIC) for the detected pathogens [9]. Furthermore, Nakamura et al [11] showed that the beneficial effect of macrolides on pulmonary function and general well-being is frequently found without a change in the number or type of bacterial isolates.

Macrolides have been demonstrated to reduce IL-8 and IL-1β levels in BAL-fluid of DPB patients [12]. By influencing the production of these and other cytokines, they have a dampening effect on the pro-inflammatory response. Furthermore, the majority of cells involved in both the innate and adaptive immune-response, are, one way or the other, influenced when macrolide antibiotics are administered. Their anti-bacterial effect consists of inhibition of bacterial protein synthesis, impaired bacterial biofilm synthesis, and attenuation of other bacterial virulence factors. Especially the effect on biofilm formation is suggested to be of importance in DPB-patients who are frequently colonised with biofilm-forming *P. aeruginosa*. Results of Japanese in vitro studies indicate that azithromycin and clarithromycin change the structure of bacterial biofilms via inhibition of polysaccharide synthesis, a major biofilm component [13,14]. An insufficient biofilm allows for enhanced phagocytosis and clearance of bacteria by alveolar macrophages [15,16].

The effectiveness of macrolide maintenance therapy in order to reduce disease activity, exacerbations and decline in lung function has been well proven in cystic fibrosis (CF) [17]. Since 2002, five large randomised trials, with a total of 608 patients included, have been published in which the role of macrolide maintenance treatment in CF is addressed [18-22]. These studies all used azithromycin in different dosages (250 or 500 mg daily, 250 or 500 mg three times a week, or 1200 mg once a week) with a mean duration of 200 days. All studies showed a significant increase in lung function (FEV1). Additional outcomes were a decrease in the frequency and duration of infectious exacerbations, improvement in physical condition and gain of body weight.

The most recent trial was performed by McCormack et al [22]. In their double-blind randomised study in 208 patients, improvement in lung function, CRP, days spent in hospital, admission rate and nutritional status was demonstrated after 6 months of treatment with azithromycin. Daily (250mg) and weekly (1200mg) administered azithromycin showed similar outcomes, although gastro-intestinal adverse effects where more common with weekly therapy. Nowadays macrolide maintenance therapy is considered common practice in the treatment of CF-patients, especially in those colonised with *P. aeruginosa* (PA). Colonisation with PA is associated with reduced survival and faster decline in lung function in DPB and CF [4,23,24]. The abovementioned trials also included patients without PA colonisation, but to date no randomised study in CF-patients uninfected with PA had been performed. However, in a recently published article, Saiman et al study the effect of azithromycin (250-500 mg three times weekly, 24 weeks vs. placebo) on pulmonary function in 260 children with CF and mild lung disease [25]. Only patients with negative respiratory tract cultures for PA for at least one year were included. Treatment with azithromycin did not result in improved pulmonary function in this relatively healthy patient group, On the other hand, a promising amelioration in secondary endpoints, weight and frequency of infectious exacerbations, was observed after treatment with azithromcyin.

The improvement of pulmonary function, reduction of exacerbation frequency and improvement of quality of life shown in the abovementioned trials is often contributed to an anti-inflammatory effect of macrolide treatment [18,19,22]. Macrolides have a direct anti-microbial effect, but also modulate many aspects of the immune-response. This so-called 'immune-modulatory effect' is exclusively demonstrated for 14- and 15-membered ring macrolides (erythromycin, clarithromycin, roxithromycin and azithromycin, respectively) [26-30]. The dual effect on both inflammation and bacterial colonisation appears to make 14- and 15-membered ring macrolides exceptionally suited to contribute to the treatment of chronic inflammatory diseases like COPD and bronchiectasis. The fact is that the key feature of chronic pulmonary diseases like COPD and bronchiectasis and, to a lesser extent, asthma and sinusitis is distortion of the inflammatory response, allowing for bacterial colonization. [31-33]. An overview of the biological mechanisms through which macrolides exert their immunomodulatory effect is provided in part 1 of these series of articles.

Considering the increasing popularity of macrolide maintenance therapy, concern about possible disadvantages seems appropriate. In the past, macrolides, especially erythromycin, where known for their ability to cause cardiac arrythmias and hearing loss when administered in high dosages [34,35]. Finding out whether the above also applies to long-term low-dose macrolide therapy is relevant to clinical practice. In addition, reduced susceptibility or resistance to macrolides is very likely to develop when these drugs are administered in low dosages during longer periods of time.

We performed a literature search for studies exploring clinical effectiveness of long-term macrolide therapy in COPD, chronic rhinosinusitis, non-CF bronchiectasis and asthma. We focused primarily to include randomised controlled trials, if available. An additional search was performed for studies about macrolide safety and emergence of macrolide resistance during long-term macrolide therapy. As none were found, the search was broadened to macrolide safety and resistance in general, with special attention to ototoxicity and cardiac toxicity.

Non-CF bronchiectasis and long-term, low-dose macrolide treatment

In bronchiectasis, irreversible, pathologic dilatation of the small and medium-sized bronchi results from an ongoing cycle of chronic inflammation and bacterial colonisation (vicious circle theory) [31,36,37]. Although the etiology remains unclear in a large percentage of patients (53-60%), common causes include immune defects, early childhood infections, and aspiration [38-40]. Clinically, this results in chronic and sometimes debilitating symptoms of productive cough, dyspnea and recurrent infections. As early as 1965, maintenance therapy with macrolide antibiotics (e.g. oleandomycin) was investigated as a potential remedy for

chronic symptoms in patients with hypogammaglobulinemia and bronchiectasis [41].

Three small, randomised controlled trials investigating the efficacy of macrolide antibiotics in non-CF bronchiectasis were published in the last decade. Patient numbers did not exceed thirty-five and all trials used different macrolide products, administered for relatively short periods of time. A total of 59 children and 21 adults with proven bronchiectasis were treated with roxithromycin (4 mg/kg b.i.d. for 12 weeks), erythromycin (500 mg b.i.d. for 8 weeks) or clarithromycin (15 mg/kg daily for 12 weeks). Improvement of sputum properties, a decrease in markers of airway inflammation, reduced sputum volume and decreased bronchial reactivity as measured by methacholine challenge were demonstrated in patients receiving macrolide treatment as compared to controls [42-44]. Only one randomised trial demonstrated a small but significant improvement in forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) [43]. A small open-label, crossover study, enrolling 12 patients, investigated the effect of azithromycin (500 mg two times a week) added to standard treatment during 6 months [45]. Despite the small numbers, this study showed significantly fewer infectious exacerbations and reduced sputum volume. No change in lung function measurements was found. A fairly recently performed patient-control study showed comparable results concerning exacerbation frequency. Long-term azithromycin treatment (250 mg three times a week for 4-38 months) of thirty-nine patients with bronchiectasis and frequent exacerbations (> 4 in the past 12 months) resulted in a significant reduction in infectious exacerbations. Furthermore, these patients reported a significant improvement of symptoms. An improvement of all lung function parameters was reported, but only the improvement in carbon monoxide transfer factor (TLCO) reached statistical significance. The severity of symptoms in this group of patients made the researchers abstain from a placebo-controlled study design [46].

In conclusion; data from numerous, but mainly small studies suggest that long-term macrolide treatment in patients with non-CF bronchiectasis could have a positive influence on frequency of infections, sputum volume and inflammation. The effect of macrolide maintenance therapy on lung function remains uncertain. In turn, improvement of these parameters has a potentially large effect on quality of life in this group of patients.

COPD and long-term, low-dose macrolide treatment

Most research on the role of macrolide antibiotics in COPD focuses on short treatment in acute exacerbations. Current guidelines promote the administration of a short course of antibiotics to patients with an AECOPD and increase in sputum purulence or signs of pneumonia [47]. A meta-analysis of macrolide antibiotics vs. quinolones and macrolides vs amoxicilline/clavulanate in patients with acute bacterial exacerbations of chronic bronchitis showed no difference in treatment success [48].

Based on the most recent Cochrane review, long-term, prophylactic antibiotic therapy in stable COPD is not recommended [49]. The reviewers, however, mainly included trials conducted over 30 years ago, when antibiotic susceptibility and availability may have been different. Moreover, they did not distinguish between macrolide and other groups of antibiotics.

Only two randomised trials investigate the efficacy of long-term macrolide treatment in COPD. Banerjee et al [50] investigated 67 patients in a prospective double-blind randomised placebo-controlled study using clarithromycin 500 mg during 3 months. Although a large number of different parameters were measured (health status, quantitative sputum cultures, exacerbation rate, spirometry, CRP, shuttle walk test), no significant change was seen in any of them. The authors state that this may be due to the studies' small number of included patients and the relatively short treatment time. Results of another very recent study seem to support their statement. Seemungal et al [33] established a positive effect of prophylactic macrolide treatment in moderate to severe COPD in their study with a longer treatment time and longer follow-up. In this single centre, randomised, double-blind, placebo-controlled trial they treated 109 patients with erythromycin 250 mg BD or placebo during one year. Their most important finding was a 35% reduction of exacerbations in the macrolide arm of the study compared to the placebo arm, with a tendency towards shorter and less severe exacerbations in the macrolide arm. No significant change was found in lung function parameters, sputum and airway inflammatory markers, side-effects and bacteria isolated.

Contemplating these results and the evidence of the anti-inflammatory and antibacterial effect of macrolide antibiotics, one could argue that low-dose macrolides, when applied long enough, may have beneficial effects in moderate to severe COPD.
Currently, a large multicenter study is performed, investigating the effect of chronic macrolide administration on the frequency and severity of COPD exacerbations. 1130 patients receive azithromycin (250 mg once daily) or placebo in a randomized, double blind fashion[51,52]. Results of this study will presumably provide more insight into the role of macrolide prophylaxis in COPD.

Long-term, low-dose macrolides in chronic rhinosinusitis

Chronic rhinosinusitis is characterized by hyperplasia, hyperthrophy and hypersecretion of the of the nasal and paranasal sinus mucosa. Typically, high levels of neutrophils and pro-inflammatory cytokines, especially IL-8, are found in nasal secretions. In ear, nose and throat- medicine, there appears to exist a moderate recommendation for the use of long-term macrolide treatment in chronic sinusitis [53]. This recommendation however, is

based on limited data. Most studies that focus on long-term macrolide treatment in chronic sinusitis are small, open-label series. Consistent findings across this studies have been improvement in sinusitis symptoms, shrinkage in the size of nasal polyps and a decrease in levels of pro-inflammatory cytokines in nasal secretion [54-58]. In 2006, the first double-blind randomised, controlled trial (roxithromycin 150 mg OD or placebo for three months) was published [59]. The macrolide-treated group (n=29) showed a significant improvement in symptom scores, endoscopy findings and olfactory function. Nasal lavage essays showed decreased levels of IL-8 in the roxithromycin-treated group. Significant improvements started to occur after 6 weeks of treatment. These findings are consistent with previous open-label studies that showed that a prolonged course of macrolide therapy of minimal 12 weeks is required in order to reach and maintain maximal clinical benefit [54,60]. A subgroup analysis showed that the improvement of symptoms was most distinct in patients with low levels of IgE. This phenomenon was also observed in an earlier Japanese open-label study. Out of 16 patients receiving macrolide maintenance therapy, the ones with low levels of IgE (below 250U/ml) showed a significantly higher rate of symptomatic improvement [61]. These findings suggest that long-term macrolide treatment could be beneficial in chronic rhinosinusitis, but treatment periods longer than 12 weeks are necessary to maintain the beneficial effect. Furthermore, a subgroup of patients with low levels of serum IgE can be identified that seems to have most benefit most of macrolide treatment.

Long-term, low-dose macrolides in asthma

Bronchial hyperresponsiveness in asthma is related to airway inflammation. Asthma is considered a chronic inflammatory disease of the airways which in most cases requires long-term anti inflammatory therapy [32,62]. Chronic inflammation in asthma is characterized by increased numbers of activated lymfocytes, eosinophils and variably reported increases of mast cells. The walls of the conducting airways are thickened, the lumen obscured with an infiltrate of inflammatory cells and excess mucus [63]. Published reports have demonstrated an anti-inflammatory effect of macrolide antibiotics in asthma. This class of antibiotics has been found to reduce airway hyperresponsiveness, NO-production, cytokine expression and eosinophilic infiltration [64-69]. Another mechanism of action is proposed by Takizawa et al [70]; they demonstrated an attenuation of endothelin-1 (a potent bronchoconstrictive peptide) expression by bronchial epithelial cells after in vitro treatment with erythromycin and clarithromycin.

Two ways of long-term application of macrolide antibiotics in asthma have been specifically studied.

1) Addition of troleandomycin to steroid treatment: Troleandomycin (TAO), a 14-membered ring macrolide, was first described as a treatment for steroid

dependent asthma in 1974 [71]. Besides its anti-bacterial properties, anti-inflammatory effects of TAO have been reported. However, its most important mechanism of action in steroid dependent asthma patients appears to be the increase of bioavailability of steroids. Use of TAO slows the elimination of methylprednisolone, effectively doubling the half life, due to reduced liver metabolism [72-74]. Initial research in small groups of patients showed promising results. 16 severe, corticosteroid-dependent yet resistant outpatient asthmatics were well controlled after 4 months of addition of TAO with minimal side effects [75]. Wald et al [76] introduced another protocol with a lower starting dose of TAO and rapid steroid tapering. Side effects were even less present and a marked increase in pulmonary function parameters was observed. Nevertheless, as time went on, increasing concern arose about increase in corticosteroid induced side-effects in patients receiving TAO and steroids [77].
Since 1990, three randomised trials investigating the use of TAO in patients with corticosteroid-dependent asthma have been published [78-80]. Two of these reported more steroid-related side-effects in the patients who were treated with TAO in addition to corticosteroids [79,80]. A meta-analysis of 108 patients (75 adults and 33 children) in these three studies failed to show a significant reduction in the required dose of oral steroids in patients treated with TAO. Furthermore, there was no improvement in lung function when pooled data from two of these studies were analyzed [81].

A steroid sparing effect of clarithromycin was demonstrated in a small study in three steroid-dependent asthma patients by Garey et al [82]. They noticed a significant decrease in prednisone requirements in all three patients and discontinuation of prednisone treatment in two patients due to clarithromycin treatment.

2) Treatment of bacterial infection.
Mycoplasma pneumoniae (MP) or *Chlamydophila pneumoniae* (CP) may be involved in asthma pathogenesis [83]. In 2001, the first systematic evaluation of CP and MP infection in 55 patients with stable asthma, showed the presence of either MP or CP as detected by PCR in 56.4%, as compared to 9% in healthy control subjects ($p<0,02$) [84]. The authors concluded that a significant number of patients with stable asthma are infected by one of these micro-organisms. Furthermore, a substantial number of acute exacerbations (18-21%) in asthma is caused by MP [85-87]. The presence of such micro organisms predisposes for more severe asthma and more frequent and serious exacerbations [88-90]. Certain features of asthma, such as bronchial hyper reactivity and impaired pulmonary function have been proven to persist long after *chlamydial* or *mycoplasmal* infections [91,92].

These findings formed the starting point of the randomised trials that investigated the role of macrolide treatment in subjects with serological evidence of infection with either MP or CP.

Kraft et al [93] treated 55 asthma patients with either clarithromycin (500 mg bid) or placebo. When subjects who received clarithromycin were divided by PCR status, only patients with positive PCR findings for CP or MP showed a significant increase in FEV1 and reduced expression of IL-5 (a pro-inflammatory cytokine). No improvement of any parameter was seen in PCR-negative, clarithromycin-treated patients. In a large multi-center trial, 232 subjects with asthma and high titers of IgA and/or IgG antibodies to *C. pneumoniae* were randomised to roxithromycin 150 mg bid or placebo for 6 weeks [94]. Treatment with roxithromycin lead to minimal improvement in asthma control (slight improvement in morning PEF). Due to this small effect, the calculated sample size was found to be insufficient to gain adequate power. They found no change in symptom scores and improvement in peak flow measurement disappeared after treatment was discontinued. Hahn et al [95] completed a community based pilot study in which 45 patients with mild to moderate asthma were randomised to azithromycin (600 mg weekly) or placebo for 6 weeks in addition to their usual asthma care. Not only did they find a clear correlation between asthma severity and serum IgA antibodies against CP, they also demonstrated a beneficial effect of azithromycin on overall asthma symptoms. Due to its pilot nature and the small number of participants, no significant conclusions could be drawn.

Although there is convincing evidence for a role of MP and CP in the pathogenesis of asthma, results of randomised trials investigating the effect of macrolide therapy in asthmatic patients with serologic evidence of atypical infection, are, to date, unsatisfactory. Perhaps advanced techniques using qPCR might elucidate the role of bacterial burden and potential benefit of long-term macrolide treatment in asthmatic patients with significant bacterial burdens.

The use of macrolide maintenance therapy in asthma in general has been subject of a Cochrane review in 2005 [96]. After long-term macrolide treatment no significant change in lung function and only a small symptom reduction was found, although serum markers of eosinophilic inflammation were found to be markedly decreased.

The authors concluded: 'Even though some clinical data indicate a positive effect of macrolides in asthmatic patients in the absence of relevant side effects, these data are insufficient to recommend the routine use of macrolides for control of asthma at present.'

Randomised controlled trials performed after 2005 focus on the possible immune-modulating effect of macrolides in asthma. A small Italian study investigated the effect of 8 weeks azithromycin treatment vs. placebo in 16 asthmatic children [97]. Although they found a significant decrease in inflammatory markers in the azithromycin treated group, no change in lung function was observed. Simpson et al [98] distinguished two types of asthmatic patients; allergic asthma, characterized by eosinophilic inflammation and high IgE-titers, as opposed to non-eosinophilic asthma (NEA) which features increased neutrophil numbers and high IL-8 levels in the airways. Forty-six patients with refractory asthma, randomised to clarithromycin (500 mg bid) or placebo, were stratified according to neutrophil proportion and treated for 8 weeks. The authors demonstrated a significant reduction in IL-8 and neutrophil numbers and an improvement of QOL in all patients after 8 weeks of clarithromycin treatment. In view of the fact that they found a non-significant fall in all other inflammation markers, the authors suggest that clarithromycin causes an overall down-regulation of neutrophil activation and mediator release. The anti-inflammatory effect was most marked in NEA patients (with high neutrophil proportions), suggesting that long-term macrolide therapy could prove a good add-on therapy in refractory NEA. So, in line with the previously mentioned studies on chronic rhinosinusitis, it is plausible that a subgroup of patients with high IL-8 and low IgE-levels, benefits most from long-term macrolide treatment.

Long-term macrolide therapy in cryptogenic organizing pneumonia

Organizing pneumonia is a non-specific inflammatory disorder, pathologically characterized by the presence of granulation tissue and fibrosis in the alveoli and distal bronchioles [99]. In the presence of specific clinicoradiological features, this entity is defined as cryptogenic organizing pneumonia (COP).

In 1993, Japanese researchers demonstrated that some patients with COP show clinical and radiological improvement after prolonged treatment with erythromycin [100].

A more recent report on clarithromycin treatment in three patients with COP, describes complete resolution of pulmonary and systemic symptoms in two of them, while being treated with low dosages (250 mg once or twice daily) for three months [101]. A possible mechanism of action is proposed by Hotta et al [102]. They demonstrated a significant decrease of IL-8 levels and neutrophil numbers in BAL-fluid of 8 patients with COP after treatment with erythromycin (600mg daily for three months), indicating that macrolides cause inhibition of neutrophil accumulation in the peripheral airways in patients with COP. No large-scale studies however, are available to confirm these findings. Up to now corticosteroids are advised as first choice for the treatment of COP.

Safety of long term macrolide treatment

Gastro-intestinal complaints are the most common adverse effects in patients receiving macrolide therapy. Gastro-intestinal adverse effect rates of 15-20% for erythromycin, 8.7% for clarithromycin and 9.6% for azithromycin have been observed in patients receiving short courses of macrolides [103]. In randomised trials of long term, low dose macrolide treatment in chronic pulmonary diseases, mild to moderate gastro-intestinal complaints are reported, which hardly ever cause study drug discontinuation (table 1).

*Table 1: Adverse events reported in randomised trials of long term macrolide treatment in chronic sino-pulmonary diseases. * (no of patients), **no significant difference between study drug and placebo*

Table 1	Patient group	No of patients	Intervention	Adverse events in treatment group*
Wolter 2002 [10]	CF, adults	60	azithromycin 250mg/day; 3 months	urticaria(1), neutropenia (1), rash (1).
Equi 2002 [8]	CF, children	41	azithromycin 250 or 500mg/day; 6 months	transient elevation of liver enzymes (1).
Clement 2006 [7]	CF, children	82	azithromycin 250 or 500mg, 3x/wk; 12 months	gastrointestinal (16), ENT-infections (14), headache (2)**.
McCormack 2007 [11]	CF, adults + children	208	azithromycin 250mg/day vs. azithromycin 1200mg/wk; 6 months	> 4x increase in liver enzymes (8), gastro-intestinal (36).
Steinkamp 2008 [82]	CF, adults + children	38	azithromycin 500/ 750/ 1000/ 1250 mg/wk; 8 weeks	gastrointestinal, rash.
Black 2001 [77]	Asthma, adults	232	roxithromycin 150mg bid; 6 weeks	gastrointestinal (19), transient elevation of liver enzymes (6).
Kostadima 2004 [83]	Asthma, adults	63	clarithromycin 250mg bid / tid; 8 weeks	withdrawal due to gastro-intestinal side effects (1).
Hahn 2006 [78]	Asthma, adults	45	azithromycin 600mg/week, 3 months	mild to moderate gastro-intestinal side effects (5).
Wallwork 2006 [43]	Chronic sinusitis, adults	64	roxithromycin 150mg/day; 3 months	withdrawal due to gastro-intestinal side effects (1).
Banerjee 2008 [36]	COPD, adults	46	clarithromycin 500mg/day; 3 months	withdrawal due to gastro-intestinal side effects (1).
Seemungal 2008 [19]	COPD, adults	109	erythomycin 250mg bid; 12 months	gastrointestinal (8), rash (3), tinnitus (1)**.

Other infrequently reported side effects related to macrolide use are rash (0.5 – 6%) and hepatotoxicity (most frequently a transient increase in liver enzymes or cholestasis)

[103,104]. As all macrolide-related side effects, these are more common following treatment with erythromycin than with the other 14- and 15-membered ring macrolides [103,105]. Furthermore, the incidence of adverse effects increases when macrolides are administered in larger dosages or reaching higher serum levels [22,103,106].

Ototoxicity and cardiac toxicity are familiar, though very rare, side effects of macrolide antibiotics. Because of their infrequent occurrence, these side effects are hardly ever observed in clinical trials. Few of the abovementioned trials monitored for cardiac arrhythmia or hearing loss, so no solid data are available concerning the incidence of these side effects during long term use of macrolides. The incidence and outcome of these conditions during short term use of macrolides has, however, been established. Furthermore, the potential seriousness of these two side effects makes them fit to be discussed in detail.

Ototoxicity:

Ototoxicity caused by macrolide use is typically described as reversible sensorineural bilateral hearing loss, involving the lower or speech frequencies. Most articles about this subject are case reports or review articles, mainly published more than 15 years ago [103,107-109]. Swanson et al [109] conducted the only prospective case-control study to date, in which hearing tests were carried out in 45 patients receiving intravenous antibiotic treatment for pneumonia. 21% of patients who were treated with high dosages of erythromycin developed varying degrees of hearing impairment, as opposed to none of the patients who were treated an other anti-microbial agent. They furthermore demonstrated that only renal impairment or decreased total systemic clearance was associated with the development of hearing loss in this small group of patients.

This study and other reports point out, that macrolide use causes ototoxicity in an obviously dose-dependent fashion. Only daily macrolide dosages similar to azithromycin 1500 mg or clarithomycin 2000 mg changed the cochlear response rate in guinea pigs [110]. Case reports about erythromycin ototoxity describe hearing impairment mainly in association with intravenous therapy and/or dosages > 4000 mg od [104,107,108,111].

Ototoxicity following long term application of macrolide antibiotics has been described in the treatment of *Mycobacterium avium* complex infections. In a prospective, non-blinded study, 25% of patients receiving high dosages of azithromycin (600 mg daily) complained of hearing impairment. This was confirmed by audiometry in 6 out of 10 patients. After decreasing the dosage of azithromycin to 300 mg/day or lower ototoxicity was no longer noticed, which was confirmed by audiological testing in 3 of these patients [106]. Wallace et al [35] also report reversible hearing loss in their series of patients with AIDS

and disseminated *M. avium* disease. 14 out of 21 patients who were treated with daily dosages of 500 mg azithromycin as part of a 3-drug combination regimen between 1992 and 1993, spontaneously complained of hearing loss. No more hearing loss occurred after replacement of azithromycin for clarithromycin in their standard treatment regimen. A few years later, Tseng et al [112] reported reversible ototoxicity in 17% of 46 HIV-patients treated with azithromycin 600mg daily.

No solid information is available concerning the incidence of ototoxicity during long-term macrolide use in lower dosages.

In conclusion, macrolide antibiotics are able to cause hearing loss that is almost always reversible and apparently only when administered in high dosages. The incidence of ototoxicity during low-dose long-term macrolide use is unknown, but, considering the above, probably negligible.

Cardiac toxicity

In case reports, macrolide antibiotics have been associated with prolongation of the QT-interval and torsades de pointes (TdP). The European Society of cardiology considers erythromycin (EM) and clarithromycin as drugs that are particularly associated with cardiac arrhythmias [113]. Intravenous use of erythromycin has been associated with a particular high risk of arrhythmias, probably due to the poorer absorption of oral preparations [113,114]. One large cohort study in 2004 found a twofold increased risk of sudden death from cardiac causes among patients currently using erythromycin, compared to those who had not used any of the antibiotic medications studied [34]. TdP is a polymorphic ventricular tachycardia, usually preceded by prolongation of the QT-interval, caused by altered cardiac repolarisation. It may be asymptomatic but has also been associated with syncope and sudden death. Drug induced prolongation of the QT-interval is generally regarded as a reliable predictive measure for the risk of TdP arrhythmias.

Macrolides have two potential effects on the QT-interval: 1. Intrinsic prolongation: macrolide antibiotics prolong the repolarisation period of the action potential by blocking the HERG potassium channels [115]. 2. Inhibition of metabolism of other pro-arrhythmogenic drugs by acting on cytochrome P450 in the liver. When, for instance, erythromycin and other inhibitors of cytochrome P450 are concurrently prescribed, a 5-fold greater risk of cardiac sudden death was reported [34]. Patient characteristics, associated with a greater risk of developing cardiac arrhythmias following macrolide use are shown in table 2 [114,116,117].

Table 2. Risk factors for QT-prolongation or torsade de points during use of macrolide antibiotics

Table 2.

- Age > 80 years
- Female gender
- Heart disease
- Use of other QT-prolonging medication
- Reduced drug elimination
- Hypokalaemia / Hypomagnesaemia
- Prolonged QT-interval before therapy
- Bradycardia
- Genetic predisposition

Information about the incidence of (fatal) cardiac arrhythmias associated with macrolide use most often comes from cohort studies and adverse events reporting systems. The only randomised clinical trial of long term macrolide treatment including follow-up ECG's reported no arrhythmias [50].

Retrospective evaluation of reports regarding TdP or ventricular tachycardia related to macrolides (erythromycin, clarithromycin and azithromycin) in the US Food and Drug Administration Adverse Events Reporting System (FDA-AERS) yielded 156 reports in a 13-year period of time (1987-2000). In half of these cases (n=78), a macrolide with no concomitant use of other QT-prolonging drugs was involved. Fatal outcomes were mentioned in 12% (n=9) of macrolide-only reports. Limitations of this analysis brought forth by the authors were the well known biases of spontaneous reports and the fact that TdP was not electrocardiographically confirmed in all cases [117]. Incidence of spontaneous reports of TdP marked out against the number of macrolide prescriptions from 1993-2000 is 0.06 (azithromycin) - 0.18 (clarithromycin) per million prescriptions, according to FDA databases [http://www.fda.gov/ohrms/dockets/ac/01/slides/3746s_02_shaffer_rev/sld020.htm].

The aforementioned data indicate that, although cardiac toxicity is rare, special care must be taken when administering macrolide antibiotics, especially erythromycin IV, to certain groups of patients. In female patients older than 80 years, with cardiac co-morbidity or using other pro-arrhythmogenic drugs, ECG follow-up to observe possible QT-prolongation during use of macrolide antibiotics should be considered.

Macrolide resistance

Epidemiology

Reduced susceptibility to antibiotics of respiratory pathogens can be considered an increasing global problem. In general, macrolide resistance has increased considerably over the last decade [118-121]. Both macrolide resistance rates and resistance mechanisms however, vary considerably depending on location. The highest incidence of macrolide resistance in *S. pneumoniae* is found in Japan, where 80-100% of pneumococci have been found to be macrolide resistant [118,122]. In contrast, resistance rates in Scandinavia (Norway, Sweden) are remarkably low, about 8 - 9 % [123]. A significant association between prescribing of macrolides and local resistance is observed in most studies [120,123-126].

Together with an increase of macrolide resistance in respiratory pathogens, a parallel increase in oropharyngeal carriage of macrolide resistant commensals is observed. High percentages of macrolide resistance up to 94% among viridians group streptococci are reported in healthy volunteers worldwide [127,128]. Although these commensals usually do not cause infections in healthy persons, they may be considered a threat for two reasons; they could cause infection in immune compromised hosts and they might transfer the resistance determinants to pathogenic streptococci [128,129].

Development of macrolide resistance during long term macrolide therapy

In long-term use of antibiotics, reduced susceptibility of potentially pathogenic micro organisms poses a threat. Nevertheless only a few of the randomised clinical trials mentioned in this review address this problem. The ones that did monitor for resistance found no macrolide resistant organisms during follow-up after three months of macrolide maintenance therapy in COPD (n=46), and chronic rhinosinusitis (n=64) [50,59]. A recently performed trial of erythromycin in COPD (n=109) found no influence on the microbiological profile of sputum by the use of erythromycin. Only one case of erythromycin resistance occurred in the macrolide arm of the study after 12 months [33].

In the field of CF research, where long-term macrolide therapy is often practiced, the problem of reduced susceptibility of micro-organisms following this treatment has been covered more thoroughly, though retrospectively [130]. Phaff et al [131] investigated macrolide resistance of *S. aureus* and *H. influenzae* in sputum isolates of 156 CF-patients receiving azithromycin maintenance therapy with a mean duration of 397 days. Erythromycin resistance in *S. aureus* increased from 6.9% at the commencement of study to 53,8% after 5 years of follow up. Concomitantly a tenfold increase in clarithromycin resistance in *H. influenzae* isolates (3,7 to 37.0%) was observed. Other notable results came from a retrospective study by Tramper-Stranders et al [132]. They noticed macrolide resistance in all (100%) *S. aureus* isolates

obtained from 100 CF patients after azithromycin treatment with a mean duration of 3.5 years. Emergence of macrolide-resistant *S. aureus* was not related to pulmonary function decline. Similar numbers were published concerning emergence of macrolide resistant *S. pneumoniae* following macrolide treatment. After 4 years of erythromycin or clarithromycin maintenance treatment, 98.2 % of pneumococcial isolates recovered from 57 CF patients showed macrolide resistance [133].

Based on these results, we conclude that macrolide resistance in respiratory pathogens only significantly increases when maintenance therapy is given for a long period of time (3-5 years). Ultimately resistance rates can become very high, up to 100%. A maintenance regimen that involves seasonal administration of macrolides (during the autumn and winter periods), as has already been used in some Dutch hospitals, might attenuate the emergence of resistance. Investigating the effect of such a regimen in a randomised trial might be helpful.

The impact of macrolide resistance on clinical outcome is almost exclusively described in observational studies in patients with community acquired pneumonia (CAP), the results of which are subject to dual interpretation. Although considerable numbers of macrolide-resistant organisms were found, no significantly increased morbidity and mortality was demonstrated in these patients [134-136]. Other authors however, believe that the observational study results indicate that macrolide non-susceptibility is responsible for treatment failure in these patients and may be considered a serious hazard [119,129,137-139].

Macrolide resistance in patients treated for *Mycobacterium avium* complex diseases

Long term macrolide therapy plays a key role in treatment regimens and prophylaxis for *Mycobacterium avium* complex (MAC) and other atypical mycobacteria. According to the guidelines of the American Thoracic Society published in 2007, the standard treatment regimen of MAC pulmonary disease in HIV-negative patients should contain either clarithromycin (500-1000 thrice weekly) or azithromycin 250-600 mg thrice weekly). The dosages depend on the status and/or severity of the disease [140].

Macrolide resistance is much more common in patients receiving macrolide monotherapy or macrolide plus a quinolone for more than one month during their treatment period. Griffith and colleagues [141] report the occurrence of macrolide resistant pathogens in 20% of patients who are initially treated with macrolide monotherapy for MAC lung disease. Conversely, only 4 – 6.6 % of patients with MAC lung disease become resistant when a three-dose regimen, including ethambutol and rifampin or rifabutin , is used from the start of treatment [142,143].

Macrolide resistance in MAC pulmonary disease appears to be associated with higher relapse rates and a poor prognosis. In a retrospective study in 51 patients with MAC lung disease, 1-year mortality increased from 0% to 34%, when patients remained culture positive despite adequate treatment [141]. A randomized study by Benson and coworkers [144] in patients with disseminated MAC showed a spectacular decrease in relapse rates when a three-drug regimen was used (6%), compared to macrolide monotherapy (24%).

To avoid the emergence of resistant organisms, it is recommended that MAC lung disease be treated with a macrolide in combination with two or three other medications [140,145,146].

Conclusion

In pulmonary practice, long-term macrolide maintenance therapy has become increasingly popular for the treatment of patients with other chronic inflammatory conditions, such as COPD, asthma, bronchiectasis and sinusitis, who have recurrent infections or other signs of 'badly-regulated disease'. Scientific evidence to justify this, however, is still conflicting in the case of COPD and lacking in non-CF bronchiectasis, sinusitis and asthma. Study results to date, show a beneficial effect of macrolides on exacerbation frequency, sputum volume, and inflammatory markers. Low dose macrolides should be applied for at least three months to establish and maintain these advantageous effects. Gastro-intestinal complaints are the most frequently reported adverse effects of long-term macrolide treatment.
Increased macrolide resistance among different pathogens has been documented in maintenance treatment, but the clinical significance of this reduced susceptibility to macrolides remains unknown.

