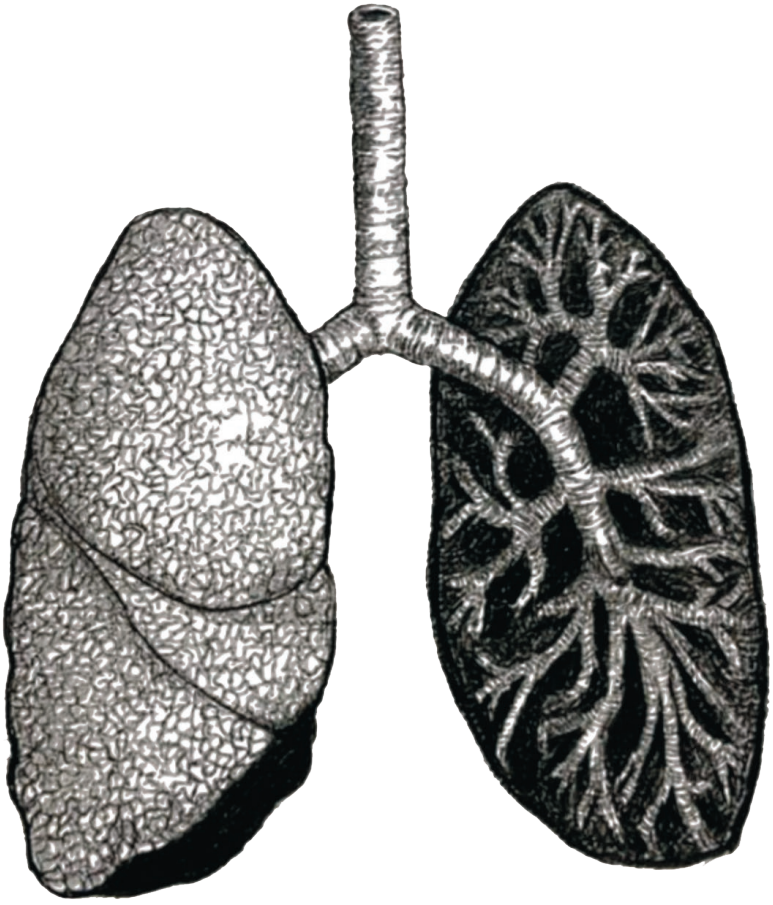
CLINICAL ASPECTS AND TREATMENT OPTIONS IN NON-CF BRONCHIECTASIS

LOTTE TERPSTRA



Clinical aspects and treatment options in non-CF bronchiectasis

Lotte Carijn Terpstra

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Clinical aspects and treatment options in non-CF bronchiectasis

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met non-CF bronchiëctasieën
(Met een samenvatting in het Nederlands)

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Lotte Carijn Terpstra
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Promotor:

Prof. dr. H.G.M. Heijerman

Copromotoren:

Dr. W.G. Boersma

Dr. I. Bronsveld

Beoordelingscommissie:

Prof. dr. H.J. Bogaard

Prof. dr. L.J. Bont

Prof. dr. M.J.M. Bonten (voorzitter)

Prof. dr. C.K. van der Ent

Prof. dr. A. Verbon

Faculteit der Geneeskunde

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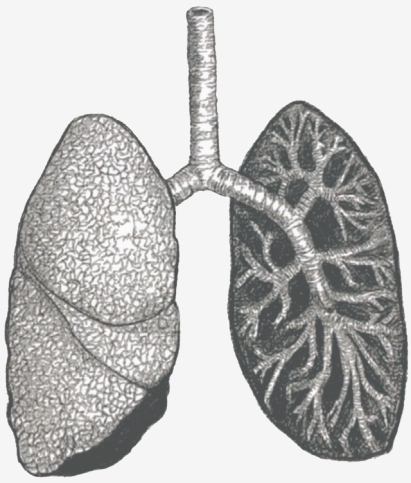
Chapter 1

General introduction and outline of thesis



GENERAL INTRODUCTION

Non-cystic fibrosis bronchiectasis (hereafter referred to as *bronchiectasis*) is a chronic respiratory disease characterized by chronic bronchial dilatation and known to cause chronic productive cough, fatigue, and recurrent respiratory tract infections in affected patients.[1]

The origin of bronchiectasis varies, but the key to development of the disease is a vicious cycle of impaired ciliary clearance, chronic inflammation, and bacterial colonization, resulting in irreversible, abnormal dilatation and thickening of the small and medium-sized bronchi. This 'vicious cycle of Cole' was originally described in 1986 and is frequently represented in more recently published studies.[2-4] A modern interpretation of Cole's vicious cycle hypothesis is shown in Figure 1.[3]

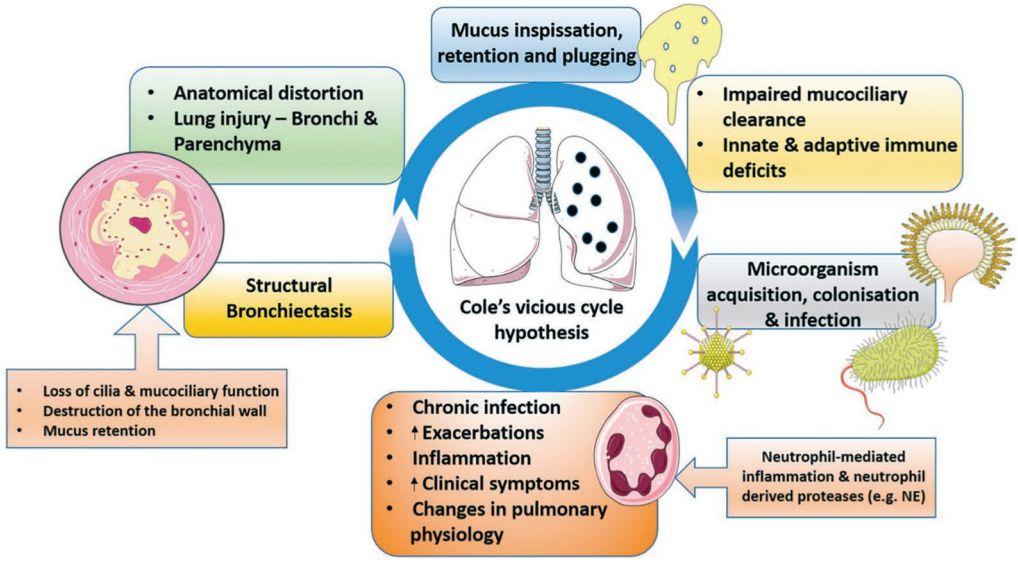


Figure 1: A modern interpretation of Cole's vicious cycle in bronchiectasis, characterized by infection, inflammation and a permanent, irreversible thickening and dilation of the bronchial wall.

Bronchiectasis has been recognized as a major clinical problem since its first description by the French physician Rene Laennec in 1819, whereby severe infections such as pneumonia and tuberculosis were the predominant cause of bronchiectasis.[5] In the last decades the incidence of bronchiectasis increased, particular in older age groups, which may be due to improved diagnostic tests, with increased use of computed tomography (CT) scans to assess patients with lung disease. In addition, bronchiectasis is associated with a markedly increased morbidity and mortality, placing an increased burden on global health care systems.[6]

Especially the frequent exacerbating bronchiectasis patients suffer from a high disease burden, which is associated with an increased morbidity and mortality.[7,8]

Reducing the number of exacerbations is the corner stone of long-term disease management, whereby favourable results were described on lung physiotherapy, sputum evacuation modalities and in addition the maintenance treatment with macro-lides.[9] However, macrolide therapy also has a downside. It is not always effective, antibiotic resistance may develop in patients with non- *Pseudomonas aeruginosa* pathogens, and relapse may occur after discontinuation. In addition, long-term treatment with macrolides is associated with gastro-intestinal side effects, and the small risk of QT prolongation, which interacts with other medication.[9-11]

An attractive alternative may be the use of inhaled antibiotics which can provide a consistent deposition of high antibiotic concentrations directly to the site of infection with a lower risk of toxicity or systemic adverse events.[12,13] In cystic fibrosis (CF) patients, chronic infection with *P. aeruginosa* is associated with and increased number of exacerbations, a decline in lung function, and an increased morbidity.[14] One of the key therapies in CF is long-term inhaled antibiotics and is associated with a reduce in lung function decline and number of exacerbations and are part of the standard care in CF with *P. aeruginosa* chronic infection.[15]

The evidence for inhaled antibiotics in non-CF bronchiectasis is limited, however the bronchiectasis guidelines recommend inhaled antibiotics in patients with *P. aeruginosa* chronic infection.[16] A recently published meta-analysis supports this recommendation, however not much is known about the ideal dosage regimen and duration of treatment, and the preference for a certain type of inhaled antibiotics.[12]

Previous studies showed that the use of inhaled antibiotics may also be a treatment option for patients without *P. aeruginosa*, but chronically infected with the other common

pathogens.[17-19] And in addition, a combination therapy of oral and inhaled antibiotics is recommended in patients with inadequate response on monotherapy.[16]

The last decade, the increasing heterogenous bronchiectasis population is a field of interest for scientists, especially after founding the EMBARC database, whereby better designed trials were executed, and promising results are expected from trials with agents specifically developed for bronchiectasis treatment.[20]

AIM OF THIS THESIS

The research described in this thesis focuses on the clinical effects and treatment options in bronchiectasis. And in this line, we would like to find answers for the undermentioned questions which probable leads to reframe our thinking for this devastating condition and optimize the treatment possibilities.

- Is there a relation between QoL (Quality of Life) and the etiology of bronchiectasis? (Chapter 2)[7]
- What is the effect of long-term azithromycin (AZM) on the radiological features in bronchiectasis? (Chapter 3)[21]

The immunological effect of AZM is not completely known and a current point of research. Previous studies have aimed to decipher these effects and reveal the potential mechanisms of action. However, there is much controversy regarding these effects and the underlying mechanisms have yet to be completely defined.[22] In Chapter 4 of this thesis we investigated the effect on sputum inflammatory markers in patients with bronchiectasis with and without AZM treatment.[23] Whereby we would like to answer the questions:

- Is there an effect of maintenance AZM on the sputum inflammatory markers in bronchiectasis?
- Is there a change in sputum inflammatory markers during an exacerbation?
- Is there a relation between the sputum inflammatory markers and the severity of the disease?

AZM is frequently prescribed in patients with bronchiectasis, and with improvement of respiratory symptoms, AZM is used for years. However, its effectiveness and safety beyond the first year of maintenance treatment is not known.

- What is the efficacy and safety of AZM maintenance therapy beyond the first year, and up to 5 years, in an observational cohort? (Chapter 5)

As mentioned before, the evidence for inhaled antibiotics in non-CF bronchiectasis is limited, whereby in this thesis, we would like to evaluate the treatment effect of inhaled tobramycin (TIS) in bronchiectasis. Chapter 6, 7 and 8 of this thesis focuses on the long-term use of TIS in the frequent exacerbating patient with bronchiectasis.[24,25]

- What is the effect of TIS once daily on the number of exacerbations?
- Is TIS once daily also a treatment option for the frequent exacerbation bronchiectasis patient with non- *P. aeruginosa* chronic infection?
- Can the suggested (in hospital) tolerance test predict the development of airway hyperresponsiveness during the treatment with the inhaled medication?
- Which side effects are frequently seen during the use of inhaled medication?

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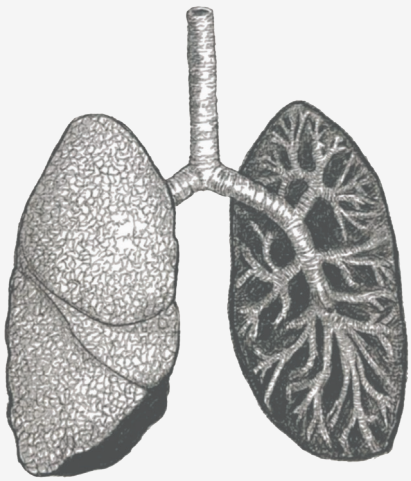
Chapter 2

Etiology and disease severity are among the determinants of quality of life in bronchiectasis

Terpstra, Lotte C., et al. "Etiology and disease severity are among the determinants of quality of life in bronchiectasis." The clinical respiratory journal 13.8 (2019): 521-529.



ABSTRACT

Introduction: Quality of life (QoL) is known to be impaired in patients with bronchiectasis, which is generally attributed to exacerbations and chronic pulmonary symptoms. The aim of this study was to determine if etiology and disease severity are associated with QoL in bronchiectasis.

Methods: We conducted a retrospective analysis of clinical stable patients with bronchiectasis. Diagnostic workup into the etiology of bronchiectasis was conducted according to the current guidelines. QoL was measured by QoL-B questionnaire (QoL-B), data on sputum microbiology, pulmonary function tests and the disease severity were obtained.

Results: The etiology of bronchiectasis was investigated in the total of 200 patients. The most identified etiologies were post-infective (39.5%) and idiopathic (12.5%). Patients with chronic obstructive pulmonary disease (COPD)- related bronchiectasis showed a significant lower QoL ($P < .05$) as compared to the other etiologies. In the total population, an increasing disease severity as measured by FACED, E-FACED and the bronchiectasis severity index was correlated with a lower QoL.

Conclusions: Our results showed that QoL in bronchiectasis is related both to etiology, with worse QoL in COPD-related bronchiectasis, and to disease severity, which suggests more attention in advance for these specific patient groups with bronchiectasis.

Keywords

Etiology of bronchiectasis, bronchiectasis severity index (BSI), FACED and E-FACED disease severity score, quality of life bronchiectasis questionnaire (QoL-B)

INTRODUCTION

Non-cystic fibrosis bronchiectasis (hereafter referred to as *bronchiectasis*) is a progressive respiratory disease characterized by chronic bronchial dilatation and inflammation leading to recurrent exacerbations, hospital visits and an impaired quality of life (QoL).[1] Previous studies have been conducted to analyze possible QoL determinants in patients with bronchiectasis; with dyspnea, daily sputum and FEV1 (forced expiratory volume in 1 s) value as most relevant health- related QoL variables, and the worst outcome in those infected with *Pseudomonas aeruginosa*.[2,3] However, treatment with long-term oral or inhaled antibiotics showed conflicting results in improvement of QoL, and suggests that there might be other factors that determine the QoL.[4,5] A wide range of disorders are known to result in bronchiectasis but in a significant proportion no cause of bronchiectasis was diagnosed.[6,7] Previous studies evaluated the etiology of bronchiectasis in relation to different levels of disease severity based on the bronchiectasis severity index (BSI) and FACED score, but failed to show significant differences, with the exception of a higher prevalence of chronic obstructive pulmonary disease (COPD)-related bronchiectasis in patients with severe disease, and an excess of mortality in both COPD and rheumatoid arthritis-related bronchiectasis.[6,8]

In this line, there might be a relation between the etiology of bronchiectasis and the QoL, suggesting a lower QoL in patients with an increasing disease severity and especially in patients with COPD-related bronchiectasis. The goal of the present study, therefore, is to determine if the QoL is related to the specific etiologies and disease severity of patients with bronchiectasis. Secondary objectives were to evaluate the correlation between QoL and the bacterial pathogens and to examine if there is a correlation between the FACED, the E-FACED, and BSI severity score itself.

METHODS

Study population

We conducted a retrospective analysis using the Northwest Clinics electronic database of patients with bronchiectasis who have participated in the European Bronchiectasis Network (EMBARC) database and completed the QoL-B questionnaire (QoL-B). The

ethics and research committee have given their approval and informed consent was required. Patients over the age of eighteen with proven bronchiectasis on (high-resolution) computed tomography ((HR) CT) were eligible for inclusion and enrolled in our database between June 2016 and September 2017. Patients were excluded if they had cystic fibrosis or an active malignancy. Demographics, radiological features, lung function, micro-biological tests during stable state and the co morbidity using the Charlson-weighted Index of Comorbidity (CCI) were recorded and estimated the 10-year survival.[9] Based on the CCI, the severity of comorbidity was categorized into three grades: mild, with CCI scores of 1-2; moderate, with CCI scores of 3-4 and severe, with CCI scores ≥ 5. For the degree of dyspnea, the Medical Research Council (MRC) score was utilized.[10] At the time of assessment, all patients were clinically stable with no exacerbation in the preceding month. These patients were managed and assessed according to the Dutch guideline and the BTS guideline for non-CF bronchiectasis.[1,11,12]

Diagnostic methods and exacerbations

The number of hospital admissions and number of exacerbations during the preceding year were registered from the electronic patient files. An exacerbation was defined as the presence of three or more of the following symptoms for at least 24 hours: increased cough, increased sputum volume or purulence, hemoptysis, increased dyspnea, increased wheezing, fever > 38.5°C and/or malaise and the clinician determines that antibiotic and/or prednisolone treatment is required.[13]

(HR)CT-scans with signs of bronchiectasis were verified by pulmonologist or radiologist and described according to the well accepted criteria for bronchiectasis.[14] The most recent lung function in clinically stable state, and within 1 year prior to enrolment, was obtained and were expressed in FEV_1 in liters and % predicted, FVC (forced vital capacity) in liters and % predicted and the FEV_1/FVC ratio.

Sputum cultures from 1 year prior to enrolment were analyzed according to the standard methods to assess the presence of pathogens.[15] Chronic colonization was defined by the isolation of potentially predominant pathogenic bacteria in a sputum culture on 2 or more occasions, at least 3 months apart in a 1-year period.[1]

Etiology of bronchiectasis

For the diagnostic evaluation of the etiology of bronchiectasis, the medical history of each patient was recorded, the serum IgG, IgM, IgA, IgG subclasses, total IgE, specific IgE and IgG Aspergillus fumigatus were determined. Autoimmunity was tested if a rheumatologic or other auto-immune disease was clinically suspected.[1,11,12]

The definition of the etiology of bronchiectasis were divided in post-infective, COPD, asthma, allergic broncho- pulmonary aspergillosis (ABPA), immunodeficiency, mixed connective tissue disease (MCTD), rheumatic disease, inflammatory bowel disease (IBD), primary ciliary dyskinesia (PCD), reflux and idiopathic.[6,16] The etiology of bronchiectasis was defined stepwise by the treating physician at the point of care.

First, the evaluation of the (HR)CT scan and the laboratory results were performed to identify a specific etiology. Secondary, if these results were negative, patients with a history of at least 10 pack years, and airflow obstruction by spirometry were diagnosed with bronchiectasis based on COPD. Bronchiectasis associated with asthma was diagnosed in patients with moderate to severe asthma according to the GINA guidelines.[17] If there were symptoms of reflux disease confirmed by gastroscopy or clinical symptoms of IBD, the etiology was defined as bronchiectasis related to reflux disease, respectively IBD. Third, post-infective bronchiectasis was diagnosed if patients reported a history of persistent respiratory symptoms with a plausible association to previous severe respiratory infections or chronic sinusitis. A patient was considered as idiopathic bronchiectasis if the laboratory tests were negative, and no association was found with severe respiratory infections or other diseases.

QoL bronchiectasis questionnaire

To assess the QoL, participants were asked to fill in the QoL-B V3.1. This self-administered, patient-reported outcome measure includes 8 scales and has been validated by Quittner et al.[18] assessing functioning, symptoms, and health-related QoL for patients with bronchiectasis.

Severity of disease

The disease severity was calculated using the validated BSI-score and FACED score.[19,20] In addition, the recently published E-FACED score was also used to assess the disease severity.[21] The BSI scoring system identifies individuals at risk of mortality, hospital

admissions, and exacerbations, while the FACED score predicts the probability of all-cause mortality after 5 years of follow up.[22] Based on the BSI, patients were classified into mild, with range between 0 and 4; moderate, with a range from 5 to 8; and severe (≥ 9) BSI scores. The FACED score is ranged from 0 to 7 points and is also classified into 3 severity classes: mild bronchiectasis (overall score 0-2 points), moderate bronchiectasis (overall score 3-4 points) and severe bronchiectasis (overall score 5-7 points).

Recently, Martinez-García[21] published the E-FACED, and incorporated the number of severe exacerbations (hospitalizations) in 1 year into the FACED. The classification of the E-FACED score is defined as mild: 0-3 points, moderate: 4-6 points and severe: 7-9 points.

Statistical analysis

Statistical analysis was conducted using IBM SPSS 20 for Windows. Discrete variables are presented as counts (percentage) and continuous variables as means ± SD or medians with IQR (interquartile range). Data among groups were compared using ANOVA with post-hoc analyses to test between group differences in case of parametric distributions, and a Kruskal-Wallis test with additional Mann-Whitney U tests for nonparametric distributions. In both cases, the Bonferroni method was used to correct for multiple testing. Multivariable linear regression analysis was used to correct for possible confounding factor. A variable was considered a confounder if it was significantly associated with etiology in univariable analysis; tested using the chi-square test in case of nominal variables and ANOVA in case of continuous variables. Correlations between parameters were assessed with Pearson's r and Spearman's ρ. A correlation ≥ 0,7 was considered as clinically relevant. A *P* value < 0.05 was considered significant.

RESULTS

Baseline characteristics

A total of 200 (72%) patients from the 277 patients included in our EMBARC database were randomly admitted in this study and included in the analysis. Demographic data and other patient characteristics are shown in Table 1. The mean age was 69 years and 57% (*n* = 114) of the population was female. The number of exacerbations in the

preceding year were in the range from 0 to 5 with a median of 1 (IQR 1). The median number of hospitalizations in the preceding year was 0 (range 0-4 IQR 0). The mean FEV_1 and FVC were, respectively, 84% and 99% of predicted. Eighty percent of this population had a CCI score of 1-2, with a mean MRC score of 2.7 (SD ± 1.1), and a BSI score of 6 (SD ± 3.2). The mean FACED and E-FACED score were, respectively, 3 (SD ± 1.4) and 3 (SD ± 1.6).

Table 1: Study population

Total	**200**
Female	114 (57)
Age, yr	69.5 ± 10.3
BMI, kg/m^2	25.3 ± 3.9
Smoker	107 (53.3)
Pack years	19.9 ± 18.8
Exacerbations	1 (IQR 1)
Hospitalizations	0 (IQR 0)
FVC (% predicted)	99.3 ± 19.8
FEV_1 (% predicted)	84.2 ± 24.3
FEV_1/FVC ratio	64.3 ± 13.3
MRC-score	2.68 ± 1.09
Charlson Comorbidity Index	
CCI 1-2	160 (80)
CCI 3-4	29 (14.5)
CCI ≥ 5	11 (5.5)
Radiology, N of lobes	2.7 ± 1.3
1	33 (16.5)
2	76 (38)
3	37 (18.5)
4	26 (13)
5	22 (11)
6	6 (3)
BSI-score	6.0 ± 3.2
Mild (0-4)	69 (34.5)
Moderate (5-8)	91 (45.5)
Severe (≥ 9)	40 (20)

Total	200
FACED score	3.0 ± 1.4
Mild (0-2)	83 (41.5)
Moderate (3-4)	97 (48.5)
Severe (5-7)	20 (10)
E-FACED	3.3 ± 1.6
Mild (0-3)	107 (53.5)
Moderate (4-6)	85 (42.5)
Severe (7-9)	8 (4.0)

Data are presented as *n* (%), mean (± SD) or median (IQR). *Abbreviations:* BMI, body mass index; BSI, bronchiectasis severity index; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; MRC, Medical Research Council.

The etiology of bronchiectasis

The etiology of bronchiectasis was determined in the total of 200 patients, of which 25 patients with idiopathic bronchiectasis. The five most identified etiologies were post-infective (39.5%), asthma (14.5%), idiopathic (12.5%), immunodeficiency (12.5%) and COPD (12%). The groups with a smaller number of patients were excluded in our final analysis (Table 2).

Table 2: Etiology of bronchiectasis in 200 patients with bronchiectasis

Etiology	
Post-infective	79 (39.5)
Asthma	29 (14.5)
Idiopathic	25 (12.5)
Immunodeficiency	25 (12.5)
COPD	24 (12)
Rheumatic disease	5 (2.5)
ABPA	4 (2)
Reflux	4 (2)
PCD	2 (1)
IBD	2 (1)
MCTD	1 (0.5)

Data are presented as *n* (%). *Abbreviations:* ABPA, allergic bronchopulmonary aspergillosis; COPD, chronic obstructive pulmonary disease; IBD, inflammatory bowel disease; MCTD, mixed connective tissue disease; PCD, primary ciliary dyskinesia.

QoL Bronchiectasis questionnaire

QoL and symptom data were available for almost all patients (98.5%), with the lowest score of 50.4 (SD ± 22.9) on the health-related subscale, and the highest score of 80.6 (SD ± 16.9) at the emotion subscale (supplemental 1). The association between the specific etiology and the score on every subscale of the QoL-B is shown in Figure 1. Overall, patients with COPD and bronchiectasis showed a lower QoL on every subscale in relation to the other etiologies, with significant differences on the physical subscale (P = .000 – P = .030), the health-related subscale (P = .005 – P = .042), and the social functioning subscale (P = .041 – P = .050). Univariable analyses showed that the baseline variables age, smoking status, BMI, FEV_1% of predicted and the severity scores were significantly associated with COPD. After correction for these variables using multivariable linear regression, a significant lower QoL persisted on the physical and the health-related subscale in patients with COPD-related bronchiectasis as compared to asthma and post-infective. For the social subscale, these differences were significant between COPD-related bronchiectasis and the etiologies post-infective and immunodeficiency.

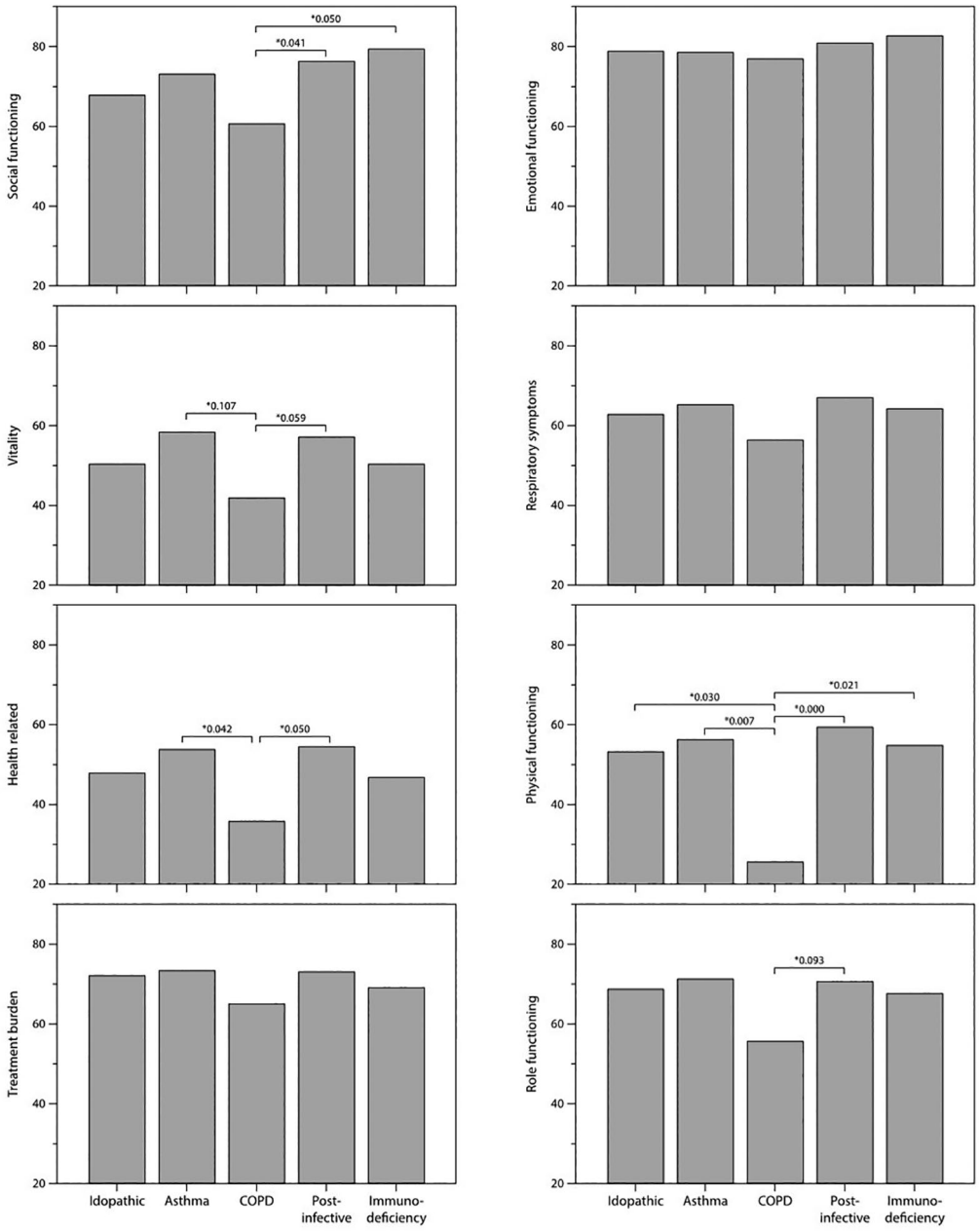


Figure 1: The association between the specific etiology and the score on every subscale of the QoL-B

Data are presented as bar charts. *X*-axis: the etiology of bronchiectasis. *Y*-axis: mean of the 8 domains of the QoL-B questionnaire, with a range from 0-100. *Represent statistical significance between COPD and another etiology. *Abbreviation:* COPD, chronic obstructive pulmonary disease

Sputum culture and QoL-B score

Sputum cultures were positive in 127 (86%) of the 148 available samples in which the most common pre-dominant pathogens were *Haemophilus influenzae, Staphylococcus aureus, P. aeruginosa* or *Escherichia coli* (Table 3). Due to the small numbers of other pathogens, only the mentioned pathogens were compared with the QoL-B. No significant difference was found between the QoL and one of these specific pathogens, but patients colonized with *P. aeruginosa* (*P* = .169) showed a not significant lower QoL on the treatment burden subscale and lower levels at the respiration domain were found in patients with *H. influenzae (P* = .967) colonization (supplemental 2).

Table 3: The results of sputum cultures of 200 patients with bronchiectasis

Sputum culture	
Total number of cultures	148 (74)
Absence of pathogens	21 (14)
Haemophilus influenzae	36 (24)
Staphylococcus aureus	22 (15)
Pseudomonas aeruginosa	20 (13)
Escherichia coli	11 (7)
Moraxella catarrhalis	8 (6)
Stenotrophomonas maltophilia	7 (5)
Klebsiella oxytoca	7 (5)
Streptococcus pneumoniae	5 (3)
Klebsiella pneumoniae	5 (3)
Other Gram-negative bacteria	7 (5)

Data are presented as *n* (%). Absence of culture in 52 patients (26%).

Disease severity

Among the total study population, 69 patients (34.5%) were classified according to the BSI as having a mild disease, 45.5% as moderate disease and 20% as severe disease. The numbers were slightly different compared to the FACED score; 41.5% were classified as having mild disease, 48.5% as moderate disease and 10% as severe disease. For the E-FACED score, 53.5% were classified as mild disease, 42.5% as moderate disease and 4% as severe disease.

An increasing disease severity as measured by FACED, E-FACED and BSI was correlated with a lower QoL in the total population (Table 4), with significant differences observed between the disease severity in relation to the eight subscales of the QoL-B. Using the BSI, these differences were found almost at every subscale with exception of social functioning domain. Both FACED and E-FACED demonstrated less discrimination in terms of QoL, especially in the respiratory, treatment burden and health-related domains of the QoL-B. A weak correlation between the FACED and the QoL-B was found with a maximum of r = .41 (P = .000) at the physical functioning subscale, with a comparable weak correlation at the physical subscale using the E-FACED score (r = .41 (P = .000)).

The correlation observed between the BSI and the QoL-B is also weak, with a maximum of r = .47 (P = .000) as well at the physical subscale. The clinically relevant correlation determined between the BSI and FACED, the BSI and E-FACED, and, respectively, the E-FACED and FACED were r = .54 (P = .000); r = .72 (P = .000); and r = .92 (P = .000).