Reference list

1. Kudoh S, Uetake T, Hagiwara K: [Clinical effects of low-dose long-term erythromycin chemotherapy on diffuse panbronchiolitis]. Nihon Kyobu Shikkan Gakkai Zasshi 1987; 25: 632-42.
2. Kudoh S, Azuma A, Yamamoto M: Improvement of survival in patients with diffuse panbronchiolitis treated with low-dose erythromycin. Am J Respir Crit Care Med 1998; 157: 1829-32.
3. Martinez FJ, Simon RH: Clinical implications of macrolide therapy in chronic sinopulmonary diseases. Curr.Pharm.Des 2004; 10: 3095-110.
4. Poletti V, Casoni G, Chilosi M: Diffuse panbronchiolitis. Eur Respir J 2006; 28: 862-71.
5. Sakito O, Kadota J, Kohno S: Interleukin 1 beta, tumor necrosis factor alpha, and interleukin 8 in bronchoalveolar lavage fluid of patients with diffuse panbronchiolitis: a potential mechanism of macrolide therapy. Respiration 1996; 63: 42-48.
6. Homma H, Yamanaka A, Tanimoto S: Diffuse panbronchiolitis. A disease of the transitional zone of the lung. Chest 1983; 83: 63-69.
7. Sugiyama Y, Kudoh S, Maeda H: Analysis of HLA antigens in patients with diffuse panbronchiolitis. Am Rev Respir Dis 1990; 141: 1459-62.
8. Fujii T, Kadota J, Kawakami K: Long term effect of erythromycin therapy in patients with chronic Pseudomonas aeruginosa infection. Thorax 1995; 50: 1246-52.
9. Nagai H, Shishido H, Yoneda R: Long-term low-dose administration of erythromycin to patients with diffuse panbronchiolitis. Respiration 1991; 58: 145-49.
10. Tateda K, Ishii Y, Kimura S: Suppression of Pseudomonas aeruginosa quorum-sensing systems by macrolides: a promising strategy or an oriental mystery? J Infect. Chemother. 2007; 13: 357-67.
11. Nakamura H, Fujishima S, Inoue T: Clinical and immunoregulatory effects of roxithromycin therapy for chronic respiratory tract infection. Eur Respir J 1999; 13: 1371-79.
12. Hiratsuka T, Mukae H, Iiboshi H: Increased concentrations of human beta-defensins in plasma and bronchoalveolar lavage fluid of patients with diffuse panbronchiolitis. Thorax 2003; 58: 425-30.
13. Ichimiya T, Takeoka K, Hiramatsu K: The influence of azithromycin on the biofilm formation of Pseudomonas aeruginosa in vitro. Chemotherapy 1996; 42: 186-91.
14. Kondoh K, Hashiba M, Baba S: Inhibitory activity of clarithromycin on biofilm synthesis with Pseudomonas aeruginosa. Acta Otolaryngol.Suppl 1996; 525: 56-60.
15. Ichimiya T, Yamasaki T, Nasu M: In-vitro effects of antimicrobial agents on Pseudomonas aeruginosa biofilm formation. J Antimicrob.Chemother. 1994; 34: 331-41.
16. Takeoka K, Ichimiya T, Yamasaki T: The in vitro effect of macrolides on the interaction of human polymorphonuclear leukocytes with Pseudomonas aeruginosa in biofilm. Chemotherapy 1998; 44: 190-97.

17. Bilton D: Update on non-cystic fibrosis bronchiectasis. Curr.Opin.Pulm.Med 2008; 14: 595-99.
18. Clement A, Tamalet A, Leroux E: Long term effects of azithromycin in patients with cystic fibrosis: A double blind, placebo controlled trial. Thorax 2006; 61: 895-902.
19. Equi A, Balfour-Lynn IM, Bush A: Long term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled crossover trial. Lancet 2002; 360: 978-84.
20. Saiman L, Marshall BC, Mayer-Hamblett N: Azithromycin in patients with cystic fibrosis chronically infected with Pseudomonas aeruginosa: a randomized controlled trial. JAMA 2003; 290: 1749-56.
21. Wolter J, Seeney S, Bell S: Effect of long term treatment with azithromycin on disease parameters in cystic fibrosis: a randomised trial. Thorax 2002; 57: 212-16.
22. McCormack J, Bell S, Senini S: Daily versus weekly azithromycin in cystic fibrosis patients. Eur Respir J 2007; 30: 487-95.
23. Courtney JM, Bradley J, Mccaughan J: Predictors of mortality in adults with cystic fibrosis. Pediatr.Pulmonol. 2007; 42: 525-32.
24. Navarro J, Rainisio M, Harms HK: Factors associated with poor pulmonary function: cross-sectional analysis of data from the ERCF. European Epidemiologic Registry of Cystic Fibrosis. Eur Respir J 2001; 18: 298-305.
25. Saiman L, Anstead M, Mayer-Hamblett N: Effect of azithromycin on pulmonary function in patients with cystic fibrosis uninfected with Pseudomonas aeruginosa: a randomized controlled trial. JAMA 2010; 303: 1707-15.
26. Bush A, Rubin BK: Macrolides as biological response modifiers in cystic fibrosis and bronchiectasis. Semin.Respir Crit Care Med 2003; 24: 737-48.
27. Giamarellos-Bourboulis EJ: Macrolides beyond the conventional antimicrobials: a class of potent immunomodulators. Int J Antimicrob.Agents 2008; 31: 12-20.
28. Healy DP: Macrolide immunomodulation of chronic respiratory diseases. Curr.Infect. Dis Rep 2007; 9: 7-13.
29. Idris SF, Chilvers ER, Haworth C: Azithromycin therapy for neutrophilic airways disease: myth or magic? Thorax 2009; 64: 186-89.
30. Tamaoki J: The effects of macrolides on inflammatory cells. Chest 2004; 125: 41S-50S.
31. Cole PJ: Inflammation: a two-edged sword--the model of bronchiectasis. Eur J Respir Dis Suppl 1986; 147: 6-15.
32. Hanania NA: Targeting airway inflammation in asthma: current and future therapies. Chest 2008; 133: 989-98.
33. Seemungal TA, Wilkinson TM, Hurst JR: Long-term erythromycin therapy is associated with decreased chronic obstructive pulmonary disease exacerbations. Am J Respir Crit Care Med 2008; 178: 1139-47.
34. Ray WA, Murray KT, Meredith S: Oral erythromycin and the risk of sudden death from cardiac causes. N Engl J Med 2004; 351: 1089-96.
35. Wallace MR, Miller LK, Nguyen MT: Ototoxicity with azithromycin. Lancet 1994; 343: 241.

36. Nicotra MB: Bronchiectasis. Semin.Respir Infect. 1994; 9: 31-40.
37. van Haren EH, Mannes GP: [Diagnosis and treatment of bronchiectasis]. Ned.Tijdschr. Geneeskd. 2004; 148: 120-25.
38. Cohen M, Sahn SA: Bronchiectasis in systemic diseases. Chest 1999; 116: 1063-74.
39. Barker AF: Bronchiectasis. N Engl J Med 2002; 346: 1383-93.
40. Pasteur MC, Helliwell SM, Houghton SJ: An investigation into causative factors in patients with bronchiectasis. Am J Respir Crit Care Med 2000; 162: 1277-84.
41. Suhs RH, Dowling HF, Jackson GG: Hypogammaglobulinemia with chronic bronchitis or bronchiectasis; treatment of five patients with long-term antibiotic therapy. Arch Intern Med 1965; 116: 29-38.
42. Koh YY, Lee MH, Sun YH: Effect of roxithromycin on airway responsiveness in children with bronchiectasis: a double-blind, placebo-controlled study. Eur Respir J 1997; 10: 994-99.
43. Tsang KW, Ho PI, Chan KN: A pilot study of low-dose erythromycin in bronchiectasis. Eur Respir J 1999; 13: 361-64.
44. Yalcin E, Kiper N, Ozcelik U: Effects of claritromycin on inflammatory parameters and clinical conditions in children with bronchiectasis. J Clin Pharm.Ther. 2006; 31: 49-55.
45. Cymbala AA, Edmonds LC, Bauer MA: The disease-modifying effects of twice-weekly oral azithromycin in patients with bronchiectasis. Treat.Respir Med 2005; 4: 117-22.
46. Davies G, Wilson R: Prophylactic antibiotic treatment of bronchiectasis with azithromycin. Thorax 2004; 59: 540-41.
47. Chronic obstructive pulmonary disease. National clinical guideline on management of chronic obstructive pulmonary disease in adults in primary and secondary care. Thorax 2004; 59 Suppl 1: 1-232.
48. Siempos II, Dimopoulos G, Korbila IP: Macrolides, quinolones and amoxicillin/clavulanate for chronic bronchitis: a meta-analysis. Eur Respir J 2007; 29: 1127-37.
49. Black P, Staykova T, Chacko E: Prophylactic antibiotic therapy for chronic bronchitis. Cochrane.Database.Syst.Rev 2003; CD004105.
50. Banerjee D, Khair OA, Honeybourne D: The effect of oral clarithromycin on health status and sputum bacteriology in stable COPD. Respir Med 2005; 99: 208-15.
51. Macrolide Azithromycin to Prevent Rapid Worsening of Symptoms Associated With Chronic Obstructive Pulmonary Disease. clinicaltrials.gov identifier: NCT00325897.
52. Kunisaki KM, Niewoehner DE: Antibiotic prophylaxis for chronic obstructive pulmonary disease: resurrecting an old idea. Am J Respir Crit Care Med 2008; 178: 1098-99.
53. Desrosiers MY, Kilty SJ: Treatment alternatives for chronic rhinosinusitis persisting after ESS: what to do when antibiotics, steroids and surgery fail. Rhinology 2008; 46: 3-14.
54. Hashiba M, Baba S: Efficacy of long-term administration of clarithromycin in the treatment of intractable chronic sinusitis. Acta Otolaryngol.Suppl 1996; 525: 73-78.
55. Kimura N, Nishioka K, Nishizaki K: Clinical effect of low-dose, long-term roxithromycin

chemotherapy in patients with chronic sinusitis. Acta Med Okayama 1997; 51: 33-37.
56. Suzuki H, Shimomura A, Ikeda K: Effects of long-term low-dose macrolide administration on neutrophil recruitment and IL-8 in the nasal discharge of chronic sinusitis patients. Tohoku J Exp Med 1997; 182: 115-24.
57. Cervin A, Wallwork B: Anti-inflammatory effects of macrolide antibiotics in the treatment of chronic rhinosinusitis. Otolaryngol.Clin North Am 2005; 38: 1339-50.
58. Yamada T, Fujieda S, Mori S: Macrolide treatment decreased the size of nasal polyps and IL-8 levels in nasal lavage. Am J Rhinol. 2000; 14: 143-48.
59. Wallwork B, Coman W, Mackay-Sim A: A double-blind, randomized, placebo-controlled trial of macrolide in the treatment of chronic rhinosinusitis. Laryngoscope 2006; 116: 189-93.
60. Cervin A, Kalm O, Sandkull P: One-year low-dose erythromycin treatment of persistent chronic sinusitis after sinus surgery: clinical outcome and effects on mucociliary parameters and nasal nitric oxide. Otolaryngol.Head Neck Surg 2002; 126: 481-89.
61. Suzuki H, Ikeda K, Honma R: Prognostic factors of chronic rhinosinusitis under long-term low-dose macrolide therapy. ORL J Otorhinolaryngol.Relat Spec. 2000; 62: 121-27.
62. Chapman ID, Foster A, Morley J: The relationship between inflammation and hyperreactivity of the airways in asthma. Clin Exp Allergy 1993; 23: 168-71.
63. Jeffery PK: Remodeling in asthma and chronic obstructive lung disease. Am J Respir Crit Care Med 2001; 164: S28-S38.
64. Amayasu H, Yoshida S, Ebana S: Clarithromycin suppresses bronchial hyperresponsiveness associated with eosinophilic inflammation in patients with asthma. Ann Allergy Asthma Immunol. 2000; 84: 594-98.
65. Beigelman A, Gunsten S, Mikols CL: Azithromycin Attenuates Airway Inflammation in a Noninfectious Mouse Model of Allergic Asthma. Chest 2009.
66. Ekici A, Ekici M, Erdemoglu AK: Effect of azithromycin on the severity of bronchial hyperresponsiveness in patients with mild asthma. J Asthma 2002; 39: 181-85.
67. Kamoi H, Kurihara N, Fujiwara H: The macrolide antibacterial roxithromycin reduces bronchial hyperresponsiveness and superoxide anion production by polymorphonuclear leukocytes in patients with asthma. J Asthma 1995; 32: 191-97.
68. Miyatake H, Taki F, Taniguchi H: Erythromycin reduces the severity of bronchial hyperresponsiveness in asthma. Chest 1991; 99: 670-73.
69. Shimizu T, Kato M, Mochizuki H: Roxithromycin reduces the degree of bronchial hyperresponsiveness in children with asthma. Chest 1994; 106: 458-61.
70. Takizawa H, Desaki M, Ohtoshi T: Erythromycin and clarithromycin attenuate cytokine-induced endothelin-1 expression in human bronchial epithelial cells. Eur Respir J 1998; 12: 57-63.
71. Spector SL: Troleandomycin; effectiveness in steroid dependent asthma and bronchitis. Journal of Allergy and Clinical Immunology 1974; 54: 367-79.

72. Szefler SJ, Rose JQ, Ellis EF: The effect of troleandomycin on methylprednisolone elimination. J Allergy Clin Immunol. 1980; 66: 447-51.
73. Hill JM, Tattersfield AE: Corticosteroid sparing agents in asthma. Thorax 1995; 50: 577-82.
74. Szefler SJ, Brenner M, Jusko WJ: Dose- and time-related effect of troleandomycin on methylprednisolone elimination. Clin Pharmacol Ther. 1982; 32: 166-71.
75. Zeiger RS, Schatz M, Sperling W: Efficacy of troleandomycin in outpatients with severe, corticosteroid-dependent asthma. J Allergy Clin Immunol. 1980; 66: 438-46.
76. Wald JA, Friedman BF, Farr RS: An improved protocol for the use of troleandomycin (TAO) in the treatment of steroid-requiring asthma. J Allergy Clin Immunol. 1986; 78: 36-43.
77. Harris R, German D: The incidence of corticosteroid side effects in chronic steroid-dependent asthmatics on TAO (troleandomycin) and methylprednisolone. Ann Allergy 1989; 63: 110-11.
78. Ball BD, Hill MR, Brenner M: Effect of low-dose troleandomycin on glucocorticoid pharmacokinetics and airway hyperresponsiveness in severely asthmatic children. Ann Allergy 1990; 65: 37-45.
79. Kamada AK, Hill MR, Ikle DN: Efficacy and safety of low-dose troleandomycin therapy in children with severe, steroid-requiring asthma. J Allergy Clin Immunol. 1993; 91: 873-82.
80. Nelson HS, Hamilos DL, Corsello PR: A double-blind study of troleandomycin and methylprednisolone in asthmatic subjects who require daily corticosteroids. Am Rev Respir Dis 1993; 147: 398-404.
81. Evans DJ, Cullinan P, Geddes DM: Troleandomycin as an oral corticosteroid steroid sparing agent in stable asthma. Cochrane.Database.Syst.Rev 2001; CD002987.
82. Garey KW, Rubinstein I, Gotfried MH: Long-term clarithromycin decreases prednisone requirements in elderly patients with prednisone-dependent asthma. Chest 2000; 118: 1826-27.
83. Sutherland ER, Martin RJ: Asthma and atypical bacterial infection. Chest 2007; 132: 1962-66.
84. Martin RJ, Kraft M, Chu HW: A link between chronic asthma and chronic infection. J Allergy Clin Immunol. 2001; 107: 595-601.
85. Berkovich S, Millian SJ, Snyder RD: The association of viral and mycoplasma infections with recurrence of wheezing in the asthmatic child. Ann Allergy 1970; 28: 43-49.
86. Lieberman D, Lieberman D, Printz S: Atypical pathogen infection in adults with acute exacerbation of bronchial asthma. Am J Respir Crit Care Med 2003; 167: 406-10.
87. Seggev JS, Lis I, Siman-Tov R: Mycoplasma pneumoniae is a frequent cause of exacerbation of bronchial asthma in adults. Ann Allergy 1986; 57: 263-65.
88. Black PN, Scicchitano R, Jenkins CR: Serological evidence of infection with Chlamydia pneumoniae is related to the severity of asthma. Eur Respir J 2000; 15: 254-59.

89. Cook PJ, Davies P, Tunnicliffe W: Chlamydia pneumoniae and asthma. Thorax 1998; 53: 254-59.
90. Cunningham AF, Johnston SL, Julious SA: Chronic Chlamydia pneumoniae infection and asthma exacerbations in children. Eur Respir J 1998; 11: 345-49.
91. Mok JY, Waugh PR, Simpson H: Mycoplasma pneuminia infection. A follow-up study of 50 children with respiratory illness. Arch Dis Child 1979; 54: 506-11.
92. Sabato AR, Martin AJ, Marmion BP: Mycoplasma pneumoniae: acute illness, antibiotics, and subsequent pulmonary function. Arch Dis Child 1984; 59: 1034-37.
93. Kraft M, Cassell GH, Pak J: Mycoplasma pneumoniae and Chlamydia pneumoniae in asthma: effect of clarithromycin. Chest 2002; 121: 1782-88.
94. Black PN, Blasi F, Jenkins CR: Trial of roxithromycin in subjects with asthma and serological evidence of infection with Chlamydia pneumoniae. Am J Respir Crit Care Med 2001; 164: 536-41.
95. Hahn DL, Plane MB, Mahdi OS: Secondary outcomes of a pilot randomized trial of azithromycin treatment for asthma. PLoS.Clin Trials 2006; 1: e11.
96. Richeldi L, Ferrara G, Fabbri LM: Macrolides for chronic asthma. Cochrane.Database. Syst.Rev 2005; CD002997.
97. Piacentini GL, Peroni DG, Bodini A: Azithromycin reduces bronchial hyperresponsiveness and neutrophilic airway inflammation in asthmatic children: a preliminary report. Allergy Asthma Proc 2007; 28: 194-98.
98. Simpson JL, Powell H, Boyle MJ: Clarithromycin targets neutrophilic airway inflammation in refractory asthma. Am J Respir Crit Care Med 2008; 177: 148-55.
99. Cordier JF: Organising pneumonia. Thorax 2000; 55: 318-28.
100. Ichikawa Y, Ninomiya H, Katsuki M: Low-dose/long-term erythromycin for treatment of bronchiolitis obliterans organizing pneumonia (BOOP). Kurume Med J 1993; 40: 65-67.
101. Stover DE, Mangino D: Macrolides: a treatment alternative for bronchiolitis obliterans organizing pneumonia? Chest 2005; 128: 3611-17.
102. Hotta M: Neutrophil chemotactic activity in cryptogenic organizing pneumonia and the response to erythromycin. Kurume Med J 1996; 43: 207-17.
103. Periti P, Mazzei T, Mini E: Adverse effects of macrolide antibacterials. Drug Saf 1993; 9: 346-64.
104. Principi N, Esposito S: Comparative tolerability of erythromycin and newer macrolide antibacterials in paediatric patients. Drug Saf 1999; 20: 25-41.
105. Rubinstein E: Comparative safety of the different macrolides. Int J Antimicrob.Agents 2001; 18 Suppl 1: S71-S76.
106. Brown BA, Griffith DE, Girard W: Relationship of adverse events to serum drug levels in patients receiving high-dose azithromycin for mycobacterial lung disease. Clin Infect.Dis 1997; 24: 958-64.
107. McGhan LJ, Merchant SN: Erythromycin ototoxicity. Otol.Neurotol. 2003; 24: 701-02.

108. Sacristan JA, Soto JA, de Cos MA: Erythromycin-induced hypoacusis: 11 new cases and literature review. Ann Pharmacother 1993; 27: 950-55.
109. Swanson DJ, Sung RJ, Fine MJ: Erythromycin ototoxicity: prospective assessment with serum concentrations and audiograms in a study of patients with pneumonia. Am J Med 1992; 92: 61-68.
110. Uzun C, Koten M, Adali MK: Reversible ototoxic effect of azithromycin and clarithromycin on transiently evoked otoacoustic emissions in guinea pigs. J Laryngol. Otol. 2001; 115: 622-28.
111. Haydon RC, Thelin JW, Davis WE: Erythromycin ototoxicity: analysis and conclusions based on 22 case reports. Otolaryngol.Head Neck Surg 1984; 92: 678-84.
112. Tseng AL, Dolovich L, Salit IE: Azithromycin-related ototoxicity in patients infected with human immunodeficiency virus. Clin Infect.Dis 1997; 24: 76-77.
113. Simko J, Csilek A, Karaszi J: Proarrhythmic potential of antimicrobial agents. Infection 2008; 36: 194-206.
114. Owens RC, Jr., Nolin TD: Antimicrobial-associated QT interval prolongation: pointes of interest. Clin Infect.Dis 2006; 43: 1603-11.
115. Volberg WA, Koci BJ, Su W: Blockade of human cardiac potassium channel human ether-a-go-go-related gene (HERG) by macrolide antibiotics. J Pharmacol Exp Ther. 2002; 302: 320-27.
116. Paran Y, Mashav N, Henis O: Drug-induced torsades de pointes in patients aged 80 years or more. Anadolu.Kardiyol.Derg. 2008; 8: 260-65.
117. Shaffer D, Singer S, Korvick J: Concomitant risk factors in reports of torsades de pointes associated with macrolide use: review of the United States Food and Drug Administration Adverse Event Reporting System. Clin Infect.Dis 2002; 35: 197-200.
118. Inoue M, Farrell DJ, Kaneko K: Antimicrobial susceptibility of respiratory tract pathogens in Japan during PROTEKT years 1-5 (1999-2004). Microb.Drug Resist. 2008; 14: 109-17.
119. Jacobs E, Dalhoff A, Korfmann G: Susceptibility patterns of bacterial isolates from hospitalised patients with respiratory tract infections (MOXIAKTIV Study). Int J Antimicrob.Agents 2009; 33: 52-57.
120. Karlowsky JA, Lagace-Wiens PR, Low DE: Annual macrolide prescription rates and the emergence of macrolide resistance among Streptococcus pneumoniae in Canada from 1995 to 2005. Int J Antimicrob.Agents 2009; 34: 375-79.
121. Pihlajamaki M, Kaijalainen T, Huovinen P: Rapid increase in macrolide resistance among penicillin non-susceptible pneumococci in Finland, 1996-2000. J Antimicrob. Chemother. 2002; 49: 785-92.
122. Niki Y, Hanaki H, Matsumoto T: Nationwide surveillance of bacterial respiratory pathogens conducted by the Japanese Society of Chemotherapy in 2007: general view of the pathogens' antibacterial susceptibility. J Infect.Chemother. 2009; 15: 156-67.
123. Riedel S, Beekmann SE, Heilmann KP: Antimicrobial use in Europe and antimicrobial

resistance in Streptococcus pneumoniae. Eur J Clin Microbiol.Infect.Dis 2007; 26: 485-90.
124. Barkai G, Greenberg D, Givon-Lavi N: Community prescribing and resistant Streptococcus pneumoniae. Emerg.Infect.Dis 2005; 11: 829-37.
125. Bergman M, Huikko S, Huovinen P: Macrolide and azithromycin use are linked to increased macrolide resistance in Streptococcus pneumoniae. Antimicrob.Agents Chemother. 2006; 50: 3646-50.
126. Garcia-Rey C, Aguilar L, Baquero F: Importance of local variations in antibiotic consumption and geographical differences of erythromycin and penicillin resistance in Streptococcus pneumoniae. J Clin Microbiol. 2002; 40: 159-64.
127. Malhotra-Kumar S, Lammens C, Martel A: Oropharyngeal carriage of macrolide-resistant viridans group streptococci: a prevalence study among healthy adults in Belgium. J Antimicrob.Chemother. 2004; 53: 271-76.
128. Perez-Trallero E, Vicente D, Montes M: High proportion of pharyngeal carriers of commensal streptococci resistant to erythromycin in Spanish adults. J Antimicrob. Chemother. 2001; 48: 225-29.
129. Lonks JR, Garau J, Medeiros AA: Implications of antimicrobial resistance in the empirical treatment of community-acquired respiratory tract infections: the case of macrolides. J Antimicrob.Chemother. 2002; 50 Suppl S2: 87-92.
130. Hansen CR, Pressler T, Hoiby N: Long-term, low-dose azithromycin treatment reduces the incidence but increases macrolide resistance in Staphylococcus aureus in Danish CF patients. J Cyst.Fibros. 2009; 8: 58-62.
131. Phaff SJ, Tiddens HA, Verbrugh HA: Macrolide resistance of Staphylococcus aureus and Haemophilus species associated with long-term azithromycin use in cystic fibrosis. J Antimicrob.Chemother. 2006; 57: 741-46.
132. Tramper-Stranders GA, Wolfs TF, Fleer A: Maintenance azithromycin treatment in pediatric patients with cystic fibrosis: long-term outcomes related to macrolide resistance and pulmonary function. Pediatr.Infect.Dis J 2007; 26: 8-12.
133. Kasahara K, Kita E, Maeda K: Macrolide resistance of Streptococcus pneumoniae isolated during long-term macrolide therapy: difference between erythromycin and clarithromycin. J Infect.Chemother. 2005; 11: 112-14.
134. Bishai W: A testament to sustained macrolide efficacy. Clin Infect.Dis 2003; 36: 935-36.
135. Nuermberger E, Bishai WR: The clinical significance of macrolide-resistant Streptococcus pneumoniae: it's all relative. Clin Infect.Dis 2004; 38: 99-103.
136. Rothermel CD: Penicillin and macrolide resistance in pneumococcal pneumonia: does in vitro resistance affect clinical outcomes? Clin Infect.Dis 2004; 38 Suppl 4: S346-S349.
137. Ewig S, Ruiz M, Torres A: Pneumonia acquired in the community through drug-resistant Streptococcus pneumoniae. Am J Respir Crit Care Med 1999; 159: 1835-42.

138. Lonks JR, Garau J, Gomez L: Failure of macrolide antibiotic treatment in patients with bacteremia due to erythromycin-resistant Streptococcus pneumoniae. Clin Infect.Dis 2002; 35: 556-64.
139. Van Kerkhoven D, Peetermans WE, Verbist L: Breakthrough pneumococcal bacteraemia in patients treated with clarithromycin or oral beta-lactams. J Antimicrob.Chemother. 2003; 51: 691-96.
140. Griffith DE, Aksamit T, Brown-Elliott BA: An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med 2007; 175: 367-416.
141. Griffith DE, Brown-Elliott BA, Langsjoen B: Clinical and molecular analysis of macrolide resistance in Mycobacterium avium complex lung disease. Am J Respir Crit Care Med 2006; 174: 928-34.
142. Gordin FM, Sullam PM, Shafran SD: A randomized, placebo-controlled study of rifabutin added to a regimen of clarithromycin and ethambutol for treatment of disseminated infection with Mycobacterium avium complex. Clin Infect.Dis 1999; 28: 1080-85.
143. Lam PK, Griffith DE, Aksamit TR: Factors related to response to intermittent treatment of Mycobacterium avium complex lung disease. Am J Respir Crit Care Med 2006; 173: 1283-89.
144. Benson CA, Williams PL, Currier JS: A prospective, randomized trial examining the efficacy and safety of clarithromycin in combination with ethambutol, rifabutin, or both for the treatment of disseminated Mycobacterium avium complex disease in persons with acquired immunodeficiency syndrome. Clin Infect.Dis 2003; 37: 1234-43.
145. Field SK, Cowie RL: Treatment of Mycobacterium avium-intracellulare complex lung disease with a macrolide, ethambutol, and clofazimine. Chest 2003; 124: 1482-86.
146. Wallace RJ, Jr., Brown BA, Griffith DE: Initial clarithromycin monotherapy for Mycobacterium avium-intracellulare complex lung disease. Am J Respir Crit Care Med 1994; 149: 1335-41.

CHAPTER 5

Effect of azithromycin maintenance treatment on infectious exacerbations among patients with non-cystic fibrosis bronchiectasis: the BAT randomized controlled trial.

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Abstract

- Context : Macrolide antibiotics have been shown to be beneficial in cystic fibrosis (CF) and diffuse panbrochiolitis (DPB), and earlier findings also suggest a benefit in non-CF bronchiectasis.

- Objective : To determine the efficacy of macrolide maintenance treatment for adults with non-CF bronchiectasis.

- Design, Setting and Participants: A randomized double-blind, placebo-controlled multicenter trial between April 2008 and September 2010 in 89 out-patients in 14 hospitals in the Netherlands with ≥ 3 lower respiratory tract infections in the preceding year (ClinicalTrials.gov Identifier: NCT00415350).

- Intervention : Azithromycin (250 mg daily) or placebo for 12 months.

- Main Outcome Measures : The number of infectious exacerbations during 12 months of treatment. Secondary endpoints included lung function, sputum bacteriology, inflammatory markers, adverse effects, symptom scores and quality of life (QoL).

- Results : 43(52%) participants received azithromycin and 40(48%) placebo and were included in the modified intention to treat analysis. At end of study, the median number of exacerbations in the placebo group was 2(IQR 1-3), compared to 0(IQR 0-1) in the azithromycin group (p< 0.0001). 32 (80%) of the placebo- versus 20 (46%) of azithromycin-treated individuals had at least one exacerbation (HR = 0.29 (95%CI: 0.16-0.51). In a mixed model analysis, change in FEV_1(% predicted) over time differed between groups (F (1,78.8)=4.085, p=0.047), showing an increase of 1.03% per three months in the azithromycin group, while decreasing with 0.10% per three months in the placebo group. Gastrointestinal adverse effects occurred in 40% of patients in the azithromycin group, and 5% in the placebo group; RR: 7.44 (CI: 0.97-56.88), for abdominal pain; and RR 8.36 (CI: 1.10-63.15) for diarrhea, but without need for discontinuation of study treatment. A macrolide resistance rate of 88% was noted in azithromycin-treated individuals, as compared to 26% in the placebo group.

- Conclusions : Among adults with non-CF bronchiectasis, the daily use of azithromycin for 12 months compared with placebo resulted in a lower rate of infectious exacerbations.

Introduction

Bronchiectasis is radiographically characterized by pathologic dilatation and mucosal thickening of the small and medium-sized bronchi. Structural abnormality of the bronchial wall causes impaired clearance of the lower airways leading to chronic bacterial infection and inflammation, a process that has been referred to as a 'vicious circle'.[1] If progressive, this may lead to respiratory failure and the need for lung transplant or death. The course of the disease is highly variable. Nearly symptom free periods intersperse with infectious exacerbations, characterized by worsening of symptoms of productive cough, hemoptysis and dyspnea. Frequent exacerbations have a major impact on quality of life.[2] Macrolide antibiotics have anti-bacterial and anti-inflammatory properties which conceivably would provide effective treatment of bronchiectasis. The effectiveness of macrolide maintenance therapy in reducing disease activity, exacerbations and decline in lung function has been demonstrated in cystic fibrosis (CF).[3].[4-8]

Efficacy of long-term low-dose macrolide treatment in non-CF bronchiectasis had first been studied in small, mostly non-randomized studies, showing a positive effect on exacerbation frequency, sputum volume and inflammatory markers.[9-14] Recently, Wong et al [15] reported an important reduction of infectious exacerbations with azithromycin in non-CF bronchiectasis. Their 'EMBRACE' trial included 141 patients with non-CF bronchiectasis who received six months of either azithromycin (500 mg 3x/ wk) or placebo.[15]

We initiated a multicenter trial to investigate whether one year of long term low dose macrolide treatment added to standard therapy is effective in reducing exacerbation frequency in non-CF bronchiectasis.

Methods:

Study design: The **B**ronchiectasis and long term **A**zithromycin **T**reatment (BAT)**-trial** was a multicenter, double-blind, placebo-controlled, parallel-group study with equal randomization [1:1], conducted in the Netherlands (14 sites) between April 2008 and September 2010. The study protocol was reviewed and approved by the ethical review committees of all study sites and the study was performed in accordance with the Good Clinical Practice (GCP) Guidelines, the International Conference on Harmonization (ICH) Guidelines, and the most recent version of the Declaration of Helsinki. This study adhered to the consolidated standards for the reporting of randomised controlled trials (CONSORT).[16;17] The BAT trial was registered at Clinicaltrials.gov, registration no: NCT00415350.

Participants: Patients who met the inclusion criteria were ≥18 years of age and had non-CF

bronchiectasis diagnosed by plain bronchography or high resolution computed tomography (HRCT). All patients had had a minimum of three lower respiratory tract infections (LRTI) treated with oral/IV antibiotics in the preceding year and had at least one sputum culture yielding one or more bacterial respiratory pathogen(s) in the year prior to study entry.
Patients were excluded if they received prolonged macrolide therapy (> 4 weeks) during the previous three months, oral/IV courses of corticosteroids within 30 days of screening or any antimicrobial treatment for a LRTI in the last two weeks. The use of long-term maintenance antibiotics or low-dose steroids was permitted during the study. Patients with a known allergy or intolerance to macrolides; women with child-bearing potential avoiding contraceptives, as well as lactating women; and patients with liver disease or with elevated transaminases: aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) ≥ the upper limit of normal were also excluded from the study.

Setting: Patients were recruited from out-patient clinics at each of the 14 study sites by their pulmonary physician or the investigator. After patients had given written informed consent, their medical history was reviewed and if eligible, were randomized.

Interventions: Following randomisation, patients were observed for clinical stability for two weeks, after which they received either oral azithromycin 250 mg once a day or placebo for the subsequent 52 weeks. Due to lack of a standard treatment regimen, the 250 mg-regimen was chosen in order to increase patient compliance by daily administration and to minimize side effects by choosing a low daily dosage. Compliance was monitored by counting empty blisters of study medication at each study visit by the investigator.