Table 4: QoL-Bronchiectasis questionnaire in relation to BSI, FACED and E-FACED severity scores

				Mild vs. Moderate	Moderate vs.	Mild vs. Severe
	Mild	**Moderate**	**Severe**	***P* value**	***P* value**	***P* value**
Respiratory symptoms						
BSI	67.3 (18)	65.1 (19)	56.8 (19)	1.000	.071	.020
FACED	67.3 (17)	62.4 (21)	60.7 (15)	.275	1.000	.505
E-FACED	65.7 (19)	62.3 (19)	64.8 (14)	.650	1.000	1.000
Physical functioning						
BSI	69.5 (28)	51.6 (30)	28.5 (31)	.001	.000	.000
FACED	66.9 (31)	45.3 (31)	34.2 (26)	.000	.501	.000
E-FACED	63.7 (32)	41.8 (30)	33.3 (35)	.000	1.000	.043
Vitality						
BSI	61.3 (22)	53.0 (24)	44.8 (23)	.090	.208	.002
FACED	59.7 (25)	49.8 (23)	51.3 (18)	.019	1.000	.502
E-FACED	58.6 (25)	48.7 (22)	51.4 (19)	.015	1.000	1.000
Role functioning						
BSI	74.7 (22)	70.4 (22)	50.9 (25)	.729	.000	.000
FACED	74.6 (23)	64.3 (24)	58.2 (25)	.013	.869	.017
E-FACED	73.1 (23)	63.0 (24)	52.0 (24)	.012	.671	.052

	Mild	Moderate	Severe	Mild vs. Moderate *P* value	Moderate vs. *P* value	Mild vs. Severe *P* value
Health related						
BSI	57.1 (23)	50.7 (21)	37.8 (22)	.209	.008	.000
FACED	55.7 (23)	46.9 (23)	44.9 (19)	.034	1.000	.167
E-FACED	53.9 (24)	47.1 (20)	37.5 (24)	.119	.759	.144
Emotional functioning						
BSI	83.4 (17)	81.2 (16)	74.4 (17)	1.000	.116	.027
FACED	83.9 (15)	77.1 (18)	83.4 (14)	.025	.466	1.000
E-FACED	83.7 (16)	76.1 (17)	85.4 (17)	.004	.396	1.000
Social functioning						
BSI	75.1 (25)	71.9 (23)	71.8 (20)	1.000	1.000	1.000
FACED	76.5 (22)	70.6 (25)	69.9 (21)	.287	1.000	.757
E-FACED	72.7 (24)	74.7 (22)	60.4 (22)	1.000	.300	.459
Treatment burden						
BSI	74.4 (25)	74.3 (21)	62.5 (25)	1.000	.044	.071
FACED	72.8 (26)	72.9 (21)	62.7 (22)	1.000	.340	.362
E-FACED	74.6 (24)	69.1 (23)	65.3 (25)	.461	1.000	.870

Data are presented as mean (± SD). *Abbreviation:* BSI, bronchiectasis severity index.

DISCUSSION

In our cohort, 200 patients with bronchiectasis were reviewed and the etiology was determined, with post-infective (39.5%) as most common etiology, and solely 12.5% of the patients with idiopathic bronchiectasis. In previous studies, the etiology remains unidentified in the majority of patients, ranging from 26% to 74%.[6] This difference is probably due to the standard of care we followed, guided by the Dutch and European guidelines, and thereby a large proportion of these patients were treated by a bronchiectasis expert, who may conduct more extensive research to identify the etiology of bronchiectasis stepwise. Either, the group of patients in our study is possibly selected due to the retrospective nature of our study and probably geographical risk factors and variations in testing practice played a role.[1,11,12] Recently, an objective

algorithm for classification of the etiology of bronchiectasis was published; using this objective algorithm in combination with the suggested minimal bundle of etiological tests; a significant reduction of idiopathic bronchiectasis was found, and indicated that it becomes easier for the clinician to identify the etiology of bronchiectasis.[11,16]

We have demonstrated that there are clinical important differences between the etiology of bronchiectasis and the QoL, with a significant lower QoL found in patients with COPD as compared to the other etiologies of bronchiectasis. Earlier studies demonstrated a functional decline and higher mortality rate in patients affected by COPD and bronchiectasis and a higher prevalence of COPD-related bronchiectasis in patients with severe disease, but this is the first study that investigated the relation between QoL and the etiology of bronchiectasis.[6,23,24] According to the previous studies and the findings in our study it suggests that patients with COPD and bronchiectasis have a specific identity, which is earlier described as the possibility of a COPD bronchiectasis overlap syndrome.[23] Still some questions remain unanswered in this specific group, because bronchiectasis is frequently radiological diagnosed in patients with COPD, with different clinical "phenotypes". Hence, it has been suggested to move beyond the simplistic bronchiectasis -COPD clinical phenotype, to a more "endotypic" approach. In such an approach, a combination of imaging parameters, airway inflammation markers and microbiology would be used to make a distinction between "true" COPD, bronchiectasis, and the overlap syndrome.[25]

In line with earlier studies, our study showed that patients colonized with *P. aeruginosa* had, although not significant, a lower QoL, especially at the treatment burden subscale. This is probably due to the more intensive treatment to reduce exacerbations in patients with bronchiectasis and *P. aeruginosa* colonization.[26]

To our knowledge, this is one of the first studies that investigated the correlation between the QoL using the QoL-B and the disease severity based on the BSI and both the FACED and E-FACED scores. Our analysis suggests that an increasing severity of the disease, especially using the BSI, is correlated with a lower QoL measured with the QoL-B. The FACED and the E-FACED demonstrated less discrimination, which is in line with the earlier published multidimensional severity assessment conducted by McDonnell et al.[27]; suggested that the BSI is superior to FACED in predicting overall clinically important disease-related outcomes. The most important difference between the BSI and the FACED is that the BSI also incorporates BMI, hospitalizations, exacerbations, and chronic

infection with bacteria other than *P. aeruginosa*. Hereby, more patients are classified as "severe" disease using the BSI as compared to the FACED and suggests that these scores measure different things; BSI is a severity assessment tool and FACED accurately predicts risk of death.[28] Recently, Martínez-García[21] published the E-FACED, and incorporated the number of severe exacerbations in 1 year into the FACED. The E-FACED showed a greater prognostic capacity for exacerbations and hospitalizations and its capacity to discriminate degrees of severity. However, in our analysis, even more patients were classified as "severe" disease using the BSI as compared to the E-FACED, but an improved correlation was found between the BSI and the E-FACED (r = .72 P = .000) as compared to the correlation between the BSI and the FACED score (r = .54 (P = .000)). Suggesting that the E-FACED may also be useful in the same way as the BSI for predictions of short-term mortality, but it is shorter and more easily to apply.[29]

Our study has some limitations, QoL and disease severity were measured at one moment, no follow up was performed. Wherefore longitudinal data are needed to evaluate the disease severity and the QoL in association with different etiologies of bronchiectasis. Therefore, more patients are needed to evaluate these disease-related outcomes. At this moment, the EMBARC database recruited almost 12.000 patients with bronchiectasis, so hopefully, research with this robust database gives us more insight in the etiology of bronchiectasis and the determinants of the QoL during long-term follow up. In our study, a comprehensive evaluation of the QoL was done through the widely used disease-specific QoL-B, which is relatively long and do not generate a total score. Especially for clinical settings, this questionnaire remains complex and not well suitable. A new health-related questionnaire (BHQ) recently developed and validated by Spinou et al.[30] is shorter and generates an overall score. The BHQ correlated well with the St. George's Respiratory Questionnaire, the Visual Analogue Scale for breathlessness and the cough and sputum scales, however no comparison with the QoL-B was performed. Additional research is needed to assess if this questionnaire is more convenient for both research and daily clinical practice in the future.

In conclusion, our results indicate a significant lower QoL in patients with COPD-related bronchiectasis as compared to the other etiologies. In the total population, an increasing disease severity, especially based on the BSI, is correlated with a lower QoL, with a moderate correlation between the E- FACED and BSI score itself. These findings may alert clinicians to be more aware of QoL of these specific patient groups.

SUPPLEMENTAL MATERIAL

See the supplemental section at page 162.
Supplemental 1: QoL-Bronchiectasis questionnaire domains in the total of 200 patients.
Supplemental 2: Association between bacterial colonization and the QoL-B treatment burden -and respiration subscale.

Conflict of interests

No conflict of interests was reported for all authors.

Author contributions

All authors were fully involved in the preparation of this article and the material has not been submitted for publication elsewhere. LC Terpstra: Designed and performed the study. Collected and analyzed the data, wrote the paper; S Biesenbeek: Performed the study, collected the data; J. Altenburg: Contributed important reagents; WG Boersma: Designed and performed the study and contributed import- ant reagents.

Ethics

The study protocol of the EMBARC registry has been approved by the National Research Ethics Committee on human research and all patients have given their written informed consent.

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Chapter 3

The effect of maintenance azithromycin on radiological features in patients with bronchiectasis – analysis from the BAT randomized controlled trial

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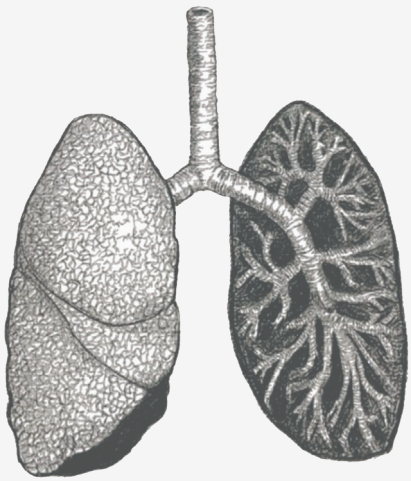

ABSTRACT

Rationale: Bronchiectasis (abnormal 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 macrolides maintenance treatment in frequent exacerbating bronchiectasis patients has been established in randomized trials.

This study was undertaken to prospectively evaluate the effect of long-term azithromycin (AZM) on radiological features in patients with bronchiectasis.

Methods: The BAT randomized controlled trial (2008-2010) investigated the effect of 1 year of AZM (250mg OD) in bronchiectasis with frequent exacerbations. Chest (HR) CT-scans at baseline and after one year of study treatment were obtained and scored by two radiologists according to the Brody – and the Bhalla scoring system.

Results: 77 (93%) patients conducted the BAT trial were evaluated in this post-hoc analysis. A significant improvement of the radiological features based on the Brody score was found after one year of AZM therapy as compared to placebo (P = 0.024), with a not significant improvement of the Bhalla score (P = 0.071). Especially the consolidation (Bhalla) and parenchymal changes (Brody) sub scores significantly improved (both p = 0.030), and even a radiological deterioration was seen on the Brody bronchiectasis sub score for the placebo treated patients (mean 14.5 (11.7) vs. 15.7 (11.9)).

Conclusions: The beneficial effect of long-term AZM treatment on radiological features was demonstrated in this randomized controlled trial. (HR)CT's can be used as an objective measure of treatment response in bronchiectasis.

Clinical trial registration number: NCT00415350

Keywords

Bronchiectasis, high resolution computed tomography (HRCT), azithromycin (AZM), immunomodulation, radiology

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, resulting in irreversible, abnormal dilatation of the small and medium-sized bronchi.[2]

High resolution computed tomography (HRCT) scanning is the method of choice in diagnosing and assessing the severity of bronchiectasis. Moreover, radiological disease severity has been identified as an independent predictor of both morbidity and mortality in these patients.[2,3] Not surprisingly, two proposed scoring systems for disease severity in bronchiectasis have included (HR)CT-scores as one of their main variables.[3-5]

In patients with cystic fibrosis (CF), the radiological extent of bronchiectasis has been found to correspond to clinical findings, *Pseudomonas aeruginosa* infection, a lower Forced Expiratory Volume in one second (FEV_1) and a higher risk of exacerbations. In addition, radiological improvement was found in CF-patients treated for pulmonary exacerbations.[6-11]

Scarce data in bronchiectasis patients suggest improvement of radiological features following macrolide maintenance treatment, which is nowadays increasingly used for treatment of frequently-exacerbating bronchiectasis patients after favourable results of three randomised trials.[12-14] However, to date this macrolide effect on radiological features has not been confirmed in larger or prospective trials.

In the current study we investigated the long-term effect of azithromycin (AZM) on radiological features in patients with bronchiectasis by using two validated radiological scoring systems for CF, the original Bhalla score and the modified Brody score.[15,16] We expect to investigate an improvement of radiological features of bronchiectasis in the patients treated with maintenance AZM therapy as compared to the placebo treated patients.

STUDY DESIGN AND METHODS

Participants

Post hoc analysis of the Bronchiectasis and long-term Azithromycin Treatment (BAT) randomized controlled trial was performed in this study. The BAT study, a multicentre, placebo-controlled trial was conducted at 14 sites in the Netherlands from 2008-2010 (Clinicaltrials.gov, number NCT00415350); detailed study protocols and results are provided elsewhere.[13] 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 sputum cultures showing evidence of airway infection. All participants were treated with AZM OD of placebo OD for 12 months and 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). The participants were familiar with routine spirometry measurements, and these were performed according to European Respiratory Society standard criteria.[20]

Radiology

At baseline – with all study participants having stable disease, without recent or current exacerbation, and within 3 months after the end of study treatment, (HR)CT scans were obtained according to local CT-protocols. Non-contrast volumetric chest CT scans, at 14 sites, were acquired on multi-detector row CT scanners in inspiration with a maximum reconstructed slice thickness of 1.5mm. All (HR)CT's were independently scored by two experienced and trained thoracic radiologists (FMH and PdJ) according to the original Bhalla scoring system and the modified Brody scoring system.[15,16]

The Bhalla scoring system is validated in CF patients and contains 9 items, representing key radiological features of bronchiectasis on segmental basis. The patient's (HR)CT score was calculated based on the severity and/or extent of these nine morphologic changes, with a maximum total score of 25. This score is subtracted from 25 to determine patients score (supplemental 1). A total score between 16-25 was defined as mild bronchiectasis, a score between 9-15 as moderate and a score < 9 as severe bronchiectasis.[17] However, for the 9 component scores of the Bhalla, a decrease in this score correspondents with a radiological improvement.

The modified Brody score (supplemental 2), a more detailed lobar scoring system, showed a good agreement and evaluates the five lung lobes and the lingula as a sixth lobe for severity and extent of central and peripheral bronchiectasis, extent of central and peripheral mucous plugging, severity and extent of central and peripheral airway wall thickening, extent of opacities (atelectasis or consolidations), extent of ground glass opacities and extent of cysts and bullae, with no major differences found in CF related bronchiectasis.[16,18,19] The focal air trapping score was excluded from scoring since not all scans had expiratory images. Scores for the presence and severity of the findings in bronchiectasis lung disease in each lobe were calculated. The following sub scores were obtained by averaging the sub scores across 6 lobes in each patient: 1) bronchiectasis, 2) mucous plugging, 3) peribronchial thickening and 4) parenchymal findings. This sub scores were ranged from 0-12 and were added(sum) to obtain the total Brody score for each patient, with a minimum overall score of 0 and a maximum overall score of 36. A higher score indicates more radiological abnormalities and radiological severity of the disease. For statistical analysis the mean total CT score and component CT scores were expressed on a scale of 0-100 (percentage of maximum possible score).

The inter-observer correlation for both scoring systems were evaluated using the intraclass correlation coefficient. The observers were blinded to study treatment, spirometry findings and exacerbation frequency.

Statistics

Descriptive statistics for patients treated with AZM or placebo were calculated for the time of the baseline CT scan. Discrete variables were presented as counts (percentage) and continuous variables as means with standard deviation (SD) if normally distributed and medians with interquartile range (IQR) if not normally distributed. The difference between the AZM and the placebo group, with respect to the CT scores after 1 year of treatment, were analyzed by multivariable linear regression analysis correcting for baseline CT scores. Residuals were tested for normality.

Inter observer agreement between both observers for CT scores were calculated by using intraclass correlation coefficient with < 0.40 reflects 'poor', 0.40 to 0.59 'fair', 0.60 to 0.74 'good', and between 0.75-1.00 'excellent' inter-observer correlation.[21] A *P* value < 0.05 was considered statistically significant. Statistical analysis was conducted by using SPSS using IBM SPSS 25 for Windows.

RESULTS

Study population

CT scans were available for 77 (92.8%) patients. Only CT-scans that were scored both by start and at the end of study treatment were included in this analysis. A total of six patients were excluded due to quality of the (HR)CT's or the inability to score (HR) CT's adequately before and after one year of study treatment. Total CT scores and item scores were available for 41 (95.3%) of AZM-treated patients and 36 (90%) of placebo-treated patients for both observers. Patient characteristics of 41 patients treated with AZM and 36 patients treated with placebo are given in Table 1.

Table 1: Patient characteristics

	Azithromycin	**Placebo**
No. (%) of patients	41 (95.3)	36 (90)
Age, mean (SD)	59.6 (12.6)	65.0 (9.4)
Woman, n (%)	27 (65.9)	23 (63.9)
Pseudomonas colonization, n (%)	6 (14.6)	6 (16.2)
Etiology of bronchiectasis, n (%)		
Post infectious	15 (37)	12 (33.3)
Idiopathic	10 (24)	12 (33.3)
Asthma	7 (17)	7 (19.4)
Auto-immune disease	3 (7.2)	2 (5.6)
Common variable immune disorder	1 (2.4)	1 (2.8)
Primary ciliary dyskinesia	1 (2.4)	0
Yellow nail syndrome	0	1 (2.8)
Aspiration	1 (2.4)	0
Mechanical obstruction	1 (2.4)	0
Allergic bronchopulmonary aspergillosis	1 (2.4)	1 (2.8)
Alpha-1-antitrypsin deficiency	1 (2.4)	0
No. of exacerbations in year before study entry, median (IQR)	4 (2)	5 (3)
No. of exacerbations during the study, median (IQR)	0 (2)	2 (2)

	Azithromycin	Placebo
Pulmonary function: % of predicted at baseline, mean (SD)		
FEV_1	77.5 (24.4)	83.0 (28.7)
FVC	91.0 (24.7)	98.4 (24.6)

All values are expressed as mean (SD) or median (IQR) unless stated otherwise. *Abbreviations:* High resolution computed tomography ((HR)CT); forced expiratory volume in one second (FEV_1); forced vital capacity (FVC); interquartile range (IQR); standard deviation (SD);

At baseline, the median annual exacerbation frequency in the total population was 4 (IQR 3) with FEV_1% of predicted of 80.1 (SD 26.5) and FVC% of predicted of 94.4 (SD 24.8) (supplemental 3). The mean overall Bhalla score (for both AZM and placebo treated patients) at baseline was 14.9 (SD 3.8), and an overall Brody score of 15.8% (SD 9.5) was found, which corresponds to mild-moderate bronchiectasis. Despite of randomisation, at baseline a significant higher total Bhalla score (15.9 (SD 3.5)) and mucous plugging sub score (1.0 (SD 1.2)) was found for the placebo population as compared to AZM (P = 0.02 and P = 0.01) (supplemental 4). The Brody score, which is a different and more specific scoring system, showed no significant differences between both groups at baseline for the total Brody score. For the sub scores mucous plugging (P = 0.04) (for both the Bhalla and Brody scoring) and parenchymal changes (P = 0.02) significant higher scores were found in de AZM group as compared to placebo and correspondents with more extensive deviations at baseline.

The intraclass correlation coefficients between both observers for scoring results for the total Bhalla score and the modified Brody score were r = 0.82 and r = 0.83 respectively, indicating an 'excellent' inter observer correlation. For all the component scores this correlation coefficient was over r = 0.72.

Effect of AZM on radiological features

Modified Brody score

Table 2 shows the modified Brody mean (SD) total score and sub scores; residuals of multivariable linear regression analysis were normally distributed. A significant improvement was found in the AZM-treated patients as compared to the placebo-treated patients (P = 0.024), with a decrease of 1.6% in the total modified Brody score after one year of AZM maintenance treatment (Figure 1).

The bronchiectasis sub score remained constant in the AZM-treated patients (respectively 21.8% and 21.9%), while the peribronchial thickening sub score (respectively 22.7% and 20.1%), and the mucous plugging sub score (respectively 16.3% and 13.8%) decreased, with a significant decrease of the parenchymal changes as compared to the placebo-treated patients (*P* = 0.030), which correspondents with an improvement of the radiological features in bronchiectasis. This improvement was not found in the placebo-treated patients, and even a deterioration was seen on the bronchiectasis sub score (respectively 14.5% and 15.7%).

Table 2: Modified Brody total score and sub scores

	Azithromycin n = 41		**Placebo n = 36**		
	Baseline	**After one year of treatment**	**Baseline**	**After one year of treatment**	***AZM vs. Placebo^ P value***
Total score#	17.8 (9.7)	16.2 (10.4)	13.5 (8.7)	13.9 (9.7)	0.02
Bronchiectasis*	21.8 (17.7)	21.9 (17.7)	14.5 (11.7)	15.7 (11.9)	0.22
Mucous plugging	16.3 (13.2)	13.8 (13.9)	12.1 (15.3)	11.9 (16.1)	0.28
Peribronchial thickening	22.7 (14.4)	20.1 (15.3)	22.3 (19.9)	22.1 (19.9)	0.14
Parenchymal changes	8.5 (8.8)	6.0 (8.4)	4.2 (4.3)	4.7 (5.2)	0.03

Data are presented as percentages of maximum possible scores (SD); decrease in percentage correspondents with improvement; Air trapping sub score was excluded from scoring since not all scans had expiratory images; standard deviation (SD); AZM: azithromycin; #Mean total score of two observers; *Extent of bronchiectasis and size; ^Results of co-variance analysis, correcting for baseline measurements.

Bhalla score

For the total Bhalla score a non-significant (*P* = 0.071) improvement of radiological features was found in the AZM-treated patients (respectively 14.0 (SD 3.9) at baseline and 14.7 (SD 4.4) after one year of treatment) as compared to the placebo-treated patients (respectively 15.9 (SD 3.4) at baseline and 15.8 (SD 4.2) after one year of treatment) (Table 3). In line with the for mentioned modified Brody sub scores, the Bhalla sub scores showed an improvement on the peribronchial thickening sub score (mean 1.3 (SD 0.5) vs. 1.2 (SD 0.5)), the mucous plugging sub score (mean 1.6 (SD 0.9) vs. 1.4 (SD 1.0)) and the bronchial generations sub score (mean 2.7 (SD 0.6) vs. 2.6 (SD 0.8)) after one year of AZM maintenance treatment (Table 3). Moreover, as compared to the placebo-treated

patients, a significant improvement was found at the consolidation sub score (P = 0.030) for the AZM-treated patients. No improvement in the sub scores was observed in the placebo-treated patients.

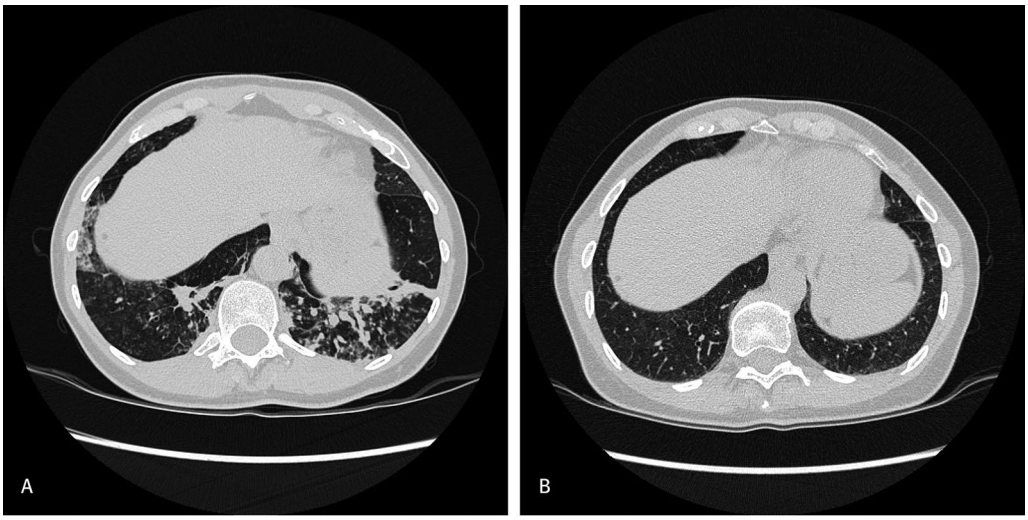

Figure 1: (HR)CT shows improvement of the radiological features of bronchiectasis; mucus plugging, parenchymal disease and peribronchial thickening.

Table 3: Bhalla total score and sub score

	Azithromycin n = 41		Placebo n = 36		
	Baseline	**After one year of treatment**	**Baseline**	**After one year of treatment**	***AZM vs. Placebo^ P-value***
Total score#	14.0 (3.9)	14.7 (4.3)	15.9 (3.4)	15.8 (4.2)	0.07
Severity of bronchiectasis	1.5 (0.8)	1.5 (0.78)	1.3 (0.6)	1.3 (0.6)	1.00
Peribronchial thickening	1.4 (0.5)	1.2 (0.5)	1.3 (0.8)	1.2 (0.8)	0.28
Bronchiectasis extension	2.1 (0.8)	2.1 (0.9)	1.9 (0.8)	1.9 (0.9)	0.46
Mucous plug extension	1.7 (0.9)	1.4 (1.0)	1.0 (1.2)	0.9 (1.1)	0.86
Sacculations and abscesses	0.3 (0.7)	0.3 (0.7)	0.2 (0.5)	0.2 (0.5)	0.88
Bronchial generations	2.7 (0.6)	2.6 (0.8)	2.5 (0.7)	2.5 (0.8)	0.54
Bullae	0.2 (0.7)	0.2 (0.7)	0.1 (0.6)	0.1 (0.6)	*
Emphysema	0.3 (0.7)	0.3 (0.7)	0.2 (0.6)	0.2 (0.6)	*
Consolidations	0.9 (0.6)	0.6 (0.7)	0.6 (0.6)	0.7 (0.7)	0.03

Data are presented as mean (SD); Increase in the total Bhalla score correspondents with improvement (total Bhalla score = 25- total points. Supplemental 1); Decrease in component scores correspondents with improvement; standard deviation (SD); AZM: azithromycin; #Mean total score of 2 observers; *No significance could be calculated, because of identical CT-scores before and after treatment; ^Results of co-variance analysis, correcting for baseline measurements.

DISCUSSION

This is the first study that prospectively evaluated the effect of long-term AZM therapy on (HR)CT features by using the Bhalla and the modified Brody scores. An improvement of the radiological features of patients with bronchiectasis was found after one year of AZM maintenance therapy as compared to placebo, with a significant improvement of the total Brody score and the parenchyma changes (Brody) and consolidation (Bhalla) sub scores. For the placebo-treated patients no improvement was observed, and even a deterioration was seen on the bronchiectasis score.

This agrees with results of Goeminne et al.,[22] who performed the only other study in 25 bronchiectasis patients focussing on CT features as surrogate markers for treatment response. In CF-patients, evidence on CT scores as parameter for CF lung disease and as

an indicator of treatment effect is slightly more robust than for bronchiectasis.[9,18,23] Few studies in CF described that CT scores can also be used measuring treatment response after exacerbations.[7,24] And in addition, it has been demonstrated that CT scores improve in response to long-term treatment with antibiotics, Rh-DNase or Ivacaftor, a CFTR potentiator in CF patients.[25-28]

Both in CF and bronchiectasis patients, favourable effects of AZM maintenance treatment have been robustly demonstrated by studies showing a significant reduction of exacerbations and an improvement of lung function and quality of life.[12,13,29] The benefits of AZM are most likely based on both the antimicrobial effect and the immunomodulatory effects. The mechanisms underlying this dual effect are not completely understood, but are thought to be partly attributable to an anti-neutrophilic mode of action as depicted by lower levels of neutrophils chemo-attractants in lavage fluid of bronchiectasis patients after macrolide treatment.[30,31]

This may indicate that CT features indicative of active bronchial inflammation, such as thickening of the airway mucosa, mucus impaction and consolidations are most responsive to change; and indeed, this was shown both in CF-patients and in patients with bronchiectasis.[22,25,27] In our study, a similar significant improvement was found for the parenchymal changes and consolidation sub score for both scores. For the mucous plugging sub scores such favourable effects were also observed, but this difference did not reach statistical significance. 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.[2] The observation that AZM treatment causes improvement of CT-features indicative of inflammation, could be another argument in favour of its intrinsic anti-inflammatory capacity. In addition, this improvement of inflammation in the small and medium sized airways may also lead to improvement of the forced expiratory volume in one second, investigated in the BAT trial.[13]

As described, a significant improvement on the modified Brody score was found for the AZM-treated patients, however this improvement in percentages (decrease of 1.6% in the Brody score) and absolute numbers (increase of 0.7 points in the Bhalla score) is small. Hence, before and after long-term AZM treatment the severity of bronchiectasis were still defined both as 'mild to moderate'. Despite of our relatively small sample size and the small differences in CT scores, previous studies showed comparable or

even smaller differences in CT scores and (HR)CT's were comparable or even more sensitive in assessing treatment effect than spirometry especially in patients with mild disease.[22,25,27,28]

In our analysis both the modified Brody and Bhalla score were used to evaluate radiological improvement after one year of AZM as compared to placebo. Both scoring systems were primary used and validated in CF, with a low between and within-observer variability of the total scores. However, the variability for the component scores differs between both scoring systems, with preference for using the more specific modified Brody score, since every lobe is systematic evaluated on specific features of bronchiectasis. By using the Bhalla score, an overall interpretation is done on 9 specific features of bronchiectasis, but not the 6 lobes independent of each other were evaluated, whereby minor changes may be missed. This could also explain the differences in the severity of bronchiectasis we measured between both scoring systems in our study population. Comparable results were described in earlier studies in CF, whereby the most common radiological scoring systems were evaluated and recommend the Brody scoring system especially in research.[9]

In our bronchiectasis study population, an 'excellent' agreement for both total CT scores was found, and both were useful for detection of relevant changes in response to therapy in bronchiectasis. However, as earlier described, the modified Brody score is a more detailed way of scoring CT scans in bronchiectasis and can probably be used as a surrogate endpoint in clinical trials. These scoring systems are, in contrast to CF-patients, not validated in bronchiectasis, and the reproducibility of the scoring is variable. Further studies are needed for this evaluation, with probably more quantitative scoring systems. Recently, in bronchiectasis the BRICS score is evaluated in idiopathic and post-infective bronchiectasis.[17] This simplified scoring system, which is somewhat comparable to the modified Reiff score,[35] can be used to predict disease severity and is simpler for clinicians to use in clinical practice. Although a more detailed scoring system (modified Brody) is desirable for the evaluation of specific (and subtle) radiological features (e.g., peribronchial thickening, mucus plugging, consolidation) in response to treatment of bronchiectasis.

Despite the 'excellent' intra correlation coefficient, this study is partly limited by the quality of the (HR)CT scans. All scans were obtained with radiological equipment available at the study sites at the time of study enrolment and according to the local

CT-protocols.[13] No expiratory images were acquired, and therefore the focal air trapping score was excluded from scoring. Despite these differences in (HR)CT-protocols and quality of (HR)CT scans, which reflects daily practice, significant differences in CT scores were found and showed the robustness of our dataset.

For evaluation of bronchiectasis and the accompanying features of inflammation, (HR)CT scanning (with expiratory) images reviewed by a trained thoracic radiologist is necessary for a good assessment of the radiological features. Even spirometer guided CT's are suggested for a more precise evaluation of bronchiectasis, and methods to quantify airway wall thickening may replace time-consuming manual annotations and visual scoring methods.[32-34]

In conclusion, this is the first study that prospectively evaluated the effect of long-term AZM on radiological features measured by both the modified Brody and the Bhalla scoring system. In the current study, as compared to placebo, one year of treatment with AZM result in a statistically significant improvement of the total Brody CT score, with improvement of specific radiological features such as peribronchial thickening, mucous plugging, parenchyma changes and consolidations, as compared to a deterioration on the bronchiectasis score for the placebo-treated patients. (HR)CT's may therefore be used as an objective measure of treatment response in bronchiectasis.

SUPPLEMENTAL MATERIAL

See the supplemental section at page: 164.
Supplemental 1: Bhalla scoring template.
Supplemental 2: Modified Brody scoring template.
Supplemental 3: Overall patient characteristics at baseline.
Supplemental 4: Bhalla and Brody score at baseline.