Procedures and outcomes: The primary outcome was the number of infectious exacerbations during the 52-week treatment period. An infectious exacerbation was defined as an increase in respiratory symptoms, requiring antibiotic treatment. Although the original protocol required the inclusion of antibiotic and steroid treated events, we decided to omit the small number of events not treated with antibiotics in the analysis of our primary endpoint. Two types of exacerbations were included in the primary endpoint: a protocol defined exacerbation (PDE) and a non-PDE (NPDE). An exacerbation was considered a PDE when at least four of the following nine symptoms, signs or findings where present: (1) change in sputum production (consistency, colour, volume, or hemoptysis); (2) increased dyspnoea (chest congestion or shortness of breath); (3) increased cough; (4) fever (>38°C); (5) increased wheezing; (6) decreased exercise tolerance, malaise, fatigue, or lethargy; (7) FEV_1 or FVC decreased by at least 10% from a previously recorded value; (8) radiographic changes indicative of a new pulmonary infectious process; or (9) changes in chest sounds. A NPDE was noted when a patient had less than four of the above abnormalities.

On weekly diary cards, patients were asked to report whether they received antibiotics for

an exacerbation in the preceding week and if so, which of the above mentioned findings 1 – 6 applied to that particular exacerbation. Findings 7-9 were evaluated by the treating physicians.

All treating physicians (general practitioners and pulmonary physicians) were instructed to report every exacerbation to the researchers by phone or fax. On every follow-up visit, patients were specifically asked about exacerbations in the past three months. At end of study, a member of the research team visited each participant in the respective hospitals. On these visits, the researcher had full insight in the patient's medical files and double-checked these for reports of infectious exacerbations and/or courses of antibiotics.

In case of an infectious exacerbation, the choice of the antibiotic regimen was left to the discretion of the attending physician who was generally not a member of the trial team and always blinded to the patient's treatment allocation. Treatment was started based on the patient's symptoms and guided by in vitro susceptibility data. Study medication was continued during an exacerbation if possible.

Secondary endpoints included lung function, serum C-reactive protein (CRP), white blood cell count (WBC), microbiological evaluation, symptoms measured by a LRTI--Visual Analogue Scale (VAS) (eFigure 2), health-related quality of life (QoL) as measured by St. George's Respiratory Questionnaire (SGRQ) and adverse events (eFigure 3).

After randomisation and a two week run in period, patients were followed every three months during the 52-week treatment period for blood sampling, lung function tests, questionnaires, sputum cultures and safety checks and once at the end of the run-out period (eFigure 1). Laboratory tests included measurement of serum CRP, WBC and measurement of ASAT and ALAT. Lung function measurements were performed according to European Respiratory Society standard criteria.[18] Sputum samples were collected at each visit and submitted for culture and susceptibility testing at the Medical Centre Alkmaar (see: eMethods).

Symptoms were measured using the LRTI-VAS (Lower Respiratory Tract Infections -Visual Analogue Scale), a symptom score, specifically designed to investigate common symptoms in patients with bronchiectasis [19;20] (eMethods; eFigure 2). This scale consists of a set of horizontal lines with two anchor points, one at each extreme, each line representing a different symptom. Each symptom is scored from 1 to 10, the subjects being unaware of the numbers. Higher scores indicate more severe symptoms. Five symptom domains were scored: dyspnoea, fatigue, cough, chest pain and sputum colour. Separate scores were calculated for each symptom with a total score consisting of all symptom scores added.

The SGRQ - a condition-specific questionnaire - was used to measure health related QoL (HRQoL) (eFigure 3). Its 76 items are partitioned into three sections (Symptoms, Activity, Impact), which are scored separately and can be added up to provide a total score, ranging from 0 to 100%, zero indicating no impairment of quality of life. A difference of 4 points or more is considered clinically significant.[21] The SGRQ requires about 10 minutes to complete; it has been validated for use in bronchiectasis patients. [22;23]

Diary cards, with weekly reports of symptoms, courses of antibiotics and adverse effects (specifically addressing gastro-intestinal, skin and 'other' adverse effects), were completed by all participants during the entire study period. An additional questionnaire evaluating hearing complaints was sent to all participants at end of study.

After one year of treatment with placebo or azithromycin, patients had variable run-out period of at least 90 days. When establishing the between-group differences for exacerbation frequency in the run-out phase, only data collected within 90 days after discontinuing study treatment was used.

Randomisation and masking: On the first study visit, all patients were seen by the investigator and sequentially assigned a subject identification code with double blinded allocation to either azithromycin or placebo treatment. Placebo tablets were manufactured by a licensed trial pharmacy and were indistinguishable from azithromycin with respect to appearance, feeling and taste.

Placebo and azithromycin tablets were provided in identical, individually numbered boxes, each box containing a year's supply of study medication for one participant. Numbers on the boxes matched a treatment allocation, in accordance with a computer-generated allocation sequence which was kept in a safe place in the pharmacy providing the study medication. We used permuted block randomization, with equally sized blocks of 10. Randomization was performed centrally, no stratification for factors such as exacerbation frequency or study center was applied.

Sample size calculation: The primary hypothesis was that prolonged treatment with azithromycin would cause ≥33% (SD1.5) reduction of the number of exacerbations per patient, decreasing the yearly number of exacerbations per patient from 3 to 2. In three small trials, exacerbations were reduced from 3-10 / year to 1-5 during azithromycin therapy [9-11]. In a study with 24 adult non-CF bronchiectasis patients, erythromycin reduced exacerbations from (4 (2-11) to 2 (0-8) year [24]. Azithromycin in CF reduced exacerbations from 3-4 to 1,5- 1,6 / year [4;7]. These limited data available combined with our clinical experience in these patients made us assume that azithromycin would at least reduce the number of exacerbations by one third.

We calculated that a sample size of 36 participants in each group was required to detect this reduction with a one-sided significance level of 0.05 and a power of 80%, and planned to include 90 patients, assuming 20% dropout. One-sided testing was considered appropriate in view of the favourable results of this treatment modality in previous, smaller trials [25].

Statistical methods

Statistical analysis was performed on the modified intent-to-treat population, defined as all randomized participants who received at least 1 dose of study drug. Patients that were randomized but afterwards appeared not to fulfil in- and exclusion criteria were were not started on study medication and were excluded from analysis. Comparisons of parameters between treatment groups were calculated with a t-test if distributed normally, otherwise with a Mann-Whitney U test. There were no patients with missing information on exacerbations during intervention.

Statistical significance in change of FEV_1% predicted and FVC % predicted due to treatment was calculated with a linear mixed-model analysis. The effect of time on outcome variables was checked for linearity by means of plots and model fits with quadratic and cubic functions of time. Change in quality of life was analysed using linear mixed-model analysis as well.

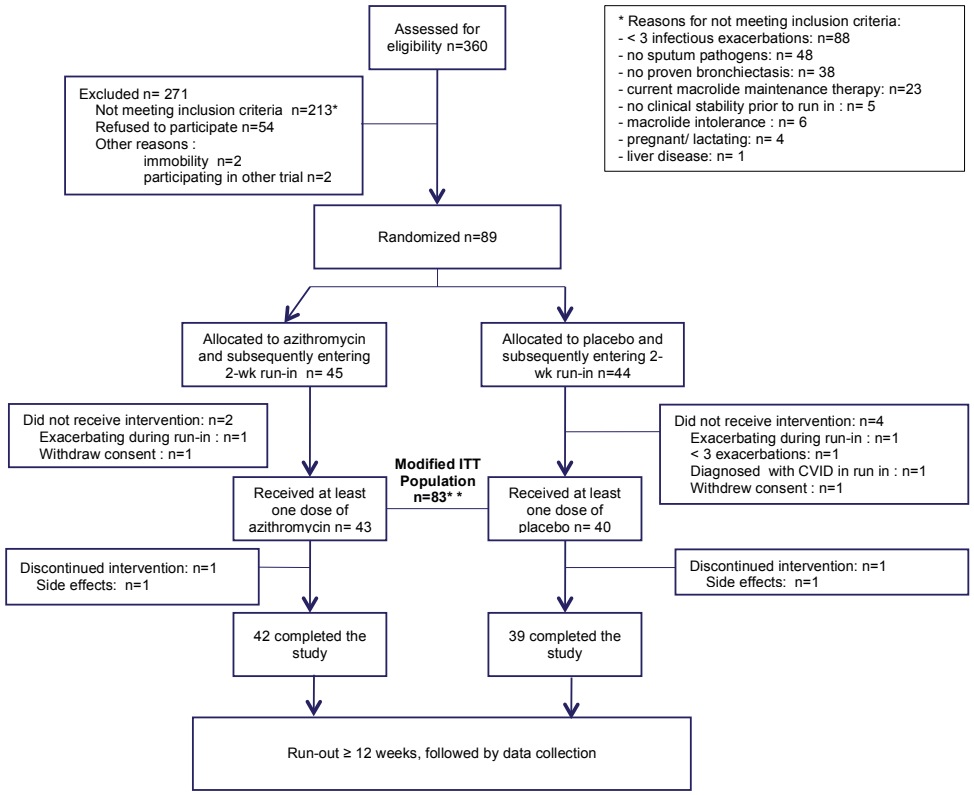
Time was entered as an interval variable. Therapy and sex were entered stepwise as fixed effects. Predictors remained in the regression equation if the model fit increased significantly. Thereafter, random effects were explored for intercept (patients) and slope (time and patients). If random effects did not change the model fit significantly, a fixed effect was assumed. Residuals followed a normal distribution. Sex and age were considered as confounder or effect modifier and reported in the results if significant. The same method was used for the evaluation of the CRP, the SGRQ and the VAS-score. $p < 0,05$ was considered statistically significant (one-sided). The software package SPSS 18 for Windows (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis.

One planned safety analysis, that was performed by an independent statistician after enrolment of 45 patients, showed no significant differences with regard to adverse effects and therefore the study was continued as planned. Between-group comparisons for adverse events were performed, but this study was not powered for safety analysis.

Results

A total of 83 patients were randomly assigned to azithromycin or placebo treatment and included in the modified intention to treat population (figure 1). Participation rate among eligible patients was 62%, reasons for exclusion are presented in figure 1. From empty blister counts we estimated that patients adhered 96.5% of the time in the azithromycin group and 98.0% of the time in the placebo group. One patient in each group discontinued intervention because of adverse effects (figure 1).

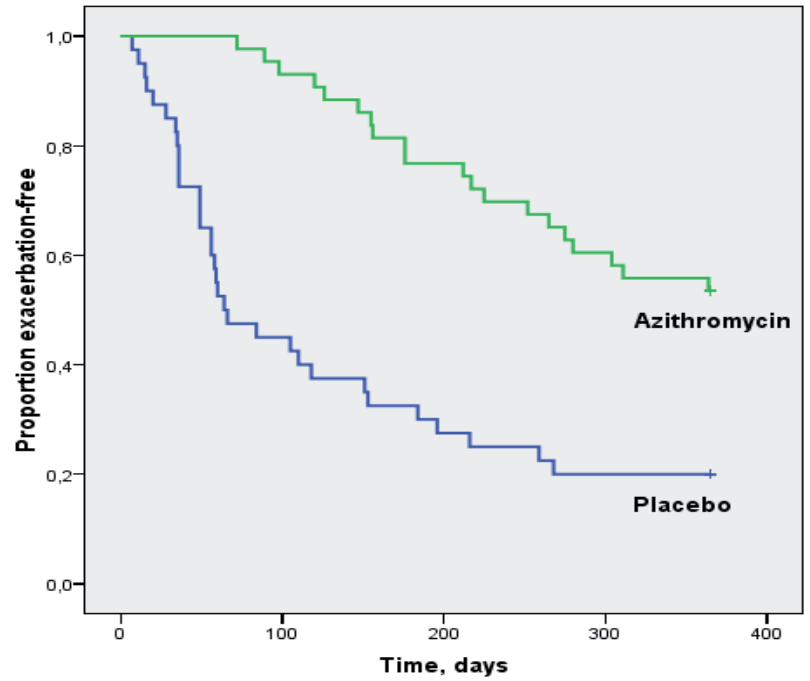
Figure 1. Patient flow chart



Primary endpoint

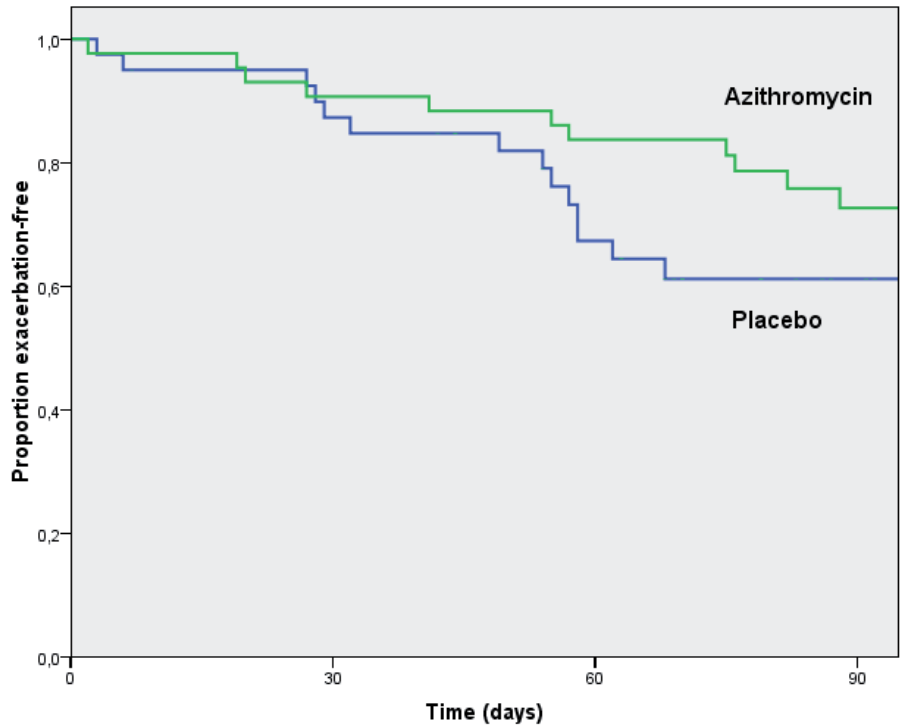
A total of 117 exacerbations (100 of which PDE, 17 non-PDE) treated with antibiotics were reported during one year of treatment, 78 of which occurred in the placebo group. The median number of exacerbations during the treatment period in the placebo group was 2 (IQR 1-3), compared to a median number of exacerbations in the azithromycin group of 0 (IQR 0-1), p< 0.0001 (MWU) (table 2). Of the 40 participants on placebo, 32 (80%) had at least one exacerbation during the study period. In the 43 participants on azithromycin, 20 (46.5%) had had at least one exacerbation in the same period, yielding an absolute risk reduction of 33.5% (95% CI 14.1- 52.9). The number of patients needed to treat with azithromycin to maintain clinical stability is 3.0. Time to a first exacerbation in a post-hoc analysis differed, with a hazard ratio of 0.29 (95%CI:0.16-0.51) for participants on azithromycin compared to placebo (figure 2A). Time until the first exacerbation did not differ in the run-out period (HR 0.56 (95% CI; 0.26-1.19) (figure 2B).

Figure 2 A. Participants remaining exacerbation free during treatment with azithromycin or placebo (day 0 – day 365). Cox proportional hazards regression . Log-rank test p< 0.0001.



No at risk	43	41	33	28
AZM exacerbations		2	8	5
No at risk	40	18	13	8
Placebo exacerbations		22	5	5

Figure 2 B. Participants remaining exacerbation free during the run-out period. Cox proportional hazards regression. Log-rank test p=0.12.



No at risk AZM	43	39	36	22
	exacerbations	4	3	4
	End follow-up	0	0	10
No at risk Placebo	40	34	23	10
	exacerbations	5	7	2
	End follow-up	1	4	11

Lung function:

Change in FEV1% predicted over time was different for patients on placebo compared to azithromycin, F (1,78.8)=4.085, p=0.047. In patients on azithromycin, FEV_1% predicted increased 1.03 per 3 months (intervals of visits). In patients on placebo FEV_1% predicted decreased 0.10 per 3 months.

Change in FVC% predicted over time was different for patients on placebo compared to azithromycin, F (1,78.6)=5.9, p=0.018. In patients on azithromycin, FVC % predicted increased 1.33 per 3 months (intervals of visits). In patients on placebo FVC% predicted decreased 0.30 per 3 months.

(details of the mixed model analysis are provided in eResults).

Inflammatory markers:

Change in serum CRP levels and white blood cell count during the study period was not significantly different between both treatment groups.

Microbiology

A total of 437 sputum samples were cultured for microbiology, which yielded one or more pathogens on 339 occasions. The microbiological profile did not differ significantly between azithromycin-treated and placebo-treated patients at baseline and after 1 year of treatment. Numbers of cultures positive for *P aeruginosa* did not differ between treatment groups or between start and end of study (table 2)

H. influenzae, S. pneumoniae, S. aureus, M. catarrhalis, H. parainfluenzae were most frequently encountered, together comprising 87% of the total number of pathogens. 75% of these pathogens were tested for macrolide resistance.

At baseline, resistance patterns were comparable between both groups (35% macrolide resistance in 8 patients in the azithromycin group against 27.5% in 9 patients in the placebo group; p = 0.75). During treatment, 53 out of 60 (88%) pathogens tested for sensitivity in 20 patients in the azithromycin group became macrolide resistant against 29 out of 112 (26%) in 22 patients in the placebo group (p < 0.0001; Student's T-test; eResults table 1).

Table 1. Baseline patient characteristics

	Azithromycin (n=43)	Placebo (n=40)	P value
Age (years, SD)	59.9 (12.3)	64.6 (9.1)	0.051[1]
Female sex (No,%)	25 (63)	28 (65)	
Nr of exacerbations in year before study entry (median, IQR)	4.0 (3-9)	5.0 (3-12)	0.32[2]
Aetiology of bronchiectasis:*			
Post infectious	15 (35)	13 (33)	
Idiopathic	12 (28)	15 (38)	
Asthma	7 (16)	7 (18)	
Auto-immune disease	3 (7)	2 (5)	
Common variable immune disorder (CVID)	1 (2)	1 (3)	
Primary ciliary dyskinesia (PCD)	1 (2)	0	
Yellow Nail Syndrome	0	1 (3)	
Aspiration	1 (2)	0	
Mechanical obstruction	1 (2)	0	
Allergic bronchopulmonary aspergillosis	1 (2)	1 (3)	
Alpha-1- antitrypsin deficiency	1 (2)	0	
Percent predicted FEV1	77.7 (24.4)	82.7 (27.2)	0.40[1]
Percent predicted FVC	91.9 (24.4)	98.5 (23.6)	0.23[1]
CRP (mmol/l) (median, IQR)	5.0 (2- 11,3)	4.5 (2- 15,3)	0.43[2]
WBC count	8.1 (2.7)	8.1 (3.3)	0.99[1]
SGRQ total score	40.6 (19.4)	40.2 (20.9)	0.94[1]
LRTI-VAS total score	17.5 (10)	17.9 (8.0)	0.88[1]
Baseline sputum microbiology:			
Haemophilus influenzae	13 (30)	9 (23)	
Staphylococcus aureus	4 (9)	9 (23)	
Pseudomonas aeruginosa	6 (14)	6 (15)	
Hearing impairment previous to study entry: †	12 (28)	11 (28)	
Body mass index	23.0 (3.4)	24.5 (4.0)	0.068[1]
Abnormalities on auscultation:			
Crackles	20 (47)	11 (28)	
Rhonchi	8 (19)	10 (25)	
Wheezing	7 (16)	6 (15)	
Dullness	0	1 (3)	
Smoker			
Current	1 (2)	1 (3)	
Former	19 (44)	17 (43)	
Treatment previous to study entry (No,%):			
Inhaled corticosteroids‡	38 (88.4)	32 (80)	
Long-acting β-agonist‡	34 (79)	30 (75)	

Table 1. Continued

Oral corticosteroids‡	4 (9)	5 (13)	
Inhaled antibiotics‡	0 (0)	2 (5)	
Longterm oral antibiotic treatment‡	4 (9)	4 (10)	
Airway clearance techniques: §			
Daily	11 (26)	11 (28)	
Weekly	3 (7)	1 (3)	
During exacerbation	4 (9)	3 (8)	

Data are n(%) or mean (SD) unless otherwise indicated. FEV1 = forced expiratory volume in 1 sec. FVC = forced vital capacity. SGRQ= St George's respiratory questionnaire. LRTI-VAS= lower respiratory tract infection- visual analogue score.
* As described by the treating pulmonary physician
† patient reported hearing impairment
‡ Treatment started before study entry and continued during the study period.
§ Any technique taught by a physiotherapist and performed by the patient in order to evacuate sputum
1. student's T-test
2. Mann Whitney U test

Quality of life (QoL) and patient-reported symptoms

Quality of Life (QoL) as measured by SGRQ showed a larger decrease of the total score (indicating better QoL) in patients on azithromycin at the end of treatment as compared to patients on placebo (p= 0.046). QoL by SGRQ was measured at the start of intervention and after 6 and 12 months. In patients on azithromycin, SGRQ total score decreased -6.09 per 6 months. This means that in the mixed model patients had average decrease in SGRQ score of 2 * 6.09 = 12.18 after one year of treatment. In patients on placebo, SGRQ total score decreased -2.06 per 6 months. In a post-hoc analysis, when comparing SGRQ total scores at start of treatment with total scores after one year, 28 (64%) patients in the azithromycin group had an improvement of 4 units, as compared to 18 (46%) in the placebo group.

Quality of life as measured by the LRTI-VAS score showed a larger decrease of the total score (indicating less symptoms) in patients on azithromycin at the end of treatment as compared to patients on placebo (p= 0.047). In patients on azithromycin, total VAS score decreased with 1.11 per 3 months. This means that in the mixed model patients had an mean decrease of total VAS score of 4 (follow-up visits until end of treatment) * 1.11 = 4.44 after one year of treatment. In patients on placebo VAS total score decreased with 0.056 per 3 months. (for more detailed results of the mixed model analysis , see eResults).

Safety

Among the adverse events reported, only abdominal pain and diarrhea showed an elevated relative risk (table 2). These complaints, mostly occurring in the first weeks of treatment and subsequently subsiding, were mild and did not result in discontinuation of treatment. One patient in each group discontinued intervention because of a suspected adverse effect (2,3% vs 2,5%); one patient in the placebo group (2,5%) developed a severe rash and was subsequently diagnosed with psoriasis, one patient in the azithromycin group (2.3%) complained about progressive fatigue, which did not resolve after discontinuation of treatment. There were no differences in ASAT or ALAT between study groups during treatment.

During treatment, 1 patient in the azithromycin group had an infectious exacerbation requiring admission, 2 patients had surgery (sinus surgery (FESS) and because of uterus prolapse), 1 was diagnosed with hyperthyroidism and 1 with insulin dependent diabetes mellitus. In the placebo group, 2 patients had an infectious exacerbation requiring admission, 3 had surgery (2 FESS and 1 cholecystectomy), 1 was diagnosed with lung carcinoma, 1 hospitalized for suspected malignancy and 1 received pharmacological treatment for depression.

Table 2. *Exacerbation frequency and secondary outcomes after one year of study treatment by treatment group*

		Azithromycin (n=43)	Placebo (n=40)	
No. of exacerbations	Total	36 (31)	81 (69)	
	- of which PDE	31 (86)	69 (85)	
	- of which non PDE	5 (14)	12 (15)	
	Median (IQR)	0 (0-1)	2 (1-3)	p< 0.0001
	Mean	0.84 (1.13)	2.05 (1.6)	p< 0.0001, 95% CI -1.8 - - 0.6 [1]
No. of exacerbations per patient				
	0	23 (54)	8 (20)	p = 0.0015 [2]
	1	10 (23)	8 (20)	p = 0.46 [2]
	2	6 (14)	10 (25)	p = 0.16 [2]
	3	2 (5)	6 (15)	p = 0.11 [2]
	4	2 (5)	5 (13)	p = 0.19 [2]
	5	0	2 (5)	p = 0.23 [2]
	6	0	1 (3)	p = 0.48 [2]
CRP level (mg/L) (median, IQR) .		2.6 (1,5- 7.0)	3.9 (2.0 – 6.15)	

Table 2. *Continued*

WBC count (x 10^e9/l) (mean, SD)		7.6 (2.6)	7.5 (2.7)	
No. of cultures during study treatment		201 (46)	236 (54)	
Microbiological profile	*Pseudomonas aeruginosa*	5 (12)	4 (10)	
	Haemophilus influenzae	10 (23)	6 (15)	
	Moraxella catarrhalis	1 (2)	3 (8)	
	Staphylococcus aureus	2 (5)	4 (10)	
	Aspergillus fumigatus	2 (5)	1 (3)	
	Haemophilus parainfluenzae	1 (2)	0	
	Streptococcus pneumoniae	0	6 (15)	
	Xanthomonas malthophilia	1 (2)	0	
	Serratia marcescens	0	1 (3)	
	Escherichia coli	1 (2)	0	
	Enterobacter cloacae	0	1 (3)	
	Achromobacter xylosoxidans	1 (2)	1 (3)	
				Relative risk (95% CI)
Adverse events	No adverse events	25 (58)	23 (58)	1,01 (0,70-1,46)
	Nausea	6 (14)	6 (15)	0,93 (0,33-2,65)
	Rash	8 (19)	4 (10)	1,86 (0,61-5,70)
	Diarrhoea	9 (21)	1 (3)	8,36 (1,10-63,15)
	Abdominal pain	8 (19)	1 (3)	7,44 (0,97-56,88)
	Auditive complaints *	5 (12)	4 (10)	1.16 (0.34-4.03)
	Itching	2 (5)	3 (8)	0,62 (0,11-3,52)
	Palpitations	1 (2)	1 (3)	0,93 (0,06-14,38)
	Headache	0 (0)	2 (5)	1.05 (0.98- 1.13)

PDE= protocol defined exacerbation (*) self-reported mild hearing loss / tinnitus. All data are expressed in No (%) unless otherwise stated.
1. Mann Whitney U test
2. Fisher's exact test

Discussion

We found a significant difference in exacerbation frequency – our primary endpoint – after one year among 83 adult patients who were randomly assigned to either azithromycin or placebo.

Azithromycin was superior to placebo with respect to lung function (FEV_1 and FVC), disease symptoms and QoL measurements. Exacerbation frequency is considered as one of the most important causes of reduced QoL in non-CF bronchiectasis patients [23 26].

The current study is the first to evaluate the effect of azithromycin maintenance treatment during a full year, thereby reducing seasonal influences on exacerbation frequency and wellbeing. Testing for macrolide resistance was done in most of the sputum pathogens, and this provides important additional information to earlier reports in this field, particularly because the emergence of resistant organisms was not mirrored by loss of efficacy in the subsequent months.

A recently published trial in non-CF bronchiectasis (the 'EMBRACE' trial), found a similar significant reduction in exacerbation frequency with 6 months of macrolide treatment [15]. Lung functional improvement and better QoL was not maintained during the 6 months after the intervention concluded. In contrast to the EMBRACE investigators, who found a small but significant reduction of the already low baseline CRP values, CRP values in our study did not change significantly, probably because of lack of power for this secondary endpoint.

Analysis of our secondary endpoints demonstrated a modest but statistically significant improvement of FEV_1 in the azithromycin group as compared to placebo. Apart from Tsang et al [13] who found improvement in FEV_1 in children, no other study showed functional improvement with macrolide therapy. HRCT-scans in bronchiectasis do not exclusively show dilated bronchi, but also signs of infection like mucus plugging, consolidation and tree-in-bud sign, potentially resulting in air flow limitation and air trapping. Improvement in pulmonary function may eventually impact survival as lung function impairment has been identified as an independent risk factor for mortality in bronchiectasis [27].

Most macrolides are active against gram-positive organisms and some anaerobes but have limited gram-negative activity. Saiman et al [28] failed to demonstrate a positive effect on lung function in their trial of azithromycin (250-500 mg three times weekly, 24 weeks vs. placebo) in 260 *Pseudomonas*-free children with CF and mild lung disease. The positive effect of macrolide treatment in CF has therefore been attributed to an inhibitory effect on Pseudomonas, rather than to an anti-inflammatory effect.

Only around 10% of the patients in the present study were infected by *P. aeruginosa* and colonization rates with *P. aeruginosa* did not importantly change during treatment, pointing towards a favorable effect of macrolide therapy apart from its proposed anti-pseudomonal effect in non-CF bronchiectasis.

Although absolute numbers of sputum pathogens were importantly lower in the azithromycin group, susceptibility testing showed 88% (53 out of 60) macrolide resistance in pathogens from these patients, as compared to 26% (29 out of 112) in the placebo group. A similar trial of macrolides in COPD patients, in which 30% of the pathogens were not available for susceptibility testing, found 81% of macrolide resistance in the azithromycin group as compared to 41% in the placebo group, the latter percentage being lower in our group, due to a lower local baseline rate of macrolide resistance [29]. Other evidence on induction of macrolide resistance comes from CF-studies which report resistance rates up to 100% associated with long-term macrolide treatment.[28] Emergence of macrolide-resistance however, was never linked to pulmonary function decline.[28;30-34] Since numerous alternative antimicrobial agents are available to treat airway pathogens and since azithromycin is not considered first choice in patients with exacerbations of non-CF bronchiectasis, macrolide resistance might not necessarily be deleterious in this patient group. However, an important risk of induction of macrolide resistance is linked to an increase of macrolide-resistant micro-organisms (pathogens and commensals alike) in the community. Since macrolides are often recommended as first-line agents for the treatment of community-acquired pneumonia (CAP), macrolide resistance could be a potential cause for treatment failure in CAP. Although the exceptional tissue penetration of macrolides causes differences in in vivo and in vitro resistance results –macrolides have been shown to be effective against micro-organisms with low-level macrolide resistance (MIC 8-16)- the increasing prevalence of macrolide resistance in 'innocent bystander' organisms is a matter of concern. Macrolide maintenance therapy should therefore exclusively be prescribed to bronchiectasis patients with at least three exacerbations annually.

Patients in the azithromycin group reported more gastro-intestinal adverse effects – comparable to other trials of macrolide maintenance therapy- but none were serious and never a reason for treatment discontinuation [15]. In the trial of macrolides in COPD-patients a slight increase of hearing decrements with audiometry was detected. Using a post-study questionnaire, which is less sensitive - a limitation of our study - we could not detect hearing loss.[29]

Our study has other limitations. First, our hypothesis – long term macrolide treatment is effective in reducing infectious exacerbations- was tested one-sided. This appeared legitimate in the light of positive treatment results with minimal adverse effects in earlier trials [4-7;9-14]. By choosing this approach we minimized the number of participants and, by

doing so, made efficient use of our resources. Furthermore, this study was not powered for toxicity.

Second, the incidence of infectious exacerbations in the placebo group was substantially lower during treatment as compared to the year before study entry (median 2 vs 5). Apart from a placebo effect, we believe that there could be two possible study-related factors that might have contributed. First of all, during the trial period patients were encouraged to report directly to their pulmonary physician in case of an increase of symptoms rather than visiting their general practitioner. One could argue that a pulmonary specialist would be less inclined to treat relatively mild symptoms with antibiotics, however we did not screen for this effect in the current trial. Moreover, since patients had to produce 3-monthly sputum samples during the trial period, current information about airway pathogens was readily available when patients presented with an exacerbation. Culture-guided therapy might have prolonged the time until the next exacerbation.
Third, we did not routinely screen for mycobacterial infection at baseline or exclude patients with evidence of a non-tuberculous mycobacterial (NTM) infection. Clinical improvement in participants with NTM infection might therefore be the result of direct anti-mycobacterial action of macrolides. However, since standard treatment for NTM infection in the participating hospitals included macrolide treatment and recent use of macrolides was an exclusion criterion, these patients were not expected to be eligible for randomization. In addition, sputum cultures obtained at baseline did not yield NTM.

Finally, we did not undertake ECG recording before administering study medication. Considering the results of a recent cohort study reporting an increased risk of cardiovascular death during azithromycin use, especially in patients in the highest decile of risk for cardiovascular disorders, we might have done so in participants at risk (a marginal percentage of our participants) [35]. Neither our study, nor other clinical trials on macrolide treatment demonstrated an increased risk of death of cardiovascular events [15;29;36].

We conclude that macrolide maintenance therapy was effective in reducing exacerbations in non-CF bronchiectasis. In this trial, azithromycin-treatment resulted in improved lung function and better quality of life but involved an increase in gastro-intestinal adverse effects and high rates of macrolide resistance.