DECLARATIONS

Author's contributions: Conceptualization

W.G. Boersma, J. Altenburg and L.C. Terpstra.

Data curation, Formal analysis, Investigation, and methodology

W.G. Boersma, L.C. Terpstra, J. Altenburg with help of and independent statistician J. Doodeman. F.A. Mohamed Hoesein and P.A. De Jong independently scored the (HR)CT's for both the Bhalla and the Brody score. S. Go and P.A.C van Rijn independently scored the (HR)CTs for the Bhalla score.

Project administration

L.C. Terpstra

Supervision

W.G Boersma and H.G.M Heijerman

Writing – original draft

L.C. Terpstra

Critical review on the original draft

W.G. Boersma, J. Altenburg, F.A. Mohamed Hoesein and P.A. De Jong, I. Bronsveld, H.G.M Heijerman

Writing – review and editing

L.C. Terpstra and W.G. Boersma

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Conflicts of interest

All authors declare no competing interests.

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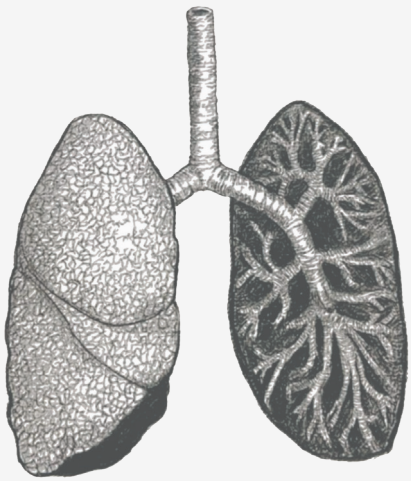
Chapter 4

The effect of azithromycin on sputum inflammatory markers in bronchiectasis – analysis from the BAT randomized controlled trial

Terpstra, L. C., et al. "The effect of azithromycin on sputum inflammatory markers in bronchiectasis." BMC Pulmonary Medicine 23.1 (2023): 1-12.



ABSTRACT

Background: Long term macrolide treatment has been found beneficial in bronchiectasis (BE) –pathogical bronchial dilatation– possibly due to a combined anti-bacterial and immunomodulatory effect. The exact mechanism of inflammatory response is unknown. Here, we investigated the effect of maintenance macrolide treatment on the inflammatory response in BE. In addition, we assessed the inflammatory profile in BE in relation to disease severity.

Methods: During the BAT randomized controlled trial (investigating the effect of 1 year of azithromycin (AZM) in 83 BE patients), data on BE severity, lung function and sputum microbiology was collected. For the current study, a wide range of inflammatory markers were analysed in 3-monthly sputum samples in all participants.

Results: At baseline, marked neutrophilic but also eosinophilic inflammation was present in both groups, which remained stable throughout the study and was not affected by AZM treatment. Significant upregulation of pro-inflammatory markers correlated with FEV_1 < 50% (TNFα, ECP, IL-21, IL-1, P = 0.01 – 0.05), *H. influenzae (HI)* colonization (MPO, ECP, MIP-1, TNFα, IL-21, Il-8, IL-1, IL-1α, P < 0.001 – 0.04) and number of exacerbations (MPO, ECP, VEGF, MMP-9, P = 0.003 – 0.01). Surprisingly, colonization with *P. aeruginosa (PA)* was found to correlate with an attenuated inflammatory response compared to non-*PA* colonized. In placebo-treated patients, presence of an infectious exacerbation was reflected by a significant excessive increase in inflammation as compared to a non-significant upregulation in the AZM-treated patients.

Conclusion: One year of AZM treatment did not result in attenuation of the inflammatory response in BE. Increasing disease severity and the presence of an exacerbation were reflected by upregulation of pro-inflammatory markers**.**

INTRODUCTION

Non-cystic fibrosis bronchiectasis (hereafter referred to as 'bronchiectasis') is characterized by a vicious cycle of bacterial colonization, airway inflammation and airway structural damage, resulting in bronchial dilatation, with recurrent infections, chronic symptoms of cough and sputum production, and an increase in severity of the disease.[1-3] The pathogenesis is poorly understood, but airway neutrophil dysfunction is considered a key component of this vicious circle of lung damage, and might result from a combination of host-derived mediators, bacterial virulence factors, and changes induced by incomplete attempts to clear biofilm-shielded bacteria.[4-6]

Previous studies revealed elevated levels of several pro-inflammatory, neutrophil driven cytokines, even in stable bronchiectasis airway secretions.[4,7] A few studies investigated the association between sputum inflammatory products and severity of the disease. Neutrophil elastase (NE) was proposed as a biomarker for exacerbations and lung function decline, and IL-8 and IL-13 were correlated with measurements of disease severity.[8,9] Also a heterogeneity of systemic inflammation was found in bronchiectasis, with higher levels of CRP, IL-6 and plasma fibrinogen during an exacerbation.[10] Plasma fibrinogen was also associated with the severity of bronchiectasis, a worse health status and with *Pseudomonas aeruginosa (PA)* colonization.[10,11] In addition, Neutrophil extracellular trap (NET) formations were recently identified as a key marker of disease severity and treatment response in bronchiectasis.[12]

For the frequent exacerbating bronchiectasis patients, macrolide maintenance treatment has shown favourable results and is nowadays part of the standard treatment in patients with bronchiectasis.[13-16] The benefits of macrolides are believed to be based on both the antimicrobial effect and the immunomodulatory effects. The mechanisms underlying this dual effect are not completely understood but are thought to be part attributable to an anti-neutrophilic mode of action as depicted by lower levels of neutrophils chemo-attractants in sputum of bronchiectasis patients after macrolide treatment.[17,18]

In the present study we investigated the relation between the inflammatory profile in spontaneous sputum samples and the severity of bronchiectasis. In addition, we studied the inflammatory effect of azithromycin (AZM) treatment om airway inflammation markers during maintenance treatment and during an exacerbation, and we explored

if higher levels of particular inflammatory markers at baseline may be predictive of an enhanced effect of AZM maintenance treatment with respect to number of exacerbations, quality of life and lung function.

MATERIALS AND METHODS

Participants

The Bronchiectasis and Long-term Azithromycin Treatment (BAT) randomized controlled trial, was a multicentre, placebo-controlled trial conducted at 14 sites in the Netherlands from 2008-2010 (Clinicaltrials.gov, number NCT00415350; Ethical approval METC Noord Holland: M07-002, CCMO: NL16025.094.07); detailed study protocols and results are provided elsewhere.[13] Participants were eligible for randomization if they had radiologically confirmed bronchiectasis, with three or more lower respiratory tract infections treated with antibiotics in the preceding year, and at least one positive sputum culture for bacterial respiratory pathogens. Patients were randomized to receive either AZM (250mg OD) or placebo for 12 months and underwent a follow up every 3 months at the outpatient ward.

Sputum cultures and immunological analysis

Sputum samples were collected at start of the treatment period till the end of the study at three-month intervals, and after the run-out period of 3 months. This spontaneously expectorated sputum used in the present analysis was frozen and stored at -80°C till processing.[19] Due to the beneficial effect of AZM some patients did not expectorate sputum anymore at the end of the study period.

In short, frozen samples were quickly thawed, the volume of the sputum was set equal to its weight and 10mM dithiothreitol (DTT) solution was added in a 1:1 ratio, followed by incubation. When not all mucus was liquified, the same volume of DDT was added again, followed by another incubation step till all mucus was liquified. After mucus was liquefied, we often found cell aggregates which were dispersed by incubation with DNase (Sigma D-5025; 150,000 U). This step was repeated when necessary. Finally, the processed samples were centrifuged after which supernatant was collected and aliquoted. As DTT reduces sulphur bridges that may affect antigenic

epitopes and antibodies, we diluted samples at least 50-fold to minimize the effect of DTT as was confirmed by further serial dilutions. Additional information about the assays and sputum analysis is shown in supplemental 3. A separate sputum sample was also collected for bacteriology.

Lung function, quality of life questionnaire and exacerbations

During the 52-week treatment period, lung function tests and QoL questionnaires were obtained every 3 months. Lung function measurements were performed according to the European Respiratory Society standard criteria.[20] The St George's Respiratory Questionnaire (SGRQ) –a condition specific questionnaire– was used to measure health-related QOL and has been validated in bronchiectasis, with a minimal important difference of -4.[21,22] An infectious exacerbation, before and during study treatment, was defined as an increase in respiratory symptoms, requiring antibiotic treatment.[13] 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.

Bronchiectasis severity index and radiological severity

The disease severity at baseline was calculated by using the bronchiectasis severity index (BSI).[2] The BSI scoring system is a mortality prediction score and identifies individuals at risk of mortality, hospital admissions, and exacerbations. This scoring system has been developed and validated in multiple, large cohorts.[2,23] At the time of the BAT trial[13] the BSI did not exist, therefore, in our analysis, the severity of the disease could only be calculated based on components of the BSI and not the total BSI score. However, these component scores of the BSI were also described as an independent predictor of the disease severity.[2] Beside the BSI, the radiological severity was calculated by using the bronchiectasis radiological indexed CT score (BRICS).[24] The BRICS score is derived from combining the bronchial dilatation and the number of segments with emphysema on (high resolution) computed tomography (HRCT) and is validated in idiopathic and post-infective bronchiectasis**.**

Statistical analysis

Statistical analysis was conducted by using IBM SPSS 25 for Windows. Descriptive statistics for patients treated with AZM or placebo were calculated at baseline. Discrete variables were presented as counts (percentage) and continuous variables as means with standard deviation (SD) if normally distributed and medians with interquartile range (IQR) if not normally distributed. Differences in the distribution of sputum markers and components of the severity index were compared using the Mann-Whitney U Test for two independent samples or Kruskal-Wallis test for comparisons of 3 or more. Differences in distribution of sputum markers at baseline and during an exacerbation were compared by using the Wilcoxon Signed Rank test for 2-related samples. The long-term effect of AZM on airway inflammation was compared to that of placebo using linear mixed model analyses. The adjusted associations between the inflammatory markers at baseline and treatment response, based on exacerbation frequency, lung function and quality of life, was shown in forest plots with 95% confidence interval (CI). A P value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics

A total of the 83 patients participated in the BAT trial and were included in this analysis. Baseline characteristics of the study population are shown in Table 1 according to the treatment group. In total 399 sputa were obtained, eight of which were limited in volume and therefore 391 sputa could be analysed. An overview of the number of sputum samples per visit is shown in supplemental 1. From the 25 inflammatory markers analysed in each sample, a total of nine markers were excluded (Fractalkine, GMCSF, IFN-γ, IL-10, IL-12p70, IL-13, IL-17A, Il-4, IL-5) because no inflammatory activity was measured in these samples at baseline and during the study treatment (Table 2).

Table 1: Patient characteristics

	AZM	Placebo	*P* value
Total of patients	43	40	
Age, mean (SD)	59.6 (12.3)	64.6 (9.1)	0.051
Female, n (%)	25 (63)	28 (65)	0.804
Aetiology of bronchiectasis, n (%)			0.850
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 deficiency	1 (2)	1 (3)	
Primary ciliary dyskinesia	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	
Baseline sputum microbiology, n (%)			
Haemophilus influenzae	13 (30)	9 (23)	0.617
Pseudomonas aeruginosa	6 (14)	6 (15)	0.855
Other(s)	24 (56)	25 (62)	0.874
No. Of exacerbations in year before study entry, median (IQR)	4 (2)	5 (3)	0.318
No. Of exacerbations during the study, median (IQR)	0 (1)	2 (2)	0.000
Pulmonary function tests, mean (SD)			
Δ (End-Start) FEV_1, % pred.	4.4 (9.1)	-0.9 (12.6)	0.046
Δ (End-Start) FVC, % pred.	5.5 (14.0)	-2.6 (12.6)	0.013
Quality of life questionnaire's, mean (SD)			
Δ (End-Start) Total score SGRQ	-11.3 (16.7)	-3.3 (15.8)	0.057

All values are expressed as mean (SD) or median (IQR) unless stated otherwise. *Abbreviations:* Azithromycin (AZM); Forced Expiratory Volume in one second (FEV_1); Forced Vital Capacity (FVC); interquartile range (IQR); standard deviation (SD); St George's Respiratory Questionnaire (SGRQ);

Table 2: Biomarkers in sputum specimen at baseline

	Overall n = 54	AZM n = 25	Placebo n = 29	*P* value
ECP (ug)	3.75 (8.8)	3.8 (8.1)	3.6 (9)	0.828
Fractalkine (pg)	0 (0)	0 (0)	0 (0)	0.392
GCSF (pg)	0 (100.2)	0 (128.8)	0 (26.1)	0.307
GMCSF (pg)	0 (0)	0 (0)	0 (0)	1.000
GRO-α (pg)	14 (297.9)	13.9 (387)	17 (170)	0.668
IFN-γ (pg)	0 (0)	0 (0)	0 (0)	0.369
IL-10 (pg)	0 (0)	0 (0)	0 (0)	1.000
IL-12p70 (pg)	0 (0)	0 (0)	0 (0)	0.185
IL-13 (pg)	0 (0)	0 (0)	0 (0)	0.031
IL-17A (pg)	0 (0)	0 (0)	0 (0)	0.471
IL-1α (pg)	285.3 (871.7)	335.7 (660.3)	285.3 (1071.9)	0.869
IL-1β (ng)	6.5 (22.0)	6.4 (17.9)	7.5 (41.8)	0.735
IL-1RA (pg)	606.7 (1716.7)	549.9 (1993.2)	606.7 (1578)	0.842
IL-21 (pg)	7.61 (62)	3.1 (44)	8.4 (90)	0.503
IL-4 (pg)	0 (0)	0 (0)	0 (3)	0.737
IL-5 (pg)	0 (0)	0 (0)	0 (0)	0.879
IL-6 (pg)	5.5 (548.5)	0 (837.1)	63.9 (504.8)	0.623
IL-8 (ng)	22.1 (84.4)	25 (101.4)	22.1 (70.3)	0.381
IP-10 (pg)	53.5 (149.2)	42.6 (136.9)	76.3 (149.2)	0.695
MIP-1β (ng)	5.7 (9.7)	4.2 (6.5)	7.2 (14.4)	0.504
MIP-3α (pg)	0 (69)	0 (175)	0 (64)	0.496
MMP-9 (ng)	178.0 (329.7)	178 (316.9)	177.1 (375.1)	0.869
MPO (ug)	76.31 (240.4)	86.4 (178)	53.3 (270.2)	0.842
TNF-α (pg)	129.9 (1334)	238.4 (1019)	129.9 (1841)	0.854
VEGF (pg)	1665.3 (3794.6)	1221.3 (3789.3)	1700.1 (3985.2)	0.683

Baseline sputum inflammatory profile expressed per gram of sputum of study subjects. All values are expressed as median with inter quartile range (IQR); *P* value: difference in inflammatory profile between the AZM treated patients and the placebo-treated patients at baseline.

Inflammatory profiles and the severity of bronchiectasis

At baseline, the severity of bronchiectasis was obtained using the following components of the BSI: age, FEV_1% of predicted, exacerbation frequency, and bacterial colonisation.[2]

A significant upregulation of pro-inflammatory markers was found in patients with a low FEV_1 (FEV_1 < 50% of predicted) (ECP *P* = 0.021; TNF-α *P* = 0.007; IL-21 *P* = 0.041), and a higher number of exacerbations (≥ 3, ≥ 5, ≥ 7) in the year prior to the start of the study (MPO *P* = 0.004; ECP *P* = 0.003; IP-10 *P* = 0.011; VEGF *P* = 0.008; MMP-9 *P* = 0.041 (data not shown)). Surprisingly, patients colonized with *PA* showed no upregulation in inflammatory markers. Even a significant reduction was seen in this population as compared to non-*PA* for VEGF (*P* = 0.031), IL-8 (*P* = 0.01), and MMP-9 (*P* = 0.001) (Table 3). For patients colonised with *Haemophilus influenzae (HI),* a significant upregulation of pro-inflammatory markers was markedly found, expect for IL1-RA and GRO-α (Table 3). The inflammatory profile at baseline was not related to the component score 'age' of the BSI, and the radiological severity based on the BRICS (data not shown).[24]

Table 3: Baseline sputum inflammatory profile grouped according to *Pseudomonas aeruginosa* or *Haemophilus influenzae* colonization

	Pseudomonas aeruginosa			**Haemophilus influenzae**		
	Yes (n = 10)	**No (n = 44)**	***P* value**	**Yes (n = 16)**	**No (n = 38)**	***P* value**
MPO (ug)	87.5 (121.1)	75.9 (264.4)	0.373	249.9 (483.8)	49.5 (110.3)	**0.004**
ECP (ug)	3.0 (7.8)	4.3 (9.5)	0.449	7.7 (17.7)	2.6 (7.0)	**0.043**
IP-10 (pg)	19.3 (72.3)	70.2 (162.7)	0.180	77 (172.5)	43 (122.6)	0.133
MIP-1β (ng)	2.2 (6.4)	6.6 (10.9)	0.084	9.7 (32.2)	4.2 (8.2)	**0.022**
VEGF (pg)	567.9 (1327.7)	1719.5 (4025.6)	**0.031**	2261.7 (4656.8)	1332.7 (2840.7)	0.185
TNF-α (pg)	78.8 (157)	554.7 (1846)	0.120	1334.2 (4014)	37.1 (549)	**0.000**
IL-1RA (pg)	100.9 (2529.9)	640.0 (1579.0)	0.082	414.5 (920.3)	616.4 (2686.4)	0.405
IL-21 (pg)	0.28 (18)	16.3 (80)	0.215	44.1 (99)	0.0 (24)	**0.001**
IL-8 (ng)	5.6 (14.7)	38.4 (98.9)	**0.010**	82.3 (110.8)	17.4 (65.1)	**0.036**
IL-1β (ng)	5.1 (7.4)	7.5 (29.1)	0.161	30.5 (58.7)	3.7 (7.6)	**0.000**
IL-6 (pg)	0.0 (244.8)	38.4 (637.8)	0.341	6.5 (993.3)	2.8 (547.2)	0.935
GCSF (pg)	0.0 (18.4)	0.0 (129.9)	0.440	0.0 (218.1)	0.0 (67.2)	0.647
GRO-α (pg)	40 (177.9)	15.5 (418.4)	0.981	0.0 (129.6)	23.7 (463.1)	0.309
IL-1α (pg)	98 (410)	344.8 (945.4)	0.160	888.5 (1072.3)	176.3 (437.8)	**0.000**
MIP-3α (pg)	0.0 (20)	0.0 (118)	0.546	0.0 (1711)	0.0 (42)	0.309
MMP-9 (ng)	64.7 (68.8)	236.2 (455.1)	**0.001**	242.2 (608.3)	176.7 (306)	0.415

Data are presented as median with inter quartile range (IQR). *Pseudomonas aeruginosa* or *Haemophilus influenzae* colonisation at baseline (V1).

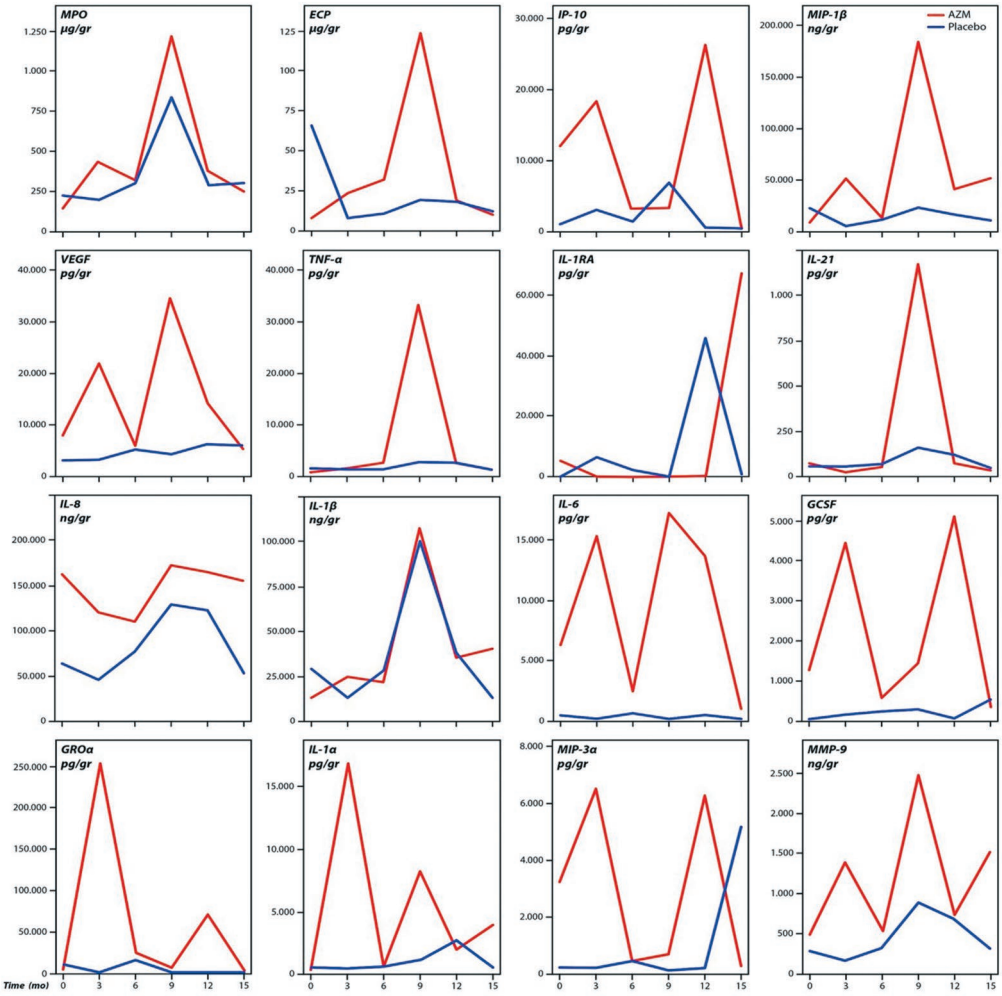


Figure 1: The effect of AZM maintenance treatment (per visit) on the inflammatory markers in sputum as compared to placebo

Sputum samples were obtained during stable disease at the outpatient ward. AZM or placebo treatment was stopped after 12 months. The last visit a 15 months is without maintenance treatment. Blue line: Placebo; Red line: AZM; *X*-axis: months; *Y*-axis: inflammatory marker in ug/gram sputum, pg/gram sputum or ng/gram sputum; AZM: azithromycin

The effect of AZM on the inflammatory profiles

To verify the presumed immunomodulatory, anti-inflammatory effect of AZM over the time, stable-state sputum inflammatory markers from AZM-treated patients were compared to the placebo-treated patients using mixed models. An overview of the effect of AZM on the inflammatory markers during maintenance treatment per visit is shown in Figure 1. No significant difference in pro-inflammatory cytokines were found in the AZM-treated patients as compared to the placebo-treated patients, and even higher levels of inflammation, except IL-1RA, were found during maintenance AZM treatment (supplemental 2).

Sputum inflammatory profiles during an exacerbation

Out of the 117 exacerbations treated with antibiotics during the study, only 29 (25%) sputum samples were collected and analysed. Of these 29 sputum samples, nine (31%) patients were treated with AZM, and 20 (69%) patients were treated with placebo. In the total population (both AZM- and placebo-treated patients) a significant upregulation of the inflammatory profile was found during an exacerbation as compared to the baseline inflammatory profile. (MPO $P = 0.003$; ECP $P = 0.004$; MIP-1β $P = 0.006$; VEGF $P = 0.027$; TNF-α $P = 0.005$; IL-21 $P = 0.010$; IL-8 $P = 0.013$; IL-1β $P = 0.003$; IL-1α $P = 0.005$; MMP-9 $P = 0.024$). When this population is divided into AZM-treated patients and placebo-treated patients, the presence of an infectious exacerbation was reflected by an excessive and significant increase in inflammation especially in the placebo-treated patients ($P = 0.012$ – $P = 0.046$) as compared to a non-significant upregulation of the inflammatory markers in the AZM-treated patients (Table 4).

Table 4: Sputum inflammatory profile in stable state and during an exacerbation grouped according to AZM treatment or placebo treatment

	AZM			Placebo		
	Baseline (n = 25)	**Exacerbation (n = 9)**	***P* value**	**Baseline (n = 29)**	**Exacerbation (n = 20)**	***P* value**
MPO (ug)	81.4 (203.1)	706.9 (1718.2)	0.068	53.3 (270.2)	222.3 (2260.1)	**0.015**
ECP (ug)	4.0 (9.0)	27.1 (151.2)	0.068	3.6 (9)	9.3 (67.8)	**0.015**
IP-10 (pg)	43 (167.6)	362.6 (1299)	0.144	76.3 (149.2)	108.4 (1270.7)	0.125
MIP-1β (ng)	4.3 (6.3)	54.8 (132.5)	0.144	7.1 (14.4)	19688.2 (83389.0)	**0.020**
VEGF (pg)	1443.3 (3936.9)	14802.6 (29729.1)	0.144	1700.1 (3985.2)	3990.8 (41378.1)	0.078
TNF-α (pg)	175.7 (1025)	3629.5 (22676.3)	0.068	129.9 (1841)	2287.0 (7732.4)	**0.020**
IL-1RA (pg)	617.2 (2112.2)	2760.9 (3649.9)	0.465	606.7 (1578)	1867.5 (17356.4)	**0.020**
IL-21 (pg)	1.8 (47)	306.2 (680.1)	0.109	8.4 (90)	59.7 (1392.0)	**0.046**
IL-8 (ng)	25 (101.4)	148 (407.5)	0.068	22.1 (70.3)	80.9 (239.4)	0.053
IL-1β (ng)	6 (14.7)	72.8 (140)	0.068	7.5 (41.8)	39 (392.2)	**0.012**
IL-6 (pg)	0 (922.3)	290.5 (1705.2)	0.109	63.9 (504.8)	29.2 (809)	0.937
GCSF (pg)	0 (129.9)	0 (992.9)	0.180	0 (26.1)	0 (35.6)	0.173
GRO-α (pg)	9 (418.4)	155.8 (1753.3)	1.000	17 (170)	0 (289.7)	0.594
IL-1α (pg)	286.8 (717.4)	1829.7 (3282.2)	0.068	285.3 (1071.9)	1069.1 (3207.4)	**0.015**
MIP-3α (pg)	0 (236)	101 (1754.3)	0.180	0 (64)	67.6 (215.4)	0.463
MMP-9 (ng)	194.9 (328.5)	851.8 (2389.9)	0.144	177.1 (375.1)	337 (2311.3)	0.061

Data are presented as median with inter quartile range (IQR) *Abbreviations:* Azithromycin (AZM)

The relation between baseline inflammation and the effect of AZM maintenance treatment based on number of exacerbations, quality of life (QoL) and lung function

In this analysis the inflammatory markers at baseline were divided into values above the median and under the median to evaluate the relation between the levels of inflammation at baseline and the response on AZM maintenance treatment based on number of exacerbations, FEV_1% of predicted and QoL.

Number of exacerbations

During AZM maintenance treatment a decrease in number of exacerbations was not related to a specific inflammatory marker at baseline. However, in patients with lower levels (under the median) of VEGF, IL1-RA, IL-6, GCSF, GRO-α, IL-1α at baseline a lower number of exacerbations during AZM maintenance treatment was found. These differences were marginal, and no specific inflammatory response is predictive for the effect of AZM maintenance treatment based on number of exacerbations. Figure 2 shows an overview of the inflammatory markers at baseline in relation to a decrease in number of exacerbations during maintenance AZM.

Quality of life

Figure 3 shows the relation between the inflammatory marker at baseline and the effect of maintenance AZM based on QoL by using the SGRQ. Overall, a decrease in the SGRQ total score was found during AZM maintenance treatment, representing a clinically relevant improvement of the QoL, expect for patients with, at baseline, higher levels of IL-8, IL-6, GRO-α, IL-1α, VEGF and lower levels of IP-10. When looking at distinct groups with higher or lower (compared to median) levels of inflammatory markers, however, no significant difference with respect to the effect of AZM treatment on QoL was found.

Lung function

Figure 4 shows the relation between the inflammatory markers at baseline and the improvement of FEV_1% of predicted during maintenance AZM treatment. A significant improvement in FEV_1% of predicted was found for patients with lower levels of the inflammatory markers VEGF (P = 0.011) IL-8 (P = 0.019), IL1-α (P = 0.034) and MMP-9 (P = 0.03) at baseline as compared to the higher levels (above the median) of these inflammatory markers at baseline.

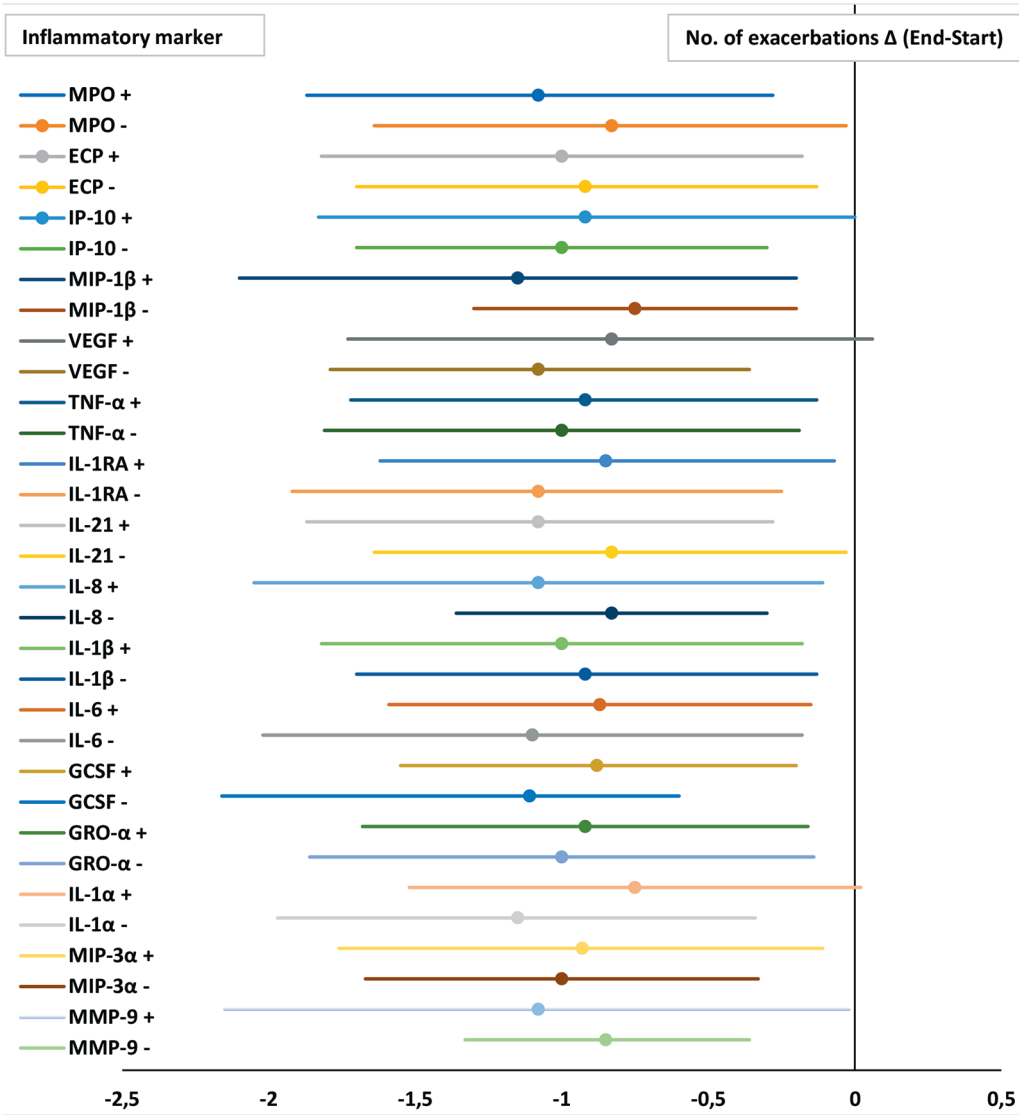


Figure 2: Association between baseline inflammatory profile and the effect of AZM maintenance treatment on the number of exacerbations in bronchiectasis

Forest plot: data are presented as Δ in number of exacerbations (End-Start) with 95% CI. *X*-axis: Decrease in number of exacerbations during 1-year AZM treatment. *Y*-axis: The + or – mentioned by the inflammatory markers represent respectively values above or below the median of the specific inflammatory marker at baseline

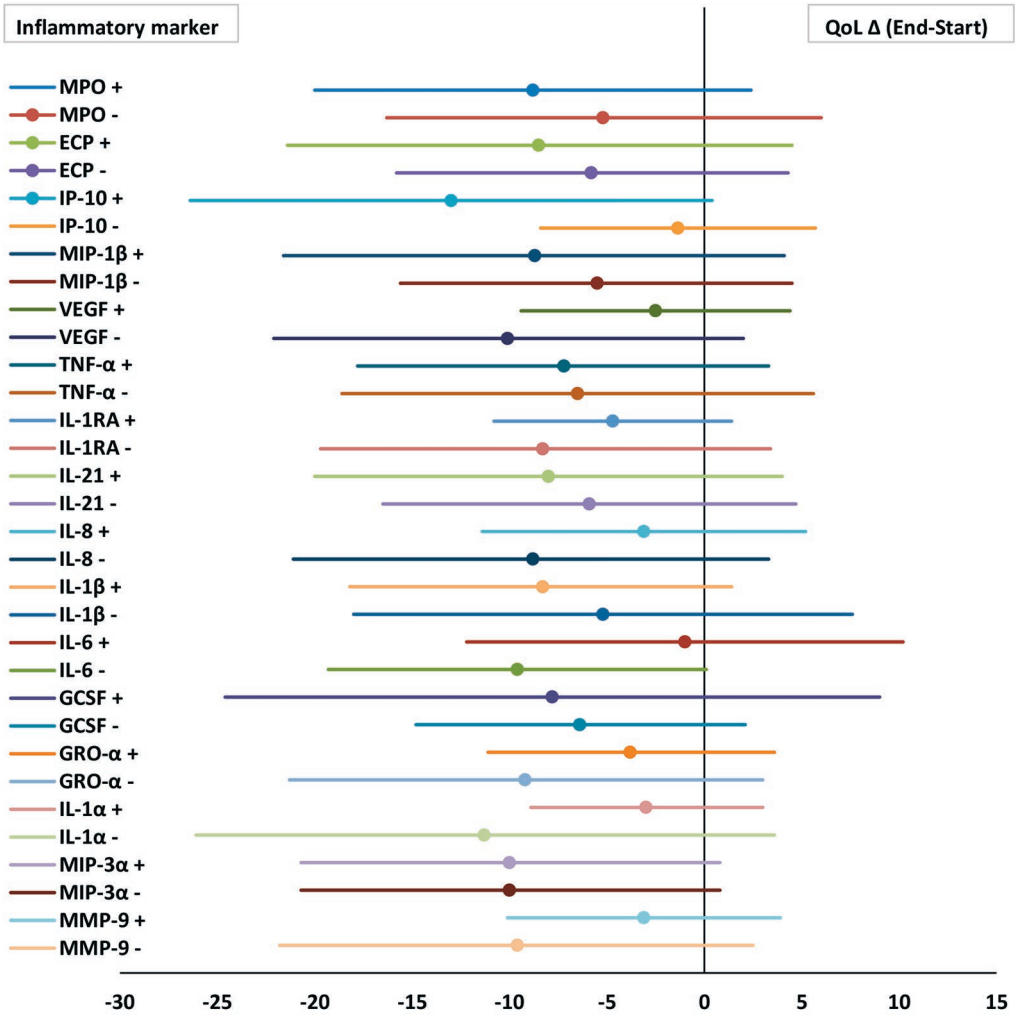


Figure 3: Association between baseline inflammatory profile and the effect of AZM maintenance treatment on SGRQ-total score in bronchiectasis

Forest plot: data are presented as Δ QoL (End-Start) with 95% CI. *X*-axis: Increase in QoL during 1-year AZM treatment based on a decrease of the SGRQ; St. George Respiratory Questionnaire. Minimal important difference of -4 correspondence with clinical relevance; *Y*-axis: The + or – represent respectively values above or below the median of the specific inflammatory marker at baseline.

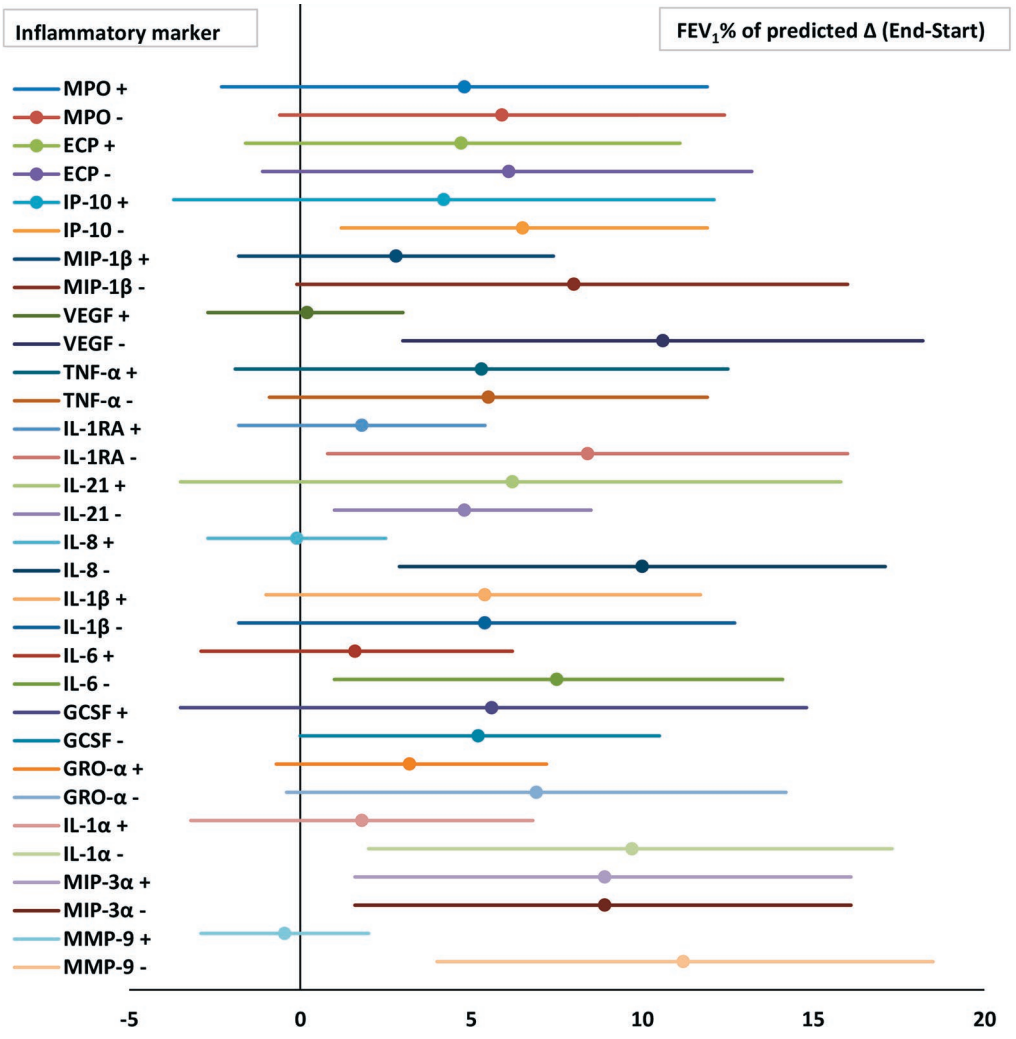


Figure 4: Association between baseline inflammatory profile and the effect of AZM maintenance treatment on change in lung function in bronchiectasis

Forest plot: data are presented as Δ FEV_1% of predicted (End-Start) with 95% CI. *X*-axis: Increase in FEV1% of predicted during 1-year AZM treatment. *Y*-axis: The + or – represent respectively values above or below the median of the specific inflammatory marker at baseline.

DISCUSSION

In the present investigator-initiated study we evaluated the inflammatory profile in expectorated sputum of patients with bronchiectasis participating in the BAT trial and treated with maintenance AZM or placebo for one year.[13] Our most remarkable finding was the fact that markers of airway inflammation remained stable or even increased during long-term macrolide treatment, suggesting that the clinically beneficial effect of macrolide treatment may not, or not as much, be driven by an anti-inflammatory effect as generally assumed. However, our results contrast with a recently published observational study whereby NETs were identified as a key marker of treatment response in bronchiectasis.[12] In this study, lower concentrations of NET proteins were found after one year of maintenance AZM, especially in patients with non-eosinophilic asthma and in patients with *PA* infection. NETs were also identified as a key marker of disease severity.[12] In our study, the inflammatory profile was examined at baseline in relation to the severity of the disease based on components of the BSI.[2] Similar results were found, whereby an increase in disease severity based on a FEV_1 of < 50% predicted as well as an increase in exacerbation rate in the year prior to the start of maintenance treatment were reflected by an upregulation of the pro-inflammatory markers at baseline.

In contrast to previous studies, the patients colonized with *PA* in our analysis showed no inflammatory upregulation.[2,25,26] Moreover, levels of VEGF, IL-8 and MMP-9 were significant lower as compared to non-*PA* patients, probably due to the diversity of *PA* strains in bronchiectasis patients.[27] Another speculative hypothesis is that long-term colonization with *PA* would lead to a more chronic inflammation and, to a lesser extent, active inflammation. In our analysis, only 10 patients were colonized with *PA*, and in addition, only 16 patients with *HI*. Due to the small number of samples, the results must be interpreted by caution. Surprisingly, the presence of *HI* in our sputum samples at baseline showed a significant upregulation of the inflammatory markers, suggesting a more marked inflammation in these patients, with probably even more clinical signs of active inflammation**.** In our study, patients with *HI* had more exacerbations in the year prior to the study as compared to patients colonized with *PA*. Both in COPD and bronchiectasis patients, *HI* is related to an increase in inflammation with higher levels of IL-6, IL-8, IL-1β and MPO and is an independent predictor for future exacerbations.[28,29]

These higher levels of inflammation were also found in our analyses, as compared to both non-*HI* and *PA* colonization.

Our analysis showed that during an exacerbation the inflammatory response increased, with an excessive and significant increase in patients treated with placebo as compared to a non-significant increase in the AZM-treated patients. This may suggest that there is indeed a dampening effect on the inflammatory response with macrolide treatment, but exclusively during exacerbations and, at least in the current study not picked up during stable state. In addition, this finding of reduced upregulation of inflammatory markers during an exacerbation may be driving the clinical finding of a marked reduction in the number of exacerbations in the active treatment group during the BAT trial.[13] However, for this sub analysis the sample size was low, with a total of 29 sputum cultures collected during the exacerbation.

Prior to this study, we hypothesized that the inflammatory profile at baseline might be predictive of the effect of macrolide treatment, with higher levels of inflammation predicting better outcome, because of its supposed anti-inflammatory mode of action. However, in the current study, upregulation of no specific inflammatory marker at baseline was predictive for the treatment effect of AZM on exacerbations, FEV_1% of predicted or QoL based on the SGRQ. Instead, lower values of (neutrophilic) inflammation expressed as based on VEGF, IL-8, IL-1α and MMP-9 were significantly associated with an increased improvement in FEV_1% of predicted during AZM maintenance treatment. This may reflect reduced disease severity at baseline with a higher tendency to regenerate and improve during treatment. However, an association between downregulation of these specific inflammatory markers was not for other outcome measures such as exacerbation frequency and QoL, so the importance of this finding remains unsure.

Contrary to what is generally believed, the current study failed to show an attenuation of the inflammatory response in bronchiectasis patients with AZM treatment. A previous published systematic review of Zimmermann et al.[18] described an overall decrease in inflammatory markers in both sputum and serum samples of patients with various kinds of respiratory tract infections/inflammation, and skin and eye infections treated with macrolides. However, AZM treatment was more frequently associated with no influence on the immunological markers as compared to the other macrolides. In contrast to these results, a review of Huckle et al.[30] included 12 RCT's of patients with stable COPD and described that prophylactic use of AZM (as compared to non-macrolides) is of benefit

in reducing exacerbation frequency with reduced levels of a wide range of inflammatory markers in sputum. The effect of macrolide maintenance treatment on the inflammatory markers in the heterogeneous group of bronchiectasis patients has been studied in detail in a limited number of previous studies. Conflicting results were found, with a decrease of concentrations of IL-8, NE, and MMP-9 after treatment with clarithromycin (for 3 months) or roxithromycin (for 6 months) in two small open label studies.[31,32] And in addition, one cohort study in patients with bronchiectasis and *PA* infection treated with maintenance AZM for one year found an decrease in NET concentrations.[12] However, in one other RCT included 20 patients, treatment with low- dose erythromycin for 2 months did not effected inflammatory markers as IL-1α, IL-8, and TNF-α.[33] Difference in treatment doses and duration of maintenance treatment of macrolides could be an explanation for these discordant results. Additionally, although we have measured a wide array of inflammatory markers, the effect of macrolide treatment on the immune system shows high complexity and is not fully understood yet. Therefore, one could argue that some effects may have been missed due underrepresentation of certain types of markers. However, this appears not very likely when considering the extensive panel, with markers representing different immunomodulatory pathways.[18] In light of the above; other factors likely contribute to the observed clinical benefit of macrolides treatment in bronchiectasis. A previous study showed that AZM attenuated IL-8 without attenuating neutrophilic inflammation, which is suggestive for the inflammatory response due to viral infections too.[34]

This is to our knowledge the first study investigating the effect of macrolide treatment on airway inflammation during exacerbations. Interestingly, during an exacerbation AZM treatment appeared to have a dampening effect on the upregulated inflammatory response during an exacerbation, as compared to placebo. However, due to the small number of samples collected during an exacerbation, these results should be interpreted by caution. Nevertheless, this agrees very well with the most important clinical effect of long-term macrolide treatment, which is a marked reduction of the exacerbation frequency.[13-15]

In our study the inflammatory profile was related to the severity of the disease expressed as an increase in number of exacerbations and a FEV_1 < 50% of predicted. This is in concordance with previous studies, mentioning upregulation of inflammatory markers (IL-8, IL-13, and NET proteins) as correlated to functional measurements of

disease severity and an increase in exacerbations.[8,9,12] In our study mainly neutrophilic inflammation was found with a significant upregulation of MPO, ECP, IP-10, TNF-α, IL-21, VEGF, MMP-9 in relation to the severity of the disease, however higher levels of ECP suggested also an eosinophilic component. These results are in line with earlier findings suggesting that in bronchiectasis patients the inflammatory profiles are dominated by neutrophilic inflammation, but there is also a role for eosinophilic inflammation, and other innate immune mediators.[4,10,12]

The results of our study are derived from a post-hoc analysis of the BAT trial[13] and showed additional information about sputum inflammation in bronchiectasis. However, there are some limitations to mention. From the total 83 patients conducted the BAT trial[13], a total of 391 sputa were collected during the study, from a maximum of 60 patients per visit (73%). During AZM treatment, the availability of sputum samples gradually decreased in contrast to the placebo-treated patients. Only the patients with persistent respiratory symptoms and probably a reduced response on AZM could still expectorate sputum at the end of the study. Second, mainly for logistical reasons, only a small number of sputum samples were collected during an exacerbation. And therefore, our subgroup analysis of the inflammatory profile during an exacerbation in the AZM-treated patients as compared to the placebo-treated patients must be interpreted carefully. In addition, the inflammatory markers were measured in spontaneously expectorated sputum, and not in bronchial alveolar lavage fluid, causing possible oral contamination and represent not exactly the inflammatory profile of the lower respiratory tract.

In conclusion, in this study, we investigated the longitudinal effect of macrolide maintenance treatment on airway inflammation in bronchiectasis patients during stable state disease and exacerbations and inflammation in relation to disease severity. We found that disease severity was related to a higher mainly neutrophilic inflammatory response at baseline, with a significant upregulation in patients especially colonized by *HI* and not in patients with *PA*. Maintenance AZM treatment did not attenuate the inflammatory response as compared to placebo, but a dampening effect on the immune response during exacerbation was seen in AZM-treated patients, which may be responsible for the observed clinical benefit of macrolide maintenance treatment.

For a better understanding of the pathways through which macrolides exert their effect in bronchiectasis, more research is needed. This may need to be more aimed at understanding an anti-bacterial or anti-viral effect and type of anti-inflammatory effect.

Also, our observation of reduced upregulation of the inflammatory response during an exacerbation warrants further research.

SUPPLEMENTAL MATERIAL

See the supplemental section at page 167.
Supplemental 1: Number of samples per visit in the total population.
Supplemental 2. The effect of maintenance AZM on the inflammatory profile in sputum. Results after mixed model analysis.
Supplemental 3: Assays.

DECLARATIONS

Ethics approval and consent to participate

This study is a sub analysis of the BAT study. The BAT study protocol was reviewed and approved by independent ethics committees and institutional review boards from all the 14 participating centers. The study was performed in accordance with the Good Clinical Practice guidelines, the International Conference on Harmonization guidelines, and the most recent version of the Declaration of Helsinki. Written informed consent for participation and publication of our results was obtained from all the participants at the screenings visit. The BAT study is registered on Clinical trials.gov number: NCT00415350.

Consent for publication and acknowledgements

Not applicable

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For the BAT study, WGB received an unrestricted grant from GlaxoSmithKline and the azithromycin was supplied by TEVA pharmaceutics. For this sub study of the BAT no funding was received.

Author's contributions

WGB, JA and LCT wrote the protocol and designed the manuscript. YS and RL did the immunological analysis. LCT, JA, HJD and WGB wrote the manuscript. LCT and HJD did the statistical analysis. LCT and HJD prepared the figures and tables. All authors (LCT, JA, HJD, YS, RL, HH and WGB) reviewed the manuscript and has given final approval. All authors read and approved the final manuscript.

Conflict of interest statement

RL reported grants paid to his institution from Foresee, Nutrileads, Longfonds, Chiesi and AstraZeneca. WGB reported grants paid to his institution from GlaxoSmithKline and reported consulting fee for adviesraad 2021 SOBI. JA reported grants paid to her institution from Longfonds. HH, LCT, HJD, YSP have nothing to disclose.

Data sharing statement

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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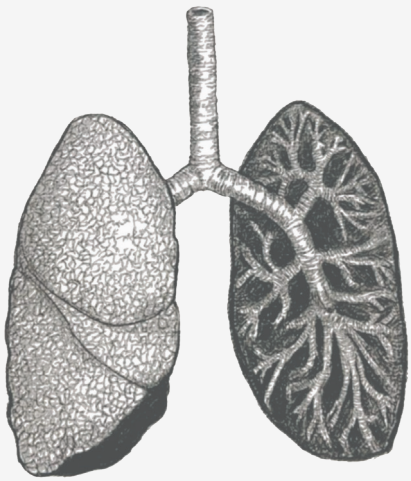
Chapter 5

Efficacy and safety of long-term azithromycin in bronchiectasis, analysis up to 5 years of maintenance treatment

Submitted



ABSTRACT

Introduction: For the frequent exacerbating bronchiectasis patients, macrolide maintenance treatment has shown favourable results and is nowadays increasingly used.[1] However, its effectiveness and safety beyond the first year is unknown. Therefore, the goal of this study is to evaluate whether the efficacy of azithromycin (AZM) is maintained after the first year, and up to 5 years of long-term treatment.

Methods: 101 patients were included in this retrospective analysis. Exacerbation frequency, lung function and sputum cultures were obtained. The prescribed dose, adverse events and reasons for discontinuation were investigated.

Results: A total of 34 (33.7%) patients were treated with AZM up to 24 months (median (IQR) of 67 (47) weeks), and 51 (50.5%) patients with AZM for at least 24 months and up to 5 years or even longer (median (IQR) 270 (138) weeks). 16 (15.8%) patients from the BAT study[2] never used AZM. The number of exacerbations per year remained low (median of 1 (IQR 3)). A decrease in number of sputum cultures was found at the end of the follow up period for both groups (63.5% (n = 47)). From the collected cultures, an increase in resistant pathogens was found (up to 100%), whereby the presence of *Pseudomonas aeruginosa* persisted and slightly increased from 6 (13.3%) to 10 (38.5%) cultures after 5 years. No differences in spirometry measurements were observed. Side effects were mild, and no cardiotoxicity was seen.

Conclusion: A prolonged beneficial effect, i.e., up to 5 years, of maintenance AZM was found. However, this is accompanied by an increase in *P. aeruginosa* isolation and microbial resistance. Further studies are needed to define an optimal treatment regime, with minimal development of resistance.

INTRODUCTION

Non-cystic fibrosis bronchiectasis (hereafter referred to as 'bronchiectasis') is a chronic progressive respiratory disease characterized by chronic bronchial dilatation and inflammation leading to recurrent exacerbations, hospital visits and an impaired quality of life (QoL).[3-5]

The current therapeutic interventions are aimed at reducing exacerbations and reducing chronic symptoms such as cough, sputum production, dyspnea, and fatigue. Among the best studies interventions is macrolide treatment, with convincing evidence in favor of long-term treatment in frequently exacerbating bronchiectasis patients.[2,6,7] In line with this, current bronchiectasis guidelines suggest offering long-term macrolide treatment to patients with three or more exacerbations per year.[3,4] In case first line treatment options (optimizing sputum clearance, treating the underlying disease) are not sufficient.

Momentarily, azithromycin (AZM) is one of the most widely used macrolide agents for maintenance treatment in bronchiectasis at this moment. However, chronic macrolide treatment has also been shown important downsides. Macrolide treatment of any duration has been associated with an increased risk of cardiovascular death and long-term use has been associated with an increase in microbial resistance.[8,9] In addition, side effects such as ototoxicity and gastro-intestinal complaints have been reported.[10]

Although studies have convincingly shown a reduction in exacerbations and chronic symptoms; its effectiveness and safety beyond the first year of maintenance treatment is not known.

The current study evaluates the efficacy and safety of AZM maintenance therapy beyond the first year, and up to 5 years, of maintenance treatment.

MATERIALS AND METHODS

Study population

Two cohorts of patients were combined in this retrospective analysis: 69 patients who participated in the Bronchiectasis and long-term Azithromycin Treatment (BAT) randomized controlled trial[2] and 32 AZM-treated patients from our outpatient clinic

were included. Patients were considered eligible when they had bronchiectasis on computed tomography (CT), had been using macrolide maintenance treatment for at least 6 months, and had chronic respiratory symptoms.

Study design

In this retrospective analysis, patients were divided in 3 groups: 1) no AZM treatment (patients from the BAT trial, which were treated with placebo), 2) AZM treatment for a period up to 24 months (patients from the BAT trial and the outpatient clinic), and 3) AZM treatment for 24 months or longer (patients from the BAT trial and the outpatient clinic).

Data was collected from the (electronic) patient files. Number of exacerbations, lung function measurements and sputum cultures were collected from the year prior to the start of AZM and every year during the 5 years of follow up. Duration and dose of macrolide treatment and/or other antibiotic use were registered for each patient, as well as side effects, reasons for AZM discontinuation, mortality, and cause of death. Unfortunately, Quality of life measurements were not available during the 5 years of follow up.

An exacerbation was considered if patients had progressive respiratory symptoms, with deterioration in three or more of the following key symptoms for at least 48h: cough, sputum volume and/or consistency, sputum purulence, breathlessness and/ or exercise tolerance, fatigue and/ or malaise, and hemoptysis. And if the clinician prescribes antibiotics and/or prednisolone for these respiratory symptoms.[11]

Lung function measurements, expressed in forced expiratory volume in one second (FEV_1) in liters and % of predicted and forced vital capacity (FVC) in liters and % of predicted, were obtained in the year prior to the start of AZM and every year during the 5 years of follow up.[12]

Sputum cultures were collected from spontaneously expectorated sputum and/ or broncho alveolar lavage. These sputum cultures were analyzed according to the standard methods to assess the presence of pathogens.[13] The predominant pathogen and macrolide resistance were obtained for every culture. Chronic infection was defined by the isolation of pathogens in a sputum culture on 2 or more occasions, at least 3 months apart in a 1-year period.

Percentages of side effects as gastrointestinal symptoms, pruritus and/or rash, abnormal liver function tests, nephrotoxicity, auditory symptoms, dizziness and/or

cardiotoxicity were obtained for each patient. Electrocardiographs (ECG) were observed and the QTC time was noted. Furthermore, reasons for discontinuation, mortality and cause of death were investigated in the study population.

Statistical analysis

Statistical analysis was conducted by using IBM SPSS 27 for MacOS. Discrete variables were presented as counts (percentage) and continuous variables as means ± SD if normally distributed and medians with (interquartile range) if not normally distributed. Data among groups were compared using T-test or ANOVA in case of parametric distributions, and a Mann-Whitney U test or Kruskal-Wallis test for nonparametric distributions. For comparison between groups with multiple variables a chi-square test was used. A *P* value of $P < 0.05$ was considered significant.

RESULTS

Baseline characteristics

One hundred and one patients were included in this retrospective analysis. From the 83 (100%) patients who participated in the BAT trial, a total of 14 (16.9%) patients were lost to follow up. The remaining 69 (68.3%) patients from the BAT trial, with 32 (31.7%) patients from our outpatient clinic were enrolled in the final analysis. The patient characteristics of the study population are shown in Table 1.

A total of 16 (15.8%) patients never used AZM, these patients were treated with placebo during the BAT study[2] and whereby AZM not was introduced after the study. Thirty-four (33.7%) patients were treated with AZM up to 24 months, with a median (IQR) of 67 (47) weeks. Fifty-one (50.5%) patients were treated with AZM for at least 24 months and up to 5 years or even longer (median (IQR) 270 (138) weeks).

The most prescribed dose of AZM was once daily 250mg (n = 77, 76.2%), whereas 8 (7.9%) patients were treated with AZM 3 times a week 500mg. AZM was mostly prescribed for the frequent exacerbating bronchiectasis patient, with at least 2 exacerbations in the preceding year (81.2%).[3,14] Four (4.7%) patients were treated with AZM without any previous exacerbation, however these patients had chronic cough with purulent sputum. For another 4 (4.7%) patients the number of exacerbations in the preceding year was not noted.

Table 1: Patient characteristics

	No AZM	**AZM up to 24 months**	**AZM 24 months or longer**
Total of patients, n (%)	16 (15.8)	34 (33.7)	51 (50.5)
Age, mean (SD)	77 (7.9)	70.7 (10.4)	70.4 (11.6)
Woman, n (%)	8 (50)	21 (61.8)	31 (60.8)
Current or former smoker, n (%)	10 (62.5)	17 (50)	23 (45.1)
No. of weeks of AZM maintenance treatment, median (IQR)	0 (0)	67 (47)	270 (138)
No. of exacerbations in year before study entry, median (IQR)	3 (0) min 3 – max 12	3 (2) min 0 – max 7	4 (3) min 0 – max 9
Lung function, mean (SD)			
FEV_1 % of predicted	85.3 (29.6)	83.4 (23.1)	79.7 (21.3)
FVC % pf predicted	104.1 (22.6)	95.2 (19.8)	97.2 (17.2)
Baseline sputum microbiology, n (%)			
No culture	3 (18.8)	5 (14.7)	6 (11.8)
No pathogen	5 (31.3)	6 (17.6)	5 (9.8)
Haemophilus influenzae	2 (12.5)	11 (32.4)	16 (31.4)
Pseudomonas aeruginosa	2 (12.5)	4 (11.8)	6 (11.8)
Moraxella catarrhalis	2 (12.5)	2 (5.9)	3 (5.9)
Staphylococcus aureus	1 (6.3)	1 (2.9)	6 (11.8)
Streptococcus pneumoniae	0	2 (5.9)	4 (7.8)
Escherichia coli	1 (6.3)	0	0
Klebsiella pneumoniae	0	1 (2.9)	1 (2.0)
Other	0	2 (5.9)	4 (7.8)
Aetiology of bronchiectasis			
Idiopathic	8 (50)	9 (26.5)	7 (13.7)
Post-infective	4 (25)	7 (20.6)	17 (33.3)
Yellow nail syndrome	1 (6.3)	0	0
α1 antitrypsin deficiency	1 (6.3)	1 (2.9)	0
Asthma	2 (12.5)	6 (17.6)	9 (17.6)
COPD	0	1 (2.9)	3 (5.9)
Immunodeficiency	0	8 (23.5)	6 (11.8)
Auto-immune disease	0	1 (2.9)	4 (7.8)

	No AZM	AZM up to 24 months	AZM 24 months or longer
PCD	0	1 (2.9)	2 (3.9)
Reflux	0	0	1 (2.0)
ABPA	0	0	2 (3.9)

All values are expressed as mean (SD) or median (IQR) unless stated otherwise. *Abbreviations:* forced expiratory volume in one second (FEV_1); forced vital capacity (FVC); interquartile range (IQR); standard deviation (SD); Chronic obstructive pulmonary disease (COPD); Primary ciliary dyskinesia (PCD); Allergic bronchopulmonary aspergillosis (ABPA).

Exacerbations

An overview of the number of exacerbations every year during 5 years after the start of AZM is shown in Table 2. During AZM maintenance treatment the number of exacerbations remains low, with a maximum of 1 exacerbation during each year. The number of exacerbations for patients treated with AZM up to 24 months remains even low after AZM discontinuation.

Table 2: Number of exacerbations during 5 years of AZM maintenance treatment

	No. patients	AZM up to 24 months (n = 34)	No. patients	AZM 24 months or longer (n = 51)	*P* value
No. of exacerbations in the year prior to the start of AZM treatment	31	3 (2-4)	50	4 (2)	0.35
No. of exacerbations in the first year	33	0 (0-1)	51	1 (0-1)	0.16
No. of exacerbations in the second year	26	0 (0-1)	48	1 (0-1)	0.06
No. of exacerbations in the third year	18	1 (0-1)*	48	0 (0-1.75)	0.61
No. of exacerbations in the fourth year	16	0 (0-1.75)*	42	1 (0-2)	0.18
No. of exacerbations in the fifth year	14	1 (0-2)*	35	1 (0-2)	0.47
Total no. of exacerbations in five years	34	1 (0-4)	51	3 (1-7)	

Data are presented as median with IQR. n = number of patients; Interquartile range (IQR); * Without AZM, AZM is already stopped in the 3rd, 4th, and 5th year.

Lung function

The lung function measurements showed no differences during the years of AZM treatment. A mean FEV_1% of predicted of 83.4% (SD 23.0) and a mean FVC of 95.2% (SD 19.8) was found before the introduction of AZM treatment for patients treated with AZM up to 24 months. During the years of AZM treatment no differences in lung function were found (FEV_1 85.2% SD 24.3; FVC 98.7% SD 25.7), with also no difference after discontinuation of AZM (FEV_1 80.0% SD 17; FVC 93.4% SD 23.1).

For the AZM-treated patients for 24 months or longer, also no differences were found in FEV_1% and FVC% of predicted with a mean of 77.1% (SD 19.1) in FEV_1 and 93.2% (SD 16.8) in FVC during AZM. These values were slightly the same before introduction of AZM with a mean FEV_1 of 79.7% (SD 21.3) and a mean FVC of 97.2% (SD 17.2) (supplemental 1).

Sputum cultures of the predominant pathogens

An overview of the available sputum cultures during 5 years of follow up is shown in Figure 1. Due to the retrospective design of the study, in combination with the beneficial effect of AZM, a total of 63.5% (n = 47) sputum cultures were available at the end of the follow up period as compared to baseline. For the AZM-treated patients up to 24 months, a decrease in *H. influenzae* was found during the years, with 11 (37.9%) positive cultures at baseline and 1 (5.9%) positive culture after 2 years of AZM treatment. The number of cultures (n = 4 (23.5%)) with *P. aeruginosa* remained stable during 2 years of AZM treatment.

For the AZM-treated patients for 24 months or longer the number of positive sputum cultures with *H. influenzae* was 16 (35.6%) at baseline and decreased during the 5 years of AZM treatment (n = 6 23.1%). However, the presence of *P. aeruginosa* persisted and slightly increased in this population from 6 (13.3%) to 10 (38.5%) positive cultures at the end of the follow up period. An overview of the development of macrolide resistance is shown in Table 3, whereby an increase in macrolide resistance was found, especially in the patients treated for AZM for 24 months or longer. Macrolide resistance for *S. pneumoniae* was not found in this study population, however the number of positive sputum cultures with *S. pneumoniae* was small. In addition, no positive sputum cultures were found for non-tuberculous mycobacterium (NTM).