Reference List

1 Cole PJ. Inflammation: a two-edged sword--the model of bronchiectasis. Eur J Respir Dis Suppl 1986;147:6-15.
2 ten Hacken NH, Wijkstra PJ, Kerstjens HA. Treatment of bronchiectasis in adults. BMJ 2007;335:1089-1093.
3 Bilton D. Update on non-cystic fibrosis bronchiectasis. Curr Opin Pulm Med 2008;14:595-599.
4 Clement A, Tamalet A, Leroux E, Ravilly S, Fauroux B, Jais JP. Long term effects of azithromycin in patients with cystic fibrosis: A double blind, placebo controlled trial. Thorax 2006;61:895-902.
5 Equi A, Balfour-Lynn IM, Bush A, Rosenthal M. Long term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled crossover trial. Lancet 2002;360:978-984.
6 Saiman L, Marshall BC, Mayer-Hamblett N et al. Azithromycin in patients with cystic fibrosis chronically infected with Pseudomonas aeruginosa: a randomized controlled trial. JAMA 2003;290:1749-1756.
7 Wolter J, Seeney S, Bell S, Bowler S, Masel P, McCormack J. Effect of long term treatment with azithromycin on disease parameters in cystic fibrosis: a randomised trial. Thorax 2002;57:212-216.
8 McCormack J, Bell S, Senini S et al. Daily versus weekly azithromycin in cystic fibrosis patients. Eur Respir J 2007;30:487-495.
9 Anwar GA, Bourke SC, Afolabi G, Middleton P, Ward C, Rutherford RM. Effects of long-term low-dose azithromycin in patients with non-CF bronchiectasis. Respir Med 2008;102:1494-1496.
10 Cymbala AA, Edmonds LC, Bauer MA et al. The disease-modifying effects of twice-weekly oral azithromycin in patients with bronchiectasis. Treat Respir Med 2005;4:117-122.
11 Davies G, Wilson R. Prophylactic antibiotic treatment of bronchiectasis with azithromycin. Thorax 2004;59:540-541.
12 Koh YY, Lee MH, Sun YH, Sung KW, Chae JH. Effect of roxithromycin on airway responsiveness in children with bronchiectasis: a double-blind, placebo-controlled study. Eur Respir J 1997;10:994-999.
13 Tsang KW, Ho PI, Chan KN et al. A pilot study of low-dose erythromycin in bronchiectasis. Eur Respir J 1999;13:361-364.
14 Yalcin E, Kiper N, Ozcelik U et al. Effects of claritromycin on inflammatory parameters and clinical conditions in children with bronchiectasis. J Clin Pharm Ther 2006;31:49-55.
15 Wong C, Jayaram L, Karalus N et al. Azithromycin for prevention of exacerbations in non-cystic fibrosis bronchiectasis (EMBRACE): a randomised, double-blind, placebo-

controlled trial. Lancet 2012;380:660-667.
16 Moher D, Hopewell S, Schulz KF et al. CONSORT 2010 Explanation and Elaboration: Updated guidelines for reporting parallel group randomised trials. J Clin Epidemiol 2010;63:e1-37.
17 Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. PLoS Med 2010;7:e1000251.
18 Miller MR, Hankinson J, Brusasco V et al. Standardisation of spirometry. Eur Respir J 2005;26:319-338.
19 Snijders D, Daniels JM, de Graaff CS, van der Werf TS, Boersma WG. Efficacy of corticosteroids in community-acquired pneumonia: a randomized double-blinded clinical trial. Am J Respir Crit Care Med 2010;181:975-982.
20 Daniels JM, Snijders D, de Graaff CS, Vlaspolder F, Jansen HM, Boersma WG. Antibiotics in addition to systemic corticosteroids for acute exacerbations of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2010;181:150-157.
21 Jones PW. St. George's Respiratory Questionnaire: MCID. COPD 2005;2:75-79.
22 Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure of health status for chronic airflow limitation. The St. George's Respiratory Questionnaire. Am Rev Respir Dis 1992;145:1321-1327.
23 Wilson CB, Jones PW, O'Leary CJ, Cole PJ, Wilson R. Validation of the St. George's Respiratory Questionnaire in bronchiectasis. Am J Respir Crit Care Med 1997;156:536-541.
24 Serisier DJ, Martin ML. Long-term, low-dose erythromycin in bronchiectasis subjects with frequent infective exacerbations. Respir Med 2011;105:946-949.
25 Knottnerus JA, Bouter LM. The ethics of sample size: two-sided testing and one-sided thinking. J Clin Epidemiol 2001;54:109-110.
26 Martinez-Garcia MA, Perpina-Tordera M, Roman-Sanchez P, Soler-Cataluna JJ. Quality-of-life determinants in patients with clinically stable bronchiectasis. Chest 2005;128:739-745.
27 Loebinger MR, Wells AU, Hansell DM et al. Mortality in bronchiectasis: a long-term study assessing the factors influencing survival. Eur Respir J 2009;34:843-849.
28 Saiman L, Anstead M, Mayer-Hamblett N et al. Effect of azithromycin on pulmonary function in patients with cystic fibrosis uninfected with Pseudomonas aeruginosa: a randomized controlled trial. JAMA 2010;303:1707-1715.
29 Albert RK, Connett J, Bailey WC et al. Azithromycin for prevention of exacerbations of COPD. N Engl J Med 2011;365:689-698.
30 Altenburg J, de Graaff CS, van der Werf TS, Boersma WG. Immunomodulatory effects of macrolide antibiotics - part 2: advantages and disadvantages of long-term, low-dose macrolide therapy. Respiration 2011;81:75-87.
31 Hansen CR, Pressler T, Hoiby N, Johansen HK. Long-term, low-dose azithromycin treatment reduces the incidence but increases macrolide resistance in Staphylococcus aureus in Danish CF patients. J Cyst Fibros 2009;8:58-62.
32 Kasahara K, Kita E, Maeda K et al. Macrolide resistance of Streptococcus pneumoniae isolated during long-term macrolide therapy: difference between erythromycin and clarithromycin. J Infect Chemother 2005;11:112-114.
33 Tramper-Stranders GA, Wolfs TF, Fleer A, Kimpen JL, van der Ent CK. Maintenance azithromycin treatment in pediatric patients with cystic fibrosis: long-term outcomes related to macrolide resistance and pulmonary function. Pediatr Infect Dis J 2007;26:8-12.
34 Phaff SJ, Tiddens HA, Verbrugh HA, Ott A. Macrolide resistance of Staphylococcus aureus and Haemophilus species associated with long-term azithromycin use in cystic fibrosis. J Antimicrob Chemother 2006;57:741-746.
35 Ray WA, Murray KT, Hall K, Arbogast PG, Stein CM. Azithromycin and the risk of cardiovascular death. N Engl J Med 2012;366:1881-1890.
36 Banerjee D, Khair OA, Honeybourne D. The effect of oral clarithromycin on health status and sputum bacteriology in stable COPD. Respir Med 2005;99:208-215.

CHAPTER 6

Changes of computed tomography features in patients with bronchiectasis following one year of azithromycin treatment.

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ABSTRACT

Background: Bronchiectasis (pathological dilatation of bronchi) is usually diagnosed by high resolution computed tomography (HRCT) and radiological severity has been found to correspond with clinical outcome. A beneficial effect of azithromycin maintenance treatment in patients with bronchiectasis and frequent exacerbations has lately been established in three randomized trials.

Aim: First, to evaluate longitudinal changes of radiological abnormalities in bronchiectasis; second, to explore whether azithromycin (AZM) maintenance treatment might revert some of these abnormalities.

Methods: The 'BAT' trial, a multicentre, randomized, placebo-controlled trial in the Netherlands (2008-2010) investigated the effect of 1 year of AZM treatment (250 mg OD) in bronchiectasis with frequent exacerbations. Chest HRCT-scans at baseline and after one year were scored by two radiologists according to a scoring system based on the Bhalla-system, but omitting three of the original item scores because of limited availability of two comparable imaging sets for each patient. CT scores were correlated to patient characteristics and longitudinal change was evaluated.

Results: Internal consistency for the 6 remaining items of the Bhalla score and interobserver agreement were sufficient (Cronbach's α 0.71, ICC 0.89). Median baseline CT scores were 3.0 (IQR3.5) for AZM (n=31) and 2.0 (IQR2.25) for placebo (n=29; p=0.2), and decreased after one year of treatment to 2.5 (3.5) in the AZM group as compared to 2.0 (3.5) in placebo-treated patients (respectively, p=0.5 and 0.9). Higher baseline CT scores were found in pseudomonas-infected patients and in responders to treatment, with statistical significance in the former (p= 0.04) Moderate-good negative correlation was found between CT-score and lung function parameters at baseline (r=0.4–0.5).

Conclusion: CT severity in bronchiectasis reflects lung function impairment and *Pseudomonas* status but the beneficial effect of long-term azithromycin treatment on exacerbation frequency, lung function and quality of life in this randomized trial was not convincingly mirrored by radiological improvements.

Introduction

Non-cystic fibrosis bronchiectasis (hereafter referred to as 'bronchiectasis') is a chronic respiratory condition featuring dilated bronchi and known to cause chronic productive cough, fatigue and recurrent respiratory tract infections in affected patients [1]. Key to development of the disease is a vicious circle of impaired ciliary clearance, chronic, predominantly neutrophilic inflammation and bacterial colonization, which results in irreversible, pathologic dilatation of the small and medium-sized bronchi [2]. High resolution CT scanning is the method of choice in diagnosing bronchiectasis and radiological disease severity was disclosed as an independent predictor of both morbidity and mortality in these patients [2;3]. Not surprisingly, two recently proposed scoring systems for disease severity in bronchiectasis have included CT-scores as one of their main variables [3;4].

The extent and type of radiological abnormalities in bronchiectasis has been found to correspond to FEV1 and other parameters of disease in some studies on the subject, but this relationship is disputed by other authors [5;6]. In addition, bronchiectasis and its accompanying radiological features are traditionally considered irreversible. However, in 2012 Goeminne et al found a small but significant improvement of CT features when retrospectively investigating patients who received long-term macrolide therapy [7]. The clinical benefits of long-term macrolide treatment as found in three recently performed trials, led to widespread use of this treatment modality for patients with bronchiectasis and frequent exacerbations [8-10]. To date however, no prospective data was available on the effect of long term macrolide treatment on CT features of bronchiectasis and which CT features are predictive of a favourable effect of long term macrolide treatment.

The current study uses data from a recently performed randomized interventional trial to investigate the correlation between radiological disease severity and clinical outcome measures during one year of macrolide or placebo treatment.

We hypothesize that the severity of bronchiectasis as measured by a validated CT scoring system will correspond to exacerbation frequency and lung function, among other parameters of disease. In addition, we expect long term macrolide treatment to cause improvement of CT features of bronchiectasis, in particular of those indicative of active inflammation (e.g., airway thickness, mucus plugging).

Materials and methods

The 'BAT' trial, a multicentre, randomized, placebo-controlled trial was conducted at 14 sites in the Netherlands from 2008- 2010 (Clinicaltrials.gov, registration no: NCT00415350).

Detailed study protocols are provided elsewhere. [8] Participants were eligible for randomization if they had non-CF bronchiectasis and three or more lower respiratory tract infections treated with antibiotics in the preceding year, with sputum cultures showing evidence of chronic airways infection.

All participants gave informed consent and ethical approval was provided by the Institutional review board of Alkmaar Medical Centre: 'METC Noord Holland' (Approval no: M07-002, CCMO: NL16025.094.07).

Patients were randomized to receive either azithromycin (250 mg daily) or placebo for 12 months, during which the number of infectious exacerbations (the primary endpoint), lung function parameters, sputum bacteriology, inflammatory markers, adverse effects, symptom scores and quality of life (QoL) were recorded.

At baseline – with all study participants having stable disease, without recent or current exacerbation, and after one year of study treatment, HRCT scans were obtained with the radiological equipment available at the study sites at the time of study enrolment and according to the local CT-protocols. All HRCT's were independently scored by two radiologists (radiology consultants with 15 (RW) and 10 (SG) years of experience in chest radiology, respectively, according to the scoring system Bhalla et al [11]. The original, validated scoring system contains 9 items, representing key radiological features of bronchiectasis, which are independently scored to add up to a maximum total score of 25 (figure 1).

However, differences in scanning techniques and protocols between study sites caused the image quality to vary. Only a small minority of patients had 2 consecutive CT scans of sufficient quality to allow for proper use of the Bhalla CT scoring system. In many cases, slice thickness and intervals were too large to allow proper evaluation of lung tissue according to the segmental anatomy. This was particularly bothersome in items which required assessment of the exact number of segments/ bronchial generations (Bhalla items no. 3, 4 and 6). We therefore decided to apply a modification to the Bhalla scoring system, omitting these 3 items (figure 1). The two observers were blinded to clinical severity of disease, spirometric findings and quality of life scores of all patients. The mean of the two readers was used for the total Bhalla score and also for score of the individual items.

All patients were familiar with routine spirometry measurements and these were performed according to European Respiratory Society standard criteria. [12] Total lung capacity (TLC) and its subdivisions were assessed with body plethysmography (Master Screen Body, Care Fusion, San Diego, California, USA). Carbon monoxide transfer factor (TLCO) was determined by the single breath-hold method (Master Screen Diff, Care Fusion, San Diego, California, USA). Reference values for spirometry, static lung volumes and TLCO are from the European Coal and Steel Community [13]

Laboratory tests included measurement of serum C-reactive protein (CRP) and white blood cell count (WBC) and sputum samples were collected at each visit and sent for culture and susceptibility testing at Alkmaar Medical Centre.

Symptoms were measured using analogue scales (VAS) for dyspnoea, cough, fatigue, pain and sputum purulence. Each symptom was scored from 1 to 10 on the lower respiratory tract infections-visual analogue scale (LRTI-VAS), higher scores indicating more severe symptoms and domain- and total scores were provided [14].

Saint George's Respiratory Questionnaire (SGRQ) was used to measure health related QoL (HRQoL). Its 76 items are partitioned into three sections (Symptoms, Activity, Impact), yielding domain- and total scores, ranging from 0 to 100%, zero indicating no impairment of quality of life. A difference of 4 points or more is considered clinically significant.[15 16;17]

An infectious exacerbation was defined as an increase in respiratory symptoms, requiring antibiotic treatment. Exacerbation frequency was reported on diary cards by the participants, documented by the treating physicians and double-checked through chart review by the principal researcher.

Statistics

Nominal and ordinal variables were expressed using median and interquartile range (IQR). In those cases that IQR=0, the range from lowest to highest value was used, instead of the 25-75% range. Interval/ratio variables were expressed in terms of mean, SD and confidence intervals.

Pearson's correlation coefficient and the ICC (intra-class correlation coefficient) were used to calculate consistency between both observers. The final CT total- and item scores as used in the analysis was the mean score of both observers. Internal consistency of the Bhalla CT-score was measured by applying Cronbach's alpha to each of the component scores at entry; accepting >0.7 as sufficient.

Between group differences were calculated with a student's t-test in case of normally distributed variables and with Mann-Whitney U or Wilcoxon signed ranks test in case of a skewed distribution. Continuous variables were checked for normality by means of Kolmogorov-Smirnov test of equality.

Spearman's correlation was used to examine the association between average CT scores and other parameters of disease severity (number of exacerbations, quality of life (QoL), symptom score, lung function (FEV1, FVC, TLCO, TLC, RV)).

When comparing two variables, P values of < 0.05 were considered statistically significant. The software package SPSS 20 for Windows (SPSS Inc. Chicago, IL, USA) was available for statistical analysis.

Results

After omitting three items of the original Bhalla CT-score, the 6 remaining items showed sufficient internal consistency (Crohnbach's Alpha 0.71).

The intraclass correlation coefficient between scoring results of the two observers was 0.89, and Pearson's correlation coefficient 0.79, indicating good correlation and allowing us to take the average score of both observations. Only CT-scans that were scored by both observers were included in the analysis.

Although all 83 patients had CT-scans performed at baseline and at end of study treatment, due to logistical difficulties both observers were not able to score all CT's for all patients. Observer SG had 2/83 (2,4%) missing scores at baseline and 8/ 83 (9.6%) at end of treatment. Observer RW had 16/83 (19,0%) of scores missing for both baseline and end of treatment.

As such, total CT scores and item scores were available for 31/43 (72%) of azithromycin-treated and 29/40 (72%) of placebo-treated patients at baseline. At end of treatment CT scores were available for 36/43 (84%) azithromycin-treated patient and 30/40 (75%) patients in the placebo group.

Median total CT score at baseline for the whole group (n=60) was 2.25 (IQR 3.0). The scores on separate items of the CT score and other disease parameters for both the azithromycin and placebo group are depicted in table 1.

Table 1. Baseline patient characteristics

	Azithromycin n=43	Placebo n=40	p
Age	63 (12.3)	67 (9.4)	0.1
Female	29 (65)	27 (60)	0.4
BMI	22.9 (3.3)	24.6 (4.0)	
Smoking status Current Former	 1 (2) 19 (44)	 1 (3) 17 (43)	
No. of exacerbations in year before study entry (median, IQR)	4 (3-9)	5 (3-12)	
FEV_1 (percentage predicted)	77.2 (24.4)	83.1 (27)	0.3
FVC (percentage predicted)	3.1 (1.1)	3.3 (1.1)	0.6
TLCO (percentage predicted)	76.3 (16.7)	73.9 (16.6)	0.6
TLC (percentage predicted)	101.2 (22.0)	107.2 (20.1)	0.2
RV (percentage predicted)	126.1 (44.9)	130.1 (30.4)	0.7
CRP mg/dL mg/L	13.0 (25.3)	9.2 (11.9)	0.4
WBC $x10^9$/L	8.1 (2.8)	8.2 (3.3)	1.0
CT total score* (median, IQR)	3.0 (3.5)	2.0 (2.25)	0.2
- Severity of bronchiectasis (median, IQR)	1.5 (0.9)	1.0 (1.25)	0.3
- Peribronchial thickening (median, IQR)	0.5 (1.0)	0.0 (0.75)	0.02
- Sacculation or abscesses (median, range)	0.0 (0.9)	0.0 (0.0-2.5)	0.1
- Bullae (median, range)	0.0 (0.0)	0.0 (0.0-2.5)	0.9
- Emphysema (median, IQR)	0.0 (1.0)	0.0 (1.0)	0.8
- Consolidation (median, IQR)	0.25 (0.5)	0.0 (0.88)	0.9
SGRQ total score	40.8 (19.6)	40.0 (20.7)	0.9
LRTI-VAS total score	17.7 (10.1)	17.6 (7.9)	1.0
Baseline sputum microbiology Haemophilus influenzae Staphylococcus aureus Pseudomonas aeruginosa	 13 (30) 4 (9) 6 (14)	 9 (23) 9 (23) 6 (15)	

All values are expressed as mean (SD) unless stated otherwise. *Mean score of two observers according to a modified Bhalla CT scoring system. (SD).
Abbreviations: CRP, C-reactive protein; FEV_1, forced expiratory volume in the first second of expiration; FVC, forced vital capacity; IQR, interquartile range; LRTI-VAS, lower respiratory tract infection–visual analogue score; SGRQ, St George's Respiratory Questionnaire; WBC, white blood cell.
SI conversion factor: To convert CRP levels to nmol/L, multiply by 9.524.

Relationship between CT-scores and other parameters of disease severity.

At baseline, the modified Bhalla total score correlated well with lung function parameters (FEV_1, r= -0.4,FVC r=-0.4 and TLCO r=-0.4). Poor correlation was found between CT scores and exacerbation frequency, BMI, CRP, WBC, RV, TLC, reversibility of FEV1 and SGRQ and VAS total scores.

When discerning patients with respect to pseudomonas status at baseline, pseudomonas-infected patients (n=10, 17%) had a median total CT score of 4.25 (IQR 5.63) as compared to 2.0 (2.5) in non-pseudomonas patients (n=50, 83%), (p=0.3). Pseudomonas-infected patients scored significantly higher on the 'consolidation' item score (0.75 (1.0) versus 0.0 (0.5), p=0.04) and the 'peribronchial thickening' item score [1.0 (1.5) versus 0.0 (1.0) p=0.04] in non-pseudomonas -infected patients

Change of CT-scores during treatment

CT total scores and item scores at baseline and after one year of study treatment are shown in table 2.

CT scores in responders and non-responders to azithromycin treatment

In the BAT trial protocol, response to treatment was defined as reduction of one exacerbation or more during study treatment as compared to the number of exacerbations in the year before study inclusion. In the current study we explored radiological severity in responders and non-responders. Since no fixed definition of 'treatment response' exists for the evaluation of azithromycin maintenance treatment, we also looked at between-group differences when using a stricter definition of response, that is, a reduction of two or three exacerbations during treatment.

When using the less strict definition of a reduction of at least one or two exacerbations during treatment, only 2 participants qualified as non-responders. When treatment response was defined as a reduction of at least three exacerbations, 7 non-responders were identified.

At baseline, CT total scores for responders were consistently higher than for non-responders and this was true for both response definitions, but the difference between both groups did not reach statistical significance (p=0.12 and 0.25 respectively) (figure 2a+b). The between group difference could mainly be explained by differences in the 'severity' and 'peribronchial thickening' item scores which were 1.5 (2.5) and 0.5 (2.5) in responders and 1.0 (0.0) and 0.0 (0.0) in non-responders (median (IQR) p=0.47 and 0.13 respectively) for patients with a reduction of one or two exacerbations.

Table 2. *CT scores and item scores (average score of two observers) for baseline and at one year for azithromycin- and placebo treated patients.*

Azithromycin group (n=31)				
	Baseline	End of treatment	Delta end-baseline	p*
CT total score	3.0 (3.5)	2.5 (3.5)	-0.1	0.5
- Severity of bronchiectasis	1.5 (0.9)	1.25 (1.0)	0.0	0.3
- Peribronchial thickening	0.5 (1.0)	0.5 (1.0)	-0.1	0.09
- Sacculation or abscesses	0.0 (0.9)	0.0 (0.0-2.0)	0	0.5
- Bullae (median, range)	0.0 (0.0)	0.0 (0.0-1.5)	0	0.3
- Emphysema	0.0 (1.0)	0.0 (1.0)	0	0.8
- Consolidation	0.25 (0.5)	0.0 (1.0)	0	0.8

Placebo group (n=29)				
	Baseline	End of treatment	Delta end-baseline	p*
CT total score	2.0 (2.25)	2.0 (3.5)	0	0.9
- Severity of bronchiectasis	1.0 (1.25)	1.0 (1.5)	0.1	0.6
- Peribronchial thickening	0.0 (0.75)	0.0 (0.6)	0	0.8
- Sacculation or abscesses (median, range)	0.0 (0.0-2.5)	0.0 (0.0-2.5)	-0.1	0.05
- Bullae (median, range)	0.0 (0.0-2.5)	0.0 (0.0-2.5)	0.1	0.4
- Emphysema	0.0 (1.0)	0.0 (1.0)	0	1
- Consolidation	0.0 (0.88)	0.25 (0.6)	0.1	0.6

* Wilcoxon signed ranks test. All values are expressed using median (IQR) unless stated otherwise.

Discussion

This is the first study in patients with bronchiectasis to prospectively evaluate changes in CT features during azithromycin maintenance therapy. Our main finding was that although the CT score did not change significantly, a trend towards an improvement (=reduction) of CT scores was noted in the azithromycin group, as compared to stability in the placebo group. Radiological disease severity corresponded very well with dynamic lung function parameters and CO-diffusion capacity and proved to be a tool for discerning pseudomonas-infected patients, who had higher scores for 'consolidation' and 'peribronchial thickening'.

In addition, responders to azithromycin treatment could be discriminated, based on their baseline CT scores which were higher as compared to those that did not respond favourably.

The favourable effect of azithromycin maintenance treatment in bronchiectasis has been demonstrated in two randomised trials [8;9], showing a significant reduction of exacerbations (number needed to treat = 3). Other beneficial effects included preserved lung function and better quality of life in patients on azithromycin treatment.

The anti-inflammatory effect of azithromycin is in part attributable to its anti-neutrophilic mode of action as depicted by lower levels of neutrophils chemo-attractants (e.g. IL-8, TNF-alpha) and markedly decreased airway neutrophilia after macrolide treatment. Besides, other anti-inflammatory effects of azithromycin are proposed; from a direct anti-bacterial effect through inhibition of bacterial protein synthesis in Gram-positive pathogens to marked effects on the secretory function of airway epithelial cells [18].

In cystic fibrosis patients, evidence on CT scores as indicators of treatment effect is slightly more robust than for non-CF bronchiectasis. A few studies describe CT scores as an objective measure of improvement after treatment of exacerbations [19]. In addition, two CF studies describe the use of CT scores to evaluate longitudinal changes during long term treatment with inhaled antibiotics or ivacaftor, a CFTR potentiator [20;21]. The latter trial demonstrated significant changes in bronchiectasis, mucous plugging, peribronchial thickening and total Brody score, a CT scoring system used in CF,[20].

Apart from the current study, the retrospective trial of Goeminne et al [7] is the only study in non-CF bronchiectasis focussing on CT features as surrogate markers for treatment response. These investigators used the modified Brody score to evaluate a treatment effect of different macrolide types, dosages and durations in 131 bronchiectasis patients, and found significant improvement of the total score and the 'bronchiectasis', 'mucus plugging', 'parenchyma' and 'peribronchial thickening' subscores.

Thus, items indicative of active bronchial inflammation, such as thickening of the airway mucosa and mucus impaction appear the CT features most responsive to change; this responsiveness was shown both in CF as in non-CF bronchiectasis. Bronchial inflammation is one of the components of the 'vicious circle' of structural airway damage, bacterial colonization and exaggerated bronchial inflammation, often quoted when describing the emergence of bronchiectasis. The observation that azithromycin treatment causes improvement of CT-features indicative of inflammation, would be another argument in favour of its intrinsic anti-inflammatory capacity.

However, such effects were not convincingly observed in the current study; although some change was noted of the peribronchial thickening item score when comparing pre-and post-treatment values, this difference did not reach statistical significance.

In our exploratory analyses we made the observation that participants who showed a reduction of exacerbations during treatment had higher baseline CT total scores as compared to non-responders. The difference between both groups was mainly accounted for by higher scores on 'peribronchial thickening' and 'severity' item scores in the responders. These findings have to be interpreted with care since, even with the somewhat 'liberal' definition of response, only a small number of non-responders could be identified which importantly limited the reliability of our statistical analyses.

In the current study, CT total scores showed good correlation to dynamic lung function parameters and the gas transfer factor. A good (negative) correlation between FEV_1 and CT scores has been demonstrated earlier; this finding can be interpreted as air flow limitation caused by thickening of the bronchial mucosa and an increase in bronchial secretions [5;6;22]. Correlation between TLCO and CT score was only investigated in one other study, which also found a negative correlation between the two parameters [23]. Small airways disease, often present in bronchiectasis, might account for the reduced CO-diffusion capacity in patients with high radiological severity scores.

Interesting, although not completely surprising, findings in the current study were the higher CT scores in patients colonized with *P. aeruginosa* as compared to those without, indicating more severe disease in the former group. Despite the fact that total CT scores showed no more than a trend towards more severe disease in patients infected with *P. aeruginosa*, the item score for consolidation and peribronchial thickness in this group was significantly higher. *Pseudomonas* presence is traditionally linked to faster lung function decline and worse prognosis in both CF and non-CF bronchiectasis.

There are certain limitations to a wider applicability of the findings in this study. In this analysis we focused on those items in the Bhalla score for which we had two comparable imaging sets. By limiting our analysis to the sub-set of items of the Bhalla score, we did not use the validated scoring system as such. However, because internal consistency of the score was still sufficient after omission of the three items and because we found a good interobserver agreement between the results on the remaining 6 items, we still felt confident using this shortened version of the Bhalla score. The study is further limited by the fact that almost one quarter of participants did not have their CT's scored, which might influence the results, although missing scans were evenly distributed between azithromycin- and placebo treated patients.

These limitations imply that the findings of the current study must be interpreted with care and that further research is necessary to confirm these results.

Despite these limitations, this study is the first prospective attempt to evaluate longitudinal

CT changes during azithromycin treatment in non-CF bronchiectasis and generates interesting material for further contemplation. In the current study, one year of treatment with azithromycin did not result in a statistically significant improvement of CT features. An interesting finding which needs further study, is the radiological finding of more severe disease in patients who were responsive to azithromycin treatment. If this finding is replicated in larger series with the same CT settings, CT scores, which include items indicative for bronchial inflammation might be useful tools to select patients with a potency to a favourable response to macrolide treatment.

Reference list

1. McShane, P. J., E. T. Naureckas, G. Tino, and M. E. Strek. 2013. Non-cystic fibrosis bronchiectasis. Am.J.Respir.Crit Care Med. 188:647-656.
2. Cole, P. J. 1986. Inflammation: a two-edged sword--the model of bronchiectasis. Eur J Respir Dis Suppl 147:6-15.
3. Chalmers, J. D., P. Goeminne, S. Aliberti, M. J. McDonnell, S. Lonni, J. Davidson, L. Poppelwell, W. Salih, A. Pesci, L. J. Dupont, et al. 2014. The bronchiectasis severity index. An international derivation and validation study. Am.J.Respir.Crit Care Med. 189:576-585.
4. Martinez-Garcia, M. A., G. J. de, R. M. Vendrell, R. Giron, C. L. Maiz, C. D. de la Rosa, and C. Olveira. 2013. Multidimensional approach to non-cystic fibrosis bronchiectasis. The FACED score. Eur.Respir.J.
5. Habesoglu, M. A., A. O. Ugurlu, and F. O. Eyuboglu. 2011. Clinical, radiologic, and functional evaluation of 304 patients with bronchiectasis. Ann.Thorac.Med. 6:131-136.
6. Alzeer, A. H. 2008. HRCT score in bronchiectasis: correlation with pulmonary function tests and pulmonary artery pressure. Ann.Thorac.Med. 3:82-86.
7. Goeminne, P. C., J. Soens, H. Scheers, W. W. De, and L. Dupont. 2012. Effect of macrolide on lung function and computed tomography (CT) score in non-cystic fibrosis bronchiectasis. Acta Clin.Belg. 67:338-346.
8. Altenburg, J., C. S. de Graaff, Y. Stienstra, J. H. Sloos, E. H. van Haren, R. J. Koppers, T. S. van der Werf, and W. G. Boersma. 2013. Effect of azithromycin maintenance treatment on infectious exacerbations among patients with non-cystic fibrosis bronchiectasis: the BAT randomized controlled trial. JAMA 309:1251-1259.
9. Wong, C., L. Jayaram, N. Karalus, T. Eaton, C. Tong, H. Hockey, D. Milne, W. Fergusson, C. Tuffery, P. Sexton, et al. 2012. Azithromycin for prevention of exacerbations in non-cystic fibrosis bronchiectasis (EMBRACE): a randomised, double-blind, placebo-controlled trial. Lancet 380:660-667.
10. Serisier, D. J., M. L. Martin, M. A. McGuckin, R. Lourie, A. C. Chen, B. Brain, S. Biga, S. Schlebusch, P. Dash, and S. D. Bowler. 2013. Effect of long-term, low-dose erythromycin on pulmonary exacerbations among patients with non-cystic fibrosis bronchiectasis: the BLESS randomized controlled trial. JAMA 309:1260-1267.
11. Bhalla, M., N. Turcios, V. Aponte, M. Jenkins, B. S. Leitman, D. I. McCauley, and D. P. Naidich. 1991. Cystic fibrosis: scoring system with thin-section CT. Radiology 179:783-788.
12. Miller, M. R., J. Hankinson, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, R. Crapo, P. Enright, C. P. van der Grinten, P. Gustafsson, et al. 2005. Standardisation of spirometry. Eur.Respir.J. 26:319-338.
13. Quanjer, P. H., G. J. Tammeling, J. E. Cotes, O. F. Pedersen, R. Peslin, and J. C. Yernault.

1993. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur.Respir.J.Suppl 16:5-40.

14. Altenburg, J., K. Wortel, C. S. de Graaff, T. S. van der Werf, and W. G. Boersma. 2014. Validation of a visual analogue score (LRTI-VAS) in non-CF bronchiectasis. Clin.Respir.J.
15. Jones, P. W. 2005. St. George's Respiratory Questionnaire: MCID. COPD. 2:75-79.
16. Jones, P. W., F. H. Quirk, C. M. Baveystock, and P. Littlejohns. 1992. A self-complete measure of health status for chronic airflow limitation. The St. George's Respiratory Questionnaire. Am Rev Respir Dis 145:1321-1327.
17. Wilson, C. B., P. W. Jones, C. J. O'Leary, P. J. Cole, and R. Wilson. 1997. Validation of the St. George's Respiratory Questionnaire in bronchiectasis. Am J Respir Crit Care Med 156:536-541.
18. Altenburg, J., C. S. de Graaff, T. S. van der Werf, and W. G. Boersma. 2011. Immunomodulatory effects of macrolide antibiotics - part 1: biological mechanisms. Respiration 81:67-74.
19. Sheikh, S. I., F. R. Long, R. Flucke, N. A. Ryan-Wenger, D. Hayes, Jr., and K. S. McCoy. 2015. Changes in Pulmonary Function and Controlled Ventilation-High Resolution CT of Chest After Antibiotic Therapy in Infants and Young Children with Cystic Fibrosis. Lung 193:421-428.
20. Sheikh, S. I., F. R. Long, K. S. McCoy, T. Johnson, N. A. Ryan-Wenger, and D. Hayes, Jr. 2015. Computed tomography correlates with improvement with ivacaftor in cystic fibrosis patients with G551D mutation. J.Cyst.Fibros. 14:84-89.
21. Nasr, S. Z., E. Sakmar, E. Christodoulou, B. P. Eckhardt, D. S. Streetman, and P. J. Strouse. 2010. The use of high resolution computerized tomography (HRCT) of the chest in evaluating the effect of tobramycin solution for inhalation in cystic fibrosis lung disease. Pediatr.Pulmonol. 45:440-449.
22. Lynch, D. A., J. Newell, V. Hale, D. Dyer, K. Corkery, N. L. Fox, P. Gerend, and R. Fick. 1999. Correlation of CT findings with clinical evaluations in 261 patients with symptomatic bronchiectasis. AJR Am J Roentgenol. 173:53-58.
23. Koulouris, N. G., S. Retsou, E. Kosmas, K. Dimakou, K. Malagari, G. Mantzikopoulos, A. Koutsoukou, J. Milic-Emili, and J. Jordanoglou. 2003. Tidal expiratory flow limitation, dyspnoea and exercise capacity in patients with bilateral bronchiectasis. Eur.Respir.J. 21:743-748.

CHAPTER 7

The relationship between serum- and sputum levels of azithromycin and clinical endpoints in patients with bronchiectasis using azithromycin maintenance treatment.

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Abstract

Background Azithromycin (AZM) is a macrolide antibiotic with distinct pharmacokinetic properties and is increasingly used as maintenance treatment in patients with bronchiectasis in order to reduce infectious exacerbations and improve pulmonary symptoms. The exact mechanism of action is not known and the relation between azithromycin dose level, local and systemic drug levels and clinical effect however, has not been extensively studied yet.

Objectives To explore the relation between AZM serum and sputum concentrations, clinical effect parameters and side effects.

Methods Azithromycin concentrations were measured in serum and sputum samples of bronchiectasis patients receiving one year of AZM treatment (250mg OD) enrolled in the Bronchiectasis and Azithromycin Treatment (BAT) trial, a double blind, randomised placebo-controlled trial. Results were correlated with data on AZM dose level, exacerbation frequency, lung function (forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC), quality of life and symptoms collected within the same year.

Results 83 sputum samples from 31 patients and 151 serum samples from 43 patients were available for analysis. Mean AZM dose-level ranged from 18.8 to 39.8 mg/kg body weight/ week, generating mean AZM concentrations of 7.57 mg/L (SD 9.49) in sputum and 0.11 mg/L (SD 0.085) in serum. No correlation was found between side effects and AZM dose level, sputum- or serum concentrations. Significant correlation was found between AZM sputum concentration and CRP-level (r= -0.6).