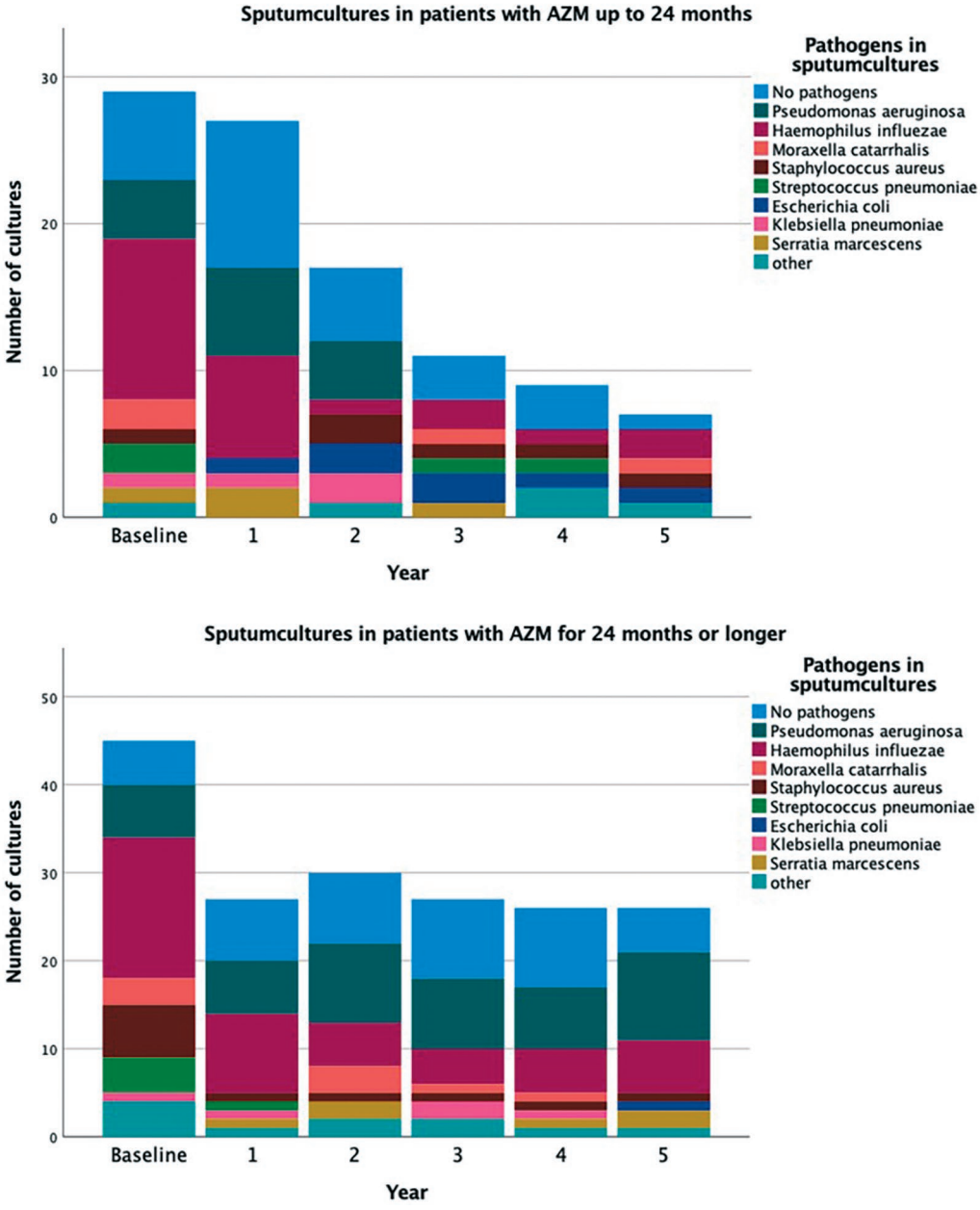


Figure 1: Overview of the sputum cultures during 5 years after the start of AZM maintenance treatment

Table 3: Number of resistant pathogens during AZM maintenance treatment up to 5 years

	Number of resistant pathogens for AZM up to 24 months (% of total)	Number of resistant pathogens for AZM 24 months or longer (% of total)
Baseline pathogens		
Haemophilus influenzae	6 (54.5)	7 (43.8)
Pseudomonas species	4 (100)	6 (100)
Staphylococcus aureus	–	1 (16.7)
Klebsiella pneumoniae	1 (100)	1 (100)
Other	2 (100)	–
Pathogens in the first year		
Haemophilus influenzae	3 (42.9)	3 (33.3)
Pseudomonas species	6 (100)	6 (100)
Escherichia coli	1 (100)	–
Klebsiella pneumoniae	1 (100)	1 (100)
Other	2(100)	2 (100)
Pathogens in the second year		
Haemophilus influenzae	–	3 (60)
Pseudomonas species	4 (100)	9 (100)
Moraxella catarrhalis	–	1 (33.3)
Staphylococcus aureus	–	1 (100)
Klebsiella pneumoniae	1 (50)	–
Other	–	1 (25)
Pathogens in the third year		
Haemophilus influenzae	2 (100)	3 (75)
Pseudomonas species	–	8 (100)
Moraxella catarrhalis	–	1 (100)
Staphylococcus aureus	–	1 (100)
Escherichia coli	1 (50)	–
Pathogens in the fourth year		
Haemophilus influenzae	1 (100)	3 (60)
Pseudomonas species	–	7 (100)
Moraxella catarrhalis	–	1 (100)
Staphylococcus aureus	–	1 (100)
Streptococcus pneumoniae	–	–

	Number of resistant pathogens for AZM up to 24 months (% of total)	Number of resistant pathogens for AZM 24 months or longer (% of total)
Escherichia coli	1 (100)	–
Klebsiella pneumoniae	–	1 (100)
Other	1 (50)	–
Pathogens in the fifth year		
Haemophilus influenzae	1 (50)	3 (50)
Pseudomonas species	–	10 (100)
Moraxella catarrhalis	1 (50)	–
Staphylococcus aureus	–	1 (100)
Streptococcus pneumoniae	–	–
Escherichia coli	1 (100)	1 (100)
Klebsiella pneumoniae	–	–
Other	1 (100)	–

Data are presented as number with percentages (%). N: number of patients; *Without AZM, AZM has been stopped in the AZM-treated patients up to 24 months.

Adverse effects

The side effects and reasons for discontinuation of AZM treatment are shown in Table 4. Twenty-one (24.7%) AZM-treated patients mentioned adverse effects, whereof 52.4% (n = 11) with gastro-intestinal side effects and 14.3% (n = 3) with pruritus and/or rash. No cardiotoxicity and/or prolonged QTc times were observed, however an ECG was performed in only 28% (n = 24) of the AZM-treated patients. A total of 37 (43.5%) patients discontinued the AZM maintenance treatment, whereby 4 (10.8%) patients discontinued due to side effects (Table 4). Of these, only 1 (2.7%) patient had gastro-intestinal complains.

A mortality rate of 4.7% (n = 4) was observed during the 5 years of follow up and was not directly related to AZM treatment or prolonged QTc intervals. One of the patients died due to pre-existent liver cirrhosis, which was not medication related.

Table 4: Adverse effects and reason for discontinuation

	Total AZM population n = 85	AZM up to 24 months n = 34	AZM 24 months or longer n = 51
Total number of adverse effects	21 (24.7)	8 (23.5)	13 (25.4)
Gastrointestinal symptoms	11 (52.4)	6 (75)	5 (38.5)
Pruritus/ Rash	3 (14.3)	0	3 (23.1)
Auditory complains and/or tinnitus	2 (9.5)	1 (12.5)	1 (7.7)
Dizziness	2 (9.5)	0	2 (15.4)
Liver function disorder	2 (9.5)	1 (12.5)	1 (7.7)
Fatigue	1 (4.8)	0	1 (7.7)
Renal dysfunction	0	0	0
Cardiotoxicity, prolonged QTC	0	0	0
Total number of ECG's during AZM treatment	24 (28)		
Number of patients discontinued AZM therapy	37 (43.5)	25 (73.5)	12 (23.5)
Reason for discontinuation due to adverse effects	4 (10.8)	4 (16)	0
No clinical improvement	6 (16.2)	3 (12)	3 (25)
Other treatment regime, inhaled antibiotics	1 (2.7)	1 (4)	0
Absence of respiratory infections	8 (21.6)	4 (16)	4 (33.3)
End of BAT study treatment	7 (18.9)	7 (28)	0
Hospitalization	1 (2.7)	1 (4)	0
Unknown	9 (24.3)	5 (20)	4 (33.3)
Death	1 (2.7)	0	1 (8.3)
Mortality rate	4 (4.7)	2 (5.9)	2 (3.9)
Malignancy	1 (25)	0	1 (50)
Pulmonary infection	1 (25)	0	1 (50)
COPD	1 (25)	1 (50)	0
Liver cirrhosis	1 (25)	1 (50)	0

Data are presented as number with percentages (%). N: number of patients; COPD: chronic obstructive pulmonary disease. *Without AZM, AZM has been stopped in the AZM-treated patients up to 24 months.

DISCUSSION

To our knowledge, this is the first study in bronchiectasis that evaluated the safety and effectiveness of long term, *i.e.,* up to 5 years, of maintenance AZM treatment. Previous studies demonstrated a significant reduction in exacerbations during the first 6 to 12 months of AZM maintenance treatment, and in our analysis this effect continued, even after 5 years of AZM maintenance treatment.[1,2,6,7] Comparable results were found in a retrospective analysis in patients with COPD, whereby the number of exacerbations remained low, even after 36 months of AZM maintenance therapy.[15] However, in one pediatric study in cystic fibrosis, this beneficial effect on number of exacerbations beyond the first year of AZM maintenance therapy was not observed, in which the loss of efficacy over time was explained by pathogen replacement and resistance emergence, and whereby AZM therapy was recommend for a maximum of 6 months.[16]

In our study, due to the retrospective design of the study, and probably the beneficial effect of AZM, the number of sputum cultures reduced during these 5 years. At baseline, *H. influenzae* was the most common observed pathogen, which decreased during AZM treatment. However, the presence of *P. aeruginosa* persisted and slightly increased at the end of the follow up period. The increase in *P. aeruginosa* positive sputum cultures during maintenance AZM treatment confirmed the hypothesis that macrolides may affect the normal microbiome an promotes the development of *P. aeruginosa*.[10,17] However, this development was not related to an increase in number of exacerbations, which can probably be explained by the 'breakdown' effect of AZM on the biofilm formation by mucoid *P. aeruginosa*.[18]

In addition, an increase in macrolide resistant was found, and is in line with earlier published data, whereby this increased risk of the development of macrolide resistance was described.[19,20] Previous studies in CF showed an increase in macrolide resistance of *S. aureus,* which was also seen in our population, especially in patients treated for 24 months or longer.[21] In contrast to numerous previous studies, macrolide resistance for *S. pneumoniae* was not found in this study population, however the number of sputum cultures positive for pneumococci was small.[9,22] No positive sputum cultures specific for NTM were found before the introduction of AZM, however during maintenance treatment, NTM were not consequently requested.

However, this increase in microbial resistance found in our study was not related to an increase in exacerbations or a decline in lung function, which is possibly based on the immunomodulating and anti-inflammatory effect of AZM.[23,24] New treatment modalities that targets the neutrophils may play an important role in the treatment of bronchiectasis. Promising results were recently published in a phase 2 trial of brensocatib, an inhibitor of dipeptidyl peptidase 1 (dpp-1), whereby a reduction of neutrophil serine protease activity was observed and was associated with a prolonged time to the first exacerbation and a decrease in number of exacerbations.[25]

AZM was mostly well tolerated in the present retrospective analysis. Mild side effects occurred, whereby gastro-intestinal disorders, mainly diarrhea, was the most frequent adverse event. Only 1 (1.2%) patient discontinued AZM maintenance treatment, due to severe gastro-intestinal symptoms. Hearing loss and tinnitus were not mentioned very often, however, no audiograms were performed during maintenance AZM treatment. Previous observational studies described an increased cardiovascular risk of AZM, which was not observed in this analysis.[26] Reasons for AZM discontinuation were mostly related to improvement of respiratory symptoms (not necessary anymore) or was not mentioned in the patient records.

This limited data found in the patient's records is unfortunately a main limitation of our study. Due to the retrospective design of the study and the long follow up period, no complete data is available for the patients during the 5 years of follow up. In the fifth year, data about the number of exacerbations was only available in 49 (57.6%) patients, and 49 (48.5%) sputum cultures were collected, whereby the conclusions must be interpreted by caution. However, no earlier studies in bronchiectasis analyzed up to 5 years of AZM therapy and reflects the current therapy in clinical practice.

The results of our study confirmed the known concerns about the increase in *P. aeruginosa* development and microbial resistance, whereby discontinuation of AZM should be considered after a treatment period for probably one year, or even shorter when in absence of respiratory complains. Other maintenance treatment regimens, for instance, an antibiotic holiday during the summer should be evaluated, with the hypothesize that the effect of AZM persisted, without development of microbial resistance.

In conclusion, in this retrospective analysis, a prolonged beneficial effect, i.e., up to 5 years, of maintenance AZM was investigated. Though, this is accompanied by a moderate increase in *P. aeruginosa* isolation and microbial resistance. Side effects of long-term AZM therapy were mild and mostly gastro-intestinal related, no cardiotoxicity was seen. Further studies are needed to define an optimal treatment regime, with a minimal development of resistance.

SUPPLEMENTAL MATERIAL

See the supplemental section at page 171.

Supplemental 1: Spirometry measurements during 5 years of AZM maintenance treatment.

DECLARATIONS

Author contribution

LC and WB conceived the study. JB and LC analyzed and interpreted the data and wrote the manuscript. WB and JA provided critical review. All authors approved the final manuscript.

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Conflicts of interest

All authors declare no competing interests.

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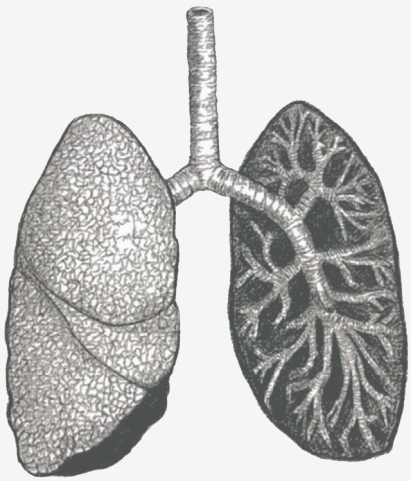
Chapter 6

The BATTLE study: effects of long-term tobramycin inhalation solution (TIS) once daily on exacerbation rate in patients with non-cystic fibrosis bronchiectasis -study protocol of a double blind, randomized, placebo-controlled trial

Terpstra, L. C., et al. "The BATTLE study: Effects of long-term tobramycin inhalation solution (TIS) once daily on exacerbation rate in patients with non-cystic fibrosis bronchiectasis. Study protocol of a double blind, randomized, placebo-controlled trial: study protocol." Contemporary Clinical Trials Communications 30 (2022): 101045.



ABSTRACT

Background: Patients with bronchiectasis typically suffer from chronic symptoms such as a productive cough with or without exacerbations leading to hospitalization, causing reduced quality of life (QoL) and mortality. Long-term inhaled antibiotics to treat chronic bronchial infection is registered for use in cystic fibrosis (CF) bronchiectasis. However, in patients with non-CF bronchiectasis data on long-term antibiotics are limited.

Objective: To investigate the effectiveness of maintenance tobramycin inhalation solution (TIS) in bronchiectasis patients without cystic fibrosis.

Study design: The BATTLE study is a randomized, double-blind placebo controlled, multicenter study in the Netherlands performed in patients aged ≥ 18-year-old with confirmed bronchiectasis, at least two exacerbations in the preceding year, and minimal one positive sputum culture with gram negative pathogens or *Staphylococcus aureus*, sensitive to tobramycin in the preceding year and at baseline. Patients will be treated with TIS once daily (OD) or placebo (saline 0.9%) OD for 52 weeks followed by a run-out period of 4 weeks after the last dose. The primary outcome is the yearly rate of pulmonary exacerbations. Among secondary outcome parameters are time to exacerbation, lung function, QoL, microbiological evaluation and safety.

Discussion: The BATTLE study is designed to determine the efficacy and safety of maintenance TIS OD in bronchiectasis patients colonized by different pathogens and could lead to important new evidence for TIS therapy in this population.

The BATTLE study is registered in Clinical trials.gov with registration number: NCT02657473.

BACKGROUND

Non-cystic fibrosis bronchiectasis (hereafter referred to as 'bronchiectasis') is chronic lung disease characterized by a vicious cycle of bacterial colonization, airway inflammation and airway structural damage, resulting in bronchial dilatation.[1] Patients commonly develop chronic symptoms of cough and sputum production, with recurrent infections, exacerbations and hospitalizations, accompanied by a reduced quality of life (QoL).[2,3] The origin of bronchiectasis varies, but the presence of microbial infection and a persistent inflammatory response are characteristic for the disease.[4] Bacteria isolated from the sputum of patients with bronchiectasis include *Streptococcus pneumoniae (S. pneumoniae)*, *Staphylococcus aureus (S. aureus)*, *Haemophilus influenzae (HI)*, and other gram-negative bacteria including *Pseudomonas aeruginosa (PA)*.[5] Chronic infections and colonization with these organisms, particularly with *PA* are associated with an increased number of exacerbations and hospital admissions, a reduced QoL and an increased morbidity and mortality.[6-8]

Reducing the number of exacerbations is the corner stone of long-term disease management, particularly for frequent exacerbating bronchiectasis patients.[9-12] Long-term systemic antibiotic treatment in bronchiectasis has shown favourable results, however antibiotic resistance may develop, relapse may occur when the antibiotics are stopped, and systemic antibiotics frequently fail to eradicate lung infections despite intensive therapy.[6,13]

An attractive alternative is the use of inhaled antibiotics which can provide a consistent deposition of high antibiotic concentrations directly to the site of infection with a lower risk of systemic toxicity and systemic adverse events like gastrointestinal side effects.[14,15] Inhaled antibiotics are part of the standard care in cystic fibrosis (CF) with *PA* colonization.[16-18] Nowadays the international bronchiectasis guidelines recommend inhaled antibiotics in patients with *PA* colonization.[9] However this is based on limited and conflicting data and not much is known about the ideal dosage regimen and duration of treatment. In addition, the population of patients with bronchiectasis not due to CF is older, has different comorbidities, and the risk of adverse events may differ from the CF population.[8,19,20] A few small studies with aerosolized tobramycin inhalation solution (TIS) are conducted in bronchiectasis patients colonized with *PA,* and found a decrease in *PA* density in sputum, with improvement of the secondary outcomes respiratory symptoms

and number of hospital admissions. In these studies, the duration ranged from 6 weeks to 13 months, TIS was given twice daily and the most common primary outcome was *PA* density in sputum.[8,20-22] No exact data is available of the effect of maintenance use of TIS once daily (OD) on exacerbation frequency, and especially in bronchiectasis patients colonized by *non-PA* Gram negative bacteria or *S. aureus*.

The present double-blind randomized placebo-controlled trial is designed to answer whether maintenance treatment with TIS OD may reduce the number of exacerbations in bronchiectasis as compared to placebo. This paper describes the study design of this randomized controlled trial: Effects of long-term To**B**r**A**mycin Inhala**T**ion Solu**T**ion (TIS) once dai**L**y on **E**xacerbation rate (BATTLE study) in patients with non-cystic fibrosis bronchiectasis.

METHODS

Objectives

The primary objective of the study is to determine whether maintenance use of TIS once daily (OD) as compared to placebo may reduce the number of exacerbations per year in patients with bronchiectasis. Secondary objectives are time to next exacerbation, lung function, QoL, laboratory test and microbiological evaluation in patients with bronchiectasis treated with TIS or placebo.

Study design

This study is a prospective, randomized, double-blinded, multicenter, placebo-controlled trial conducted in the Netherlands. The efficacy and safety of TIS OD will be evaluated as compared to placebo during a 52-week treatment period followed by a 4 weeks off-treatment follow up after the last study dose (Figure 1). Patients with bronchiectasis with recurrent exacerbations (≥ 2 per year) colonized by Gram-negative bacteria or *S. aureus* will be included and evaluated. Whereby colonization is defined as at least two results of sputum culture separated by at least 3 months in a year. Subjects meeting all study eligibility criteria (Table 1), including a sputum culture with the predefined bacterial pathogens at baseline, are randomized 1:1 to receive OD treatment with either TIS or placebo at visit 1 (week 0).

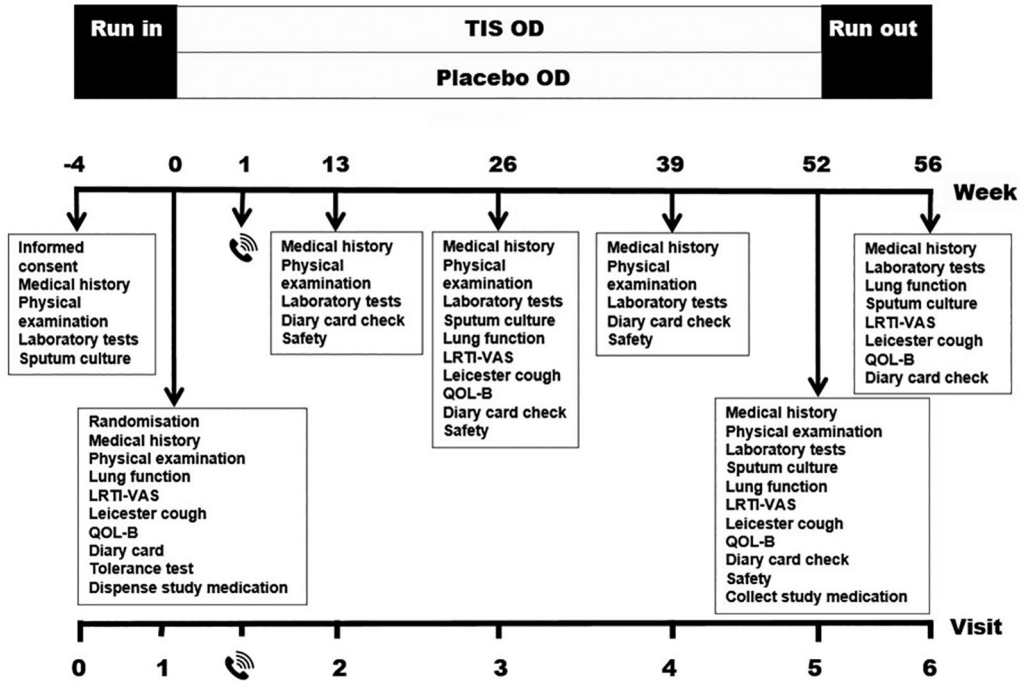


Figure 1: Study schedule

Abbreviations: Tobramycin inhalation solution (TIS); Once daily (OD); Lower respiratory tract infections – Visual Analogue Scale (LRTI-VAS); Leicester cough questionnaire (Leicester cough); Quality of life bronchiectasis questionnaire (QoL-B).

At visit 1 (week 0) a tolerance test will be performed with lung function examination before and after the first dose of the study medication to assess the occurrence of local intolerability. Patients are instructed to use the diary card weekly to examine the respiratory symptoms and side effects, and if so, to notice an exacerbation. Patients are asked to contact the study staff if the experience shortness of breath, cough, tinnitus, or other side effects. If needed patients are referred to otolaryngologist for hearing examination. Study visits are planned at the outpatient ward and consists of an up to 4 weeks screening phase (run-in period), a treatment phase of 52 weeks, and a washout phase (run out period) of 4 weeks (Figure 1). Throughout the study, visits are planned every 3 months, and clinical, QoL questionnaire's, bacteriological and laboratory examinations will be performed including lung function tests. Documented approval from the Independent Ethics Committees and Institutional Review Boards was obtained from all participating centers before start of the study, according to Good Clinical

Practice and local laws and regulations. Written informed consent was obtained from all participants.

Table 1: In and exclusion criteria for the BATTLE study

Inclusion criteria	Exclusion criteria
1. Age ≥ 18 years	1. Any exacerbation within the month prior to the start of the study
2. The presence of chronic respiratory symptoms such as cough, dyspnea, expectoration of sputum	2. Diagnosis of CF
3. Confirmed bronchiectasis by (HR)CT	3. Diagnosis of ABPA
4. Documented history of at least 2 pulmonary exacerbations treated with courses of antibiotics and/or prednisolone within 12 months before inclusion	4. Any oral, IV or inhaled antibiotics (except for macrolides) within 1 month prior to the start of the study
5. No course of antibiotics or maintenance antibiotics (except for macrolides) 1 month prior to the start of the study	5. Any IV or IM corticosteroids or change in oral corticosteroids (> 10mg) within 1 month prior to the start of the study
6. Minimal one documented sputum or BAL-fluid culture with gram-negative bacteria or *S. aureus* within 12 months	6. Any change/start treatment regimens of macrolides, hypertonic saline, inhaled mannitol or other mucolytics, or corticosteroids within 1 month prior to the start of the study
7. Growth of protocol defined pathogens (gram-negative bacteria or *S. aureus*) in sputum at randomization	7. Severe immunosuppression or active malignancy
	8. Active tuberculosis or NTM
	9. Chronic renal insufficiency (eGFR < 30ml/min)
	10. Use of loop diuretics, urea or mannitol
	11. Earlier diagnosed hearing impairment, balance disorders or neuromuscular disorders
	12. Serious active haemoptysis
	13. Have received an investigational drug or device within 1 month prior to the start of the study
	14. Serious or active medical or psychiatric illness
	15. Pregnancy and childbearing
	16. History of poor cooperation or non-compliance
	17. Unable to use nebulizers
	18. Allergic for tobramycin (or Saline 0.9%)

Abbreviations: Active allergic bronchopulmonary aspergillosis (ABPA); cystic fibrosis (CF); non-tuberculous mycobacterial infection (NTM); High resolution computed tomography (HRCT).

Intervention

TIS 300mg (TEVA pharmaceutics) OD and matched placebo (saline 0.9%) are provided in small plastic ampules of 5ml and are packed in identical sealed boxes and will be dispended on the regular visits every 3 months. The investigators are blinded for the content of the boxes. An OD dosing schedule, and not a twice daily (BID) dosing schedule is administered, which promotes adherence of the relatively intensive and time-consuming treatment schedule, whereby probably no increase in side effects or the development of tobramycin resistance. The study medication is delivered using the InnoSpire Deluxe air compressor (Philips Respironics) with a SideSteam Plus nebulizer with filter and mouthpiece (Figure 2). Salbutamol aerosol with aerochamber is administered every day at a dose of 200mcg before the study medication. The nebulizer will be used for about 10 minutes until the reservoir is empty. The whole procedure: preparation of the nebulizer, inhalation and cleaning takes about 20 minutes to complete. Afterwards the patient should rinse their mouth three times. The study drug administration is performed OD in the morning after completion of the patient's regular bronchiectasis treatment.

Figure 2: InnoSpire Deluxe with side stream plus with filter and mouthpiece

Study population

Patients are included with proven bronchiectasis on high resolution computed tomography ((HR)CT), at least two exacerbations in the year prior to the study and a positive sputum culture for gram negative pathogens or *S. aureus* in the preceding year, as well at the screening visit (week 0, visit -4). The inclusion and exclusion criteria for the BATTLE study are shown in Table 1. Patients with known CF, active allergic bronchopulmonary aspergillosis (ABPA), tuberculosis or non-tuberculous mycobacterial infection (NTM) are excluded. All co-medications are allowed, except for any oral (except for macrolides), IV or inhaled antibiotics or corticosteroids (> 10mg) within 1 month prior to the start of the study. Other exclusion criteria are the use of immunosuppressive agents or any change or start of treatment regimens with macrolides, hypertonic saline, inhaled mannitol or other mucolytics within 1 month prior to the start of the study. Because of the potential interaction with tobramycin the use of loop diuretics or mannitol are prohibited during the study.

Sample size

We hypothesize that maintenance treatment with TIS reduces the number of exacerbations per patient by 50%. This reduction seems clinically relevant and assumes that maintenance treatment with TIS OD as well as intermittent TIS BID is comparable to that of maintenance AZM treatment. This assumption is derived from data of the BAT (Bronchiectasis and Long-term Azithromycin Treatment) trial (placebo: mean 2,1 exacerbations (SD 1.6); azithromycin: mean 0,8 exacerbations (SD 1.1).[23] The reduction in percentage is used for the determination of sample size. A Poisson regression model is used to determine group size.[24] For type I error and type II error 0.05 and 0.2 are used respectively. The hypothesis is tested two sided. With a baseline exacerbation rate of 2.1 in the placebo group and an expected response rate ratio of 0.5 with an exposure time of 1 year a total of 18 evaluable patients are required to be on each treatment arm. With a drop-out percentage of 30% we must include totally 48 (24 patients per group) patients.[20,21] Due to unforeseen reasons 2 extra patients per arm are included. So, a total of 52 patients are included in the study.

Interim analysis

After inclusion and follow up of 50% of the randomized patients, an interim analysis will be conducted.

The interim analysis will be used to calculate the predetermined effect size, and whether more patients should be included depending on this effect size. Depending on the frequency of exacerbations at baseline and the effect size, a power analyses will be performed by using the Poisson regression model.[24]

Randomization

Block randomization of 4 will be performed centrally with an allocation ratio of 1:1 between groups. The numbers are anonymously dispensed in closed envelopes and stored at the pharmacy of the Northwest Clinics location Alkmaar and at an independent medical doctor. At visit 1 (week 0) patients receive the unique randomization number that allows subsequent identification of their randomized treatment group allocation. The study is blinded for treatment assignment and regimen.

STUDY ASSESSMENTS

Efficacy assessments

The primary efficacy endpoint of the BATTLE study is the number of exacerbations per year in patients with bronchiectasis. An overview of the efficacy and safety assessments are shown in Table 2. Secondary efficacy endpoints of the study are time to next exacerbation, lung function (FVC% predicted, FEV_1% predicted) and QoL measurements based on Lower respiratory tract infections – Visual Analogue Scale (LRTI-VAS), Quality of Life-Bronchiectasis questionnaire (QOL-B) and the Leicester cough score.[25-27] In addition, laboratory assessments and bacterial load in sputum with the development of tobramycin resistance will be evaluated in both groups.

Table 2: Overview of the study assessments for the BATTLE study

Primary assessment	**Secondary assessments**
1. Reduce in number of exacerbations	1. Time to next exacerbation
	2. Change in lung function (FEV_1% and FVC%)
	3. Change in QoL measurements (QoL-B, LRTI-VAS, Leicester cough)
Safety assessments	**Additional assessments**
1. Occurrence of any AE or SAE	1. Development of tobramycin resistance in sputum (if possible, with MIC values)
2. Occurrence of bronchospasm during the tolerance test	2. Bacterial load in sputum and pathogen eradication
3. Occurrence of bronchospasm, dyspnea, cough or other respiratory symptoms during the study	3. Occurrence of new pathogens
4. Occurrence of hearing impairment/ tinnitus	4. Change in inflammatory markers in serum
5. Change in safety laboratory values (renal and liver function)	5. Analyses of the use of inhaled medication (time consuming, treatment burden)

Abbreviations: Adverse event (AE); Serious adverse event (SAE); non-tuberculous mycobacterial infection (NTM); Forced expiratory volume in one second (FEV_1); Forced vital capacity (FVC); Quality of life (QoL); quality of life bronchiectasis questionnaire (QoL-B); Lower respiratory tract infections – Visual Analogue Scale (LRTI-VAS); Leicester cough questionnaire (Leicester cough); Minimum Inhibitory Concentration (MIC).

Safety assessments

Safety analysis include the occurrence of AE's and SAE's, with special interest to bronchospasm, hemoptysis, and hypersensitivity reactions. In addition, renal and liver function disorders, and the occurrence of hearing impairment and/or tinnitus probably due to the use of TIS will be evaluated.

The development of tobramycin resistant pathogens in sputum will be observed in both groups, including the occurrence of NTM and/or *Aspergillus fumigatus*.

A tolerance test will be performed with the first dose of study medication to evaluate the occurrence of inhalation induced bronchospasms. Defined as a decrease in FEV_1% of predicted of 20% following the study drug, and/or saturation < 90%. A safety analysis will be performed by an independent expert every six months during the study and might recommend termination of the study if there are any safety concerns, outstanding benefit and/or futility.

Exacerbations

All participants are provided with 24-hour contact details and invited to contact their respiratory physician or general practitioner and/or study staff when they experience worsening of respiratory signs and symptoms, to ensure that these symptoms are evaluated prospectively. In case of an exacerbation, participants are asked to provide a fresh sputum sample and are instructed to ensure that antibiotic and/or prednisolone prescriptions are provided by the own respiratory physician of the centres or the general practitioner. Criteria for a protocol defined pulmonary exacerbation (PDPE) are adjudicated prospectively by the treating respiratory physician. Deteriorations in respiratory symptoms that do not meet criteria for PDPEs will be termed non-protocol-defined pulmonary exacerbations (non-PDPEs). In these circumstances, participants are advised not require antibiotics, only if clinically indicated determined by the treating physician or the general practitioner. During an exacerbation, the study treatment is continued, if possible, unless the study medication is not tolerated, or the exacerbation is believed to be related to the study drug.

Exacerbation definition

A PDPE is defined as the presence of three or more of the following symptoms or signs for at least 24 hours:

1. increased cough
2. increased sputum volume and or/purulence
3. haemoptysis
4. increased dyspnoea
5. increased wheezing
6. fever (> 38.5°C) or malaise

AND the treating physician agreed that antibiotic and/or prednisolone therapy is required.

Study discontinuation

All participants who receive any study medication are encouraged to complete all the study assessments. However, participants can terminate the study at any time for any reason without any consequences. The investigator/treating physician can also decide

to withdraw a subject from the study for urgent medical reasons. We estimate a drop-out of 30% patients based on previous studies with inhaled antibiotics

STATISTICAL ANALYSIS

Efficacy analysis

Efficacy analysis will be performed in the intention to treat (ITT) population, defined as all randomized patients, and the modified intention to treat population (mITT). The mITT population excludes the randomized patients who dropped out directly after the tolerance test, or in the first two weeks of study treatment (non-evaluable). Analysis in the per protocol population (PP), defined as all randomized patients who received and completed treatment according to the study protocol for at least nine months, will serve as supporting evidence.

Methods of analysis

Descriptive statistics for patients treated with TIS or placebo will be calculated at baseline in the ITT population. Discrete variables will be presented as counts (percentage) and continuous variables as means with standard deviation (SD) if normally distributed and medians with interquartile range (IQR) if not normally distributed. Between groups differences will be tested using the students T-test or the Mann-Whitney U Test depending on the distribution. The effect of TIS as compared to placebo on exacerbation frequency will be analyzed by using the Poisson regression analysis. Linear mixed model analysis will be used to analyze the effects on lung function and QoL over the time. The minimal important difference (MID) of the QoL-questionnaires in bronchiectasis is previously reported only for the QoL-B respiratory symptom scale, with an increase of 8 points representing clinical relevance. The MID for the total score of the Leicester cough questionnaire is 1.3 points.[25,27] Time to first exacerbation during the treatment period, as well as during the wash-out period, will be assessed using Cox proportional hazards regression. A P value < 0.05 is considered statistically significant. The data will be collected in the online Electronic Case Report Form (ECRF) of Castor EDC – Medical Research and the analysis will be conducted by using IBM SPSS 25 for Windows.

DISCUSSION

The BATTLE study is designed to determine the efficacy and safety of maintenance TIS OD in bronchiectasis patients infected by tobramycin sensitive pathogens, with an expected reduction in number of exacerbations of 50% as compared to placebo (saline 0.9%). Secondary objectives of the study are time to next exacerbation, change in lung function, QoL measurements and safety assessments.

As mentioned, in CF bronchiectasis; a more homogenous and younger population; long-term inhaled antibiotics are part of the standard care with sufficient evidence, especially in *PA* colonized patients.[17] In bronchiectasis not due to CF, previous studies of inhaled TIS are small and limited, but showed some promising results with a decrease of *PA* density in sputum and improvement of respiratory symptoms.[8,20,21] Other studies with inhaled antibiotics in bronchiectasis found also trends towards clinical benefit of the inhaled treatment regime, however the primary endpoints were not met.[28-30] Though, a recently published meta-analysis of inhaled antibiotics demonstrated a significant reduction in exacerbations with no significant improvement of respiratory symptoms or QoL.[14] The results of this BATTLE study can complement the current evidence in this heterogenous and older population of bronchiectasis patients, with different etiologies and more comorbidities.

The strengths of our RCT are the multicentre study design, with the strict inclusion and exclusion criteria, and a predefined clear definition of an exacerbation, whereby the impact of variability between the clinical assessments should be reduced. Safety will be monitored closely, and all patients underwent a tolerance test with the randomized study medication at visit 1, to observe the development of bronchospasms.

In addition, not only patients with *PA* colonization will be included, but also patients colonized with other gram-negative and gram-positive pathogens. Beside *PA,* also other pathogens often colonize bronchiectasis patients and may also be associated with poorer outcomes.[2] It is therefore important to determine the effect of inhaled tobramycin on different pathogens, and could provide insight into treatment with TIS in *non-PA*. All aetiologies are included in our study, excluding the bronchiectasis patients diagnosed with ABPA or active tuberculosis or NTM, which reflects the daily patient population of non-CF bronchiectasis.

A unique feature of our BATTLE design is the OD maintenance treatment with TIS or placebo.

Twice daily on/off dosing was originally chosen for TIS in CF with the underlying idea to maximize the treatment effect and reduce the development of tobramycin resistance pathogens, however there is a lack of evidence for this specific treatment schedule.[31] During daily practice, a decrease in adherence to therapy developed due to the BID cycle of this intensive therapy, with an increase of treatment burden. The use of antibiotic inhalation solution is time consuming and will take about 20 minutes a day, including preparation of the InnoSpire and the cleaning protocol afterwards.

Intravenous administration of aminoglycosides have shown that OD dosing is equivalent in terms of antimicrobial efficacy compared to more frequent dosing.[35-37] And in addition, the OD dosing is supported by knowledge that its bactericidal activity is concentration-dependent with a long post-exposure antibiotic effect.[32-34]

A possible disadvantage of this continuous OD treatment instead of the on/off schedule is probably an earlier development of tobramycin resistance or an increase of side effects, for example the occurrence of local intolerability.

In conclusion, the BATTLE study is designed to determine the efficacy and safety of maintenance TIS OD in bronchiectasis patients infected by different pathogens and could lead to advances in the treatment of bronchiectasis, including an optimal treatment regime for TIS therapy in this population.

SUPPLEMENTAL MATERIAL

No supplemental material for this chapter.

DECLARATIONS

Ethics approval and consent to participate

Approval of Independent Ethics Committees and Institutional Review Boards was obtained from all the participating centers. Clinical trials.gov number of the BATTLE

study: NCT02657473. EudraCT number of the BATTLE study: 2016-000166-35. Written informed consent was obtained from all the participants at the screening visit.

Funding

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Authors contributions

WGB and LCT designed and drafted the manuscript. All authors were involved in revising the manuscript and have given final approval of the version to be published.

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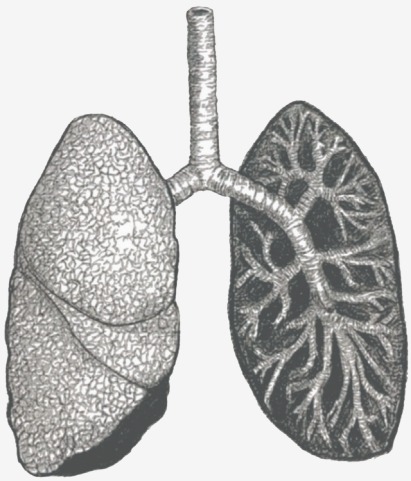
Chapter 7

Effects of longterm tobramycin inhalation solution (TIS) once daily on exacerbation rate in patients with non-cystic fibrosis bronchiectasis. The BATTLE randomized controlled trial

Terpstra, Lotte C., et al. "Effects of long-term tobramycin inhalation solution (TIS) once daily on exacerbation rate in patients with non-cystic fibrosis bronchiectasis." Respiratory Research 23.1 (2022): 330.



ABSTRACT

Background: Use of long-term tobramycin inhalation solution (TIS) has been shown beneficial in cystic fibrosis (CF) and earlier findings also suggest a benefit in non-CF bronchiectasis.

We investigated the efficacy and safety of maintenance TIS once daily (OD) in frequent exacerbating bronchiectasis patients chronically infected by different pathogens sensitive for tobramycin.

Objective: The primary outcome was the frequency of exacerbations during the 12-month study period. Secondary outcomes were time to first exacerbation, change in lung function and quality of life (QoL), bacterial analysis and safety.

Materials/patients: In this multicenter RCT patients aged ≥ 18-year-old were included with confirmed bronchiectasis and ≥ 2 exacerbations in the preceding year. Patients were assigned (1:1) to receive TIS or placebo OD for 1-year.

Results: 58 patients were included of which 52 were analyzed in the mITT analysis. TIS reduced exacerbation frequency with a RR of 0.74 (95% CI 0.49 – 1.14) (P = 0.15). Within the TIS population a decrease in number of exacerbations was found (2; P = 0.00), which was also seen in the placebo-treated patients (1.5; P = 0.00). In the TIS-treated patients the QoL improved (LRTI-VAS P = 0.02 Leicester Cough P = 0.02) without additional safety concerns. No differences were found for the other secondary outcomes.

Conclusion: Long-term TIS OD is a safe treatment modality and showed a non-significant reduced exacerbation frequency of 0.74 as compared to placebo in bronchiectasis patients chronically infected by tobramycin sensitive pathogens. TIS OD may be a potential therapeutic strategy in selected patients with bronchiectasis suffering from a high burden of disease.

The BATTLE study was registered at Clinical trials.gov number: NCT02657473.

INTRODUCTION

Non-cystic fibrosis bronchiectasis is a heterogenous respiratory disease characterized by chronic symptoms of cough and sputum production, with recurrent infections, exacerbations and hospitalizations, resulting in a reduced quality of life (QoL).[1,2] Exacerbations are associated with an increased morbidity and mortality, and are more common in patients with chronic bacterial infection.[1,3-5] Particularly in patients chronically infected with *Pseudomonas aeruginosa,* and to a lesser extent with *Haemophilus influenzae.*[1,6] Reducing the number of exacerbations is the corner stone of long-term disease management, with favourable results for long-term macrolide treatment.[7-11] However macrolide therapy is not always as effective, antibiotic resistance may develop and relapse may occur after discontinuation. In addition, long-term treatment with macrolides is associated with gastro-intestinal side effects, and the risk of QT prolongation, which frequently interacts with other medication. [3,12,13]

An attractive alternative may be the use of inhaled antibiotics which can provide a consistent deposition of high antibiotic concentrations directly to the site of infection with a lower risk of toxicity or systemic adverse events.[8,14] In cystic fibrosis (CF) long term inhaled antibiotics have been shown to reduce lung function decline and exacerbations and are part of the standard care in CF with *P. aeruginosa* chronic infection.[15-17] The evidence for inhaled antibiotics in non-CF bronchiectasis is limited, however the bronchiectasis guidelines recommend inhaled antibiotics in patients with *P. aeruginosa* chronic infection.[18,19] A recently published meta-analysis supports this recommendation, however not much is known about the ideal dosage regimen and duration of treatment, and the preference for a certain type of inhaled antibiotics.[8]

For aerosolized tobramycin inhalation solution (TIS) a few small studies were conducted in bronchiectasis patients colonized with *P. aeruginosa* and described a decrease in *P. aeruginosa* density in sputum, with an improvement of the respiratory symptoms. In these studies, the duration ranged from 6 weeks to 13 months, TIS was in the majority given twice daily 28 days on-off, and the most common primary outcome was *P. aeruginosa* density in sputum.[20-23] No data have been published about the effect of maintenance use of TIS once daily (OD) on exacerbation frequency, and especially in bronchiectasis patients with chronic infection by *non- P. aeruginosa* Gram-negative bacteria or *Staphylococcus aureus (S. aureus).*

In the present multicentre randomized controlled trial, effects of long-term toBrAmycin inhalaTion soluTion once daiLy on Exacerbation rate, The BATTLE study, we investigated the effect of TIS OD on exacerbation frequency in bronchiectasis patients during one-year maintenance treatment.

METHODS

Study design

The BATTLE study was a multicenter, double-blind, placebo-controlled randomized controlled trial, conducted in 6 hospitals in the Netherlands between September 2016 and December 2019. Full details of the study design are described in Chapter 6 of this thesis. Documented approval from the Independent Ethics Committees and Institutional Review Boards was obtained from all participating centers before start of the study and after the interim analysis.

Study population

Patients with proven bronchiectasis on HRCT, aged ≥ 18-year-old, chronic respiratory symptoms and at least two respiratory exacerbations treated with antibiotics and/or prednisolone in the preceding year were recruited from the outpatient clinic. All participants had one or more positive sputum cultures for gram-negative pathogens or *S. aureus* in the preceding year, as well as one positive sputum culture with the predefined pathogens at baseline. Patients with known CF, active allergic bronchopulmonary aspergillosis, tuberculosis, or non-tuberculous mycobacterial infection were excluded. Other exclusion criteria were the use of maintenance antibiotics, except for maintenance treatment with macrolides if treatment was not initiated within 1 month prior to study entry. We also excluded patients treated with prednisolone > 10mg per day for > 1 month, and/or patients treated with mucolytics. For safety reasons, patients with chronic renal insufficiency (GFR< 30ml/min), earlier diagnosed tinnitus, hearing impairment, balance disorders or neuromuscular disorders were excluded. An overview of the complete inclusion and exclusion criteria are shown in supplemental 1.

Procedures

After the run-in period of 4 weeks, stable bronchiectasis patients were randomly assigned (1:1) to receive TIS 300mg/5ml OD or placebo (NaCl 0.9%) OD for 52 weeks by using the InnoSpire Deluxe compressor (Philips Respironics) with a SideSteam Plus nebulizer with filter and mouthpiece.[24]

All patients underwent a tolerance test with spirometry before and after the first dose of the study medication to assess the occurrence of local intolerability. Airway hyperresponsiveness was defined as a decrease in FEV_1% of predicted of 20% following the study medication and/or saturation < 90%, and/or signs of bronchospasm. Study visits were planned at the outpatient ward every 3 months, and clinical, QoL questionnaire's, bacteriological and laboratory examinations were performed as well as spirometry. The study medication was delivered from a central pharmacy to each study center in batches, stored at 2-8°C. Medication was dispensed to the patient at each visit for a period of 3 months. The empty ampules were collected at each visit in blinded sachets. Patients were asked to use the diary card weekly to examine the respiratory symptoms and if so, to notice an exacerbation. The final visit was conducted 4 weeks (run-out period) after the end of the treatment period. See Chapter 6 of this thesis for an overview of the study schedule.

Outcomes

The primary outcome of the study was the number of exacerbations during the 1-year treatment period. A protocol defined pulmonary exacerbation (PDPE) was defined as the presence of three or more of the following symptoms or signs for at least 24 hours: 1) increased cough; 2) increased sputum volume and/or purulence; 3) haemoptysis; 4) increased dyspnoea; 5) increased wheezing; 6) fever (> 38.5°C) or malaise AND the treating physician agreed that antibiotic and/or prednisolone therapy was required. Secondary outcomes were time to next exacerbation, change in lung function (FVC% predicted, FEV_1% predicted) and QoL measurements based on lower respiratory tract infections- visual analogue scale (LRTI-VAS), Quality of Life-Bronchiectasis (QOL-B) and the Leicester cough score.[25-27] In addition, change in biomarkers, liver function and renal function, and bacterial diversity in sputum with the development of tobramycin resistance were evaluated in both groups. Additional safety assessments were assessed in both groups. For an overview of the study assessments see supplemental 2.

Statistical analysis

Details of the randomization process, sample size calculations and statistical analyses were described in Chapter 6 of this thesis. Using an expected baseline exacerbation frequency and effect size, a power analyses was done based on a Poisson regression model. After inclusion and follow up of 50% of the randomized patients an interim analysis was conducted to test the assumptions used in the sample size calculation. The interim analysis revealed that the assumptions made for the sample size calculation were appropriate. However, the drop-out rate was 34%, which was higher than the expected drop-out rate of 30% and made it necessary to increase the sample size by 6 patients.

The primary efficacy analysis was performed on the modified intention to treat (mITT) population. Patients who were randomized but dropped out directly after the tolerance test due to a moderate-severe bronchial obstruction, or during the first 2 weeks of the study treatment, were excluded from the mITT analysis. All randomized patients who received and completed treatment according to the study protocol for at least 9 months were included in the per protocol (PP) analysis. Early termination of the study or incomplete data collection was defined as non-evaluable. Statistical analysis was performed using SPSS version 25. The analyses were performed on the mITT- and the PP-population; discrete variables were presented as counts (percentage) and continuous variables as means with standard deviation (SD) if normally distributed and medians with interquartile range (IQR) if not normally distributed. Between groups differences were tested using the chi-square or Fisher exact test if appropriate in case of nominal or ordinal variables. In case of continuous variables, the student T-test or the Mann-Whitney U Test depending on the distribution was used. The effect of TIS as compared to placebo on exacerbation frequency was analyzed by means of negative binomial regression analysis correcting for the exacerbation rate in the year prior to the study. The association between use of TIS and exacerbation rate was expressed as a rate ratio with 95% confidence interval (RR (95% CI)). Linear mixed model analysis was used to analyze the effects on lung function and QoL over time. Results were presented as differences with *P* values. Time to first exacerbation during the treatment period was analyzed using a Cox regression analysis. Resulting associations were presented as hazard ratios with 95% confidence intervals (HR (95% CI). A P value < 0.05 was considered statistically significant.

RESULTS

Patients

A total of 58 patients were randomly assigned to receive either TIS or placebo OD and were included in the ITT-population. A total of 6 patients (3 patients in each group) dropped out in the first 2 weeks of study treatment (Figure 1). These patients were excluded from the mITT population. Baseline patient characteristics of the mITT population were well balanced between TIS and placebo (Table 1), except for maintenance stable doses of prednisolone < 10mg, which was significantly more frequent in the TIS population (P = 0.04). All patients passed the tolerance test at visit 1, with no occurrence of severe bronchus obstruction (defined as a decrease in FEV_1% of predicted of > 20% and/or saturation < 90%) after the first dose of the study medication. Treatment compliance was high; from empty ampules count, we estimated that patients adhered 94% over the time in the TIS group and 95% in the placebo group.

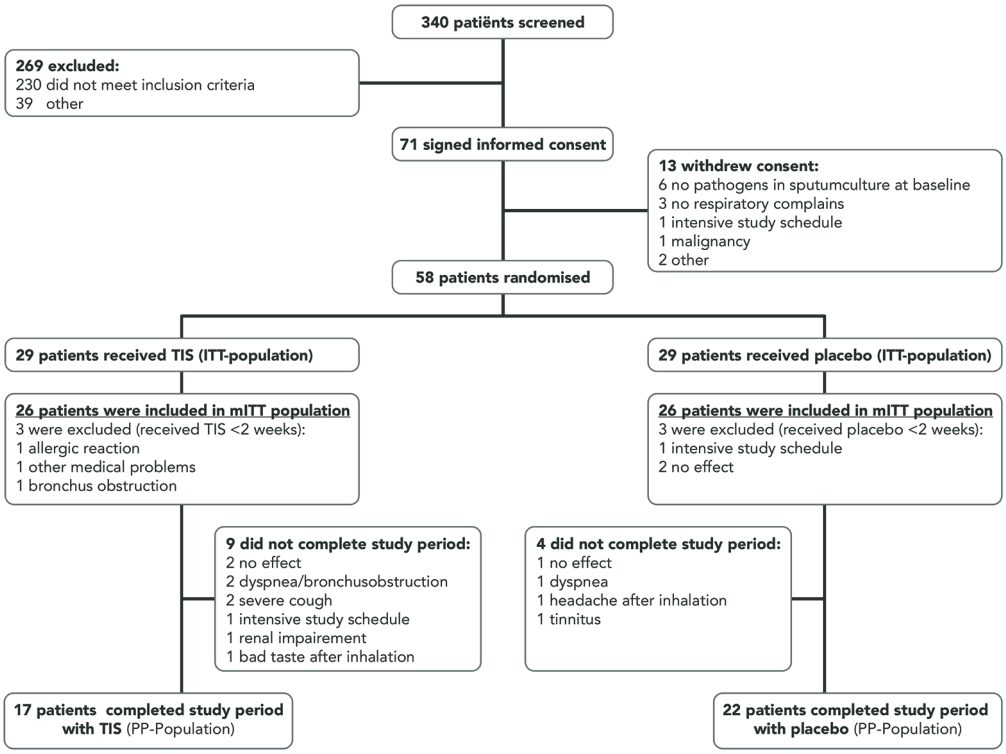


Figure 1: Study flow chart

Abbreviations: Tobramycin inhalation solution (TIS); Intention to treat population (ITT); modified intention to treat population (mITT); per protocol population (PP); Patients were screened in 6 hospitals

Table 1: Baseline patient characteristics of the modified intention to treat (mITT) population

	TIS n = 26	**Placebo n = 26**
Age, mean (SD)	67.9 (6.6)	64.1 (14.0)
Female, n (%)	13 (50)	17 (65.4)
No. exacerbations in the year before study entry, median (IQR)	4 (3-5)	3.5 (2-5.25)
Smoking status, n (%)		
Current	1 (3.8)	1 (3.8)
Former	13 (50)	15 (57.7)
Never	12 (46.2)	10 (38.5)
Etiology, n (%)		
Post-infective	4 (15.4)	8 (30.8)
Idiopathic	6 (23.1)	3 (11.5)
COPD	6 (23.1)	6 (23.1)
Asthma	3 (11.5)	4 (15.4)
Immunodeficiency	3 (11.5)	2 (7.7)
Rheumatic disease	1 (3.8)	1 (3.8)
Primary ciliary dyskinesia	0	1 (3.8)
Alpha-1 antitrypsin deficiency	0	1 (3.8)
Yellow nail syndrome	1 (3.8)	0
Aspiration	2 (7.7)	0
Charlson comorbidity index, n (%)		
1 to 2	19 (73.1)	23 (88.5)
3 to 4	7 (26.9)	3 (11.5)
Pulmonary function % predicted at baseline, mean (SD)		
FEV_1	65.9 (24.9)	70.5 (24.0)
FVC	84.4 (20.1)	90.2 (18.6)
Maintenance AZM, n (%)	7 (26.9)	4 (15.4)
Duration of AZM therapy in weeks, median (IQR)	87.8 (3-500)	49.7 (4-100)
Maintenance prednisolone during the study < 10mg, n (%)	5 (19.2)	0
Maintenance immunoglobulin therapy, n (%)	1 (3.8)	1 (3.8)
Physiotherapy, n (%)	7 (26.9)	8 (30.8)

	TIS n = 26	Placebo n = 26
Inhaled medication, n (%)		
SABA	15 (57.7)	18 (69.2)
SAMA	3 (11.5)	8 (30.8)
LABA	17 (65.4)	18 (69.2)
LAMA	10 (38.5)	10 (38.5)
ICS	17 (65.4)	19 (73.1)
Mucolytics (hypertonic saline) in the years before study entry	5 (19.2)	4 (15.4)
Pathogens in sputum at baseline, n (%)		
Pseudomonas aeruginosa	5 (19.2)	9 (34.6)
Haemophilus influenzae	7 (26.9)	9 (34.6)
Staphylococcus aureus	4 (15.4)	2 (7.7)
Streptococcus pneumoniae	0	1 (3.8)
Other	10 (38.4)	5 (19.3)
QoL questionnaires, mean (SD)		
QoL-B Physical	49.8 (30.0)	42.5 (34.8)
QoL-B Role	64.4 (24.7)	59.2 (23.9)
QoL-B Vitality	53.1 (17.4)	48.7 (17.6)
QoL-B Emotional	87.9 (10.5)	82.4 (16.5)
QoL-B Social	65.0 (20.9)	60.1 (23.4)
QoL-B Treatment Burden	66.4 (17.9)	64.0 (19.4)
QoL-B Health perceptions	42.4 (16.8)	43.2 (18.9)
QoL-B Respiratory symptoms	56.1 (17.8)	57.8 (16.7)
LRTI-VAS Total score	21.0 (7.3)	21.0 (8.5)
Leicester Cough Total score	13.4 (2.9)	13.8 (4.2)

Data are presented as n (%), mean (SD) or median (IQR). *Abbreviations:* Tobramycin Inhalation Solution (TIS); Forced expiratory volume in one second (FEV_1); Forced vital capacity (FVC); Azithromycin (AZM); short acting β agonist (SABA); Short acting anticholinergics (SAMA); long acting β agonist (LABA); long acting anticholinergics (LAMA); inhalation corticosteroids (ICS); Quality of life (QoL); Quality of life bronchiectasis questionnaire (QoL-B); Lower respiratory tract infections – Visual Analogue Scale (LRTI-VAS); Leicester cough questionnaire (Leicester cough).

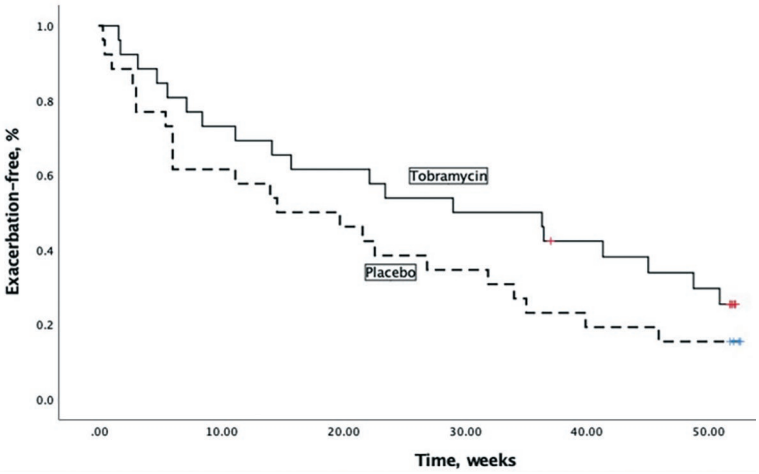
Exacerbations

In the mITT population, a total of 99 protocol defined exacerbations were reported during the study, 41 (41.4%) of which occurred in the TIS-treated patients and 58 (58.6%) in the placebo-treated patients. During the study, a median number of 2 (IQR 0-2) exacerbations was found in the TIS treatment group and 2 (IQR 1-3) in the placebo treatment group. Negative binomial regression analysis correcting for baseline exacerbation rate showed a RR of 0.74 (95% CI 0.49 – 1.14) suggesting a lower, non-significant exacerbation incidence rate in TIS group (P = 0.15). For the PP-population similar results in exacerbation incidence were found, with a RR of 0.77 (95% CI 0.42 – 1.17) (supplemental 3).

The total number of exacerbations of the mITT population also included number of exacerbations during the study period from patients who terminated the study medication.

Additional post-hoc calculations were performed, looking only at exacerbations which occurred during the weeks that patients were on study medication (median 52 (IQR 12-52)). During the use of TIS, the exacerbation rate was reduced by 41% (P = 0.02) as compared to placebo.

The secondary endpoint time to first exacerbation differed between both groups with a mean of 29 (SD19.6) weeks for patients treated with TIS and a mean of 21 (SD 18.7) weeks for the placebo-treated patients, resulting in a hazard ratio of 0.64 (P = 0.15) (Figure 2).



Number at risk (numbers censored)

Tobramycin	26 (0)	19 (0)	16 (0)	13 (0)	10 (1)	7 (1)
Placebo	26 (0)	16 (0)	12 (0)	9 (0)	5 (0)	4 (0)

Figure 2: Time to next exacerbation

Kaplan Meier plot of time to fist exacerbation in the mITT population; *X*-axis: Time in weeks. *Y*-axis: Percentage of exacerbation free patients. Hazard ratio: 0.64 (95% CI 0.35 – 1.19).

Lung function

The longitudinal analysis of lung function in the mITT-population showed no statistically significant differences between both groups (supplemental 4). Comparisons within the TIS treatment group showed a non-significant improvement in FEV_1% of predicted with a mean of 65.9% (SD 24.9) at baseline and a mean of 72.7% (SD 23) at the end of the study (P = 0.58). A non-significant improvement was also found in the placebo population, with a mean of 70.5% (SD 24.0) at baseline and 73.2% (SD 23.5) at the end of the study (P = 0.21). The FVC % of predicted was stable during the study for both groups (Table 2).

Table 2: Lung function and QoL-questionnaires

Lung function and QoL-questionnaires	TIS			Placebo		
	Start	**End**	***P* value**	**Start**	**End**	***P* value**
Pulmonary function						
FEV_1% predicted, mean (SD)	65.9 (24.9)	72.7 (23.0)	0.58	70.5 (24.0)	73.2 (23.5)	0.21
FVC% predicted, mean (SD)	84.4 (20.1)	85.0 (24.3)	0.32	90.2 (18.6)	90.3 (19.5)	0.52
QoL-Bronchiectasis questionnaire, mean (SD)						
QoL-B Physical	49.8 (30.0)	47.6 (27.4)	0.75	42.5 (34.8)	42.7 (38.4)	0.47
QoL-B Role	64.4 (24.7)	65.1 (20.7)	0.81	59.2 (23.9)	57.9 (23.9)	0.77
QoL-B Vitality	53.1 (17.4)	51.5 (18.7)	0.85	48.7 (17.6)	45.5 (24.5)	0.25
QoL-B Emotional	87.9 (10.5)	83.3 (11.2)	0.05	82.4 (16.5)	84.1 (12.8)	0.57
QoL-B Social	65.0 (20.9)	64.8 (16.8)	0.81	60.1 (23.4)	67.2 (18.6)	0.10
QoL-B Treatment Burden	66.4 (17.9)	63.9 (24.2)	0.66	64.0 (19.4)	62.8 (21.7)	0.75
QoL-B Health perceptions	42.4 (16.8)	48.1 (19.7)	0.08	43.2 (18.9)	46.1 (17.5)	0.24
QoL-B respiratory symptoms	56.1 (17.8)	61.0 (17.4)	0.05	57.8 (16.7)	59.8 (17.9)	0.14
LRTI-VAS total score, mean (SD)	21.0 (7.3)	18.0 (7.5)	0.02	21.0 (8.5)	20.0 (7.1)	0.18
Leicester Cough total score, mean (SD)	13.4 (2.9)	14.5 (3.2)	0.02	13.8 (4.2)	15.1 (2.6)	0.09

Lung function and QoL questionnaires of the mITT population. Data are presented as mean (SD). A decrease in LRTI-VAS score corresponds to clinical improvement. *Abbreviations:* Tobramycin Inhalation Solution (TIS); Forced expiratory volume in one second (FEV_1); Forced vital capacity (FVC); Quality of life (QoL); Quality of life bronchiectasis questionnaire (QoL-B); Lower respiratory tract infections – Visual Analogue Scale (LRTI-VAS); Leicester cough questionnaire (Leicester cough);

Quality of life

The QoL was measured during the study period by using the LRTI-VAS, the Leicester Cough questionnaire, and the QoL-B questionnaire in both groups.[25-27] Longitudinal analysis of the QoL measurements showed no significant improvement for both the mITT and the PP population (supplemental 3 and 4).

However, within the TIS population significant improvement of the total LRTI-VAS score (improvement is reflected by a reduction in LRTI-VAS score) and the total Leicester Cough questionnaire score were found after 1-year of study treatment ($P = 0.02$, $P = 0.02$) (Table 2). These differences were not observed into the placebo population ($P = 0.17$;

P = 0.08) (Table 2). Within groups comparisons showed no significant differences for the 8 QoL-B subscales. However, in the TIS treatment group, the QoL-B respiratory symptom scale (RSS) improved from 56.1 (SD 17.8) to 61.0 (SD 17.4) points after 1-year (*P* = 0.05), with also improvement of the QoL-B health perceptions scale from 42.4 (SD 16.8) to 48.1 (SD 19.7) points at the end of the study (*P* = 0.08), while the QoL treatment burden scale and the emotional scale decreased (*P* = 0.65; *P* = 0.05) (Table 2).