Conclusions High and stable AZM sputum levels were reached during long term treatment, as opposed to low AZM levels in serum. Apart from CRP-levels to AZM sputum concentration, no other outcome parameter showed significant correlation to AZM serum- or sputum levels. AZM dose- or exposure levels were not predictive for the occurrence of side effects.

Introduction

Azithromycin (AZM) is a 15-membered azalide antibiotic exhibiting a bacteriostatic effect towards susceptible pathogens (mainly gram positive organisms) through inhibition of the RNA-dependent protein synthesis, attenuation of the bacterial biofilm and a deleterious effect on bacterial virulence factors. In addition, an anti-inflammatory effect is described, involving reduced cytokine production and anti-neutrophilic action among other effects, which remains incompletely understood.[1]

AZM has distinct pharmacokinetic properties which set it apart from other macrolide antibiotics. Its large volume of distribution due to rapid uptake and accumulation in phagocytic cells is followed by slow release and accounts for an exceptionally long half-life and high intracellular concentrations in the presence of low plasma levels.[1, 2] Short courses (3 to 5 days) of AZM are frequently used to treat a variety of community acquired infections. Long term azithromycin treatment is a key element of Cystic Fibrosis (CF) treatment and is increasingly used to treat other chronic respiratory infections, such as non-CF bronchiectasis (hereafter referred to as 'bronchiectasis') and COPD after favourable results of clinical trials. [3-6]

Bronchiectasis –abnormal dilated bronchi, resulting from a vicious circle of mucus retention, bacterial colonization and inflammation- is a chronic lung disease, characterized by a variable course. Stable periods with a mild productive cough are interspersed with infectious exacerbations which importantly contribute to reduced quality of life. Since 2012 three randomised clinical trials have confirmed the efficacy of long term macrolide treatment in bronchiectasis.[7-9] Patients treated with azithromycin (250 OD or 500 mg three times weekly) or erythromycin (400 mg BD) showed a marked reduction of infectious exacerbations annually. Favourable effects were also noted with respect to lung function and quality of life but these were not consistent between studies.

The pharmacokinetics (PK) and exposure after a single dose or short courses of azithromycin are well known. Exposure after chronic use in CF patients has been investigated by measuring azithromycin in blood and in sputum. CF patients on chronic azithromycin show a wide inter-individual variation in clinical efficacy but also in blood-, sputum and tissue concentrations of AZM, even at the same dose level.[10] The intra individual variation in sputum concentration showed a stable concentration when measured at monthly intervals during 3 months.[20] A relationship between exposure in blood and sputum and clinical efficacy has never been investigated neither in CF treatment nor in treatment of bronchiectasis. We report the results of a study towards the relationship between individual exposure and clinical efficacy of chronic azithromycin therapy in patients with bronchiectasis.

Objectives

In the current study the authors explore the relation between AZM concentrations both in plasma and sputum and clinical effect parameters: exacerbation frequency, lung function (Forced Expiratory Volume in 1 second (FEV1) and Forced Vital Capacity (FVC), High Resolution Computed Tomography (HRCT) scores, quality of life and symptoms. Additionally we investigated the relationship between azithromycin dose level in mg/kg bodyweight and exposure, clinical efficacy parameters and side effects.

Patients and Methods

The 'BAT' (Bronchiectasis and Azithromycin Treatment) trial, a multicentre, 1:1 randomised, placebo-controlled trial was conducted at 14 sites in the Netherlands from 2008- 2010 (Clinicaltrials.gov, registration no: NCT00415350). Detailed study protocols are provided elsewhere.[7] Participants were eligible for randomization if they had bronchiectasis and three or more lower respiratory tract infections treated with antibiotics in the preceding year, with positive sputum cultures.

All participants gave informed consent and ethical approval was provided by the Institutional review board of Alkmaar Medical Centre: 'METC Noord Holland' (Approval no: M07-002, CCMO: NL16025.094.07).

Patients were randomised to receive either azithromycin (250 mg once daily) or placebo for 12 months, during which the number of infectious exacerbations (the primary endpoint), lung function parameters, sputum bacteriology, HRCT-scores, inflammatory markers, adverse effects, symptom scores and quality of life (QOL) were recorded. During the BAT trial an infectious exacerbation was defined as the prescription of a course of antibiotics because of the presence of at least 4 of the following 9 symptoms, signs, or findings: (1) change in sputum production (consistency, colour, volume, or haemoptysis); (2) increased dyspnoea (chest congestion or shortness of breath); (3) increased cough; (4) fever (>38°C); (5) increased wheezing; (6) decreased exercise tolerance, malaise, fatigue, or lethargy; (7) FEV1 or FVC decreased by at least 10% from a previously recorded value; (8) radiographic changes indicative of a new pulmonary infectious process; or (9) changes in chest sounds.[7]

All patients were familiar with routine spirometry measurements and these were performed according to European Respiratory Society standard criteria.[11] Reference values for spirometry were from the European Coal and Steel Community.[12]

Symptoms were measured using visual analogue scales (VAS) for dyspnoea, cough, fatigue,

chest pain and sputum purulence. Each symptom was scored from 1 to 10, higher scores indicating more severe symptoms and domain- and total scores were provided.[13]

Saint George's Respiratory Questionnaire (SGRQ) was used to measure health related QoL (HRQoL). Its 76 items are partitioned into three sections (Symptoms, Activity, Impact), yielding domain- and total scores, ranging from 0 to 100%, zero indicating no impairment of quality of life. A difference of 4 points or more is considered clinically significant.[14 15, 16]

At baseline and after one year of study treatment, HRCT scans were obtained and independently scored by two radiologists according to the validated scoring system designed by Bhalla et al.[17] Scores range from 0 for no abnormalities to a maximum score of 25, measuring the presence and extent of key morphologic features of bronchiectasis.

At three-monthly intervals serum samples and samples of spontaneously expectorated sputum were collected and stored at -70 °C. In the current study samples obtained from patients on azithromycin were included after unblinding the study data and reporting the clinical outcome of the study.[7] Samples at 3, 6, 9 and 12 months from start of treatment and 3 months after treatment discontinuation were used. In case of a missing sample at one of these visits, samples from directly previous or subsequent visits were used. Azithromycin was quantified in serum and in sputum using liquid chromatography, triple quad tandem mass spectrometry (LCMS/MS Agilent Technologies) and 13CD3 azithromycin as internal standard. Serum and sputum samples were kept at -70 ^{0}C until quantification. Azithromycin proved stable under these conditions. Before quantification sputum samples were homogenised by vortexing after addition of glass pearls. After addition of the internal standard quantification was performed in duplo. The method proved linear between 0094 and 18.9 mg/L in sputum and between: 0.0189 and 0.944 mg/L in serum. The limit of quantification was 0.1 mg/L in sputum and 0.02 mg/L in serum with a reproducibility of 1.4% in sputum (at 0.472 mg/L and 9.44 mg/L) and between 2.8% (at 0.028 mg/l) and 7.4% (at 0.472 mg/l) in serum.

Statistics

Comparisons of parameters between groups were calculated with a t test if normally distributed and with a Mann- Whitney U test if not.

When analysing the relation between azithromycin levels and clinical endpoints, we started by calculating Crohnbach's alpha including measurements at 3, 6, 9 and 12 months for azithromycin serum and sputum levels in order to ascertain if it was justified to calculate means over time, accepting Crohnbach's alpha >0.7 as sufficient. Change in clinical endpoint

during one year of treatment was expressed by delta's (measurement at 12 months minus baseline). The relationship between azithromycin concentrations and clinical endpoints was explored dually by calculating both Pearson's correlation coefficient and performing linear regression for each variable . When calculating Pearson's correlation coefficient r (p), $r \geq 0.7$ was interpreted as indicating very strong correlation, 0.4- 0.69 as strong correlation, 0.3- 0.39 as moderate correlation, 0.2-0.29 as weak correlation and < 0.2 as no or negligible correlation. $P < 0.05$ was considered statistically significant. SPSS version 20 (SPSS inc.) was available for statistical analysis.

Results

A total of 83 sputum samples from 31 patients were available for analysis. The percentage of patients able to produce spontaneous sputum decreased from 51% at baseline to 23% after one year of AZM treatment and increased to 47% after treatment discontinuation, which indicates a treatment effect of azithromycin. Serum samples were available for all AZM-treated patients (n= 43), yielding 151 serum samples for analysis. Baseline patient characteristics are described in table 1.

Crohnbach's alpha for measurements of AZM levels at 3, 6, 9 and 12 months was 0.71 for serum AZM levels and 0.74 for sputum AZM, indicating sufficient correlation between different measurements and allowing us to calculate means.

Table 1. *Baseline patient characteristics*

Baseline patient characteristics (n=43)	
Age (years, SD)	59.9 (12.3)
Female sex (No,%)	25 (63)
Body mass index	23.0 (3.4)
Smoker	
Current	1 (2)
Former	19 (44)
Aetiology of bronchiectasis:*	
Post infectious	15 (35)
Idiopathic	12 (28)
Asthma	7 (16)
Auto-immune disease	3 (7)
Common variable immune disorder (CVID)	1 (2)
Primary ciliary dyskinesia (PCD)	1 (2)

Table 1. *Continued*

Yellow Nail Syndrome	0
Aspiration	1 (2)
Mechanical obstruction	1 (2)
Allergic bronchopulmonary aspergillosis	1 (2)
Alpha-1- antitrypsin deficiency	1 (2)
No of exacerbations in year before study entry (median, IQR)	4.0 (3-9)
HRCT score	9.0 (3.0)
SGRQ total score	40.6 (19.4)
LRTI-VAS total score	17.5 (10)
Abnormalities on auscultation:	
Crackles	20 (47)
Rhonchi	8 (19)
Wheezing	7 (16)
Dullness	0
CRP (mmol/l) (median, IQR)	5.0 (2- 11,3)
WBC count ($x10^9$/L)	8.1 (2.7)
Percent predicted FEV1	77.7 (24.4)
Percent predicted FVC	91.9 (24.4)
Baseline sputum microbiology:	
Haemophilus influenzae	13 (30)
Staphylococcus aureus	4 (9)
Pseudomonas aeruginosa	6 (14)
Treatment previous to study entry:	
Inhaled corticosteroids‡	38 (88.4)
Long-acting β-agonist‡	34 (79)
Oral corticosteroids‡	4 (9)
Inhaled antibiotics‡	0 (0)
Longterm oral antibiotic treatment‡	4 (9)
Airway clearance techniques: §	
Daily	11 (26)
Weekly	3 (7)
During exacerbation	4 (9)

Data are n(%) or mean (SD) unless otherwise indicated. FEV1 = forced expiratory volume in 1 sec. FVC = forced vital capacity. SGRQ= St George's respiratory questionnaire. LRTI-VAS= lower respiratory tract infection- visual analogue score. * As described by the treating pulmonary physician †patient reported hearing impairment; ‡Treatment started before study entry and continued during the study period. § Any technique taught by a physiotherapist and performed by the patient in order to evacuate sputum.

Clinical endpoints

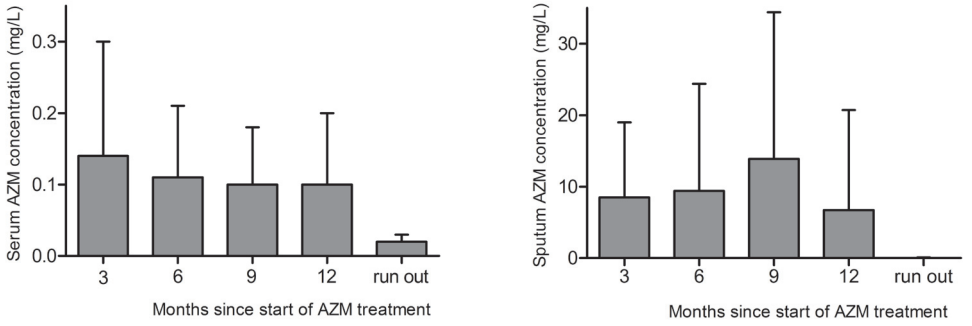
A mean number of 4.47 (SD 1.55) exacerbations for each patient were noted during the year before start of study as compared to 0.84 (SD 1.13) while receiving azithromycin ($p < 0.001$). During one year of azithromycin treatment changes in other clinical parameters were as follows:

FEV1 and FVC at baseline were 77.7 (SD 24.4) and 91.9 (SD 24.4) % of predicted as compared to 81.9 (SD 24.6) and 97.2 (SD 26.1) % pred. at 12 months. VAS total score decreased from 17.5 (SD 10.0) at baseline to 12.4 (SD 8.95) at 12 months and SGRQ total score declined from 40.6 (SD 19.4) to 29.3 (SD 20.4). CRP and leukocytes at baseline were 12.8 (SD 25.0) and 8.14 (SD 2.76) respectively as compared to 5.5 (SD 9.2) and 7.7 (SD 2.7) at end of treatment (e-figure 1).

Azithromycin serum- and sputum levels

Mean azithromycin concentration for all visits was 7.57 mg/L (SD 9.49 mg/L) in sputum and 0.11 mg/L (SD 0.085 mg/L) in serum (Figure 1 and Table 2).

Figure 1. *Mean azithromycin (AZM) concentrations in sputum and serum during –and three months after discontinuation- of AZM 250 OD maintenance treatment. Error bars represent standard deviations.*



There was moderate correlation between sputum and serum levels in this patient group ($r=0.4$ (Pearson)) (figure 2). Three months after discontinuation of treatment sputum samples from all but one patient and serum samples from all but one other patient were negative for azithromycin (sputum < 0.1 mg/L, serum < 0.02 mg/L). Correlations between AZM concentration in serum and sputum and change of clinical endpoints such as lung function, HRCT score and exacerbation frequency are shown in table 3. Sputum AZM levels showed moderate-good correlation with change of VAS total score, leukocyte count and CRP levels, but only the correlation between sputum AZM and CRP reached statistical significance when performing regression analysis ($p=0.001$). No correlation was found between AZM serum concentrations and all clinical endpoints ($p>0.05$).

Table 2. *Mean azithromycin concentrations (mg/L) in sputum and serum during one year of maintenance treatment (azithromycin 250 mg once daily)*

Months from start of treatment	No. of patients	Mean serum concentration (SD)	No. of patients	Mean sputum concentration (SD)
3	43	0.14 (0.16)	22	8.49 (10.5)
6	43	0.11 (0.10)	27	9.41 (15)
9	43	0.10 (0.08)	24	13.9 (20.5)
12	43	0.10 (0.10)	10	6.71 (14)
3 months after treatment discontinuation	43	0.002 (0.014)	20	0.02 (0.09)

When comparing AZM serum- and sputum levels in different patient groups (classified by smoking habit, etiology (idiopathic/ post infectious/ other diagnosis) or gender, no between group-difference was found. In addition, no correlation between AZM levels and age or weight existed.

Figure 2. *Correlation between mean azithromycin sputum- and serum levels in 43 patients receiving one year of maintenance treatment (azithromycin 250 mg once daily). r represents Pearson's correlation coefficient.*

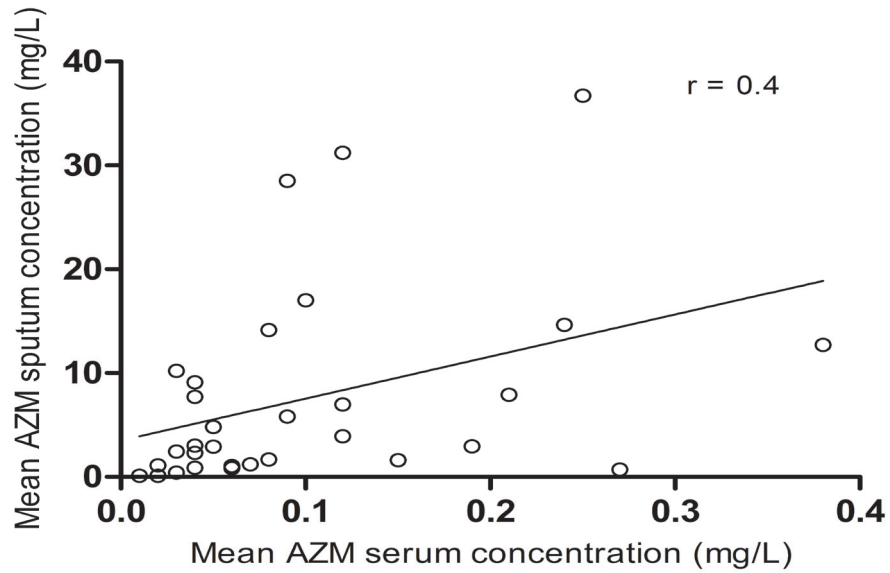


Table 3. Correlations between mean AZM serum and sputum levels and change of clinical endpoints during one year of maintenance treatment.*

	Serum			Sputum		
	n	r (p)	Regression coefficient (SD)	n	r (p)	Regression coefficient (SD)
Exacerbation frequency	43	0.02 (0.9)	0.4 (3.1)	31	0.1 (0.6)	0.02 (0.03)
FEV1 (percent predicted)	37	-0.03 (0.85)	-3,3 (17)	26	0.2 (0.2)	0.2 (0.2)
FVC (percent predicted)	36	0.09 (0.61)	13.8 (26.9)	25	0.4 (0.08)	0.5 (0.3)
VAS total score	34	-0.1 (0.5)	-16.5 (24)	24	-0.4 (0.07)	-0.5 (0.3)
SGRQ total score	36	0.2 (0.3)	38.6 (33.1)	25	0.04 (0.86)	0.07 (0.4)
CRP	40	-0.2 (0.3)	-41.7 (41.8)	28	-0.6 (0.001)	-1.3 (0.4)
Leukocyte count	40	-0.03 (0.8)	-1.2 (5.8)	28	-0.4 (0.07)	-0.1 (0.06)

* Δ visit 6 (12 months) – visit 2 (baseline)

Dose level

Mean body weight of the participants was 66,6 kg (SD 12.8) and a mean dose-level of azithromycin of 26,3 mg/kg bodyweight/week (bw/wk) (range 18.8 to 39.8 mg/kg bw/ wk) was calculated. No or very weak correlation was found between dose level and change in exacerbation frequency (r=0.14), FEV1 (r=0.21), FVC (r=0.23),SGRQ- and LRTI-VAS scores (r=-0.05 and -0.1 respectively) and HRCT-scores (r=-0.04). Only weak correlation was found between dose level and AZM sputum levels (r=0.3) and no correlation between dose level and AZM serum levels.

Side effects

During AZM treatment 23 of 43 (53%) patients reported any side effects, mostly mild gastro-intestinal complaints. When comparing dose levels in patients with or without any side effects, no significant difference between groups was found (p=0.57). When comparing AZM serum and sputum concentrations in patients with or without any side effects, no significant difference between groups was found (p=0.85 and 0.84 respectively).

Discussion

In this study we quantified AZM concentrations in serum and sputum of bronchiectasis patients receiving AZM maintenance treatment (250 mg OD). To our knowledge this is the first study to report this type of data on AZM maintenance treatment in a large group of patients with bronchiectasis without CF. Much more is known on kinetics of AZM in CF patients, for whom it is often presumed that bioavailability and pharmacokinetics of azithromycin and possibly other drugs differ from non-CF patients. However, already in 2005 Beringer et al [18] reported that –in comparison with healthy volunteers- the bioavailability, absorption rate and pharmacokinetics of single dosages of AZM in CF patients taking pancreatic enzyme suppletions were no different.

We found azithromycin sputum concentrations ranging from 6.71 mg/L to 13.9 mg/L, about 70 times higher than in serum. Reports in CF patients taking either 500 OD or 1000 mg once weekly describe higher sputum concentrations (26.6 mg/L (SD15.6) at 500 mg OD and 9.6 (SD7.1) at 1000 mg weekly), but the level of accumulation is comparable.[19]

Only moderate correlation was found between serum and sputum concentrations of AZM. This is in concordance with the results of Wilms et al [20] who also failed to demonstrate a strong relationship between sputum and blood concentrations in CF patients. Their hypothesis that sputum concentrations might be influenced by the availability of neutrophils in the lungs and the amount of sputum that is produced, might also apply to non-CF bronchiectasis. In this view, neutrophils, with their high intracellular level of AZM would act as so-called 'vehicles' for AZM transportation, delivering relatively large amounts of antibiotics to the site of inflammation. This is further supported by the finding of higher concentrations of azithromycin in infected versus uninfected tissue in a mouse thigh infection model and in inflamed versus non inflamed blisters in humans.[21, 22] This might especially be true for bronchiectasis, because its clinical course is characterized by periods with usually mild chronic complaints, interspersed with infectious exacerbations. During stable disease, and even more so during an exacerbation, markedly raised numbers of neutrophils are found in the airways of bronchiectasis patients, not necessarily accompanied by raised systemic inflammation markers. This airway-predominant inflammation may in part account for the differences between AZM serum- and sputum levels in this study.

As reported earlier, favourable changes in clinical parameters were noted during one year of azithromycin treatment [7]. Apart from an evident reduction of infectious exacerbations, a small improvement in lung function was seen together with an improvement of quality of life as measured by SGRQ. In addition, symptoms and inflammatory parameters were reduced. No earlier studies have reported the relationship between clinical efficacy and the individual exposure to azithromycin during maintenance treatment. Since AZM shows

multiple pharmacodynamic effects of which the contribution to the clinical efficacy has not been fully understood, a clear concentration-exposure relationship was not expected. However, in earlier reports, macrolides have been described to suppress sputum production through inhibition of chloride secretion by airway epithelial cells.[23] Tagaya et al [24] described a dose dependent effect of erythromycin, on chloride diffusion in an animal model. In our study the exposure-effect relationship was less distinct; although changes in FVC, VAS total score, CRP level and leukocyte count showed moderate-good correlation to AZM sputum levels, only the correlation with CRP-level reached statistical significance. The current study failed to demonstrate any significant correlation between response parameters and serum AZM concentrations.

In the current study a standard dosing regimen of AZM 250 mg daily resulted in a wide range of azithromycin dose levels (dose per kg bodyweight). Dose level did not appear to influence systemic exposure to the study drug since correlation between dose level and AZM-levels in serum and sputum was moderate at best. In CF patients azithromycin maintenance therapy leads intra-individually, to concentrations in bronchial secretion approximately linearly related to the oral dose and irrespective of the azithromycin dosing frequency and interval. The inter-individual variability in drug concentrations is therefore likely due to patient-specific parameters. However, when analysing the available data on age, weight, etiology, smoking status or gender in relation to AZM levels, no such parameter was identified. Other factors, such as bioavailability, therapy adherence, number of neutrophils in sputum and sputum kinetics might importantly influence local and systemic drug levels.

An unexpected finding in the current study is the absence of a relation between the occurrence of side effects and AZM concentrations or dose level. In the past decade, several authors reported an increased incidence of adverse effects with larger dosages of macrolides or higher AZM serum concentrations in CF or *Mycobacterium avium* complex (MAC)-disease.[25, 26] A similar dose-dependent occurrence of side effects is observed when comparing adverse events in the BAT trial and the EMBRACE trial by Wong and colleagues.[9] During treatment with AZM 250 mg daily, 40% of participants in the BAT trial experienced gastro-intestinal side effects as compared to 27% in the latter trial using 500 mg AZM thrice weekly.[7, 9]

In the current study, clinical improvement appears to be unrelated to AZM dose level, therefore one could speculate if the dose of 250mg/day chosen in the BAT trial might not be unnecessarily high in patients with lower body weight. In these patients a dosing regimen of 250 every other day - as is already frequently used in pulmonary clinics – might be sufficient. To date, no randomised trials comparing different dosing regimens are available. For patients with cystic fibrosis one of the current authors recently proposed a dose advice of AZM 22-30 mg/kg/wk, based on efficacy in clinical trials.[10] In the current trial, the lowest

dose level inducing a clinically relevant response was 18,85 which corresponds to a daily dose of AZM of approximately 150 mg for patients with a body weight between 55 and 60 kilos.

Although the current study provides interesting and new information on the relations between AZM dose level, serum- and sputum concentration and clinical effect parameters, the authors wish to point out a number of weaknesses, mostly related to the study design.

The original BAT trial was not designed to measure pharmacokinetic parameters, which means that no information was available about the exact timing of sputum expectoration or blood sampling in relation to drug ingestion. Especially the serum AZM levels have to be interpreted with caution, since AZM concentrations in blood show a distinct pattern characterized by a peak within hours after ingestion, followed by quick distribution into the tissue.[20] Therefore, the timing of blood sampling will importantly contribute to variations in AZM levels. This methodological problem may also be one of the reasons that no correlation was found between AZM serum levels and other parameters in the current study. AZM sputum concentrations are more robust, since earlier studies showed that accumulation of AZM in bronchial secretions still occurs after 5 days of treatment, reaching stable values in about 1 month of treatment, yielding stable values throughout time and small intra-individual variations.[10]
Second, the availability of sputum samples gradually decreased during AZM treatment. Results for visit 9 and 12 might therefore be less robust when data from sputum analysis are involved. Finally when quantifying the total azithromycin concentration in sputum we were not able to distinguish between intra- and extracellularly, bound and unbound azithromycin.

In conclusion: one year of AZM maintenance treatment resulted in high levels of sputum AZM as opposed to serum levels which were about 70 times lower. Higher sputum concentrations of AZM only coincided with a reduction of serum CRP, but showed poor correlation to other response parameters. Contrary to findings in the literature and our own clinical experience, we failed to demonstrate a relation between adverse events and AZM concentrations or dose level. Considering the favourable response to treatment in patients with a relatively low dose level of AZM, it may be justified to apply reduced dosage regimens for patients with low body weight.

Reference List

1. Van BF, Tulkens PM. Macrolides: pharmacokinetics and pharmacodynamics. Int J Antimicrob Agents 2001; 18 Suppl 1: S17-S23.
2. Di PA, Barbara C, Chella A, et al. Pharmacokinetics of azithromycin in lung tissue, bronchial washing, and plasma in patients given multiple oral doses of 500 and 1000 mg daily. Pharmacol Res 2002; 46: 545-50.
3. Albert RK, Connett J, Bailey WC, et al. Azithromycin for prevention of exacerbations of COPD. N Engl J Med 2011; 365: 689-98.
4. Uzun S, Djamin RS, Kluytmans JA, et al. Azithromycin maintenance treatment in patients with frequent exacerbations of chronic obstructive pulmonary disease (COLUMBUS): a randomised, double-blind, placebo-controlled trial. Lancet Respir Med 2014; 2: 361-8.
5. Brusselle GG, Vanderstichele C, Jordens P, et al. Azithromycin for prevention of exacerbations in severe asthma (AZISAST): a multicentre randomised double-blind placebo-controlled trial. Thorax 2013; 68: 322-9.
6. Chalmers JD, Elborn JS. Reclaiming the name 'bronchiectasis'. Thorax 2015; 70: 399-400.
7. Altenburg J, de Graaff CS, Stienstra Y, et al. Effect of azithromycin maintenance treatment on infectious exacerbations among patients with non-cystic fibrosis bronchiectasis: the BAT randomized controlled trial. JAMA 2013; 309: 1251-9.
8. Serisier DJ, Martin ML, McGuckin MA, et al. Effect of long-term, low-dose erythromycin on pulmonary exacerbations among patients with non-cystic fibrosis bronchiectasis: the BLESS randomized controlled trial. JAMA 2013; 309: 1260-7.
9. Wong C, Jayaram L, Karalus N, et al. Azithromycin for prevention of exacerbations in non-cystic fibrosis bronchiectasis (EMBRACE): a randomised, double-blind, placebo-controlled trial. Lancet 2012; 380: 660-7.
10. Wilms EB, Touw DJ, Heijerman HG, et al. Azithromycin maintenance therapy in patients with cystic fibrosis: a dose advice based on a review of pharmacokinetics, efficacy, and side effects. Pediatr Pulmonol 2012; 47: 658-65.
11. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J 2005; 26: 319-38.
12. Quanjer PH, Tammeling GJ, Cotes JE, et al. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16: 5-40.
13. Altenburg J, Wortel K, de Graaff CS, et al. Validation of a visual analogue score (LRTI-VAS) in non-CF bronchiectasis. Clin Respir J 2014.
14. Jones PW. St. George's Respiratory Questionnaire: MCID. COPD 2005; 2: 75-9.
15. Jones PW, Quirk FH, Baveystock CM, et al. A self-complete measure of health status for chronic airflow limitation. The St. George's Respiratory Questionnaire. Am Rev Respir Dis 1992; 145: 1321-7.
16. Wilson CB, Jones PW, O'Leary CJ, et al. Validation of the St. George's Respiratory Questionnaire in bronchiectasis. Am J Respir Crit Care Med 1997; 156: 536-41.
17. Bhalla M, Turcios N, Aponte V, et al. Cystic fibrosis: scoring system with thin-section CT. Radiology 1991; 179: 783-8.
18. Beringer P, Huynh KM, Kriengkauykiat J, et al. Absolute bioavailability and intracellular pharmacokinetics of azithromycin in patients with cystic fibrosis. Antimicrob Agents Chemother 2005; 49: 5013-7.
19. Wilms EB, Touw DJ, Heijerman HG. Pharmacokinetics and sputum penetration of azithromycin during once weekly dosing in cystic fibrosis patients. J Cyst Fibros 2008; 7: 79-84.
20. Wilms EB, Touw DJ, Heijerman HG. Pharmacokinetics of azithromycin in plasma, blood, polymorphonuclear neutrophils and sputum during long-term therapy in patients with cystic fibrosis. Ther Drug Monit 2006; 28: 219-25.
21. Carbon C. Clinical relevance of intracellular and extracellular concentrations of macrolides. Infection 1995; 23 Suppl 1: S10-S14.
22. Liu P, Allaudeen H, Chandra R, et al. Comparative pharmacokinetics of azithromycin in serum and white blood cells of healthy subjects receiving a single-dose extended-release regimen versus a 3-day immediate-release regimen. Antimicrob Agents Chemother 2007; 51: 103-9.
23. Tamaoki J, Isono K, Sakai N, et al. Erythromycin inhibits Cl secretion across canine tracheal epithelial cells. Eur Respir J 1992; 5: 234-8.
24. Tagaya E, Tamaoki J, Kondo M, et al. Effect of a short course of clarithromycin therapy on sputum production in patients with chronic airway hypersecretion. Chest 2002; 122: 213-8.
25. McCormack J, Bell S, Senini S, et al. Daily versus weekly azithromycin in cystic fibrosis patients. Eur Respir J 2007; 30: 487-95.
26. Brown BA, Griffith DE, Girard W, et al. Relationship of adverse events to serum drug levels in patients receiving high-dose azithromycin for mycobacterial lung disease. Clin Infect Dis 1997; 24: 958-64.

CHAPTER 8

Validation of a visual analogue score (LRTI-VAS) in non-CF bronchiectasis.

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Abstract

Introduction: Quality of life in patients with non-cystic fibrosis (non-CF) bronchiectasis is largely defined by respiratory symptoms. To date no disease specific tool for symptom measurement in this patient group was available. We developed the Lower Respiratory Tract Infections –Visual Analogue Scale (LRTI-VAS) in order to quickly and conveniently quantify symptoms in non-CF bronchiectasis.

Objectives: This study aimed to validate LRTI-VAS for use in non-CF bronchiectasis.
Methods: This study included out-patients with radiologically proven bronchiectasis and no evidence of CF. Results of LRTI-VAS were compared to other markers of disease activity (lung function parameters, oxygen saturation and three health-related quality of life questionnaires (SF-36, SGRQ and LCQ) and validity, reliability and responsiveness were assessed.

Results: 30 stable and 30 exacerbating participants completed the LRTI-VAS questionnaire. When testing for repeatability on two separate occasions, no statistically significant difference between total scores was found (1.4 (SD 5.3) , p= 0.16). Internal consistency was high across items (Cronbach's alpha 0.86). Correlation with SGRQ-, SF36- and LCQ total scores was high. Following antibiotic treatment, mean (SD) LRTI-VAS total score improved from 18.1 (SD 9.9) to 26.1 (SD 6.6) (p< 0.001).

Conclusions: LRTI-VAS showed excellent validity, reliability and responsiveness to change and therefore appears a reliable tool for symptom measurement in non-CF bronchiectasis.

Introduction

Bronchiectasis (BE), first described by Laennec (1) in 1819, is characterized by irreversible, pathologic dilatation of the small and medium-sized bronchi, resulting from a vicious cycle of inflammation and bacterial colonization. Impaired clearance of the lower airways leads to chronic bacterial infection and inflammation, a process that has been referred to as a 'vicious circle", leading to the occurrence and progression of BE. (2) Although the aetiology remains unclear in a large proportion of patients (25-53%), common causes include immune defects, previous severe infections and aspiration.(3;4)

The course of the disease is highly variable, including nearly symptom free periods interspersed with infectious exacerbations. Many patients with BE however, suffer from chronic complaints, such as a productive cough, dyspnoea and fatigue.(5;6) Infectious exacerbations are characterized by worsening of symptoms and signs of airways infection, sometimes complicated by pneumonia.(7) The disease was considered to be offensive and untreatable in the pre-antibiotic era, but infections and symptoms are nowadays relatively well controlled with antibiotics and supportive therapy.(8) However, many patients with bronchiectasis today still experience feelings of embarrassment about their coughing or bronchorrhoea, sometimes leading towards social isolation. As the impact of symptoms on quality of life (QOL) may be considerable, improving QOL through symptom reduction is one of the main goals of non-CF BE management.(9)

We developed the LRTI-VAS (Lower Respiratory Tract Infections – Visual Analogue Scale), a symptom scale that can be used to quantify the degree of dyspnoea, fatigue, cough, pain and sputum colour in patients with non-CF BE. These five domains of the LRTI-VAS reflect the most frequently encountered symptoms reported by individuals with BE in clinical practice.(5-8) The LRTI-VAS is significantly less time consuming than other disease specific questionnaires, has a low administrative burden and a simple design.

Objective: The aim of this study was to validate the LRTI-VAS for assessment of symptoms in non-CF BE.