Microbiology and inflammation

A total of 162 sputum cultures were collected during the study. At baseline, the most common isolated pathogens were *H. influenzae* (16 (30.8%)) and *P. aeruginosa* (14 (26.9%)). At the end of the study, no pathogens were isolated from sputum in 10 (38.5%) TIS-treated patients as compared to 4 (15.4%) placebo-treated patients (Table 3). In 80% of patients with *P. aeruginosa* chronic infection treated with maintenance TIS (n = 5 (19.2%)), no *P. aeruginosa* was isolated in sputum at the end of the study, as compared to 33.3% after placebo (n = 9 (34.6%)). In one (3.8%) TIS-treated patient with *Escherichia Coli (E. coli)* infection tobramycin resistance occurred during the study treatment. After the run-out phase, 4 weeks after the last dose of the study medication, *Aspergillus fumigatus* was isolated from two sputum cultures, 1 (3.8%) in the placebo population and 1 (3.8%) in the TIS population, both with no clinical signs of *Aspergillus* infection. No other 'opportunistic' pathogens were found during the study. In addition, serum inflammatory markers were collected every 3 months during the study. No differences were found in CRP, leucocytes, and eosinophil counts (supplemental 6). During an exacerbation the inflammatory markers were not collected on regulatory base (not shown).

Table 3: Microbiological evaluation at the end of the study treatment

Microbiological evaluation	TIS (n = 26)*	Placebo (n = 26)*
No pathogens	10 (38.5)	4 (15.4)
No sputum culture	5 (19.2)	7 (26.9)
Pseudomonas aeruginosa	1 (3.8)	6 (23.1)
Haemophilus influenzae	3 (11.5)	4 (15.4)
Staphylococcus aureus	2 (7.7)	2 (7.7)
Streptococcus pneumoniae	1 (3.8)	0
Other gram-negative pathogens	4 (15.4)	3 (11.5)

Data are presented as n (%). *No significant differences were found between both groups. *Abbreviations:* Tobramycin Inhalation Solution (TIS);

Safety

All patients underwent a tolerance test at the start of the study. Spirometry measurements were performed before and after the first dose of the study medication. No occurrence of severe bronchus obstruction was found directly after the first dose. However, 3 (8.8%) TIS-treated patients developed respiratory symptoms in the first 4 weeks of study treatment (none in the placebo-treated patients) and withdrew from the study. Unfortunately, no spirometry was performed during the development of these respiratory symptoms. Two out of 29 (6.8%) TIS-treated patient and 3 out of 29 (10.3%) placebo-treated patients experienced no effect of the study treatment and withdrew from the study. Two out of 58 (3.4%) patients (1 TIS and 1 placebo) mentioned intensive study treatment as a reason for discontinuing the study (Figure 1). In the mITT population, 7 (26.9%) TIS-treated patients mentioned cough as compared to 5 (19.2%) placebo-treated patients (P = 0.47). An overview of all the side effects is shown in Table 4. A total of 28 serious adverse events (SAE) were reported during the study, all related to hospital admissions, of which 24 to a pulmonary exacerbation, 2 patients with known cardiac diseases ,1 patient with a near collapse and 1 patient with an anaphylactic reaction on amoxicillin clavulanate. One hundred and fifty-seven adverse events were reported, mostly related to a PDPE or non-PDPE (supplemental 5).

DISCUSSION

This multicenter, double blinded, randomized controlled trial is the first study with exacerbation frequency as primary outcome in bronchiectasis patients chronically infected by different pathogens and treated with maintenance TIS OD for one year. A non-significant decrease in incidence of exacerbations was found for the TIS-treated patients with a RR of 0.74 (95% CI 0.49 – 1.14) as compared to placebo. This decrease in number of exacerbations due to TIS in bronchiectasis confirmed the previous published reduced exacerbation frequency of RR 0.81 (0.67-0.97) in a meta-analysis of Laska et al.[8] However, in this systemic review including 15 randomized controlled trials, different inhaled devices and different types of inhaled antibiotics were analyzed. Resulted in an even more heterogenic bronchiectasis population, which makes comparison with the present study difficult.

In our study, a significant reduction of 2 exacerbations/year was found within the TIS population (P = 0.00). However, a similar reduction was noticed in patients receiving placebo treatment, which might be due to the positive effect of saline on sputum evacuation in combination with the known 'placebo effect' in randomized controlled trials. This 'placebo-effect' may be partially explained by 'regression to the mean'-effects or may be due to improved bronchiectasis care or adherence during study participation. In addition, inhaled saline as was used by placebo participants is likely to have improved airway clearance. This phenomenon has been described by other authors in the last couple of years and has been hampering other interventional studies in bronchiectasis, especially in RCT's with inhaled antibiotics.[28,29] Considering this, our study may have been relatively underpowered since we did not take this substantial placebo-effect into account when designing the study.

In view of the clear trend towards a reduction of exacerbations in the current study, one might assume that our prespecified assumption of a 50% reduction of yearly exacerbations, could have been reached when a larger number of participants had been included. Though, the assumption of a reduction of 50% in number of exacerbations was probably too high in relation to the recent meta-analysis with inhaled antibiotics whereby a rate ratio of 0.81 was observed.[8] On the other hand, a 50% reduction seems clinically relevant because the long-term use of inhaled antibiotics is an intensive and time-consuming therapy and therefore a solid decrease was desirable.

Discontinuation of the TIS treatment was noted in our study population, which influenced our results. When only considering exacerbations while patients where adherent to treatment showed a much larger reduction as compared to placebo-treated patients. However, the reasons for TIS discontinuation were in line with previous studies and presumable reflects real-life adherence in bronchiectasis patients. [21,22]

Differences in effect on lung function were not found, however previous trials showed that lung function is poorly responsive and poorly correlated with other key outcome measures, and they suggested the need to develop biomarkers to identify responders.[30]

In our study, the QoL improved, with a significant improvement of the LRTI-VAS total score and the Leicester Cough total score for the TIS population, which was not found for the placebo-treated patients. In addition, the QoL-B measurements showed improvement of the RSS and the health perception scale, with a small decrease of the treatment burden and emotional subscale for the TIS population. This is presumably due to the time-consuming therapy of inhaled antibiotics, which takes about 20 to 30 minutes a day, and includes the preparation of the InnoSpire, the use of the study medication and the cleaning protocol afterwards.

Other treatment devices, like mesh nebulizers or powder inhalators provide a faster treatment modality and may therefore reduce treatment burden.[31] However, the use of dry powder inhalation is also related to airway irritation, especially in the older and frailer patients, of which the bronchiectasis population mainly consist of.[55,31,32]

A unique feature of our study is the OD dosing schedule and may have contributed to the high adherence to therapy during study treatment (95% in both groups, based on empty ampules count). Twice daily (BID) on/off dosing every month was originally chosen for TIS with the underlying idea to maximize the treatment effect and to reduce the development of toxic side effects and tobramycin resistance pathogens, however there is a lack of evidence for this specific treatment schedule.[33-36] In addition, previous studies with intravenous administration of aminoglycosides have shown that the OD dosing schedule is equivalent in terms of antimicrobial efficacy as compared to frequent dosing, with an long-term concentration-dependent post-antibiotic effect.[37-39] Our study showed that OD continuous treatment of TIS was well tolerated, with no increase in tobramycin resistance or side effects as compared to earlier trials with the BID 28 days on-off treatment schedule.[20-22] A recently published study in bronchiectasis with

P. aeruginosa chronic infection showed similar results and described that continuous regimes have advantage over cyclic regimes in reducing *P. aeruginosa* density.[32]

In our study safety was monitored closely. All patients underwent a tolerance test at the start of the study, with no observation of severe respiratory symptoms or a decrease in FEV_1 directly after the tolerance test. However, two TIS-treated patients withdrew from the study in the first two weeks after randomization, one with bronchus obstruction after inhalation and one patient with a local allergic reaction. Patients in the TIS group reported more side effects, which is in line with previous studies with tobramycin.[21,22,34,40] Cough and dyspnea were mentioned mostly during the study, which were reasons for study discontinuation in both groups. No significant differences were found between both groups for the frequently described side effects due to inhaled administration and showed that the use of TIS OD on a continues base can be prescribed safely.

In our study we have focused on all common gram-negative pathogens (and *S. aureus*), causing chronic infection in bronchiectasis, which reflects the daily population of bronchiectasis patients.[1,2] Tobramycin is a broad-spectrum antibiotic and is expected to affect not only *P. aeruginosa* chronic infection, but chronic infection with other pathogens as well. Indeed, a decrease in all types of pathogens were found for the TIS population at the end of the study, with no significant increase in tobramycin resistance or overgrow of other pathogens. Inhaled tobramycin is currently registered for chronic *P. aeruginosa* infection in CF, but our findings suggest that TIS treatment may also be a treatment option for patients without *P. aeruginosa* chronic infection. However, due to the treatment effect, only a small number of sputum cultures could be obtained at the end of the study, with no significant differences between both groups. Though focusing on the patients with *P. aeruginosa* chronic infection treated with TIS in our population, eradication was achieved in 80% of sputum cultures at the end of the study, as compared to 52% with other pathogens (57% in patients with *Haemophilus influenzae* chronic infection). These results are in line with the previously published studies in CF and non-CF bronchiectasis whereby only patients were included with *P. aeruginosa* chronic infection.[32,40,41] However, in our population more patients (9 vs. 5 $P = 0.625$) with *P. aeruginosa* chronic infection were randomly included in the placebo population, which may also have led to a limited effect of TIS as compared to placebo.

Another limitation of our study was the small number of included patients based on our power calculation. The earlier mentioned 'placebo effect' and the unexpected

positive effect of saline nebulization was insufficiently considered in the power analysis, which meant that more patients should have been included. In addition, probably a higher number of patients was required due to the wide range of etiologies in non-CF bronchiectasis.[42] Unfortunately, this makes the study underpowered for evaluating the primary endpoint and resulted in a non-significant reduced exacerbation frequency as compared to placebo.

A strength of our study is the investigator initiated multi center double blinded design, including the heterogenous bronchiectasis population which reflects daily practice, and resulted in an extensive database about this study population.

In conclusion, this is the first study that evaluated the effect of long-term TIS OD on exacerbation frequency in non-CF bronchiectasis patients infected by different pathogens. It showed a non-significant decrease in number of exacerbations with a RR of 0.74 (95% CI 0.49 – 1.14) as compared to placebo, and an improvement in QoL. Long-term TIS OD was well tolerated with no additional safety concerns. Therefore, TIS OD may be a potential therapeutic strategy in selected patients with bronchiectasis suffering from a high burden of disease.

SUPPLEMENTAL MATERIAL

See the supplemental material section at page 173.

Supplemental 1: Overview of the in- and exclusion criteria of the BATTLE study.

Supplemental 2: Overview of the study and safety assessments for the BATTLE study.

Supplemental 3: Results of the longitudinal analysis, exacerbation, lung function and QoL for the PP-population.

Supplemental 4: Results of the longitudinal analysis of lung function and QoL for the mITT population.

Supplemental 5: Overview of adverse events and serious adverse events.

Supplemental 6: Overview of the inflammatory markers in serum.

DECLARATIONS

Ethics approval and consent to participate

Approval of Independent Ethics Committees and Institutional Review Boards was obtained from all the participating centers. Clinical trials.gov number of the BATTLE study: NCT02657473. EudraCT number of the BATTLE study: 2016-000166-35. Written informed consent for participation and publication of our results was obtained from all the participants at the screenings visit.

Consent for publication and acknowledgements

Not applicable.

Competing interest

WGB received an unrestricted grant from TEVA pharmaceutics and TIS was supplied by TEVA pharmaceutics. The other authors have no competing interest.

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Author's contributions

WGB, JA and LCT wrote the protocol and designed the manuscript. LCT and WGB wrote the manuscript and prepared the figures and tables. LCT did the statistical analysis. WR did the microbiological analysis. All authors (LCT, JA, IB, MDK, YB, DS, WR, HGMH and WGB) reviewed the manuscript and has given final approval of the version to be published.

Data sharing statement

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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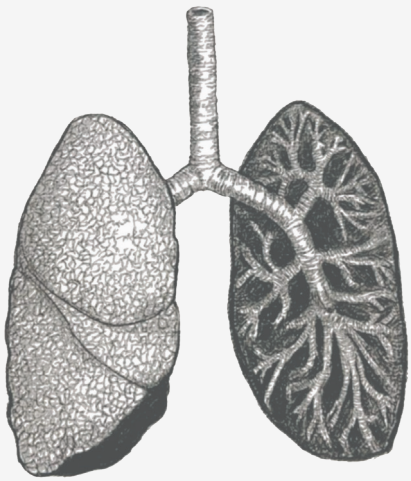
Chapter 8

Treatment with inhaled antibiotics in bronchiectasis, side effects and evaluation of the tolerance test – analysis from the BATTLE randomized controlled trial

Terpstra, Lotte., et al. Treatment with inhaled antibiotics in bronchiectasis, side effects, and evaluation of the tolerance test; analysis from the BATTLE randomized controlled trial. Clin Respir J. 2023; 1- 6.



ABSTRACT

Introduction: Tobramycin inhalation solution (TIS) is a treatment option for the frequent exacerbation bronchiectasis patient. A possible side effect of TIS is the development of chronic cough and bronchospasm, whereby the guidelines suggest a (in hospital) tolerance test with the first dose of TIS. However, data on the respiratory adverse events are not consistent. In the present analysis from the BATTLE study (NCT02657473), we evaluated the added value of the tolerance test and aimed to observe the development of inhaled treatment related bronchial hyperreactivity.

Methods: 57 patients from the BATTLE study were analyzed. Patients were randomized to receive TIS or placebo OD for one year. A tolerance test was performed with spirometry measurements before -and after the first dose, and with a bronchodilator in advance. Adverse events we strictly monitored.

Results: 57 patients (100%) passed the tolerance test with no decrease in spirometry measurements or development of local intolerability. During the study treatment a total 5 TIS-treated patients (17.8%) withdrew due to airway hyperresponsiveness after a mean of 9.2 (SD 13.9) weeks, and 1 placebo-treated (3.5%) after 2 weeks. (TIS vs. placebo; $P = 0.66$). The other TIS related adverse events were not clinically significant.

Conclusion: The use of inhaled medication is well tolerated in the heterogenous bronchiectasis population, without signs of airway hyperresponsiveness after the first dose of inhaled medication. From this observation it can be concluded that there is no additional value for this advised tolerance test. However, closely monitoring on adverse effects during the first weeks after starting TIS is recommended.

INTRODUCTION

Bronchiectasis is characterized by the presence of dilated bronchi and chronic inflammation, which leads to persistent cough and sputum production with recurrent exacerbations.[1] Long-term inhaled antibiotics are recommended for the frequent exacerbating bronchiectasis patients, with additional evidence for treatment with Tobramycin Inhalation Solution (TIS).[1-3] The inhaled administration of antibiotics can provide a consistent deposition of high antibiotic concentrations directly to the side of infection, with lower risk of toxicity or systemic adverse events.[2,4] However, possible side effects of the use of inhaled TIS are chronic cough and bronchospasm, and is reported in up to 70% of patients treated with TIS in previous studies.[5-7] However, data on the respiratory adverse events were not consistent, and in contrast to these studies two recent conducted randomized controlled trials observed no significant bronchial hyperreactivity after the use of TIS.[3,8] In addition, no significant increase in the development of bronchospasms was found in a recent published meta-analysis, whereby randomized controlled trials (RCT's) were included with TIS, but also other variants of inhaled antibiotics.[2] Due to the lack of consistent data, our current available guidelines suggest a clinical (in hospital) tolerance test with the first dose of the inhaled antibiotics to observe the possible occurrence of a bronchospasm.[1,9] This observation often includes spirometry measurements before and after a supervised test-dose of inhaled antibiotics. Furthermore, inhalation of a short-acting bronchodilator before the use of inhaled antibiotics is advised to prevent bronchospasm. However, it remains unclear whether conducting this tolerance test with the first dose of inhaled antibiotics can prevent patients from the chance of occurring persistent cough and/or airway obstruction directly or later during the inhaled treatment. In the present analysis from the BATTLE study,[10] we evaluated the added value of the suggested (in hospital) tolerance test and aimed to observe and analyze the development of inhaled treatment related bronchial hyperreactivity and other side effects.

METHODS

Study population

In the present analysis data from the BATTLE randomized controlled trial were included.[10,11] The BATTLE randomized controlled trial (clinical trials.gov number: NCT02657473) was conducted in the Netherlands between 2016 and 2019.[10,11] A total of 58 bronchiectasis patients with frequent exacerbations were included and randomized to receive TIS (300mg/5ml) once daily (OD) or placebo OD (5ml saline 0.9%) for one year by using the InnoSpire Deluxe compressor (Philips Respironics) with a SideSteam Plus nebulizer with filter and mouthpiece, which is generally used in the Netherlands for inhaled antibiotics.[12] The study protocol and the results of the BATTLE study are previously published.[10,11]

Objectives

The primary objective of this study is to evaluate the presence of airway hyperresponsiveness during the tolerance test for patients treated with TIS as compared to placebo. Secondary objectives were time to first signs of airway hyperresponsiveness for patients treated with TIS as compared to placebo, number of treatment related adverse events, and etiology of bronchiectasis patients in which intolerability of the inhaled medication occurs.

Tolerance test

Patients were clinically stable at the start of the study, a tolerance test with the first dose of the study medication was performed for each patient at the outpatient ward. Patients were excluded if they failed the tolerance test. Study visits were planned every 3 months for one year and a diary card was used every week to obtain the development of possible side effects. (See Chapter 6 of this thesis).

All patients underwent this supervised test dose by using the InnoSpire deluxe at the outpatient ward at visit 0 (start of the study) to assess the occurrence of local intolerability. Patients continued their own maintenance inhaled medication during the tolerance test and next to the study medication.

A spirometry measurement was performed 20 minutes before the first dose of the study medication. All patients received a short-acting beta agonist (200mcg salbutamol dose aerosol with aerochamber) 5 minutes before the study medication. Spirometry measurements were repeated 20 minutes after the use of the inhaled study

medication (supplemental 1). Spirometry measurements were expressed in forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) in liters and percent (%) of predicted. Study discontinuation by airway hyperresponsiveness was defined as: 1) decrease in FEV_1% of predicted of 20% following the study medication, 2) and/or SO2 < 90% at fingertip, 3) and/or signs of bronchospasm. Signs of bronchospasm were defined as cough, dyspnea, wheezing and signs of airway obstruction by clinical examination. During the study period, all patients used the short-acting beta agonist (200mcg salbutamol dose aerosol with aerochamber) daily before the study medication.

Statistical analysis

Statistical analysis was conducted by using IBM SPSS 25 for Windows. Discrete variables were presented as counts (percentage) and continuous variables as mean with ± SD (standard deviation) if normally distributed or median with IQR (interquartile range) if not normally distributed. Comparison between groups was performed using independent samples test if normally distributed and Mann-Whitney U test if not normally distributed. For comparison between groups with multiple variables a chi-square test was used. Within each treatment group, change in FEV_1 and FVC pre and post study medication was analyzed using Wilcoxon signed rank test. Mixed model analysis was conducted for differences between both groups. A *P* value $P < 0.05$ was considered statistically significant.

RESULTS

Patients

Fifty-seven patients out of 58 patients conducted the BATTLE study[10] were included in this analysis. One patient (1.7%) was excluded during the first visit of the study due to other medical problems. An overview of the patient characteristics is shown in Table 1. Baseline patient characteristics were well balanced, except for maintenance (stable) doses of prednisolone <10mg, which was more often used in the TIS group ($P = 0.02$). The mean age of the study population was 68.6 yr. (SD 7.2) for patients treated with TIS and 65.8 yr. (SD 14.3) for the placebo-treated patients. No differences were found in smoking status, etiology of bronchiectasis and the use of inhaled medication (Table 1).

Table 1: Patient characteristics

	Tobramycin n = 28	**Placebo n = 29**
Age, mean (SD), yr.	68.6 (7.2)	65.8 (14.3)
Female, n (%)	14 (50.0)	19 (65.5)
Smoking status, n (%)		
Current	2 (7.1)	1 (3.4)
Former	14 (50.0)	16 (55.2)
Never	12 (42.9)	12 (41.4)
Etiology, n (%)		
Post-infective	5 (17.9)	8 (27.5)
Idiopathic	6 (21.4)	4 (13.8)
COPD	6 (21.4)	6 (20.7)
Asthma	3 (10.7)	5 (17.2)
Immunodeficiency	4 (14.3)	3 (10.3)
Rheumatic disease	1 (3.6)	1 (3.4)
Primary ciliary dyskinesia	0	1 (3.4)
Alpha-1 antitrypsin deficiency	0	1 (3.4)
Yellow nail syndrome	1 (3.6)	0
Aspiration	2 (7.1)	0
Charlson comorbidity index, n (%)		
1 or 2	20 (71.4)	23 (79.3)
3 or 4	8 (28.6)	6 (20.6)
Maintenance AZM during the study, n (%)	8 (28.6)	4 (13.8)
Maintenance prednisolone during the study < 10mg, n (%)	6 (21.4)	0
Maintenance immunoglobulin therapy, n (%)	1 (3.4)	1 (3.4)
Inhaled medication, n (%)		
SABA	17 (60.7)	20 (69.0)
SAMA	3 (10.7)	9 (31.0)
LABA	19 (67.9)	19 (65.5)
LAMA	10 (35.7)	12 (41.4)
ICS	19 (67.9)	21 (72.4)
No use of SABA before tolerance test	1 (3.4)	1 (3.6)

Data are presented as n (%), mean (SD) or median (IQR). *Abbreviations*: Azithromycin (AZM); short acting β agonist (SABA); Short acting anticholinergics (SAMA); long acting β agonist (LABA); long-acting anticholinergics (LAMA); inhalation corticosteroids (ICS).

Tolerance test

The tolerance test was performed in 57 patients, whereof 2 (3.5%) patients did not receive salbutamol dose aerosol in advance. All patients passed the tolerance test without severe airway obstruction (defined as a decrease in FEV_1% of predicted of > 20% and/or SO2 < 90%, measured at fingertip) after the first dose of the study medication. In the total population a minimal improvement of FEV_1% of predicted from 68.3% (SD 23.9) to 69.1% (SD 23.3) was found after the tolerance test, with no differences in FVC (before and after: 86% of predicted (SD 19.0)). One (3.5%) TIS-treated patient mentioned a bad taste after inhalation. No other treatment related adverse events were mentioned or observed during or directly after the tolerance test. An overview of the spirometry measurements during the tolerance test for the TIS- and the placebo-treated patients are shown in Table 2. Mixed model analysis was conducted to evaluate differences in spirometry measurements between TIS and placebo. However, no significant differences were found before and after the use of the inhaled medication between both treatment groups (P = 0.62 for FEV_1% of predicted and P = 0.23 for FVC% of predicted).

A total of 16 patients (28%) were known with a FEV_1% of predicted < 50% before the use of the inhaled study medication, of which 11 patients (68.8%) were treated with TIS. For this subpopulation with a FEV_1 < 50% of predicted, a significant improvement was found in FEV_1% of predicted from 39.1% (SD 7.4) to 41.5% (SD 8.9) during the tolerance test (P = 0.01) (not shown). Only, one TIS-treated patient has been withdrawn after 3 weeks due to airway hyperresponsiveness. No spirometry measurement was performed at the time of outage.

Table 2: Tolerance test

	Tobramycin			Placebo		
	Before	**After**	***P* value**	**Before**	**After**	***P* value**
FEV_1 Liters	1.8 (0.7)	1.8 (0.7)	0.28	1.9 (0.7)	1.9 (0.7)	0.45
FEV_1% of predicted	65.1 (24.2)	67.0 (23.4)	0.18	71.4 (23.7)	71.3 (23.5)	0.58
FVC Liters	2.9 (0.8)	3.0 (0.8)	0.17	3.1 (0.9)	3.1 (0.9)	0.61
FVC% of predicted	84.4 (19.4)	86.7 (19.8)	0.11	89.4 (18.3)	87 (24.6)	0.46

Spirometry measurements before and after the inhaled study medication. Tobramycin (n = 28), Placebo (n = 29). Data are presented as mean (SD). *Abbreviations*: Forced expiratory volume in one second (FEV_1); Forced vital capacity (FVC).

Reasons for study discontinuation

During the study treatment a total of 18 (31.6%) patients withdrew from the study, 11 (19.3%) TIS-treated patients after a mean of 10 weeks (SD 10.6) and 7 (12%) placebo-treated patients after a mean of 7.7 (SD 6.4) weeks (P = 0.22). An overview of the side effects is shown in Table 3. A total of 5 TIS-treated patients (17.8%) withdrawn from the study due to airway hyperresponsiveness defined as dyspnea and/or signs of airway obstruction and/or severe cough after a mean of 9.2 (SD 13.9) weeks. One placebo-treated patient (3.5%) experienced signs of airway obstruction during treatment and withdrawn from the study after 2 weeks. No spirometry measurements were performed at the time of withdrawal. Though, spirometry measurements during the tolerance test for these 5 TIS-treated patients and 1 placebo-treated patient improved, with no other signs of airway hyperresponsiveness.

Of these 6 patients who stopped the study, 1 TIS-treated patient (16.7%) was an actual smoker, whereas the other patients never smoked. For 1 TIS-treated patient, the etiology of bronchiectasis was defined as asthma, 2 patients were known with idiopathic bronchiectasis, 1 post-infective and 1 patient with immunodeficiency related bronchiectasis. The placebo-treated patient was known with asthma related bronchiectasis. None of them was known with chronic obstructive pulmonary disease (COPD) (supplemental 2).

Regarding to specific adverse events based on the aminoglycoside safety profile in the patients who withdrawn, no TIS-treated patients experienced ototoxicity, one patient (9%) showed reversible renal impairment after a treatment period of 9 months, and 1 patient (9%) showed an allergic reaction with swelling and irritation of the lips after the use of TIS, which was not seen during the tolerance test. One (9%) placebo-treated patient experienced ototoxicity with tinnitus during the treatment period. For this patient an audiogram was performed which showed no signs of medication related ototoxicity. An overview of all the adverse events and serious adverse events in the total population is shown in supplemental 3.

Table 3: Overview of reasons for study discontinuation

	Tobramycin (n = 28)	**Placebo (n = 29)**	***P* value**
Withdrawn from the study, n (%)	11 (39)	7 (24)	0.22
Number of weeks, mean (SD)	10 (10.6)	7.7 (6.4)	0.62
Range	1-34 weeks	1-18 weeks	
Reasons for study discontinuation			
Airway obstruction/Dyspnea	3 (27)	1 (14)	ns
Severe cough	2 (18)	0	
Allergic reaction	1 (9)	0	
Bad taste after inhalation	1 (9)	0	
Renal impairment	1 (9)	0	
Tinnitus	0	1 (14)	
Headache after inhalation	0	1 (14)	
No effect	2 (18)	3 (43)	
Intensive study schedule	1 (9)	1 (14)	

Data are presented as n (%) or mean (SD). *Abbreviation*: non-significant (ns).

DISCUSSION

The present sub analysis of the BATTLE study[10] showed that the use of inhaled medication (TIS or NaCl 0.9%) is well tolerated in the heterogenous bronchiectasis population, without signs of airway hyperresponsiveness after the first dose of inhaled medication.

A total of 57 patients underwent a tolerance test with the first dose of the inhaled medication (TIS or placebo) with salbutamol DA in advance. None of these patients showed a lung function decline or other signs of airway hyperresponsiveness after the tolerance test. Despite of a normal tolerance test, airway hyperresponsiveness developed especially during the first weeks of maintenance treatment, whereby in our study 6 (10.5%) out of the 57 patients showed airway obstruction, dyspnea, or chronic cough. None of the other adverse effects of TIS during the study could be predicted by the tolerance test.

Based on this observation, no additional value was seen for this advised tolerance test, however closely monitoring in the first weeks after the start of maintenance inhalation treatment seems more relevant.

Higher percentages, up to 30%, in occurrence of airway hyperresponsiveness were described in previous studies with TIS, however bronchodilation in advance was not standard used in these studies.[5,6]

Only in a few studies in bronchiectasis a tolerance test or supervised test dose was performed before the use of an inhaled treatment option.[13,14] After the first dose of inhaled mannitol, a decline in lung function, oxygen desaturation or use of bronchodilator was described up to 16.5% and were reported as screen failure.[13] In a RCT with colistin, no lung function decline was found after the first dose of inhaled colistin, though only one placebo-treated patient showed a decrease in FEV_1 > 15%. In line with the results of our study, airway hyperresponsiveness developed in the first weeks after the start of inhaled colistin in 5 (7%) patients.[14]

Maintenance use with inhaled antibiotics is time consuming, and takes about 20-30 minutes a day, and includes preparation of the device, the use of the inhaled medication and the cleaning protocol afterwards. This time-consuming therapy has been a reason for a total of 7 patients (38.9%) to withdrawn from our study. They experienced an insufficient effect of the treatment in combination with a too intensive study schedule. Other adverse events obtained in our study were (reversible) renal impairment after the use of TIS, which was in comparable rates described in the recently published iBEST study[8] in patients treated with the OD dosing schedule, the twice daily (BID) dosing schedule showed increased rates of renal impairment.[8] In our study ototoxicity which led to study discontinuation was found in one patient (9%) and was not TIS-treatment related. Two patients (7%, not shown) in the total TIS population mentioned tinnitus, but this was mild and transient and did not result in change of study medication. Previous studies showed higher rates of ototoxicity which is probably related to the twice daily dosing schedule and therefore dose depending.[7,8,15] However, in our study only patients who mentioned hearing complaints underwent an audiometry, therefore our results must be interpreted by caution.

In our study, bronchiectasis patients with pre-existent low spirometry measurements were included (FEV_1 < 50% of predicted), even in this population the use of the inhaled study medication was well tolerated. Only one patient dropped out of the study after a period of 3 weeks due to airway hyperresponsiveness despite the use of a bronchodilator.

In conclusion, the use of inhaled medication (TIS or NaCl 0.9%) is well tolerated in the heterogenous bronchiectasis population, without signs of airway hyperresponsiveness after the first dose of inhaled medication. From this observation it can be concluded that there is no additional value for this advised tolerance test. However, closely monitoring on adverse/site effects during the first weeks of TIS is recommended.

SUPPLEMENTAL MATERIAL

See the supplemental section at page 179.
Supplemental 1. Time schedule of the tolerance test.
Supplemental 2. Analysis of the sub population who withdrawn from the study due to airway hyperresponsiveness.
Supplemental 3. Overview of the adverse events and serious adverse events in the total population.

DECLARATIONS

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Author's contributions

WGB and LCT designed and drafted the manuscript. All authors were involved in revising the manuscript and have given final approval of the version to be published.