Materials and methods

Study population

From 2010 to 2011 non-CF BE patients visiting the out-patient clinic of the Department of Pulmonary Medicine of the Medical Centre Alkmaar, a large teaching hospital, were asked to participate by the primary investigator. Patients were eligible for inclusion if they had HRCT-

confirmed non-CF BE and spirometry performed less than six months prior to inclusion. Exclusion criteria were CF or inability to read or otherwise complete the questionnaires.

Reliability was measured during a clinically stable situation; measurement of responsiveness required the presence of an exacerbation. Validity was tested on both occasions.

To guarantee clinical stability, each participant was instructed to report to the researchers without delay any changes in their clinical condition, pointing towards an infectious exacerbation. In addition, each participant completed daily diary cards, asking about symptoms indicative of an infective exacerbation. Patients were excluded if clinical stability, as defined by any of the two above-mentioned criteria, was compromised/lacking.

In order to test for responsiveness, an additional inclusion criterion was added; the presence of an infectious exacerbation (meeting the criteria of an exacerbation given below) treated in- or out hospital with a course of oral or iv-antimicrobial treatment.

Study visits

All participants visited our out-patient clinic on two separate occasions, three weeks apart. On both occasions they were asked to complete the LRTI-VAS, the Medical Outcomes Study Short-Form 36 Health Survey (SF-36), the St George's Respiratory Questionnaire (SGRQ) and the Leicester Cough Questionnaire (LCQ) in a randomized order. In addition, on both study visits all patients performed flow-volume spirometry and arterial oxygen saturation was measured, using a fingertip pulse oximeter (Beurer GmbH Y23/003700). In case of an exacerbation, the first study visit was scheduled just before starting antibiotics.

Definition of an exacerbation

In this study an exacerbation was defined as abnormalities in at least four of the following eight symptoms, signs, or laboratory findings: 1) change in sputum production (consistency, colour, volume, or haemoptysis); 2) increased dyspnoea (chest congestion or shortness of breath); 3) increased cough; 4) fever (>38°C); 5) increased wheezing; 6) decreased exercise tolerance, malaise, fatigue, or lethargy; 7) FEV1 or FVC decreased by at least 10% from a previously recorded value; 8) changes in chest sounds.(10)

In order to validate the LRTI-VAS for measuring symptoms in patients with non-CF BE, the validity, reliability and responsiveness of this measure were established as follows:

Testing for reliability

30 patients with clinically stable BE were invited to repeat the LRTI-VAS 3 weeks after completion of the initial questionnaire. Reliability is defined as the extent to which a test provides consistent results across repeated measurements.(11) This is estimated by

measuring the test-retest reliability and internal consistency. The test-retest reliability is the ability of the questionnaire to produce consistent scores over a short period of time, higher consistency meaning higher reliability. Internal consistency concerns the degree of association between the questionnaire items.

Testing for validity

30 patients with clinically stable bronchiectasis and 30 patients with deterioration of symptoms because of an infectious exacerbation completed the LRTI-VAS, LCQ, SF-36 and SGRQ on two separate occasions, three weeks apart. In addition they performed spirometry and pulse oxygen saturation measurement on both occasions. Correlation of LRTI-VAS results with LCQ, SF-36, SGRQ, FEV1, FVC and oxygen saturation was calculated in order to test for validity, which is the extent to which an indicator represents the intended concept. (11) Validity can be tested by comparing the actual outcomes of a test with a theoretical expectation of these outcomes.

Testing for responsiveness

30 patients with an infectious exacerbation completed the LRTI-VAS just before starting antibiotic treatment and two weeks after completion of treatment. The responsiveness of the questionnaire was assessed by comparing changes in scores to changes in markers of disease activity and scores on the other, validated, questionnaires, at two separate points in time.

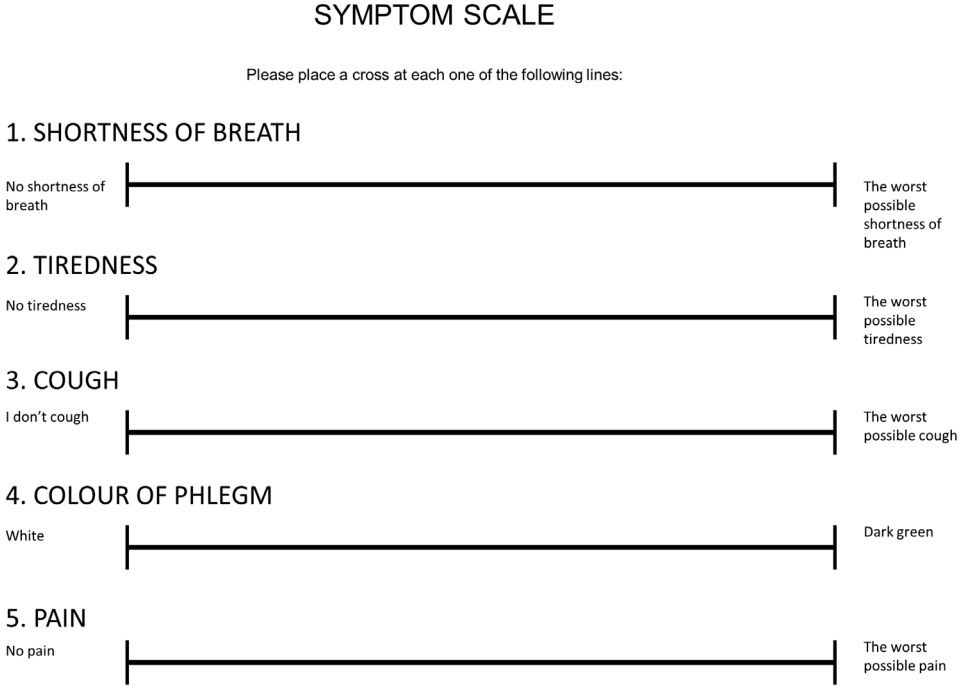
Questionnaires

Apart from the LRTI-VAS, patients were asked to complete the SGRQ, the SF-36 and the LCQ in a randomized order, generated with Graphpad Prism®. All questionnaires were adapted to ask about symptoms in the preceding week.

- LRTI-VAS: consists of a set of horizontal lines with two anchor points, one at each extreme, each line representing a different symptom (fig 1). It is scored from 1 to 10, the subjects being unaware of the numbers. Higher scores indicate more severe symptoms. Five symptom domains are scored: dyspnoea, fatigue, cough, pain and sputum colour (white – dark green). Separate scores are calculated for each symptom and a total score is provided, consisting of all symptom scores added up. Similar weight is assigned to all symptom domains. In our study, a Dutch translation of the LRTI-VAS was used; Its simple and visual design allows for use in other languages, without additional validation studies.
- SGRQ: a condition specific HRQL-measure, that consists of 76 items. These items are partitioned into three sections (Symptoms, Activity, Impact), which are scored separately and can be added up to provide a total score, ranging from 0 to 100, zero indicating no impairment of quality of life. The SGRQ requires about 10 minutes to complete.(12)

- SF-36: a self-administered, generic 36-item HRQL measure. Eight different health concepts are scored, scores ranging from 0-100. A lower score on one of these domains indicates more limitations in this specific domain.(13) Although the SF-36 is primarily designed to measure between-group differences of QoL, this survey is frequently used to measure the effectiveness of medical treatment in clinical trials.(14)
- LCQ: a HRQL questionnaire, validated for assessing chronic cough in non-CF BE.(9;15) It is a 19-item, self-completed questionnaire, exploring the impact of cough severity across three domains; physical, psychological and social.

***Figure 1**. Lower respiratory tract infections – visual analogue scale (LRTI-VAS).*



Statistical analysis

Sample size

When comparing parameters at two different time points, at least 30 participants are required at each point to be able to analyze the difference with a paired T-test assuming normality or the Wilcoxon test when there is no normality. In our study, the separate items of the LRTI-VAS were scored on 10-point scales. Standard deviation on t=1 and t=2 is 1,95. To be able to detect a mean difference of 1 point between scores on t=1 and t=2, with alpha being 0,05 and beta 0,20, a sample size of 30 patients on each measuring moment is required assuming a moderate correlation (0.5) between the scores on t=1 en t=2.

Reliability, validity and responsiveness

Paired T-tests were used to compare LRTI-VAS domain and total scores on two occasions during clinical stability and at the start and end of an exacerbation. In case of a skewed distribution, Wilcoxon's signed ranks test was used. Pearson's correlation was used to assess validity.

Internal consistency of the LRTI-VAS was measured by applying Cronbach's alpha to each of the component scores at entry; accepting >0.7 as sufficient.(16)

During statistical analysis we checked for floor and ceiling effects-. Nominal and ordinal variables were expressed using frequency tables, modus and median. Interval/ratio variables were expressed in terms of mean, standard deviation and confidence intervals. When comparing two variables, p-values of < 0.05 were considered statistically significant. The software package SPSS 16 for Windows (SPSS Inc. Chicago, Illinois, USA) was available for statistical analysis.

Results

60 patients were included in the study and were followed up according to the study protocol, thirty of whom were clinically stable. Patient characteristics are shown in table 1.

They all completed all questionnaires and had spirometry and oxygen saturation measurements on two occasions. Of these, 30 were clinically stable and 30 had an exacerbation fulfilling our criteria (figure 2) (*please find results for LRTI-VAS, SGRQ, LCQ and SF-36 domain scores in e-table 1, online data supplement).*

Table 1. Baseline patient characteristics

Patient characteristics (n=60)	
Age in years (mean (SD)	66.6 (10.3)
Gender (female)	35 (58.3)
FEV1, percentage predicted mean (SD)	82.3 (28.2)
FVC percentage predicted, mean (SD)	93.2 (25.7)
Aetiology of bronchiectasis - idiopathic - allergic bronchopulmonary aspergillosis (ABPA) - post-transplant - primary ciliary dyskinesia - chronic obstructive pulmonary disease (COPD) - rheumatoid arthritis - post- infectious (incl TBC) - common variable immune deficiency (CVID) - gastro-intestinal reflux disease (GERD)	 30 (50) 3 (5) 1 (2) 3 (5) 6 (10) 4 (7) 9 (15) 2 (3) 2 (3)
Smoking status Never Former Current	 36 (60) 23 (38) 1 (2)
Long term medication: - oral antibiotics - inhaled antibiotics - oral steroids - inhaled steroids	 19 (31) 8 (13) 8 (13) 40 (67)
Pseudomonas aeruginosa (PA) status repeated cultures positive for PA no PA	 11 (18) 49 (80)
No of exacerbations in the year of study participation: 0 1 2 3 4 > 4	 14 (22) 11 (18) 14 (23) 9 (15) 9 (15) 3 (5)

Values are no. (%) unless otherwise specified
FEV1 and FCV within 6 months prior to study inclusion.

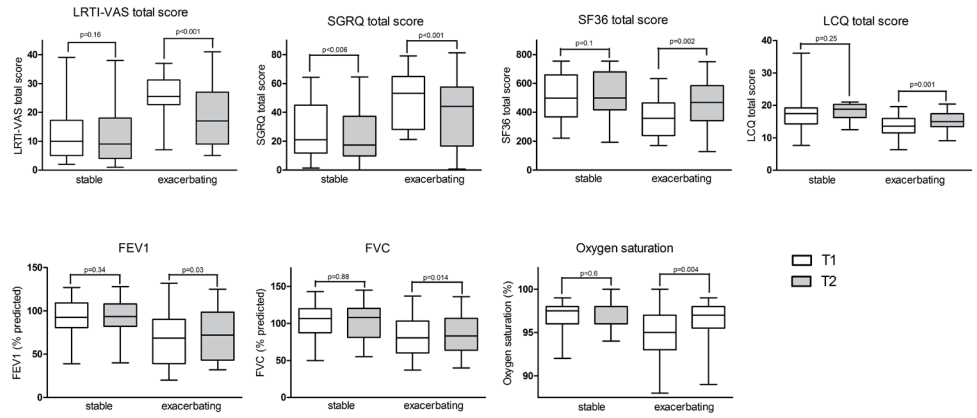
Reliability: the repeatability of the LRTI-VAS in stable bronchiectasis over a 3-wk period

30 stable patients repeated the LRTI-VAS 3 weeks after completion of the initial questionnaire. Mean difference between total scores was 1.4 (SD 5.3), p= 0.16 (fig 2).
Cronbach's alpha for internal consistency for the five LRTI-VAS domains was 0.86, indicating good consistency between items. Internal consistency decreased when one of the items was deleted.

Responsiveness:

30 exacerbations met the study criteria and were eligible for inclusion in the final analysis. LRTI-VAS total score at start of treatment was 26.1 (SD 6.6) as compared to 18.1 (SD 9.9) at end of treatment (mean difference 8.0 (SD 9.1) p< 0.001) (fig 2)

Figure 2. *Results for clinical parameters and questionnaires at baseline (T1) and three weeks later (T2) in a clinically stable situation and during an antibiotically treated exacerbation.*

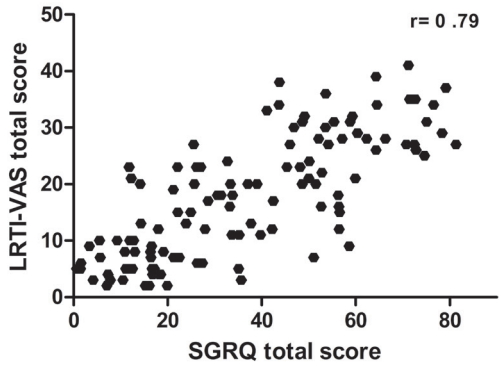


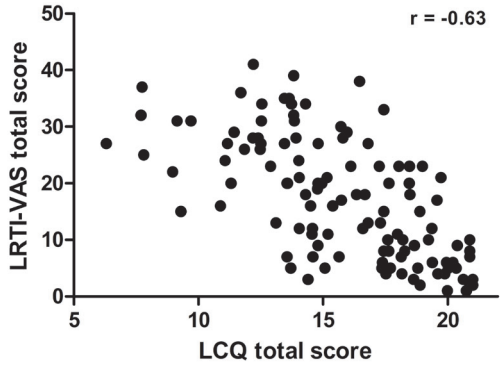
LRTI-VAS: lower respiratory tract infections – visual analogue score. SGRQ: Saint Georges Respiratory Questionnaire . SF-36: Medical Outcomes Study Short-Form 36 Health Survey. LCQ: Leicester Cough Questionnaire. FEV1: Forced expiratory volume in one second. FVC: Forced vital capacity.

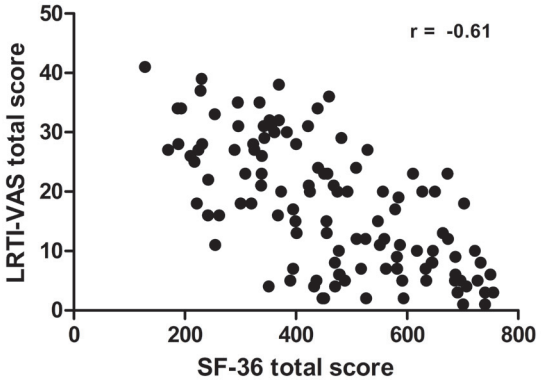
Validity: comparing the LRTI-VAS with other indicators of disease severity

The correlation coefficients between total scores on validated questionnaires (SF-36, SGRQ and LCQ), and LRTI-VAS total score are shown in figure 3. Correlation between FEV1, FVC, oxygen saturation and LRTI-VAS total score was low (r = 0.26-0.39).

Figure 3. Correlation between scores on validated questionnaires and LRTI-VAS total score. r *represents Pearson's correlation coefficient.*







LRTI-VAS: lower respiratory tract infections – visual analogue score. SGRQ: Saint Georges Respiratory Questionnaire SF-36: Medical Outcomes Study Short-Form 36 Health Survey. LCQ: Leicester Cough Questionnaire

Discussion

To our knowledge, the LRTI-VAS is the first clinical tool solely designed for quantification of symptoms in chronic respiratory diseases. Other questionnaires have also been validated for non-CF bronchiectasis patients, the best known being the SGRQ and LCQ.(9;12;15-17) Recently the Quality of Life Questionnaire for Bronchiectasis (QOL-B) was added to our armamentarium.(18) All of these are fairly comprehensive Ouality of Life questionnaires which contain a 'symptoms' domain but are not exclusively designed for measurement of symptoms. The LRTI-VAS meets a need of a faster, more simplified tool for patient-reported outcome in trials and clinical settings. In addition, our questionnaire has a simple design and therefore makes it equally acceptable for poorly educated or illiterate patients.

Visual analogue scores have been used in a variety of clinical settings since their first description in 1957, primarily applied for the assessment of variations in intensity of pain. Nowadays evidence to support their use to measure other symptoms, such as dyspnoea and fatigue, is mounting.(19;20) In clinical trials of patients with COPD, asthma, CF or BE, breathlessness or dyspnoea is frequently measured by means of a VAS.(21-26) VAS-scores have also been applied to measure sputum volume, cough frequency and fatigue in patients with a variety of chronic respiratory diseases.(22;25;27;28) Smith et al (29) disclosed VAS as the only measure to correlate well with objective cough rates in CF-patients who were hospitalized for an exacerbation. We used the LRTI-VAS before, to quantify symptoms in 223 patients with acute exacerbations of COPD and to measure clinical outcome in 213 patients with community acquired pneumonia.(30;31) On all occasions the LRTI-VAS was generally well accepted by patients, showed a high response rate and both patients and researchers appeared to quickly familiarize with this questionnaire.

Our patients scored between 2 to 3,5 points per item on the LRTI-VAS 10-point scale for dyspnea, fatigue, sputum colour and cough in a clinically stable situation. Prior to antibiotic treatment of an exacerbation LRTI-VAS scores for these symptoms increased to 5-6 points per item with a statistically significant decrement after antibiotic treatment. Scores for pain were considerably lower during clinical stability and did not change during treatment for an exacerbation, suggesting that this might be a less prominent and less responsive feature in non-CF BE. A large study in 103 patients with non-CF BE revealed that productive cough (96%), sputum expectoration (87%), dyspnea (60%), and fatigue were the most frequently encountered disease symptoms. Only 19% of their patients complained of chest pain.(32) However, leaving out 'pain' as an item reduced reliability.

In BE, clinical measures such as lung function often correlate only moderately with functional capacity and well-being.(33) This might explain why we only found low correlation between LRTI-VAS and FEV1, FVC and oxygen saturation. Health-related quality

of life (HRQL) in these patients is mainly defined by the presence, extent and severity of symptoms, such as dyspnoea and sputum expectoration. LRTI-VAS identifies and quantifies disease symptoms and could therefore be used to guide management in a clinical setting. In addition, symptoms as measured by LRTI-VAS are potentially valuable outcome measures when evaluating treatment effects in clinical trials.

Conclusion

In this study of 30 stable and 30 exacerbating non-CF bronchiectasis patients LRTI-VAS showed moderate to high correlation with other validated questionnaires. In addition LRTI-VAS responded to clinical changes and showed excellent repeatability and internal consistency. It therefore meets the three key requirements of a questionnaire to be used in monitoring disease severity over time: validity, responsiveness and reliability.

Reference List

1. Laennec RTH. De l'Auscultation Médiate ou Traité du Diagnostic des Maladies des Poumons et du Coeur. 1819. Paris, Brosson & Chaudé. 1819.
2. Cole PJ. Inflammation: a two-edged sword--the model of bronchiectasis. Eur J Respir Dis Suppl 1986;147:6-15.
3. Pasteur MC, Helliwell SM, Houghton SJ, Webb SC, Foweraker JE, Coulden RA, Flower CD, Bilton D, Keogan MT. An investigation into causative factors in patients with bronchiectasis. Am J Respir Crit Care Med 2000 October;162(4 Pt 1):1277-84.
4. Altenburg j, Wortel K, de Graaff CS, Boersma WG. Time to diagnosis and adherence to guidelines in a large cohort of patients with non-CF bronchiectasis. Abstract P2896 ERS Annual Congress Barcelona. 2010.
5. Nicotra MB, Rivera M, Dale AM, Shepherd R, Carter R. Clinical, pathophysiologic, and microbiologic characterization of bronchiectasis in an aging cohort. Chest 1995 October;108(4):955-61.
6. Silverman E, Ebright L, Kwiatkowski M, Cullina J. Current management of bronchiectasis: review and 3 case studies. Heart Lung 2003 January;32(1):59-64.
7. ten Hacken NH, Wijkstra PJ, Kerstjens HA. Treatment of bronchiectasis in adults. BMJ 2007 November 24;335(7629):1089-93.
8. Nicotra MB. Bronchiectasis. Semin Respir Infect 1994 March;9(1):31-40.
9. Murray MP, Turnbull K, MacQuarrie S, Pentland JL, Hill AT. Validation of the Leicester Cough Questionnaire in non-cystic fibrosis bronchiectasis. Eur Respir J 2009 July;34(1):125-31.
10. Altenburg J, de Graaff CS, Stienstra Y, Sloos JH, van Haren EH, Koppers RJ, van der Werf TS, Boersma WG. Effect of azithromycin maintenance treatment on infectious exacerbations among patients with non-cystic fibrosis bronchiectasis: the BAT randomized controlled trial. JAMA 2013 March 27;309(12):1251-9.
11. Carmines EG, Zeller RA. Reliability and validity assessment. London, United Kingdom: SAGE Publications, Inc.; 1979.
12. Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure of health status for chronic airflow limitation. The St. George's Respiratory Questionnaire. Am Rev Respir Dis 1992 June;145(6):1321-7.
13. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992 June;30(6):473-83.
14. Moy ML, Ingenito EP, Mentzer SJ, Evans RB, Reilly JJ, Jr. Health-related quality of life improves following pulmonary rehabilitation and lung volume reduction surgery. Chest 1999 February;115(2):383-9.
15. Birring SS, Prudon B, Carr AJ, Singh SJ, Morgan MD, Pavord ID. Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ). Thorax 2003 April;58(4):339-43.

16. Huisman AN, Wu MZ, Uil SM, van den Berg JW. Reliability and validity of a Dutch version of the Leicester Cough Questionnaire. Cough 2007;3:3.
17. Wilson CB, Jones PW, O'Leary CJ, Cole PJ, Wilson R. Validation of the St. George's Respiratory Questionnaire in bronchiectasis. Am J Respir Crit Care Med 1997 August;156(2 Pt 1):536-41.
18. Quittner AL, Marciel KK, Salathe MA, O'Donnell AE, Gotfried MH, Ilowite JS, Metersky ML, Flume PA, Lewis SA, McKevitt M, Montgomery AB, O'Riordan TG, Barker AF. A preliminary Quality of Life Questionnaire-Bronchiectasis: A patient-reported outcome measure for bronchiectasis. Chest 2014 March 13.
19. Carlsson AM. Assessment of chronic pain. I. Aspects of the reliability and validity of the visual analogue scale. Pain 1983 May;16(1):87-101.
20. Stervus SS, Galunter E.H. Ratio scales and category scales for a dozen perceptual continua. J Exper Psychol 1957 January 1;54:377-411.
21. Cooper CB. Desensitization to dyspnea in COPD with specificity for exercise training mode. Int J Chron Obstruct Pulmon Dis 2009;4:33-43.
22. Durairaj L, Launspach J, Watt JL, Mohamad Z, Kline J, Zabner J. Safety assessment of inhaled xylitol in subjects with cystic fibrosis. J Cyst Fibros 2007 January;6(1):31-4.
23. Karapolat H, Atasever A, Atamaz F, Kirazli Y, Elmas F, Erdinc E. Do the benefits gained using a short-term pulmonary rehabilitation program remain in COPD patients after participation? Lung 2007 July;185(4):221-5.
24. Rosi E, Lanini B, Ronchi MC, Romagnoli I, Stendardi L, Bianchi R, Zonefrati R, Duranti R, Scano G. Dyspnea, respiratory function and sputum profile in asthmatic patients during exacerbations. Respir Med 2002 September;96(9):745-50.
25. Saito Y, Azuma A, Morimoto T, Fujita K, Abe S, Motegi T, Usuki J, Kudoh S. Tiotropium ameliorates symptoms in patients with chronic airway mucus hypersecretion which is resistant to macrolide therapy. Intern Med 2008;47(7):585-91.
26. Suguikawa TR, Garcia CA, Martinez EZ, Vianna EO. Cough and dyspnea during bronchoconstriction: comparison of different stimuli. Cough 2009;5:6.
27. Mutalithas K, Watkin G, Willig B, Wardlaw A, Pavord ID, Birring SS. Improvement in health status following bronchopulmonary hygiene physical therapy in patients with bronchiectasis. Respir Med 2008 August;102(8):1140-4.
28. Polley L, Yaman N, Heaney L, Cardwell C, Murtagh E, Ramsey J, Macmahon J, Costello RW, McGarvey L. Impact of cough across different chronic respiratory diseases: comparison of two cough-specific health-related quality of life questionnaires. Chest 2008 August;134(2):295-302.
29. Smith JA, Owen EC, Jones AM, Dodd ME, Webb AK, Woodcock A. Objective measurement of cough during pulmonary exacerbations in adults with cystic fibrosis. Thorax 2006 May;61(5):425-9.
30. Snijders D, Daniels JM, de Graaff CS, van der Werf TS, Boersma WG. Efficacy of corticosteroids in community-acquired pneumonia: a randomized double-blinded clinical trial. Am J Respir Crit Care Med 2010 May 1;181(9):975-82.
31. Daniels JM, Snijders D, de Graaff CS, Vlaspolder F, Jansen HM, Boersma WG. Antibiotics in addition to systemic corticosteroids for acute exacerbations of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2010 January 15;181(2):150-7.
32. King PT, Holdsworth SR, Freezer NJ, Villanueva E, Holmes PW. Characterisation of the onset and presenting clinical features of adult bronchiectasis. Respir Med 2006 December;100(12):2183-9.
33. Martinez-Garcia MA, Perpina-Tordera M, Roman-Sanchez P, Soler-Cataluna JJ. Quality-of-life determinants in patients with clinically stable bronchiectasis. Chest 2005 August;128(2):739-45.

CHAPTER 9

Summary

In this thesis we are taking a step forward in understanding and applying azithromycin maintenance treatment in bronchiectasis.

Bronchiectasis, pathological widening of the small and medium sized bronchi, may result from various disorders with one common trait; they all result in a faltering airway defence system. This in turn, allows for persistent bacterial on-going low-grade infection, with intermittent exacerbations. In **chapter two** we describe epidemiology, clinical presentation, diagnostic workup and treatment options in adult bronchiectasis, using data from the cohort of bronchiectasis visiting the outpatient clinic in our own hospital. Similar to other studies on the subject, we identified infection (e.g. former pneumonia or tuberculosis) and immunodeficiencies (such as common variable immune deficiency) as the main underlying causes of bronchiectasis. After a clinical suspicion of bronchiectasis has been raised by otherwise unexplained recurrent infections, a chronic productive cough or obstructive lung disease with a unusually severe clinical course, the diagnosis is confirmed by high-resolution CT of the chest. The finding of bronchiectasis should prompt a search for the underlying cause, in order to facilitate disease-directed treatment and counselling. In this chapter we propose a diagnostic algorithm, using the (inter)national guidelines as a starting point.

Treatment of bronchiectasis is directed at symptom reduction –with a productive cough being the most obvious trait- and the reduction of infectious exacerbations. Prior to turning to medical treatment options, clearance of bronchial secretions can be improved by specific physiotherapy and inhalation of nebulised (iso- or hyperosmolar) solutions. Patients with frequent exacerbations can be considered for long-term low-dose macrolide treatment and inhaled antibiotics might be beneficial in selected patients, especially those infected with *Pseudomonas aeruginosa*. Important developments in the last decade, including the introduction of international guidelines and the proposal for a validated scoring system for disease severity are discussed.

Azithromycin, a macrolide antibiotic, is one of the most widely used agents for maintenance treatment in bronchiectasis. In **chapter three**, an overview of the multiple pathways through which azithromycin is thought to intervene in the vicious circle of inflammation and bacterial colonization underlying many chronic inflammatory airway diseases is given. Apart from a distinct antibacterial effect, mainly consisting of inhibition of bacterial protein synthesis and interfering in biofilm formation, macrolides are known for their immunomodulatory effect. The immune response in bronchiectasis is dominated by neutrophilic inflammation, as becomes evident from the high numbers of granulocytes and neutrophilic chemo attractants in the airways of affected patients. Macrolide antibiotics have been demonstrated to interfere with neutrophil accumulation, adhesion and function and as such to dampen the exaggerated inflammatory response in airways of patients with bronchiectasis. In addition, macrolides have been shown to suppress the production of pro-inflammatory cytokines

and chemokines by other cell types, such as macrophages, eosinophils and epithelial cells and to enhance macrophage function, in particular phagocytosis. Apart from the innate immune system, key components of the adaptive immune system, such as T-cell regulation and antigen presentation are apparently modulated by macrolide antibiotics.

Due to its dual mode of action, macrolide antibiotics are exceptionally suited for the treatment of chronic inflammatory airway diseases. **Chapter four** summarizes the available evidence for long term macrolide treatment in bronchiectasis among other chronic respiratory conditions. The use of macrolides in diffuse panbronchiolitis (DPB), a rapidly progressive and deleterious respiratory condition almost exclusively seen in patients from Asia, is undisputed. DPB that was once often lethal has now become a condition that can usually be managed successfully, as has been shown in several randomised clinical trials. However, no solid evidence exists for this treatment modality in asthma, COPD and chronic rhinosinusitis, although a beneficial effect of long-term macrolide therapy has been found in small clinical trials in patients with these conditions.

Multiple smaller, heterogenic studies using different macrolides as maintenance treatment in bronchiectasis showed promising results with respect to exacerbation frequency and other disease parameters such as lung function and sputum volume. In order to further establish the clinical effectiveness and safety of azithromycin maintenance treatment in bronchiectasis, we performed the Bronchiectasis and Azithromycin Treatment (BAT-) trial, captured in **chapter five**. In this randomized, double blind, placebo controlled multicentre trial, we investigated the potency of azithromycin 250 mg once daily to reduce exacerbations in 83 bronchiectasis patients with 3 or more (median 4.0-5.0) infectious exacerbations in the year preceding study inclusion. We also monitored lung function, sputum bacteriology, inflammatory markers, symptom scores, quality of life and adverse effects.

After one year, 23 (54%) of 43 patients receiving azithromycin remained free of exacerbations as compared to only 8 (20%) of the placebo-group (p= 0.02, hazard ratio, 0.29 [95% CI, 0.16-0.51]). Although both groups suffered notably less exacerbations during the trial as compared to the year before, the median number of exacerbations was significantly lower in the azithromycin-group (0 (IQR 0-1) when compared to placebo treated patients (2 (IQR 1-3) (p< 0.01). Patients receiving azithromycin gained 1% forced expiratory volume in one second (FEV1) and 1.3% forced vital capacity (FVC) each month they were treated as opposed to a small decline in both measures in the placebo group. Both treatment groups reported improvement of quality of life and symptoms during study treatment, but this increase was significantly larger in azithromycin treated patients. An important observation during the study was the development of macrolide resistance in patients who received antibiotic treatment. Although the microbiological profile did not change importantly during treatment, resistance pattern certainly did. Absolute numbers of sputum pathogens

were much lower in the azithromycin group, but almost 90% of the pathogens tested for susceptibility showed macrolide resistance *in vitro*, as compared to 26% in the placebo group (p< 0.01). Patients in the azithromycin group reported more gastrointestinal adverse effects (40 vs 5% of patients), but none were serious and were never a reason for treatment discontinuation.

High resolution CT (HRCT) scanning is the method of choice in diagnosing bronchiectasis and radiological disease severity is an independent predictor of both morbidity and mortality in these patients. Before and one year after start of treatment during the BAT trial, chest HRCT scans were obtained for each participant, and these were used in the study described in **chapter six**. At baseline and after one year these were scored by two radiologists according to a scoring system based on the Bhalla-system, but omitting three of the original item scores because of limited availability of two comparable imaging sets for each patient. CT-scores before and after treatment were compared for azithromycin- and placebo-treated participants and correlation between CT scores and clinical parameters were investigated. Baseline CT scores showed good negative correlation with lung function parameters (FEV_1, r= -0.4,FVC r=-0.4 and TLCO r=-0.4). In addition, patients infected with *Pseudomonas aeruginosa* had higher CT scores at baseline, reflecting the clinical inferiority of these patients with respect to lung function and prognosis. One year of treatment with azithromycin did not result in a statistically significant improvement of CT features and no significant difference was found when comparing post-treatment scores between azithromycin- and placebo treated patients. Baseline CT scores in patients who responded well to azithromycin treatment were higher than in non-responders. Compared to patients who did not have their number of exacerbations importantly reduced during the study, responders to treatment scored higher on 'peribronchial thickening' and 'severity of bronchiectasis'. If this finding is replicated in larger series, CT scores, which contain such items might be useful tools to select patients likely to have a favourable response to macrolide treatment.

Worldwide, many patients with bronchiectasis and other inflammatory lung diseases are treated with azithromycin or other macrolides, but not much is known about the pharmacodynamics and pharmacokinetics of this agent during long term treatment. The relationship between azithromycin sputum and serum levels and its clinical efficacy during long-term treatment in bronchiectasis is explored in **chapter seven**. In patients receiving one year of azithromycin 250 mg once daily, we found high concentrations of azithromycin in sputum, which were stable over time. Serum levels were about 70x lower and more variable. Higher levels of azithromycin in sputum were found to correlate well with a larger reduction in levels of C-reactive protein (CRP) in serum, but appeared to be unrelated to clinical endpoints such as exacerbation frequency or symptoms. Surprisingly, higher azithromycin concentrations were not associated with a higher incidence of adverse effects. During the

study, all participants received the same dose of azithromycin irrespective of body weight, thus resulting in widely scattered dose levels (the weekly amount of azithromycin per kg bodyweight). Good clinical response was observed also for the lowest dose levels. We therefore suggested to apply lower dose regimens (e.g., 150 mg daily) to patient weighing less than 60 kilograms.

During our work in a clinical research setting we were struck by the lack of simple, quick, and easy-to-process tools for the measurement of disease symptoms in these patients. In **chapter eight** we report on the development and validation of a new tool for symptom measurement in bronchiectasis patients, the '*Lower respiratory tract infections – visual analogue scale'* (LRTI-VAS). The LRTI-VAS consists of 5 ten-point scales for dyspnoea, fatigue, sputum colour, pain and cough, adding up to a total score with similar weight for all items. In our validation study, the LRTI-VAS showed good internal consistency and correlated well with other markers of disease severity. In addition, symptoms measured by LRTI-VAS proved stable during clinical stability with good responsiveness in case of an exacerbation, as such meeting the key requirements of a valid instrument.

CHAPTER 10

General discussion and future perspectives

In the early 19-th century, René Laënnec is believed to be the first physician who discovered, described and diagnosed bronchiectasis by using a now classical medical instrument that he called the stethoscope [1]. He was able to link his clinical assessment – as evidenced by the presence of abnormal chest sounds - to histopathological abnormalities of airways during autopsy and vividly described the nature of bronchiectasis, consisting of pathological dilatations with retention of purulent secretions [2].

In the two centuries that since have elapsed, bronchiectasis as a result of previous chest infection has become less common owing to the drop in tuberculosis in affluent parts of the world and due to improved hygiene and vaccination programs. Today, with chest infections still among the leading causes of bronchiectasis, immunodeficiencies and genetic disorders such as primary ciliary dyskinesia, Young's syndrome, and alpha-1-antitrypsin deficiency have been emerging as equally important underlying causes [3]. Although the morbidity and prognosis of bronchiectasis have improved, the incidence and prevalence is found to increase during the last two decades, especially in older age groups [4]. As a result still many patients suffer from this often debilitating condition that is accompanied by stigma and limited participation in social activities and that clearly impairs quality of life [5]. Not only do affected patients abstain from visiting theatres, cinemas or other social activities for fear of being frowned upon for their constant cough, but their ailment also generates high annual costs, arising from maintenance treatment, hospital admissions and days off work or school. In addition, recent work has confirmed noteworthy mortality rates [6]

Evidence based treatment modalities for bronchiectasis are sparse, and only macrolide maintenance treatment has been studied in more than one randomised clinical trial, among which the BAT trial in this thesis.

For decades, long-term macrolide treatments have been reserved for patients with relatively uncommon conditions such as cystic fibrosis and diffuse panbronchiolitis and perhaps the odd bronchiectasis patient with unusually severe symptoms. The higher awareness of bronchiectasis combined with the publication of trials which confirm the effectiveness and acceptable safety profile of long-term macrolide treatment, not just in bronchiectasis but also in patients with obstructive lung disease will expectedly result in a rapidly growing number of prescriptions for macrolide maintenance treatment [7;8]. This may be cause for concern for multiple reasons, which include cardiovascular side effects and above all the induction of macrolide resistance. Thus, since we have opened Pandora's box of apparently unrestricted possibilities for the long-term use of macrolides, the challenge we are facing now will be to keep that box no more than just ajar. For a restrictive and balanced use of macrolide treatment of bronchiectasis, a better phenotyping of bronchiectasis patients and a consensus-based definition of (a) predictor(s) of macrolide response would greatly help to advance the field.

Bronchiectasis is a disease with heterogeneous aetiology; many possible underlying causes have been described, ranging from frequently encountered conditions, such as asthma or pneumonia to rare immune- or genetic disorders. In addition, the clinical presentation and symptoms may be very different between different patients. A pseudomonas-colonised bronchiectasis patient with generalized saccular bronchiectasis, abundant sputum expectoration and frequent exacerbations may be considered a different disease phenotype and requires a different type of treatment than the patient with sparse, cylindrical bronchiectasis as a result of traction from fibrosis caused by systemic disease, with only limited sputum production occasionally growing *Haemophilus influenzae*, and without apparent exacerbations.

The introduction of a targeted treatment approach necessitates careful phenotyping of bronchiectasis patients [9]. Expectedly, better understanding of the different categories of bronchiectasis patients will also help to predict which patient group benefits the most from a certain treatment modality, and as such attribute to a judicious application of macrolide treatment. Therefore, it is important to gain more insight into which specific traits set the one bronchiectasis patient apart from the other. And in addition to define in which patient the benefits of a certain treatment are expected to outweigh the possible downsides. Once that has been made clear, the next step should be the application of tailored treatment for each of the patient groups. Looking at the studies in this thesis, a good response to macrolide treatment might be predicted by inflammatory markers, radiological characteristics or clinical correlates of disease, such as exacerbation frequency, all of which will now be discussed in further detail.

The current guidelines use the frequency of infectious exacerbations to direct the application of macrolide maintenance treatment, restricting this treatment to patients with three or more yearly exacerbations [10]. Of note, two out of three trials showing benefit of macrolide maintenance also included patients with a lower number of exacerbations (< 1 or 2 exacerbations yearly). However, the number of exacerbations in patients that were eventually included in those trials was generally much higher than suggested by the inclusion criteria (mean number of 3.6 in EMBRACE (inclusion criterion 1 or more), and 30% of patients with >5 exacerbations in BLESS (inclusion criterion 2 or more). In addition, the net reduction of exacerbations was largest in the BAT trial, which included patients with the highest number of exacerbations (mean 4.5/year). In an exploratory subgroup analysis of data from the BLESS trial the authors demonstrated the largest reduction in exacerbations in patients with 5 or more exacerbations yearly. This may suggest a tendency to macrolide response in patients with frequent exacerbations. Discerning and selecting patients for a given treatment modality by the frequency of their infectious exacerbations has the evident advantage of being a simple and widely applicable way to categorize patients without need for additional testing, which may therefore also be of use in low income countries with their

relatively high prevalence of the disease. Currently, our group is trying to shed a light on this subject by merging results from BAT, BLESS and EMBRACE trials. We hypothesise that it will be possible to define a 'frequent exacerbator' subtype with a distinct response to macrolide treatment.

Another way to characterize bronchiectasis patients would be by the type and extent of the inflammatory response(s). The 'vicious circle' hypothesis, already in the 19-eighties proposed by P.T. Cole and still generally accepted, states that airway inflammation plays a central role in bronchiectasis [11]. Macrolides are considered to have an anti-inflammatory effect and have been proven effective in bronchiectasis, however, many questions about the exact mechanism of action are still unanswered. The identification of a marker of macrolide responsiveness may not only help to gain insight in the macrolide mechanism of action but could also be of use in selecting those patients who are expected to benefit the most from this treatment modality. Blood biomarkers, such as CRP, are increasingly recognized as markers of inflammation in other diseases, but were found not to reliably reflect the extent of the inflammatory response in the airways of CF patients [12]. Our longitudinal data on CRP-levels and white blood cell count during macrolide treatment extends this finding to the bronchiectasis patients; CRP levels and WBC were overall low and not significantly different between responders (n=34) and non-responders (n=7) to treatment and may therefore not be the most promising markers.

In contrast to blood biomarkers, inflammatory markers in sputum of bronchiectasis patients were found to give a good impression of the augmented inflammatory process underlying bronchiectasis. Even in a clinically stable situation, increased levels of neutrophils and neutrophilic chemo-attractants, the most important of which are believed to be IL-8, IL-1β, IL-17, TNF-α and LTB-4 are present in the airways of patients with bronchiectasis [13;14]. Small studies have shown that markers of neutrophilic inflammation are among those that are primarily suppressed during macrolide treatment [15;16]. The quantity of the neutrophilic inflammatory response, as measured by the presence of sputum neutrophilia, neutrophil chemo attractants, neutrophilic degranulation products, or a combination, might be therefore be predictive of a favourable response to macrolide maintenance treatment. Sputum neutrophil counts are among the more readily available measurements in most clinics and may therefore be the most interesting measure for use in clinical practice, but more extensive testing for inflammatory markers may be primarily restricted to research settings as interesting endpoints for future trials on macrolide treatment. In order to facilitate widespread clinical use of sputum markers, a first requirement is the possibility to use spontaneously expectorated sputum. In CF, both spontaneously expectorated and induced sputum have been used to measure and type the inflammatory response [12]. In addition, recent work showed high levels of inflammatory markers in spontaneous sputum, which is confirmed by our own analysis of sputum samples which shows markedly elevated

levels of IL-1 β, IL-8, IL-10, MPO and TNF-α, among other inflammatory markers (Altenburg et al, non-published data) [9;17], suggesting that spontaneous sputum may be a reliable way to investigate lower airway inflammation.

Another indicator of disease severity that is readily available in clinical practice is the extent of radiological abnormalities as seen on chest CT scans and certain features may also predict macrolide responsiveness. In chapter 5 we state that CT abnormalities indicative for active inflammation, such as mucus plugging and bronchial wall thickness have been noted to improve during macrolide treatment. In addition, in an exploratory analysis of the BAT trial results we discriminated responders and non-responders based on differences in bronchial wall thickness (mucus plugging was not accounted for in our study). Since CT scanning is the gold standard for the diagnosis of bronchiectasis and will therefore be available for almost each patient with this diagnosis, CT-based guidance for the allocation of macrolide treatment would be very attractive. Future studies in this direction should focus on the above mentioned features indicative of active inflammation, ensure adequate CT-quality as to facilitate the use of validated scoring systems and use CT severity as a secondary or even primary endpoint in clinical trials of maintenance treatment. Low dose CT scans, with radiation doses similar to those used in conventional chest radiography are commonly used for the follow up of pulmonary nodules. Recently, authors have stated that dose-reduced CT scans may also be used for diagnosing and monitoring bronchiectasis [18;19]. Considering a possible rise in the number of CT-scans for clinical- and research purposes, this is a very interesting development and deserves to be further explored.

In our exploratory study of the dose-effect relationship in azithromycin maintenance treatment, we found stable concentrations of azithromycin in sputum, as opposed to variable serum levels, but these findings need to be confirmed in studies primarily designed for PK/PD purposes. Other findings, such as a relation between azithromycin sputum levels and suppression of systemic inflammation are only theoretically explained yet.

These and other gaps in our knowledge on the pharmacokinetic characteristics of long-term macrolide treatment have led to a lack of uniformity with concern to macrolide dosing regimens worldwide, which are usually copied from the field of CF and blended with local habits and traditions. In addition, there is no evidence-based information on the ideal duration of treatment as we are unaware whether, and if so, when, azithromycin's beneficial effect – or benefit/harm ratio may decrease over time.

One might get an idea on the sustainability of the treatment effect from the three randomized trials on macrolide treatment, since both BAT and BLESS trials demonstrated a sustained effect of macrolide treatment on clinical outcome parameters such as lung function and quality of life during one year of macrolide treatment. The effect subsided in the 2-4 month

run-out period of the BAT trial when treatment was discontinued as did the advantages of 6 months of macrolide treatment in the EMBRACE trial in the following 6 months of follow up. [20-22].

There is an urgent need for additional evidence in this specific area in order to further optimize the azithromycin dosing regimen and treatment duration. For a start this could be done as simple as performing a careful longitudinal cohort-follow up of the many bronchiectasis patients who are currently started on azithromycin maintenance treatment. Long-term follow up of well-defined patient groups on macrolide treatment e.g., former randomized trial participants, would provide interesting information about both the sustainability of the benefits and about the evolution of potentially deleterious effects during extended periods of macrolide treatment. Since the 'antibiotic holiday' (abstaining from macrolide treatment during the summer months) has become common practice, appreciated by both physicians and patients, ample opportunity exists for further study into this interesting and maybe promising habit.

In addition, measuring concentrations of azithromycin in serum, sputum and perhaps also lung tissue samples (if available) would provide important information about the long-term PK and PD of azithromycin during 'steady state'.

We believe that it is likely that gaining evidence on duration, dosing and frequency of azithromycin maintenance treatment will result in a more careful and tailored prescription behaviour. This is particularly important in view of induction of macrolide resistance, a major potential disadvantage of this treatment modality, but will also help in minimizing other adverse effects.

Long-term use of antibiotics is associated with the induction of microbial resistance against the agent(s) concerned. This is particularly true for azithromycin that has been shown to cause a more substantial and sustained increase in macrolide resistance among respiratory tract pathogens as compared to other macrolide antibiotics, possibly because of its long half-life [23;24]. In addition, a significant association between macrolide prescribing and resistance has been demonstrated [24-26].

Randomized trials of long-term macrolide treatment consistently report an increase in both the proportions of macrolide resistant oropharyngeal streptococci and macrolide resistance in sputum pathogens in participating patients, with macrolide resistance rates of sputum pathogens up to 90% [20-22;27]. In addition, molecular analyses of respiratory samples (16S rRNA sequencing) during erythromycin treatment for bronchiectasis showed substantial change to the airway microbiota, more specifically an increase in intrinsically macrolide-tolerant organisms [28].

But, is this emergence of resistance cause for actual concern? We believe the answer to this question is 'yes, but it depends on who and what we are looking at'. In the individual macrolide-treated patient, no additional mortality or treatment failure has been shown from infections caused by macrolide-resistant pathogens [29;30]. Moreover, macrolide maintenance treatment even caused a substantial reduction of the total number of sputum pathogens and significantly higher eradication rates [20-22;31]. But, although deleterious effects of long-term use of antibiotics on both the nasopharyngeal and faecal microbiota have been described for the individual patient, the real danger of resistance induction lies in the increase of macrolide resistance on a population level [32].

Macrolide resistant pathogens in asymptomatic (and therefore untreated) carriers may be transferred to vulnerable hosts. As such, increasing number of difficult-to-treat infections caused by macrolide resistant pathogens in children and immune-compromised patients have been reported, and additionally, transfer of resistance determinants from commensals to pathogens is likely to occur [33-35].

Most trials on macrolide maintenance treatment in bronchiectasis have focussed on development of resistance in pathogens cultured from sputum samples of participants during exacerbations when trying to quantify macrolide resistance rates. However, these pathogens may likely represent only a tip of the iceberg since the airway bacterial load in a patient on macrolide treatment will be suppressed and those patients are less likely to be able to produce sputum later on during the trial, due to a treatment effect. A more accurate way to establish the magnitude of resistance induction would be to quantify macrolide resistance in bacterial strains being carried as commensal bacteria by the individual and perhaps also in household contacts of individuals on long term macrolide treatment, since frequent transmission of commensal organisms among close contacts has been reported [36]. To determine the composition of both the respiratory and intestinal microbiota, study protocols should include nasopharyngeal swabs and collection of faeces specimen which –in view of the superior sensitivity of those techniques- should preferably be tested with molecular genetic tests e.g., fluorescence in situ hybridisation or 16S rRNA sequencing.

The antibacterial effects of azithromycin are likely to be independent of its anti-inflammatory activity. A very promising way to circumvent the problem of resistance induction is the development of novel, non-antibiotic macrolides, including the azithromycin derivate CSY0073 [37]. Although currently the evidence is limited to animal models, CSY0073 exhibits anti-inflammatory activity similar to regular azithromycin, without bacteriostatic activity [38].

Macrolide maintenance treatment has been shown to cause important reduction of respiratory symptoms and infectious exacerbations in patients with bronchiectasis [20-

22]. However, the lack of any concomitant improvement of structural airway damage in our simultaneously conducted radiological study, may suggest that the perceived clinical improvement is in fact no more than a very effective, but temporary suppression of inflammation. The structural damage to the airways itself does not appear to improve importantly [39]. In the last decade mechanisms involved in regeneration of lung tissue have been unveiled and this forms the basis of the rapidly advancing field of modern tissue engineering [40]. Great efforts are made to identify progenitor- or stem cells that have the ability to regenerate lost or damaged cells of airways or alveoli [41]. Translational research is needed to eventually repair structural lung injury, the major characteristic of bronchiectasis. Current treatment relies on symptoms relieve, reducing further structural damage to airways and lung tissue, while patients with critically advanced disease can only be salvaged by lung transplant.

Concluding remarks

Many things have changed since Dr Laennec first used his stethoscope to listen to the crackling breath sounds of a little boy suffering from bronchiectasis. Nowadays, a boy like the one Laennec examined would have a fair chance to survive into adulthood and, particularly if he happened to have severe symptoms, would probably be on azithromycin maintenance treatment right now, just as many of his fellow-patients.

Hopefully, they will grow older into a future where the individual risks and benefits of long term macrolide treatment will be known and prudential use of macrolides will be guided by either clinical traits, inflammatory markers or radiological disease severity. Only the ‘macrolide-responsive’ phenotype of bronchiectasis patients will be prescribed macrolides and other tailored treatment modalities will be available for other patient categories.

Ideally, the reduction of antibiotic use resulting from this approach will have caused a decrease in macrolide resistance rates and patients in whom macrolide treatment is justified will be periodically checked for the presence of unfavourable changes to their microbiota using modern sequencing techniques.

Ghandi himself once said “the future depends on what you do today” and this is particularly true for the rapidly changing field of *macrolide treatment for bronchiectasis.*

Reference List

1. Roguin A. Rene Theophile Hyacinthe Laennec (1781-1826): the man behind the stethoscope. Clin Med Res 2006 Sep;4(3):230-5.
2. Sakula A. R T H Laennec 1781--1826 his life and work: a bicentenary appreciation. Thorax 1981 Feb;36(2):81-90.
3. Altenburg J, Wortel K, van der Werf TS, Boersma WG. Non-cystic fibrosis bronchiectasis: clinical presentation, diagnosis and treatment, illustrated by data from a Dutch Teaching Hospital. Neth J Med 2015 May;73(4):147-54.
4. Quint JK, Millett ER, Joshi M, Navaratnam V, Thomas SL, Hurst JR, et al. Changes in the incidence, prevalence and mortality of bronchiectasis in the UK from 2004 to 2013: a population-based cohort study. Eur Respir J 2016 Jan;47(1):186-93.
5. Martinez-Garcia MA, Perpina-Tordera M, Roman-Sanchez P, Soler-Cataluna JJ. Quality-of-life determinants in patients with clinically stable bronchiectasis. Chest 2005 Aug;128(2):739-45.
6. Quint JK, Millett ER, Joshi M, Navaratnam V, Thomas SL, Hurst JR, et al. Changes in the incidence, prevalence and mortality of bronchiectasis in the UK from 2004 to 2013: a population-based cohort study. Eur Respir J 2016 Jan;47(1):186-93.
7. Uzun S, Djamin RS, Kluytmans JA, Mulder PG, van’t Veer NE, Ermens AA, et al. Azithromycin maintenance treatment in patients with frequent exacerbations of chronic obstructive pulmonary disease (COLUMBUS): a randomised, double-blind, placebo-controlled trial. Lancet Respir Med 2014 May;2(5):361-8.
8. Brusselle GG, Vanderstichele C, Jordens P, Deman R, Slabbynck H, Ringoet V, et al. Azithromycin for prevention of exacerbations in severe asthma (AZISAST): a multicentre randomised double-blind placebo-controlled trial. Thorax 2013 Apr;68(4):322-9.
9. Aliberti S, Lonni S, Dore S, McDonnell MJ, Goeminne PC, Dimakou K, et al. Clinical phenotypes in adult patients with bronchiectasis. Eur Respir J 2016 Apr;47(4):1113-22.
10. Pasteur MC, Bilton D, Hill AT. British Thoracic Society guideline for non-CF bronchiectasis. Thorax 2010 Jul;65 Suppl 1:i1-58.
11. Cole PJ. Inflammation: a two-edged sword--the model of bronchiectasis. Eur J Respir Dis Suppl 1986;147:6-15.
12. Sagel SD, Chmiel JF, Konstan MW. Sputum biomarkers of inflammation in cystic fibrosis lung disease. Proc Am Thorac Soc 2007 Aug 1;4(4):406-17.
13. Tsang KW, Chan K, Ho P, Zheng L, Ooi GC, Ho JC, et al. Sputum elastase in steady-state bronchiectasis. Chest 2000 Feb;117(2):420-6.
14. Chalmers JD, Hill AT. Mechanisms of immune dysfunction and bacterial persistence in non-cystic fibrosis bronchiectasis. Mol Immunol 2013 Aug;55(1):27-34.
15. Ratjen F, Saiman L, Mayer-Hamblett N, Lands LC, Kloster M, Thompson V, et al. Effect of azithromycin on systemic markers of inflammation in patients with cystic fibrosis

uninfected with Pseudomonas aeruginosa. Chest 2012 Nov;142(5):1259-66.
16. Hodge S, Hodge G, Jersmann H, Matthews G, Ahern J, Holmes M, et al. Azithromycin improves macrophage phagocytic function and expression of mannose receptor in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2008 Jul 15;178(2):139-48.
17. Hill A, Gompertz S, Bayley D, Stockley R. Inflammatory mediators in spontaneously produced sputum. Methods Mol Med 2001;56:317-34.
18. O'Connor OJ, Vandeleur M, McGarrigle AM, Moore N, McWilliams SR, McSweeney SE, et al. Development of low-dose protocols for thin-section CT assessment of cystic fibrosis in pediatric patients. Radiology 2010 Dec;257(3):820-9.
19. Murphy KP, Maher MM, O'Connor OJ. Imaging of Cystic Fibrosis and Pediatric Bronchiectasis. AJR Am J Roentgenol 2016 Mar;206(3):448-54.
20. Altenburg J, de Graaff CS, Stienstra Y, Sloos JH, van Haren EH, Koppers RJ, et al. Effect of azithromycin maintenance treatment on infectious exacerbations among patients with non-cystic fibrosis bronchiectasis: the BAT randomized controlled trial. JAMA 2013 Mar 27;309(12):1251-9.
21. Wong C, Jayaram L, Karalus N, Eaton T, Tong C, Hockey H, et al. Azithromycin for prevention of exacerbations in non-cystic fibrosis bronchiectasis (EMBRACE): a randomised, double-blind, placebo-controlled trial. Lancet 2012 Aug 18;380(9842):660-7.
22. Serisier DJ, Martin ML, McGuckin MA, Lourie R, Chen AC, Brain B, et al. Effect of long-term, low-dose erythromycin on pulmonary exacerbations among patients with non-cystic fibrosis bronchiectasis: the BLESS randomized controlled trial. JAMA 2013 Mar 27;309(12):1260-7.
23. Malhotra-Kumar S, Lammens C, Coenen S, Van HK, Goossens H. Effect of azithromycin and clarithromycin therapy on pharyngeal carriage of macrolide-resistant streptococci in healthy volunteers: a randomised, double-blind, placebo-controlled study. Lancet 2007 Feb 10;369(9560):482-90.
24. Serisier DJ. Risks of population antimicrobial resistance associated with chronic macrolide use for inflammatory airway diseases. Lancet Respir Med 2013 May;1(3):262-74.
25. Riedel S, Beekmann SE, Heilmann KP, Richter SS, Garcia-de-Lomas J, Ferech M, et al. Antimicrobial use in Europe and antimicrobial resistance in Streptococcus pneumoniae. Eur J Clin Microbiol Infect Dis 2007 Jul;26(7):485-90.
26. Seppala H, Klaukka T, Vuopio-Varkila J, Muotiala A, Helenius H, Lager K, et al. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland. Finnish Study Group for Antimicrobial Resistance. N Engl J Med 1997 Aug 14;337(7):441-6.
27. Li H, Liu DH, Chen LL, Zhao Q, Yu YZ, Ding JJ, et al. Meta-analysis of the adverse effects of long-term azithromycin use in patients with chronic lung diseases. Antimicrob Agents Chemother 2014;58(1):511-7.
28. Rogers GB, Bruce KD, Martin ML, Burr LD, Serisier DJ. The effect of long-term macrolide treatment on respiratory microbiota composition in non-cystic fibrosis bronchiectasis: an analysis from the randomised, double-blind, placebo-controlled BLESS trial. Lancet Respir Med 2014 Dec;2(12):988-96.
29. Bishai W. A testament to sustained macrolide efficacy. Clin Infect Dis 2003 Apr 1;36(7):935-6.
30. Nuermberger E, Bishai WR. The clinical significance of macrolide-resistant Streptococcus pneumoniae: it's all relative. Clin Infect Dis 2004 Jan 1;38(1):99-103.
31. Hansen CR, Pressler T, Hoiby N, Johansen HK. Long-term, low-dose azithromycin treatment reduces the incidence but increases macrolide resistance in Staphylococcus aureus in Danish CF patients. J Cyst Fibros 2009 Jan;8(1):58-62.
32. Jakobsson HE, Jernberg C, Andersson AF, Sjolund-Karlsson M, Jansson JK, Engstrand L. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS One 2010;5(3):e9836.
33. Malhotra-Kumar S, Lammens C, Martel A, Mallentjer C, Chapelle S, Verhoeven J, et al. Oropharyngeal carriage of macrolide-resistant viridans group streptococci: a prevalence study among healthy adults in Belgium. J Antimicrob Chemother 2004 Feb;53(2):271-6.
34. Perez-Trallero E, Vicente D, Montes M, Marimon JM, Pineiro L. High proportion of pharyngeal carriers of commensal streptococci resistant to erythromycin in Spanish adults. J Antimicrob Chemother 2001 Aug;48(2):225-9.
35. Logan LK, McAuley JB, Shulman ST. Macrolide treatment failure in streptococcal pharyngitis resulting in acute rheumatic fever. Pediatrics 2012 Mar;129(3):e798-e802.
36. Goerke C, Kraning K, Stern M, Doring G, Botzenhart K, Wolz C. Molecular epidemiology of community-acquired Staphylococcus aureus in families with and without cystic fibrosis patients. J Infect Dis 2000 Mar;181(3):984-9.
37. Mencarelli A, Distrutti E, Renga B, Cipriani S, Palladino G, Booth C, et al. Development of non-antibiotic macrolide that corrects inflammation-driven immune dysfunction in models of inflammatory bowel diseases and arthritis. Eur J Pharmacol 2011 Aug 31;665(1-3):29-39.
38. Balloy V, Deveaux A, Lebeaux D, Tabary O, le RP, Ghigo JM, et al. Azithromycin analogue CSY0073 attenuates lung inflammation induced by LPS challenge. Br J Pharmacol 2014 Apr;171(7):1783-94.
39. Altenburg j, Wolf R, Go S, et al. Changes of computed tomography findings in patients with bronchiectasis following one year of azithromycin treatment. Manuscript in preparation. 2016.
40. Kotton DN, Morrisey EE. Lung regeneration: mechanisms, applications and emerging stem cell populations. Nat Med 2014 Aug;20(8):822-32.
41. Schilders KA, Eenjes E, van RS, Poot AA, Stamatialis D, Truckenmuller R, et al. Regeneration of the lung: Lung stem cells and the development of lung mimicking devices. Respir Res 2016;17:44.

Nederlandse Samenvatting

In dit proefschrift wordt onderzoek beschreven naar het toepassen van een onderhoudsbehandeling met antibiotica bij patiënten die lijden aan beschadigde luchtwegen - of bronchiëctasieën. In dit proefschrift tonen wij aan dat zo'n behandeling effectief is. We beschrijven waarom deze behandeling zo goed werkt, wat de eventuele nadelen zijn, en hoe we patiënten zou moeten selecteren die in aanmerking komen voor deze behandeling.

In de longen worden grofweg drie onderdelen onderscheiden: de longblaasjes, waar de uitwisseling van zuurstof en koolzuur met het bloed in de haarvaten plaatsvindt; het bind- en steunweefsel, dat een fijnmazig rooster vormt waarin de longblaasjes en bloedvaten zijn ingebed, samen met het derde onderdeel; de luchtwegen of bronchiën, het lucht geleidende buizenstelsel dat begint bij de luchtpijp en dat tot aan de longblaasjes in de periferie van de long reikt. De in meer dan 20 generaties steeds verder opsplitsende bronchiën vormen een soort omgekeerde boom (de bronchiaalboom) waarvan de takken steeds kleiner worden tot ze uiteindelijk de longblaasjes bereiken.

Via de luchtwegen komt de buitenlucht, waarin zich voor het lichaam schadelijke stoffen of micro-organismen bevinden, in direct contact met de lichaamscellen en om die reden is de bronchiaalboom uitgerust met een aantal verdedigingsmechanismen. De luchtwegen zijn van binnen voorzien van een slijmlaag, waarin zich afweercellen en ontstekingsremmende stofjes bevinden. Verder is een groot gedeelte van de bronchiaalboom bekleed met cellen met trilhaartjes aan het oppervlak, die door hun continue trilbeweging zorgen dat het 'slijmtapijt' zich in een gestage stroom via de grote luchtwegen verplaatst naar de keelholte, met medeneming van schadelijke stoffen en ook ingeademde bacteriën.

Als de kleine of middelgrote luchtwegen beschadigd raken en er daardoor verwijdingen of uitstulpingen van de luchtwegwanden ontstaan, noemen wij dit bronchiëctasieën. Er zijn verschillende aandoeningen die kunnen leiden tot het ontstaan van bronchiëctasieën, waaronder longontstekingen, trilhaaraandoeningen en afweerstoornissen. Hoe verschillend deze aandoeningen ook zijn, zij hebben met elkaar gemeen dat zij ervoor zorgen dat de verdedigingsmechanismen van de luchtwegen aangetast raken, waardoor het opruimen van slijm en schadelijke stoffen verstoord wordt. De aandoening kan heel plaatselijk zijn, bijvoorbeeld als gevolg van een longontsteking op een bepaalde plek, maar ook verspreid door de hele long, bijvoorbeeld bij afweerstoornissen of in het geval van een trilhaarziekte.

De beschadiging van de luchtwegen uit zich onder andere in het dikker worden van de wanden van de luchtwegen en het ontstaan van uitstulping of verwijdingen van de luchtwegen die bronchiëctasieën genoemd worden. Een kenmerk van deze beschadigde luchtwegen is de overmatige slijmproductie als gevolg van de ruime aanwezigheid van slijmvormende cellen. Verder werken ter plaatse van de beschadigingen de trilharen minder goed, zelfs in afwezigheid van een trilhaarziekte, waardoor bronchiëctasieën een plek zijn waar bacteriën

zich makkelijk kunnen innestelen en vermenigvuldigen. Door de schadelijke stoffen die de bacteriën produceren en doordat er in de luchtwegen zelf een ontstekingsreactie ontstaat door de eigen afweer- en ontstekingscellen, waarbij die cellen schadelijke stoffen afgeven, kan ter plaatste de luchtwegschade steeds verder toenemen. Men spreekt wel van een 'vicieuze cirkel-theorie' (van luchtwegschade – bacteriële kolonisatie- ontsteking - toename van luchtwegschade) om te verklaren waardoor bronchiëctasieën in de loop van de tijd vaak steeds ernstiger worden.

Patiënten met bronchiëctasieën klagen vooral over frequent ophoesten van slijm vanuit de diepe luchtwegen. Vaak worden aanzienlijke hoeveelheden slijm opgehoest, waardoor patiënten zich erg kunnen schamen voor hun aandoening en bepaalde activiteiten, zoals het bezoeken van openbare voorstellingen (bioscoop, theater, concert, museum) gaan vermijden. Doordat het luchtwegslijmvlies bij bronchiëctasiepatiënten erg kwetsbaar is, wordt met enige regelmaat bloed opgehoest wat soms leidt tot levensbedreigende longbloedingen. Een ander veel voorkomend probleem van bronchiëctasieën is het steeds terugkerend optreden van verergeringen (exacerbaties) als gevolg van luchtweginfecties, die kunnen leiden tot frequent verzuim van werk en/of school en algeheel onwelbevinden, kortademigheid of vermoeidheid; soms zijn die exacerbaties zo ernstig dat patiënten in het ziekenhuis opgenomen moeten worden en antibiotica via het infuus nodig hebben om weer op te knappen.

De mate waarin patiënten met bronchiëctasieën klachten ervaren is erg wisselend. Soms hebben patiënten met milde bronchiëctasieën nauwelijks of geen klachten, zelfs zonder enige behandeling. Daar tegenover staan de patiënten met ernstige bronchiëctasieën, met veelvuldige infecties en ernstige chronische klachten. Die laatste groep is de groep patiënten waar dit proefschrift over gaat.

Bronchiëctasieën werden tot voor kort beschouwd als een niet-vaak voorkomende aandoening. Daarnaast vormen bronchiëctasie patiënten een 'lastige' groep voor het verrichten van wetenschappelijk onderzoek omdat de oorzaken en symptomen zo divers kunnen zijn. Dit heeft er toe geleid dat er tot een aantal jaar geleden weinig wetenschappelijk onderzoek naar deze aandoening verricht is. De laatste jaren is er om verschillende redenen echter meer aandacht voor dit ziektebeeld ontstaan en blijkt dit helemaal niet zo zeldzaam als gedacht. De meest recente onderzoeken laten zien dat bronchiëctasieën voorkomen bij 50-60 per 100.000 personen en op oudere leeftijd (> 70 jaar) zelfs nog een stuk vaker, bij > 250 per 100.000 personen. De ziekte treft met name vrouwen van middelbare leeftijd, maar leeftijden van patiënten variëren van 18 -99 jaar, waarbij bepaalde onderliggende aandoeningen vaker voorkomen in bepaalde leeftijdsgroepen.

De huidige behandeling van bronchiëctasieën bestaat uit leefstijlaanpassingen als gezonde

voeding en sport ter verbetering van de algehele conditie en verbetering in de technieken van het ophoesten van slijm. Patiënten met bronchiëctasieën hebben soms de neiging om hun lichamelijke inspanning te beperken omdat ze zich niet zo fit voelen. Ook merken ze vaak dat als ze meer gaan bewegen, bij inspanning de ademteugen haast vanzelf veel dieper worden waardoor ze meer moeten hoesten met opgeven van slijm. Toch is dat juist goed om hun luchtwegen zo schoon mogelijk te houden en in goede algemene conditie te blijven. Vaak wordt gerichte fysiotherapie ingezet, samen met inhalaties van zoute vernevelvloeistof om het 'schoonhouden' van de luchtwegen verder te optimaliseren. Als de bronchiëctasieën veroorzaakt worden door een specifieke aandoening volgt soms ook een specifieke behandeling. Als bijvoorbeeld een afweerstoornis bestaat, gepaard met een gebrek aan afweerstoffen, wordt deze apart, bijvoorbeeld door afweerstoffen van bloeddonoren via het infuus toe toegediend om de weerstand tegen luchtweginfecties te verhogen.

Ter voorkoming van luchtweginfecties bij patiënten met ernstiger bronchiëctasieën werd al jarenlang door longartsen met enige regelmaat een langere kuur van een lage dosis antibiotica, een onderhoudsbehandeling, voorgeschreven. Zo werd geprobeerd infecties te voorkomen en de slijmproductie te verlagen. Voor zo'n onderhoudsbehandeling werd vaak azitromycine gebruikt, een antibioticum dat destijds vooral bekend was uit de behandeling van patiënten met taaislijmziekte of Cystische Fibrose (CF), een zeldzamere en ernstiger verlopende ziekte waarbij de bevochtiging van slijmvliezen onvoldoende is, waardoor luchtwegen ook al op jongere leeftijd beschadigen met het ontstaan van ernstige bronchiëctasieën. Bij deze patiënten gaf een onderhoudsbehandeling met azitromycine een verbetering van de longfunctie en leidde tot minder luchtweginfecties.

In **hoofdstuk 2** wordt achtereenvolgens overzichtsinformatie gegeven over het ziektebeeld 'bronchiëctasieën', de kenmerken van een grote groep Nederlandse bronchiëctasiepatiënten en de huidige behandelmogelijkheden. Daarna worden in **hoofdstuk 3 en 4** de wetenschappelijke onderzoeken tot op heden samengevat die het effect van een onderhoudsbehandeling met azitromycine bij verschillende chronische longziekten hebben onderzocht en wordt een overzicht gegeven van wat er tot op heden bekend is over de verschillende wegen waarlangs azitromycine in de long en het lichaam zijn effect bereikt.

De gunstige werking van azitromycine wordt toegeschreven aan het feit dat het niet alleen antibacterieel werkt (zoals de meeste antibiotica) maar daarnaast ook een ontstekingsremmende werking heeft. Dit is gebleken uit studies die lieten zien dat het aantal ontstekingscellen en de hoeveelheid ontstekingsbevorderende stoffen in de luchtwegen van patiënten fors omlaag gaat als zij behandeld worden met azitromycine. Verder viel in onderzoek bij weer een andere groep patiënten op dat zij door behandeling met een middel dat erg op azitromycine lijkt een duidelijke verbetering van hun luchtwegklachten ervoeren en een veel betere longfunctie kregen. Het bijzondere bij deze patiëntengroep was dat de

bacteriën die uit hun slijm gekweekt werden helemaal niet of slechts ten dele gevoelig waren voor de azitromycine, waardoor duidelijk werd dat het gunstige effect toegeschreven moet worden aan de ontstekingsremmende werking en niet zozeer aan de antibacteriële werking.

In de ervaring van longartsen ontstond de indruk dat een behandeling met azitromycine door deze eigenschappen een goed effect had bij patiënten met bronchiëctasieën. Ervaring is nog geen bewijs; wat ontbrak was goed wetenschappelijk onderzoek waaruit duidelijk kan worden hoe groot het effect van azitromycine is, in vergelijking met een controle-behandeling zonder azitromycine; hoe lang zo'n eventueel gunstig effect aanhoudt; en wat de eventuele bezwaren en nadelen van zo'n behandeling zouden zijn. Een antwoord op zulke vragen was nodig om de behandeling te kunnen opnemen in de richtlijnen en protocollen voor de behandeling.

In de BAT- trial, de studie die beschreven wordt in **hoofdstuk 5** van dit proefschrift, hebben wij onderzocht in hoeverre een onderhoudsbehandeling met azitromycine effectief is als het gaat om het voorkomen van luchtweginfecties bij patiënten met bronchiëctasieën. De studiedeelnemers hadden voorafgaand aan het onderzoek allemaal minimaal 3 infecties, ook wel exacerbaties genoemd, per jaar. Tijdens het onderzoek werd er voor iedere deelnemer 'blind' geloot tussen een onderhoudsbehandeling met azitromycine (eenmaal daags 250 mg) of een 'nepmiddel' of placebo, voor de duur van een jaar. Na 1 jaar bleek dat patiënten die azitromycine hadden gebruikt duidelijk minder exacerbaties hadden, gemiddeld 0.5/jaar, ongeveer de helft van het aantal van patiënten in de placebogroep. Verder rapporteerden zij een betere kwaliteit van leven en werd een lichte verbetering van de longfunctie gemeten. Als bijwerking van de azitromycine werden vooral maag-darm bezwaren zoals buikpijn en diarree gerapporteerd. Dit kwam voor bij bijna de helft van de patiënten die azitromycine gebruikten, maar in geen van de gevallen zo hevig dat de medicatie gestaakt moest worden. Deze bijwerking verdween in de meeste gevallen na een aantal weken.

Een andere belangrijke bevinding tijdens de studie had betrekking op de bacteriën die uit het slijm van de luchtwegen gekweekt werden. Hoewel het totale aantal bacteriën dat bij de azitromycinegroep gekweekt werd veel kleiner was dan bij de patiënten die placebo kregen, waren bijna alle gekweekte bacteriën resistent geworden tegen azitromycine. In hoeverre dit schadelijke gevolgen kan hebben wordt verderop in deze samenvatting besproken.

Bronchiëctasieën komen aan het licht door het maken van een CT-scan van de longen en borstkas. De kenmerkende verwijding van de luchtwegen en de verdikte luchtwegwanden zijn daar vaak goed op te zien. Daarnaast geeft een CT scan informatie over de uitgebreidheid van de bronchiëctasieën en over eventuele bijkomende problemen, zoals de aanwezigheid

van slijm in de luchtwegen of gebieden van ontsteking. Alle deelnemers aan de BAT trial hebben voorafgaand aan de behandeling en na een jaar een CT scan van de longen ondergaan. Wij wilden namelijk bekijken of een behandeling met azitromycine naast het verbeteren van de symptomen van bronchiëctasieën ook de op CT zichtbare afwijkingen in de longen kan verminderen. Met behulp van een puntensysteem dat eerder met name bij CF patiënten gebruikt werd, werden de afwijkingen op de CT-scans voor en na de behandeling gescoord en vervolgens werd de azitromycine-groep met de placebogroep te vergeleken. In **hoofdstuk 6** worden de resultaten hiervan beschreven. De scores van de twee groepen bleken onderling niet significant te verschillen, niet aan de start van de behandeling, maar ook na een jaar werd er geen verschil tussen de beide groepen gezien. Behandeling met azitromycine zorgde dus niet voor een duidelijke afname van het aantal punten dat met dit score-systeem aan de CT-afwijkingen werd toegekend. Dit zou kunnen betekenen dat azitromycine inderdaad de afwijkingen op CT niet vermindert, maar gedurende het onderzoek merkten wij ook dat het scoringssysteem niet goed toepasbaar was op een deel van onze CT-scans. Wij konden daardoor niet het volledige scoringssysteem gebruiken. Onderdelen van het scoringssysteem die betrekking hadden op ontstekingsactiviteit, zoals de verdikking van de luchtwegen, bleken wel iets verbeterd na een jaar azitromycine-behandeling.

Het is ons inziens goed mogelijk dat met een beter toepasbaar scoringssysteem, dat specifiek voor bronchiëctasiepatiënten is ontworpen, wél een vermindering van CT-afwijkingen gedurende behandeling met macroliden te zien zal zijn. Inmiddels zijn er een aantal van deze scoringssystemen beschikbaar, en toekomstig onderzoek zal moeten uitwijzen of deze inderdaad een radiologisch behandeleffect kunnen meten.

Een interessante bevinding in dit onderzoek was het feit dat vooral de patiënten die een hoge CT-score hadden, goed reageerden op de behandeling. Als dit in toekomstige onderzoeken ook bevestigd wordt, zal er aan de hand van de CT-score van een patiënt misschien wel van tevoren voorspeld kunnen worden of deze patiënt goed zal reageren op een behandeling met azitromycine.

Hoewel azitromycine een vrij 'oud' middel is en een onderhoudsbehandeling met azitromycine in de praktijk al regelmatig toegepast wordt, is er nog weinig bekend over hoe het middel zich in het lichaam gedraagt gedurende een langdurige behandeling. Van korte kuren azitromycine is beschreven dat het lang in het lichaam aanwezig blijft en zich ophoopt in witte bloedcellen. Wat dat voor bronchiëctasiepatiënten tijdens een onderhoudsbehandeling betekent, bijvoorbeeld welke hoeveelheid van het middel in het bloed of in het longweefsel terecht komt (de bloed- of sputumspiegels) en of de hoogte van die spiegels iets zeggen over de werking van het middel is echter niet bekend. Verder is in het geheel niet bekend welke dosering azitromycine (bv 250 of 500 mg per keer) en

welke doseringsfrequentie (bv 3x per week, om de dag of dagelijks) het beste werkt. In het onderzoek dat beschreven wordt in **hoofdstuk 7**, hebben we gekeken naar de hoeveelheid azitromycine in het bloed en in het sputum van de 43 patiënten die geloot hadden voor de onderhoudsbehandeling en gekeken of die hoeveelheden iets zeiden over het uiteindelijke effect van de behandeling. Als eerste belangrijke observatie zagen wij dat de hoeveelheid azitromycine in het bloed van patiënten 70 maal lager was dan in het sputum. Verder waren de bloedconcentraties erg variabel, en de hoeveelheden in het sputum redelijk stabiel binnen dezelfde patiënt. Daarom hebben wij voor de verdere berekeningen vooral naar de sputumspiegels gekeken. Hoewel alle patiënten exact dezelfde dosis azitromycine kregen (1 maal daags 250 milligram), zagen wij grote verschillen in de concentraties die uiteindelijk bereikt werden. Waar dit door veroorzaakt werd, werd in het onderzoek niet duidelijk. De hoogte van de azitromycine-concentratie in het sputum bleek niet samen te hangen met de werkzaamheid van het middel; het was bijvoorbeeld niet zo dat patiënten met de hoogste concentraties of spiegels ook de minste exacerbaties of klachten en afwijkingen hadden. Ook zagen wij vreemd genoeg niet méér bijwerkingen bij patiënten die hogere bloedconcentraties hadden. Met die informatie in het achterhoofd, konden wij bekijken of we bij sommige patiënten de dosis azitromycine dan niet wat konden verlagen. Immers, lagere concentraties betekenden in dit onderzoek niet dat het middel minder goed werkte. De laagste dosis die tijdens de BAT trial voor een afname van exacerbaties zorgde, was omgerekend ongeveer 19 milligram per kilo lichaamsgewicht per week. Voor een patiënt van rond de 50 kilo komt dat overeen met een dosis van ongeveer 130 milligram per dag. Deze patiënten zouden dus mogelijk met de helft van de in de studie gebruikte dosering toe kunnen. Omdat uit eerdere onderzoeken al was gebleken dat een lagere dosis minder bijwerkingen geeft, is dit een belangrijke bevinding, mede vanwege de kosten.

Tijdens het uitvoeren van bovenstaande onderzoeken hebben wij veelvuldig gebruikt gemaakt van vragenlijsten om iets te weten te komen over het klachtenpatroon en de dagelijkse beperkingen die patiënten met bronchiëctasieën ervaren. Wat ons hierbij opviel was dat geen van de vragenlijsten die gebruikt kunnen worden bij bronchiëctasiepatiënten ons en de onderzoeksdeelnemers erg goed bevielen. Over het algemeen waren de vragenlijsten erg uitgebreid waardoor het invullen veel tijd kostte. Sommige vragen waren lastig te begrijpen en daarnaast was het verwerken van de resultaten vaak erg arbeidsintensief. Verder waren er geen vragenlijsten beschikbaar die zich beperkten tot alleen de klachten en verschijnselen die patiënten ervaren, hoewel wij dat juist graag wilden kunnen meten. Om deze redenen hebben wij een eigen meetinstrument ontwikkeld, een klachtenscorelijst, waarvan wij in **hoofdstuk 8** onderzoeken of deze voldoet aan de criteria om deze in wetenschappelijk onderzoek te kunnen gebruiken. Wij vroegen 60 bronchiëctasiepatiënten om naast onze nieuwe klachtenscorelijst, de LRTI-VAS, nog een aantal van de klassieke vragenlijsten in te vullen en we bepaalden bij deze patiënten de longfunctie en het jaarlijks aantal exacerbaties. De LRTI-VAS werd getest op drie aspecten; ten eerste werd gekeken naar de validiteit.

Hiermee wordt bedoeld of het meetinstrument daadwerkelijk meet wat het zegt te meten. Hiervoor vergeleken wij de uitslagen van de LRTI-VAS met andere parameters die iets zeggen over de ernst van de symptomen bij bronchiëctasiepatiënten, zoals de longfunctie en uitkomsten van andere vragenlijsten. De mate van overeenkomst (correlatie) tussen de verschillende uitkomstmaten geeft aan of het meetinstrument valide is. In ons onderzoek vonden wij met name een goede correlatie tussen de uitkomsten van andere gevalideerde vragenlijsten en onze LRTI-VAS, maar ook de correlatie tussen longfunctie en LRTI-VAS was voldoende. In de tweede plaats werd de responsiviteit van de LRTI-VAS gemeten. Hierbij willen onderzoekers weten of een verandering in de klachten of ziekteverschijnselen van een patiënt goed wordt 'opgepikt' door het meetinstrument. Wij namen hiervoor de LRTI-VAS tweemaal af bij bronchiëctasiepatiënten die een exacerbatie hadden; eenmaal tijdens de exacerbatie en eenmaal als zij hun behandeling afgerond hadden en aangaven zich weer als voor de exacerbatie, dus redelijk goed te voelen. Na de exacerbatie scoorden alle patiënten beduidend lager op de LRTI-VAS dan tijdens de exacerbatie, waarmee wij konden bewijzen dat de LRTI-VAS voldoende responsief is. Het laatste vereiste waaraan een meetinstrument moet voldoen voor wetenschappelijke toepassingen wordt betrouwbaarheid genoemd. De betrouwbaarheid van een meetinstrument is hoog als het instrument bij herhaalde afname bij een proefpersoon in een stabiele situatie ook dezelfde uitkomst geeft. Hiervoor vroegen wij 30 bronchiëctasiepatiënten om de LRTI-VAS tweemaal in te vullen gedurende een periode waarin zij geen toename van klachten ervoeren. De resultaten van de LRTI-VAS op beide tijdstippen werden vergeleken en bleken niet significant van elkaar te verschillen. Dit wijst op voldoende betrouwbaarheid van de LRTI-VAS.

De LRTI-VAS meet de vijf meest voorkomende symptomen van bronchiëctasiepatiënten, door middel van het plaatsen van een kruisje op een horizontale lijn die de mate van ernst van elk symptoom aangeeft. De plaats waar het kruisje gezet wordt, bepaalt het aantal punten (0-10) die een patiënt scoort. De totaalscore wordt berekend door het optellen van de punten die op elke afzonderlijke vraag gescoord worden. Het is dus een heel simpele en snel in te vullen vragenlijst, die ook nog eens erg eenvoudig te verwerken is. In ons onderzoek hebben we daarnaast dus bewezen dat het een valide meetinstrument is voor het meten van klachten en ernst van ziektelast bij bronchiëctasiepatiënten.

Een van de conclusies die uit dit proefschrift naar voren komen, is het feit dat een onderhoudsbehandeling met azitromycine zeer effectief is als het gaat om het verminderen van exacerbaties en verminderen van klachten van bronchiëctasiepatiënten. Het wijd verbreid toepassen van zo'n behandeling heeft echter ook een aantal flinke nadelen. Ten eerste leidt, zoals duidelijk werd in hoofdstuk 5, het langdurig toedienen van azitromycine tot resistentievorming. Bijna alle bacteriën (tot 90%) die gekweekt werden uit het sputum van patiënten die geloot hadden voor de azitromycine behandeling bleken resistent voor azitromycine geworden te zijn. Uit onze studie, en eerdere onderzoeken bij CF patiënten,

lijkt dit voor de patiënt zelf weinig gevolgen te hebben, deze knapt immers flink op onder de behandeling. Resistentie kan echter wel een probleem zijn voor de omgeving van de patiënt en de bevolking als geheel. In de eerste plaats kunnen resistente bacteriën worden overgedragen naar andere mensen. Bij toename van het aantal resistente bacteriën neemt ook de kans toe dat kwetsbare groepen, zoals kinderen of patiënten met een afweerstoornis, in contact komen met deze bacteriën. De infecties die zo ontstaan zijn dan vaak lastiger te behandelen als gevolg van de resistentie. Daarnaast zijn er steeds meer aanwijzingen dat langdurig antibioticagebruik toch ook voor de gebruiker zelf nadelige gevolgen kan hebben. Moderne analysetechnieken tonen aan dat er aanzienlijke veranderingen plaatsvinden in de samenstelling van natuurlijk voorkomende bacteriën (het microbioom) in de mond, darmen en luchtwegen van patiënten die een onderhoudsbehandeling met antibiotica krijgen. Een stabiel microbioom is van belang voor een groot aantal lichaamsfuncties, zoals de verwerking van voedsel in de darmen, het voorkomen van infecties en het gezond blijven van de slijmvliezen. Daarom wordt aangenomen dat verstoringen direct gevolgen kunnen hebben voor het individu. De precieze relatie tussen het gebruik van antibiotische onderhoudsbehandelingen, verstoringen in het microbioom en gevolgen voor de gebruiker zijn nog niet voldoende bekend en dit vereist nader onderzoek.

Naast het beperken van het voorschrijven van een onderhoudsbehandeling met azitromycine tot de patiënten die de meeste kans op een gunstig effect hebben, is het van belang meer te weten te komen over de optimale dosering en behandelduur, zodat er geen patiënten onnodig lang of hoog gedoseerd behandeld worden. Uit de resultaten van ons onderzoek naar azitromycinespiegels in hoofdstuk 7 bleek dat een deel van de patiënten mogelijk met een lagere dosis azitromycine toe kan. Echter, gecontroleerde studies naar de meest effectieve en veilige dosis zijn er nog niet. Verder is er ook nog bijzonder weinig bekend over de effectiviteit en veiligheid van azitromycine op de langere termijn (> 1 jaar).

Ter voorkoming van onnodige resistentievorming en andere schadelijke effecten is het van groot belang het gebruik van een onderhoudsbehandeling met azitromycine te kunnen beperken tot de patiënten bij wie de bovengenoemde nadelen minder zwaar lijken te wegen dan de voordelen. Hiervoor moeten wij te weten komen weten welke patiënten het meeste baat hebben bij zo'n behandeling. Naar aanleiding van de onderzoeken in dit proefschrift en andere recente studies over dit onderwerp lijken er patiëntkenmerken naar voren te komen die kunnen voorspellen of een bronchiëctasiepatiënt baat zal hebben bij langdurige behandeling met azitromycine. Volgens de huidige richtlijnen wordt het jaarlijks aantal exacerbaties gebruikt om de indicatie voor onderhoudsbehandeling te stellen, waarbij alleen patiënten die minimaal drie maal per jaar een exacerbatie hebben in aanmerking komen. Dit lijkt in ons onderzoek en andere recente studies bevestigd te worden. Echter, niet alle patiënten met veelvuldige exacerbaties blijken een gunstig effect van de behandeling te ondervinden. Daarnaast zouden bepaalde afwijkingen op de CT scan dus een voorspellende

waarden voor het behandeleffect van macroliden kunnen hebben, maar een CT-scan stelt patiënten bloot aan radioactieve straling en de kosten zijn hoog. Het zou daarom van voordeel kunnen zijn als we door het bepalen van een bepaalde stof in een bloedtest of in het sputum direct zou kunnen zien of iemand een grote kans heeft om goed te reageren op een onderhoudsbehandeling. Omdat azitromycine vooral een ontstekingsremmend effect heeft, ligt het voor de hand om hiervoor te zoeken naar een stof die betrokken is bij ontstekingen in het lichaam, een '*biomarker*' voor ontsteking. In de BAT studie hebben wij gekeken naar twee van die *biomarkers*, het *C-reactive protein* (CRP) en de witte bloedcellen, maar beiden waren in zeer lage concentraties in het bloed aanwezig en leken amper mee te bewegen met de behandeling. Daarmee zijn ze dus ongeschikt voor het voorspellen van een respons op de behandeling. Op dit moment onderzoeken wij in de sputummonsters van de deelnemers van de BAT studie een groot aantal andere ontstekingsgerelateerde stoffen in de hoop een *biomarker* te vinden die voor een individuele patiënt kan voorspellen of een azitromycine-behandeling effect zal hebben.

Een onderhoudsbehandeling met azitromycine heeft ontegenzeggelijk een zeer gunstig effect op het voorkomen van exacerbaties en het verminderen van symptomen bij patiënten met bronchiëctasieën. Resistentievorming en bijwerkingen vormen echter een niet te verwaarlozen schaduwzijde. Dit is reden om het gebruik in te perken tot die groepen die er maximaal van kunnen profiteren. Hopelijk zullen in de toekomst alleen nog geselecteerde groepen bronchiëctasiepatiënten langdurig behandeld worden met een onderhoudsbehandeling azitromycine. Welke eigenschappen van de patiënt de selectiecriteria zullen vormen en wat de ideale vorm en duur van de onderhoudsbehandeling zal worden, zullen toekomstige onderzoeken moeten uitwijzen.

Dankwoord

Zoals elke golf ook weer voorbij gaat, komt er nu – jaren later dan gedacht, maar daarom des te fijner - ook een eind aan dit promotie onderzoek.

O wat heb ik de beslissing om promotie onderzoek te gaan doen bij tijd en wijle vervloekt! Met een kapotte auto, tot aan de nok volgeladen met sputumpotjes, een uur stilstaan in de buitenwijken van Heerlen… Met gevoelloze handen in de -70 vriezer serumsamples selecteren… Net bevallen een JAMA deadline proberen te halen… En dat alles gecombineerd met de opleiding tot specialist, best pittig..

Maar.. wat heeft het doen van promotie onderzoek ongelooflijk veel toegevoegde waarde! Ten eerste heb ik nog steeds erg veel plezier van alle contacten die ik tijdens het uitvoeren van vooral de BAT trial heb mogen opdoen, zowel vakinhoudelijk als op de borrels na willekeurige nascholingsbijeenkomsten. En verder, ik ben op plekken gekomen en heb kansen gekregen die ik anders nooit gehad zou hebben en die mij als persoon en zeker als arts completer gemaakt hebben.

In mijn eentje zou ik dat allemaal nooit voor elkaar gekregen hebben.

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Wim, de aanstichter van dit alles.. In de wandelgangen (letterlijk!) vroeg je mij of ik er wel eens aan gedacht had om onderzoek te gaan doen. Je zocht iemand om een multicenter onderzoek op te starten en vroeg ‘jij bent wel goed in dingen regelen, toch?’. En het begin van een jaaaaarenlange samenwerking was geboren. Ik heb enorm veel bewondering voor de manier waarop jij het keiharde werken in een drukke opleidingskliniek combineert met het afleveren van nu alweer je 4e promovendus. Door jouw energie, enthousiasme, maar ook je Groningse koppigheid, krijg je het nu al jaren voor elkaar om Alkmaar als onderzoekscentrum draaiend te houden en mooie studies af te leveren, waarvan jij dan ook nog eens je promovendi alle eer gunt. Want ja, ‘wie het meeste werk heeft gedaan mag z’n naam erop zetten’ zoals jij altijd lekker nuchter zegt. Jij hebt mij altijd mijn eigen weg laten gaan en me aangemoedigd zelf ideeën voor onderzoek aan te dragen en uit te voeren, waardoor dit boekje helemaal geworden is zoals ik hoopte. Dank je wel.

En Tjip, mijn promotor uit het Hoge Noorden.. Ik geef toe, ik moest even aan je wennen. Een prof die zijn poli-assistentes (en af en toe ook zijn promovendi) aanspreekt met ‘laive skat’, mij zonder blikken of blozen midden op de grote markt de sleutel van zijn witte Audi cabrio geeft (en zelf uitstapt omdat hij al bijna te laat voor een laudatie is) en spaghettisaus uit de diepvries voor me opwarmt omdat hij het zo zielig vond dat ik anders met een lege maag de reis naar Amsterdam moest aanvangen, die kom je gewoon niet elke dag tegen. Maar al snel raakte ik gewend aan je kwinkslagen, onverwachte opmerkingen en associatieve manier van denken en heb ik juist daardoor ontzettend veel van je geleerd. Na elk overleg in Grunn –waarvoor je steeds bijna je hele werkdag uittrok!- kwam ik weer enthousiast en met een hoofd vol plannen en ideeën terug. Jouw revisies van de manuscripten waren voor mij super waardevol en tilden het stuk altijd naar een hoger plan. Daarnaast heb ik veel geleerd van jouw mooie, compacte manier van Engels schrijven. En hoe jij ondanks al je reizen naar de meest afgelegen oorden zonder internetverbinding het toch voor elkaar kreeg om steeds zo snel mijn werk gereviseerd terug te sturen, blijft me een raadsel.

Lyn en Pek, mijn paranimfen! Lyn, *we go way back*.. van de Springplank in Sassem, via het Fioretticollege samen naar de grote stad en aan de studie. Alleen je promotie kreeg je iets vlugger dan ik voor elkaar ;). Vroeger samen zwalkend over straat, nu met een wijntje aan de keukentafel, geniet ik nog steeds evenveel van je gezelschap, je rust en je grappige en slimme opmerkingen. Heel stoer dat je na je onderzoek zelf copromotor was en eigenlijk nog stoerder dat je nu op een heel andere manier voor jezelf en je gezin kiest. En Pek, sinds HoerA zijn we elkaar gelukkig nooit meer uit het oog verloren. Dank je wel voor je trouwe vriendschap, je nuchtere kijk op de wereld en dat je met je eigen drukke baan, man en kind nog tijd vond om me hierbij te helpen. ‘Doe je jurk in dezelfde kleur als je boekje, Doon’, was toch wel een van je meest waardevolle adviezen.

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Een multicenterstudie lukt alleen met veel (en vaak volstrekt belangeloze) medewerking van alle betrokkenen uit de verschillende deelnemende ziekenhuizen. Voor de hartelijke ontvangst (soms zelfs met lunch!) en jullie nauwgezetheid bij de patiëntselectie en het uitvoeren van de studiebezoeken van de BAT studie wil ik longartsen Jan Maarten van Haarst, Ivo van der Lee, Folkert Brijker, Jan Willem van den Berg, Ralph Koppers, Chiel Wijnands, Bob van den Berg, Erik Kapteijns, Eric van Haren, Menno van der Eerden en Monique Reijers en alle poli-assistentes en onderzoeksverpleegkundigen (in het bijzonder Petra, Marcella en Saskia) erg bedanken.

Onderzoek doen kan af en toe best.. nou ja... langdradig.. frustrerend...traag... zijn en dan is het heel erg fijn om daarover te kunnen klagen bij mensen die net hetzelfde hebben doorgemaakt

en je een hart onder de riem kunnen steken. Dominic Snijders, Maarten de Mulder, Mireille van Stijn en de andere promovendi uit Alkmaar die mij voorgingen, ook al werkten we niet allemaal in dezelfde vakgroep, ik kon steevast bij jullie terecht voor tips, tricks en het zoveelste kopje koffie. Heel erg fijn om het met jullie over SPSS, congresdeadlines, subsidieaanvragen maar ook over allerlei veel gezelliger zaken te kunnen hebben.

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'Als je denkt dat je tegen een berg opziet, zijn het eigenlijk meestal een paar kleine heuveltjes'

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Gek eigenlijk dat in de proefschrift de belangrijkste en meest nabije personen altijd als laatste genoemd worden.. Misschien is dat wel expres, zodat ze het langst blijven hangen in de gedachten van de lezers. En hoe terecht is dat als het over Pat en Stijn gaat, mijn liefste jongens!

Stijn, lieve vent, apie van me, al dat gewerk en gedoe lijkt onbelangrijk als jij me knuffelt, enthousiast naast me stapt of werkelijk stuk gaat van het lachen. Wel saai he, dat ik zooo vaak 'in m'n hok' moest zitten, en anders wel in het ziekenhuis in Alkmaar of Rotjeknor was. Uiteindelijk wilde je zelf ook maar dokter worden, zodat je tenminste met mij mee kon naar de zieke mensen. Je bent al zo groot, slim en stoer geworden, ik ben supertrots op je en het is fantastisch om je bij ons te hebben en al je mijlpalen met je te mogen meemaken.

Lieve pat, ik denk niet dat zonder jou dit proefschrift af gekomen was. Heel terecht ben jij er ook net zo 'klaar mee' als ik, maar ook minstens net zo trots op. Tijdens de vele uren achter de laptop en de dagen op congres, wist ik dat het thuis allemaal perfect liep, en dat helpt zo veel! Je bent de liefste papa voor Stijn die voor hem van elke dag een feestje maakt en het is fantastisch om jullie samen treinbanen te zien bouwen, voetballen of stoeien. 'Ik ben een zekerheidje hoor', zei je al toen we elkaar nog niet eens zo lang kenden en gelukkig is dat gebleken. *It's always better when we're together*, eerst samen, toen met Stijn erbij, en het voelt nog steeds zo. Vanaf nu hopelijk nog meer tijd met z'n drietjes, voor onze verbouwingsplannen, windsurfen, vakanties en om gewoon lekker samen even niks te hoeven.

About the author

Curriculum Vitae

List of publications

Curriculum vitae

Josje Altenburg werd op 7 september 1979 geboren in Leiderdorp en groeide op in Sassenheim. Zij slaagde *cum laude* aan het gymnasium van het Fioretti College te Lisse.

Na de middelbare school werd zij ingeloot voor de studie Geneeskunde aan de Vrije Universiteit (VU) in Amsterdam, waar zij in 2002 haar doctoraal examen haalde. Na haar coschappen, onder andere in het Kalafong Hospital te Zuid-Afrika, slaagde zij in 2004 voor haar artsexamen.

Zij werkte van 2004 tot 2006 als ANIOS interne geneeskunde in het Zaans Medisch Centrum en startte in 2006 de opleiding Longziekten in het Medisch Centrum Alkmaar onder leiding van Drs C.S. de Graaf en Dr J.G. van den Aardweg. Tijdens de opleiding begon zij aan haar promotie onderzoek onder leiding van Dr Wim Boersma, Alkmaar, en Prof Tjip van der Werf, verbonden aan het Universitair Medisch Centrum Groningen en de rijksuniversiteit Groningen.

Tijdens de studie en opleiding was zij actief in de studievereniging van de VU en vervolgens in de assistentensectie van de Nederlandse Vereniging voor Longartsen en Tuberculose (NVALT).

Zij volgde in 2015 een verdiepingsstage in de long-infectieziekten in het Erasmus Medisch Centrum te Rotterdam waar zij nu sinds augustus 2016 werkzaam is en als longarts deel uit maakt van het infectieteam.

Josje woont in Amsterdam, samen met Patrick van den Elshout en hun zoon Stijn (2012) met wie zij graag op reis gaat in hun 30-jaar oude camper. Zij houdt verder van hardlopen en heeft een passie voor windsurfen.

Josje Altenburg was born on September 6th 1979 in Leiderdorp and grew up in Sassenheim. She graduated secondary school *with honours* at the Fioretti College in Lisse and obtained her medical degree in 2004 at the Vrije Universiteit in Amsterdam, which included an internship in the Kalafong Hospital in South Africa.

From 2004 until 2006 she worked as a resident in internal medicine at the Zaans Medical Centre and started her training as a pulmonologist in 2006 at the Alkmaar Medical Centre (Drs C.S. de Graaf and Dr J.G. van der Aardweg). During her specialist training she started her PhD research supervised by Dr Wim Boersma, with prof Tjip van der Werf at the University Medical Centre in Groningen, University of Groningen.

During her studies and specialist training, Josje was an active member of the student society of the Vrije Universiteit and of the junior section of the Netherlands Society of Pulmonary physicians and Tuberculosis (NVALT).

After a 6-month fellowship on pulmonary infectious diseases at the Erasmus Medical Centre Rotterdam, she recently started working there as a pulmonary physician, and joined the Infectious Diseases team of the Department of Pulmonary Medicine.

Josje lives in Amsterdam, together with Patrick van den Elshout and their son Stijn (2012) with whom she likes to travel in their 30-year old campervan. She also likes running, working out and has a passion for windsurfing.

List of publications

Janssen KM, de Smit MJ, Brouwer E, de Kok FA, Kraan J, Altenburg J, Verheul MK, Trouw LA, van Winkelhoff AJ, Vissink A, Westra J. Rheumatoid arthritis-associated autoantibodies in non-rheumatoid arthritis patients with mucosal inflammation: a case-control study. Arthritis Res Ther. 2015 Jul 9;17:174

Altenburg J, Wortel K, de Graaff CS, van der Werf TS, Boersma WG. Validation of a visual analogue score (LRTI-VAS) in non-CF bronchiectasis. Clin Respir J. 2016 Mar;10(2):168-75

Altenburg, Wortel, van der Werf, Boersma. Non-CF Bronchiectasis –clinical presentation, diagnosis and treatment; illustrated by data from a Dutch Teaching Hospital. Neth J Med. 2015 May;73(4):147-54.

Altenburg J, de Graaff CS, Stienstra Y, Sloos JH, van Haren EH, Koppers RJ, van der Werf TS, Boersma WG. Effect of azithromycin maintenance treatment on infectious exacerbations among patients with non-cystic fibrosis bronchiectasis: the BAT randomized controlled trial. JAMA. 2013 Mar 7;309(12):1251-9.

Altenburg J, de Graaff CS, van der Werf TS, Boersma WG. Immunomodulatory effects of macrolide antibiotics - part 1: biological mechanisms. Respiration. 2011;81(1):67-74. Review.

Altenburg J, de Graaff CS, van der Werf TS, Boersma WG. Immunomodulatory effects of macrolide antibiotics - part 2: advantages and disadvantages of long-term, low-dose macrolide therapy. Respiration. 2011;81(1):75-87. Review.

E. Vandenbroucke, J.C. Grutters, J. Altenburg, W.G. Boersma, E.J. ter Borg, J.M.M. van den Bosch. Rituximab in life threatening antisynthetase syndrome. Rheumatology International 2009 Oct;29(12):1499-502

J. Altenburg, Dr. W.G. Boersma. Longabces. Longartsen Vademecum 2008

Altenburg J, Vermeulen RJ, Strijers RL, Fetter WP, Stam CJ. Seizure detection in the neonatal EEG with synchronization likelihood. Clinical Neurophysiology 114 (2003) 50-55