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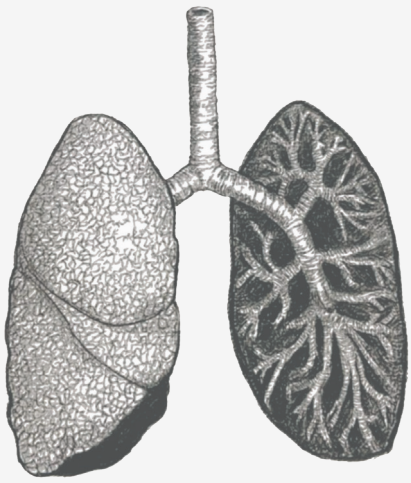
Chapter 9

General discussion and future perspectives



Bronchiectasis is a heterogenous disease and is diagnosed in patients of all ages, with geographic variability around the globe.[1,2] The prevalence of bronchiectasis worldwide rose by 40% between 2004 and 2014, and still increases with a growing recognition of the disease.[3] Using analysis from various insurance claims databases, a prevalence of 701 cases per 100,000 was reported in 2018 in the United States and was higher among women and increased with age.[4] Similar or even higher rates have been reported in Europe, but also Singapore and China.[1]

The clinical syndrome is characterized by chronic cough and sputum production in the presence of abnormal thickening and dilatation of the bronchial wall. The recognition of bronchiectasis is multifactorial, including through greater use of CT scanning to evaluate lung disease and augmented awareness among clinicians.[3,5,6] At this moment, various underlying causes are known as an explanation for the development of bronchiectasis.[6-8] Bronchiectasis is frequently seen in relation to other respiratory diseases as chronic obstructive pulmonary disease (COPD) or asthma, and complicates its treatment. In addition, bronchiectasis coexists with several congenital and hereditary diseases including Cystic Fibrosis (CF), Primary Ciliary Dyskinesia (PCD), Mounier-Kuhn syndrome, Yellow Nail Syndrome, Williams-Campbell Syndrome, and alpha-1 Antitrypsin Deficiency. Bronchiectasis may also develop in patients with immunodeficiency syndromes and auto-immune diseases (for example rheumatoid arthritis, inflammatory bowel disease and Sjogren's syndrome). But is also seen in patients with chronic rhinosinusitis, gastro-intestinal reflux, dysphagia, and recurrent aspiration. In addition, bronchiectasis may develop during the years in patients with childhood diseases like measles, mumps, whooping cough, and after premature birth.[6,8,9] Bronchiectasis is also seen after a (severe) pneumonia and after tuberculosis, however, in numerous bronchiectasis patients, the etiology remains unknown (idiopathic).[7]

The variety of known etiologies of bronchiectasis was described in chapter 2 of this thesis. In this retrospective analysis, the most common identified etiologies were post-infective (39.5%) and idiopathic (12.5%). As described, in previous studies, the etiology remains unidentified in the majority of patients ranging from 26% to 74%.[7] However the last years, and objective algorithm for classification of the etiology was developed and using this algorithm in combination with a minimal bundle of etiological tests, a significant reduction of idiopathic bronchiectasis was found.[9] Our current Dutch bronchiectasis guideline suggests also this minimal bundle of different tests to identify

the etiology of bronchiectasis stepwise. Treatable underlying conditions should be addressed to prevent disease progression and the potentially reverse bronchiectasis and facilitate improvement of the personalized treatment.[10]

This study demonstrated also the clinical important differences between the etiology of bronchiectasis and the QoL based on the QoL-B questionnaire. A significant lower QoL was found in patients with COPD as compared to the other etiologies. This is in line with previous studies whereby in patients with COPD-related bronchiectasis a higher prevalence of severe disease was described, with a functional decline and a higher mortality rate.[2]

Overall, a high disease burden is described in this heterogenous population, with and increasing morbidity and mortality, whereby awareness of bronchiectasis and its etiology is crucial in preventing a delay in diagnosis and initiating appropriate management.[11,12]

The management of bronchiectasis is complex due to the variability of symptoms that are associated with bronchiectasis and requires a personalized approach.

Educating the patients about bronchiectasis, its symptoms and the chronic entity of the disease is an important first step in the management of bronchiectasis. Goals of treatment include symptom reduction and a reduction of exacerbations, improvement of QoL, preservation of lung function and reduction of overall morbidity and mortality.[1] Treatment modalities as airway clearance techniques helps to improve sputum evacuation, supported by chest physiotherapy and physical excercises.[13] Various airway clearance techniques are known and includes specific breathing exercises as huff coughing technique or Active Cycle Breathing Technique (ACBT). Whereby support of specific devices helps to improve sputum evacuation as Positive Expiratory Pressure (PEP) therapy or airway oscillating therapy.[14,15] In addition, the inhalation of saline (isotonic or hypertonic) should be considered for further support in sputum evacuation.[10,16]

Azithromycin

The positive effect of macrolides was originally seen in diffuse panbroncholitis (DPB) and was described in early nineties.[17] DPB is a chronic inflammatory lung disease, which predominantly affects East Asians (especially Japan), and characterized by recurrent respiratory infections resulted in progressive airflow limitation. A significant improvement in the prognosis of this disease has been attributed to the long-term use of erythromycin. DPB has several features in common with Cystic fibrosis (CF), whereby

previous studies in CF have confirmed the benefits of macrolides in moderate to severe lung disease, especially in reducing the number of exacerbations.[18-20]

In the frequent exacerbating bronchiectasis patient, convincing evidence in favor of long-term macrolide treatment was also described.[21,22] In line with this, the current bronchiectasis guidelines suggest offering long-term macrolide treatment to patients with three or more exacerbations per year, in case first line treatment options (optimizing sputum clearance, treating the underlying disease) are not sufficient.[10,16]

Momentarily, AZM is one of the most widely used macrolide agents for maintenance treatment in bronchiectasis, with evidence from several randomized controlled trials, including the BAT study.[23] Data from this previous published BAT[23] randomized controlled trial was used for the analysis described in chapter 3 en 4.

In chapter 3 we studied the effect of long-term AZM treatment on radiological features of bronchiectasis.[24] Two validated radiological scoring systems for CF, the original Bhalla score and the modified Brody score, were used for this analysis.[25,26] The scoring was conducted by independent and blinded radiologists. These scoring systems are complex and especially for research developed, and difficult to use in daily practice. In addition, for a good assessment, it is necessary that images are reviewed by trained thoracic radiologists.

However, in this study an improvement of the radiological features was found after one year of AZM treatment as compared to placebo, with a significant improvement of the total Bhalla score and the parenchyma changes (Brody) and the consolidation (Bhalla) sub-scores. This is the first study that prospectively evaluated this long-term effect of AZM on radiological features and indicates that CT features indicative for active bronchial inflammation are most responsive to change. CT's may therefore be used as an objective measure of treatment response in bronchiectasis, beside the monitoring of symptoms and number of exacerbations.

The benefits of macrolides are believed to be based on a combination of antimicrobial and immunomodulatory effects, and it has been demonstrated that these drugs are therapeutically beneficial in various lung diseases. However, there is inconsistent data and an incomplete understanding of their immunomodulatory mechanisms of action. Clinical and experimental research has aimed to decipher these effects, but it is still not completely understood. The mechanisms underlying this dual effect are thought to be part attributable to an anti-neutrophilic mode of action, a reduction of immune cell

infiltration into the lungs and effects on the macrophage function by enhancing the phagocytic capacity of macrophages. In addition, macrolides can cause a breakdown of the biofilm formation by mucoid *P. aeruginosa*.[27] Previous studies also suggested an immunomodulatory effect on the adaptive immunity.[28]

The anti-neutrophilic mode of action is thought to be the fundamental mechanism behind the positive effect of macrolide treatment. As depicted by lower levels of neutrophils chemo-attractants and neutrophil extracellular traps (NET's) in sputum of bronchiectasis patients after macrolide treatment.[29-31] However conflicting results were found in previous studies, and in our analysis from the BAT[23] study population, we did not observe this effect.[30,32] The results of our inflammatory analyses are shown in chapter 4.

In this study, described in chapter 4, we evaluated the inflammatory profile in expectorated sputum of patients with bronchiectasis participating in the BAT trial and treated with maintenance AZM or placebo for one year.[23] In concordance with previous studies, an upregulation of inflammatory markers in this population was correlated to functional measurements of disease severity and an increase in exacerbations.[11,12]

However, in contrary to what is generally believed, the current study failed to show an attenuation of the inflammatory response in bronchiectasis patients with long-term AZM treatment. Our most remarkable finding was the fact that markers of airway inflammation remained stable or even increased during long-term macrolide treatment. This observation suggests that the clinically beneficial effect of AZM treatment may not as much, be driven by an anti-inflammatory effect as generally assumed. One could argue that some effects may have been missed due underrepresentation of certain types of markers. However, an extensive panel of inflammatory markers has been determined, representing different immunomodulatory pathways.[30]

Probably the antimicrobial effect is also an important factor for the clinical benefits of long-term macrolides. Whereby macrolides accumulate within tissues to concentrations above the antibacterial threshold and the possibly to target the infection through antibacterial mechanisms.[28] However as mentioned earlier, the effect of macrolide treatment on the immune system shows high complexity and is not fully understood yet.

Momentarily, AZM is one of the most widely used macrolide agents for maintenance treatment in bronchiectasis. However, chronic macrolide treatment has also been shown important downsides. Macrolide treatment of any duration has been associated with an increased risk of cardiovascular death and long-term use has been associated with an increase in microbial resistance.[21,33] In addition, side effects such as ototoxicity and gastro-intestinal complaints has been reported.[34]

Although studies have convincingly shown a reduction in exacerbations and chronic symptoms; its effectiveness and safety beyond the first year of maintenance treatment is not known.

In the retrospective analysis described in chapter 5, we evaluated the effect and safety of AZM up to 5 years of maintenance treatment. During these 5 years, the number of exacerbations remained low, with a maximum of 1 exacerbation during each year. Mild side effects occurred, whereby diarrhea was the most frequent adverse event. Auditory complains and tinnitus were not mentioned very often (n = 2 (2.4%)). However, no audiograms were performed during maintenance AZM treatment. No increased cardiovascular risk was seen in this analysis.

In addition, a prolonged beneficial effect of AZM was observed, but it's accompanied by a moderate increase in *P. aeruginosa* isolation and microbial resistance.

The results of our study confirmed the known concerns about the increase in *P. aeruginosa* development and microbial resistance, whereby discontinuation of AZM should be considered after a treatment period for probably one year, or even shorter in the absence of respiratory complaints. Thereby, other maintenance treatment regimens, for instance, an antibiotic holiday during the summer should be evaluated, with the hypothesize that the effect of AZM persisted, without development of microbial resistance.

Inhalation of antibiotics

Another treatment option for the frequent exacerbation bronchiectasis patient is the use of inhalation of antibiotics.[16] In CF, long term inhaled antibiotics have been shown to reduce exacerbations and lung function decline and are part of the standard care.[35] A twice daily dosing schedule, month on – month off, was originally chosen for the treatment in CF, with the rationale that during the treatment-free period antibiotic sensitive pathogens may repopulate the lower aiways.[35,36]

However, the inhalation of antibiotics is time consuming and takes almost 30 minutes to complete the treatment, including the cleaning protocol afterwards. Nowadays, this twice daily dosing is frequently abandoned, and a continue once daily dose is prescribed. Evidence for this once daily dosing schedule is described in previous pharmacokinetic studies whereby different dosing schedules of tobramycin inhalation solution (TIS) were compared, with the observation of a post- antibiotic effect which is also seen after intravenous aminoglycosides.[37-39]

In bronchiectasis, evidence for the use of inhaled antibiotics is limited.[40] However the bronchiectasis guidelines recommend inhaled antibiotics in patients with *P. aeruginosa* chronic infection.[16] It's a matter of debate whether this is the right recommendation since the effect of macrolides in patients with *P. aeruginosa* chronic infection is well established.[22]

For the frequent exacerbation bronchiectasis patient with intolerance for AZM, a contra indication for AZM, or a lack of response, long term treatment with inhaled antibiotics should be considered instead of AZM. And if there is still an inadequate response, a combination of AZM with inhaled antibiotics is suggested as an optional treatment modality.[16,41]

However, for the inhalation of antibiotics, not much is known about the ideal dosage regimen and duration of treatment, and the preference for a certain type of inhaled antibiotics.[40]

Tobramycin inhalation solution (TIS)

The use of TIS is investigated in a few small observational studies in bronchiectasis patients colonized with *P. aeruginosa* and described a decrease in *P. aeruginosa* density in sputum, with an improvement of the respiratory symptoms.[42-45] In these studies, the duration ranged from 6 weeks to 13 months, TIS was in the majority given twice daily 28 days on-off, and the most common primary outcome was *P. aeruginosa* density in sputum. No data have been published about the effect of maintenance use of TIS once daily (OD) on exacerbation frequency, and especially in bronchiectasis patients with chronic infection by non- *P. aeruginosa* Gram-negative bacteria or Staphylococcus aureus (*S. aureus*).

The BATTLE study is the first randomized placebo-controlled trial whereby this OD dosing is investigated with number of exacerbations as primary outcome. In addition,

not only patients chronically infected with *P. aeruginosa* were included, but also patients with a chronic infection with the other most common gram-negative bacteria and *S. aureus*. This provides more information about a possible treatment strategy also for patients without *P. aeruginosa* chronic infection. Chapter 7 describes the study protocol of the BATTLE study.[46]

The results of our BATTLE study are provided in chapter 8 of this thesis.[47] A non-significant decrease in number of exacerbations with a RR of 0.74 (95% CI 0.49 – 1.14) was found for patients treated with TIS as compared to placebo, and an improvement in QoL. Our results are in line with a previous meta-analysis about the use of inhaled antibiotics.[40] However, in this systemic review including 15 randomized controlled trials, often pharmaceutical driven, different inhaled devices and different types of inhaled antibiotics were analyzed. This resulted in an even more heterogenic bronchiectasis population, which makes comparison with the present study difficult.

Unfortunately, in our analysis, the predefined 50% reduction in number of exacerbations in patients with ≥ 2 exacerbations in the preceding year was not reached. Whereby the known 'placebo effect' and the unexpected positive effect of saline nebulization (placebo) may be an explanation.[48] These mentioned effects were insufficiently considered in the power analysis, which meant that more patients should have been included. In addition, probably a higher number of patients was required due to the wide range of etiologies in non-CF bronchiectasis.[9] Either in our power analysis we opt for a reduction of 50% in number of exacerbations, however this was probably too optimistic (or not realistic) based on observations in previous studies with inhaled antibiotics whereby a reduced exacerbation frequency up to 25% was observed.[40]

Though the results of the BATTLE study[47] gives us more information about the design for further studies in the heterogenic bronchiectasis population.

On the other hand, no previous investigator initiated multi center double blind randomized controlled trials were conducted that evaluated the effect of long term TIS OD directed to multiple pathogens in this heterogenous bronchiectasis population. Whereby the present research resulted in an extensive database about this study population and reflects our daily practice.

Long-term TIS OD was well tolerated with no additional safety concerns. These analyses are described in detail in chapter 9 of this thesis. Bronchospasm is a frequent described side effect of the inhalation of antibiotics, which is seen directly after the

first dose, but can also develop during a longer treatment period.[42] By using a bronchodilation (salbutamol DA with aerochamber) in advance of the use of TIS, no bronchospasm was seen in our population directly after the first dose. However, during a longer treatment period the development of airway hyperresponsiveness related to the inhaled medication was seen. Based on this observation, no additional value was seen for this advised tolerance test. However, closely monitoring in the first weeks after the start of maintenance inhalation treatment seems more relevant.

Other side effects were mild, and the use of inhaled medication (TIS or NaCl 0.9%) was well tolerated in the heterogenous bronchiectasis population.

In conclusion, TIS OD may be a potential and safe therapeutic strategy in selected patients with bronchiectasis suffering from a high burden of disease and is also effective in patients without *P. aeruginosa* chronic infection.

As mentioned before, for the frequent exacerbating bronchiectasis patient, a stepwise treatment strategy should be recommended. Maintenance AZM should be considered, except for patients with *S. aureus* infection, whereby antimicrobial resistance rapidly occurs. In addition, preferably not in patients with non-tuberculous mycobacterium (NTM) lung disease. Secondary, TIS OD maintenance treatment or one of the other types of inhaled antibiotics should be added in patients with intolerance for AZM, a contra indication for AZM, or a lack of response. Whereby closely monitoring is advised the first weeks of treatment.

However, both treatment modalities have side effects whereby the development of antibiotic resistance, especially in patients treated with maintenance AZM, is a major point of concern.

Further studies are needed whereby other maintenance regimes should be investigated. For instance, AZM discontinuation after a treatment period for probably one year, or an antibiotic holiday during the summer, with the hypothesis that the effect of AZM persists, without development of microbial resistance. In addition, a RCT with other maintenance antibiotics, such as doxycycline or cotrimoxazole, for bronchiectasis patients chronically infected with non- *P. aeruginosa* pathogens should be considered. And in addition, based on the results of our BATTLE study, the effect of inhalation of Nacl 0.9% should be investigated, whereby improvement of sputum evacuation leads to a possible reduction in number of exacerbations. Also, a re-evaluation of the effect of TIS OD with a sufficient power analysis based on the most recent literature would be very interesting.

Beside this, new treatment modalities that targets the neutrophils may play an important role in the treatment of bronchiectasis. Promising results are seen in the phase 2 study with brensocatib, which is an oral reversible inhibitor of dipeptidyl peptidase 1.[49]

In conclusion, the increasing heterogenous bronchiectasis population is a field of interest for scientists. And especially after founding the EMBARC database, whereby better designed trials were executed. In the future, promising results are expected from trials with agents specifically developed for bronchiectasis treatment.[49]

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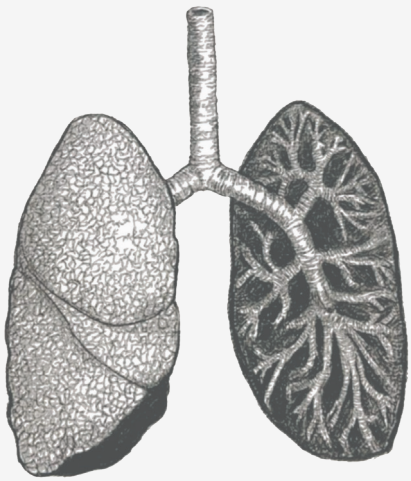
Chapter 10

Supplemental material section



Chapter 2

Etiology and disease severity are among the determinants of quality of life in bronchiectasis

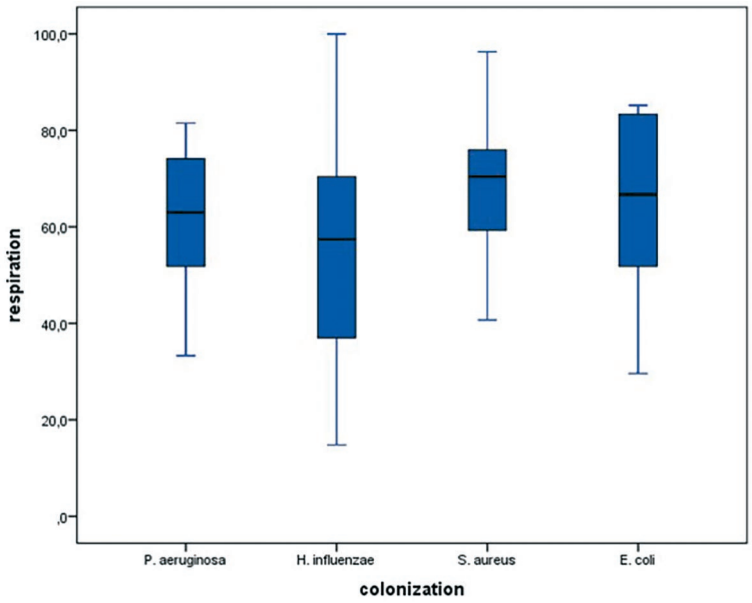
Terpstra, Lotte C., et al. "Etiology and disease severity are among the determinants of quality of life in bronchiectasis." The clinical respiratory journal 13.8 (2019): 521-529.

Supplemental 1: QoL-Bronchiectasis questionnaire domains in the total of 200 patients

QoL-Bronchiectasis	n (%)	Mean (± SD)
Physical functioning	196(98)	53.4(33.3)
Role functioning	197(98.5)	68.0(24.3)
Vitality subscale	193(96.5)	54.2(23.9)
Emotional functioning	193(96.5)	80.6(16.9)
Social functioning	197(98.5)	73.1(23.4)
Treatment burden	161(80.5)	71.8(23.8)
Health related	197(98.5)	50.4(22.9)
Respiratory symptoms	195(97.5)	64.2(19.0)

Data are presented as n (%) and mean(± SD). 8 subscales are shown, no total score is generated for the QoL–B questionnaire

Supplemental 2: Association between bacterial colonization and the QoL-B treatment



Data are presented as median with Inter quartile range (IQR); *P* value of 0.967 for H. influenzae colonisation at the respiratory symptom scale; *P* value of 0.169 for *P. aeruginosa* colonization at the treatment burden subscale

Chapter 3

The effect of maintenance azithromycin on radiological features in patients with bronchiectasis – analysis from the bat randomized controlled trial

Terpstra, Lotte C., et al. "The effect of maintenance azithromycin on radiological features in patients with bronchiectasis-Analysis from the BAT randomized controlled trial." Respiratory Medicine 192 (2022): 106718.

Supplemental 1: Bhalla scoring template[15]

BHALLA SCORE TEMPLATE

CATEGORY	SCORE			
	0	1	2	3
SEVERITY OF BRONCHIECTASIAS	NONE	MEDIUM (Luminal diameter slightly larger than the adjacent vessel.)	MODERATE (Bronchial diameter between 2 and 3 times the diameter of the adjacent vessels.)	SEVERE (Bronchus is more than 3 times the diameter of the adjacent vessel.)
PERIBRONCHIAL THICKENING	NONE	MEDIUM (Wall thickness is similar to that of surrounding vessels.)	MODERATE (Wall thickness greater, but less than twice, the diameter of the adjacent vessels.)	SEVERE (More than twice the thickness of the adjacent vessels.)
BRONCHIECTASIS EXTENSION (NUMBER OF AFFECTED SEGMENTS).	NONE	1-5	6-9	>9
MUCOUS PLUG EXTENSION (NUMBER OF SEGMENTS).	NONE	1-5	6-9	>9
SACCULATIONS AND ABCESSES EXTENSION (NUMBER OF SEGMENTS).	NONE	1-5	6-9	>9
BRONCHIAL GENERATIONS AFFECTED BY BRONCHIECTASIS OR MUCOUS PLUGS	NONE	> 4ª GE	> 5ª GE	> 6ª GE
BULLAE	NONE	UNILATERAL (NO>4)	BILATERAL (NO>4)	>4
EMPHYSEMA (NUMBER OF AFFECTED SEGMENTS).	NONE	1-5	>5	
COLLAPSE / CONSOLIDATION	NONE	SUBSEGMENTARY	SEGMENTARYS/ LOBAR	

TOTAL PUNCTUATION:…… BHALLA SCORE = 25-(TOTAL POINTS) = ……

Supplemental 2: Modified Brody scoring template[16]

Bronchiectasis score (range 0 to 12)	= (	Extent of bronchiectasis in central lung 0 = none 1 = 1/3 of lobe 2 = 1/3 to 2/3 of lobe 3 = >2/3 of lobe	+	Extent of bronchiectasis in peripheral lung 0 = none 1 = 1/3 of lobe 2 = 1/3 to 2/3 of lobe 3 = >2/3 of lobe	) x	Average bronchiectasis size multiplier 0.5 = 0 1 = 1 1.5 = 1.25 2.0 = 1.5 2.5 =1.75 3 = 2
where						
Average bronchiectasis size	= (	Size of largest dilated bronchus 1 = <2x 2 = 2x-3x 3 = >3x	+	Average size of dilated bronchi 1 = <2x 2 = 2x-3x 3 = >3x	) /	2
Mucous plugging score (range 0 to 6)	=	Extent of mucous plugging in central lung 0 = none 1 = 1/3 of lobe 2 = 1/3 to 2/3 of lobe 3 = >2/3 of lobe	+	Extent of mucous plugging in peripheral lung 0 = none 1 = 1/3 of lobe 2 = 1/3 to 2/3 of lobe 3 = >2/3 of lobe		
Peribronchial thickening score (range 0 to 9)	= (	Extent of peribronchial thickening in central lung 0 = none 1 = 1/3 of lobe 2 = 1/3 to 2/3 of lobe 3 = >2/3 of lobe	+	Extent of peribronchial thickening in peripheral lung 0 = none 1 = 1/3 of lobe 2 = 1/3 to 2/3 of lobe 3 = >2/3 of lobe	) x	Severity of peribronchial thickening 1 = mild 1.25 = moderate 1.5 = severe
Parenchyma score (range 0 to 9)	=	Extent of dense parechymal opacity 0 = none 1 = 1/3 of lobe 2 = 1/3 to 2/3 of lobe 3 = >2/3 of lobe	+	Extent of ground glass opacity 0 = none 1 = 1/3 of lobe 2 = 1/3 to 2/3 of lobe 3 = >2/3 of lobe	+	Extent of cysts or bullae 0 = none 1 = 1/3 of lobe 2 = 1/3 to 2/3 of lobe 3 = >2/3 of lobe
Air Trapping score (range 0 to 4.5)		Extent of air trapping 0 = none 1 = 1/3 of lobe 2 = 1/3 to 2/3 of lobe 3 = >2/3 of lobe	X	Appearance of air trapping 1 = subsegmental 1.5 = segmental or larger		

Supplemental 3: Overall patient characteristics at baseline

Total of patients in the BAT study (n)	83
No. (%) of patients with evaluable (HR)CT scans	77 (92.8)
Age, mean (SD)	62.1 (11.4)
Woman, n (%)	50 (64.9)
Pseudomonas colonization, n (%)	12 (15.6)
FEV_1% of predicted, mean (SD)	80.1 (26.5)
FVC% of predicted, mean (SD)	94.4 (24.8)
No. of exacerbations in year before study entry, median (IQR)	4 (3)
Total Brody score, mean (SD)*	14.9 (3.8)
Total Bhalla score, mean (SD)	15.8 (9.5)

All values are expressed as mean (SD) or median (IQR) unless stated otherwise. *Abbreviations*: forced expiratory volume in one second (FEV_1); forced vital capacity (FVC); interquartile range (IQR); standard deviation (SD); *Data are presented as percentages of maximum possible scores (SD).

Supplemental 4: Bhalla and Brody score at baseline

Bhalla score at baseline	**AZM n = 41**	**Placebo n = 36**	***P* value**
Total score[#]	14.0 (3.9)	15.9 (3.5)	0.02
Severity of bronchiectasis	1.5 (0.8)	1.3 (0.6)	0.30
Peribronchial thickening	1.4 (0.5)	1.3 (0.8)	0.48
Bronchiectasis extension	2.1 (0.8)	1.9 (0.8)	0.44
Mucous plugging extension	1.7 (0.9)	1.0 (1.2)	0.01
Sacculations and abscesses	0.3 (0.7)	0.2 (0.5)	0.34
Bronchial generations	2.7 (0.6)	2.5 (0.7)	0.13
Bullae	0.2 (0.7)	0.1 (0.6)	0.58
Emphysema	0.3 (0.7)	0.2 (0.6)	0.20
Consolidations	0.9 (0.6)	0.6 (0.6)	0.06
Brody score at baseline	**AZM n = 41**	**Placebo n = 36**	***P* value**
Total score[#]	17.8 (9.7)	13.5 (8.7)	0.05
Bronchiectasis*	21.8 (17.7)	14.5 (11.7)	0.08
Mucous plugging	16.3 (13.2)	12.1 (15.3)	0.04
Peribronchial thickening	22.7 (14.4)	22.3 (19.9)	0.59
Parenchymal changes	8.5 (8.8)	4.2 (4.3)	0.02

Data are presented as mean (SD) for the Bhalla score and percentages of maximum possible scores (SD) for the Brody score; the Air trapping sub score of the Brody score was excluded from scoring since not all scans had expiratory images; Standard deviation (SD); AZM: azithromycin; [#]Mean total score of two observers; *Extent of bronchiectasis and size

Chapter 4

The effect of azithromycin on sputum inflammatory markers in bronchiectasis – analysis from the bat randomized controlled trial

Terpstra, L. C., et al. "The effect of azithromycin on sputum inflammatory markers in bronchiectasis." BMC Pulmonary Medicine 23.1 (2023): 1-12.

Supplemental 1: Number of samples per visit in the total population

	V1 (Start study)	V2 (3 months)	V3 (6 months)	V4 (9 months)	V5 (End of study)	V6 (Run out)
For all the inflammatory markers	54 (65)	50 (60)	60 (72)	56 (67)	46 (55)	47 (57)
AZM	25 (46)	25 (50)	32 (53)	29 (52)	24 (52)	23 (49)
Placebo	29 (54)	25 (50	28 (47)	27 (48)	22 (48)	24 (51)

Data are presented as numbers with percentages of the total population. At every visit, during stable state, all inflammatory markers were obtained and analysed.

Supplemental 2: The effect of maintenance AZM on the inflammatory profile in sputum. Results after mixed model analysis

	V1# Baseline	V2* 3 months	V3* 6 months	V4* 9 months	V5* End of treatment	V6* Run-out
IL-8						
Δ (AZM-placebo)	98314	51230	36878	16875	228435	102297
P value	0.267	0.565	0.650	0.832	0.011	0.255
IL-6						
Δ (AZM-placebo)	5684	9770	14125	-667	18183	-2633
P value	0.280	0.270	0.081	0.933	0.043	0.771
GCSF						
Δ (AZM-placebo)	1176	3613	-115	-375	1543	-1297
P value	0.140	0.02	0.934	0.784	0.330	0.408

	V1# Baseline	V2* 3 months	V3* 6 months	V4* 9 months	V5* End of treatment	V6* Run-out
MMP-9						
Δ (AZM-placebo)	189	101	1620	217	903	1235
P value	0.322	0.931	0.128	0.835	0.432	0.300
TNF-α						
Δ (AZM-placebo)	-697	2174	33037	3542	4076	1410
P value	0.224	0.884	0.016	0.792	0.787	0.926
IL-1β						
Δ (AZM-placebo)	-15885	414	10837	152	33078	36550
P value	0.130	0.995	0.852	0.998	0.603	0.577
MPO						
Δ (AZM-placebo)	-82	147	407	47	700	32
P value	0.297	0.785	0.406	0.923	0.197	0.953
ECP						
Δ (AZM-placebo)	-60	3.9	107	21	88	2.3
P value	0.351	0.928	0.007	0.590	0.047	0.958
IP-10						
Δ (AZM-placebo)	1088	21409	-7726	1638	230	6.5
P value	0.327	0.062	0.459	0.873	0.984	1.000
MIP-1β						
Δ (AZM-placebo)	-14558	25018	161912	1591	66143	44325
P value	0.196	0.724	0.013	0.980	0.353	0.541
VEGF						
Δ (AZM-placebo)	4675	6402	28999	256	16117	-1272
P value	0.256	0.605	0.011	0.982	0.196	0.920
IL-1RA						
Δ (AZM-placebo)	5278007	–	–	–	–	–
P value	0.195					
IL-21						
Δ (AZM-placebo)	17	-52	1018	-17	5.2	16
P value	0.696	0.979	0.045	0.973	0.993	0.987

	V1# Baseline	V2* 3 months	V3* 6 months	V4* 9 months	V5* End of treatment	V6* Run-out
GRO-α						
Δ (AZM-placebo)	-7733	69619	4226	12487	162664	-846
P value	0.483	0.308	0.946	0.839	0.025	0.990
IL-1α						
Δ (AZM-placebo)	179	-484	7271	339	1226	2242
P value	0.581	0.948	0.282	0.959	0.866	0.771
MIP-3α						
Δ (AZM-placebo)	2973	4214	-1337	-1556	-1836	-6992
P value	0.159	0.165	0.628	0.567	0.557	0.025

Data are presented as Δ AZM-Placebo. #results after independent samples T-test; *results after mixed model analysis with correction for baseline

Supplemental 3: Assays

The following assays were performed on sputum supernatants: ECP was measured using ECP monoclonal capture antibody (clone 614, Diagnostics Development, Uppsala, Sweden), ECP standard (ImmunoCAP ECP Calibrator Nieuwegein, the Netherlands), and biotinylated polyclonal detection antibody (Diagnostics Development, Uppsala Sweden) as described elsewhere.[35]

MPO was measured using duoset reagents DY3174 (R&D) and all steps were performed as described elsewhere.[36]

The following cyto-and chemokines were measured using eBioscience reagents: IP-10/CXCL10, MIP-1/CCL4, MIP-3/CCL20, MMP9, VEGF-A, INF-, TNF-, IL-1RA, IL-21, G-CSF, GM-CSF and GRO-/CXCL1 according to the manufacturer's instructions. The plates were read on a Bioplex 200 (BioRad).

Sputum analysis by UPLC-MS/MS

$^{2}H_{4}$-succinic acid was added as internal standard to 100ul sputum. After vortexing, the sample was deproteinated using a 30kD Amicon filter and 1uL formic acid was added to the filtrate. 10uL of extract was injected to an UPLC-MS/MS (XEVO TQ-S micro, Waters, Milford, Massachusetts, USA) operated in negative ESI mode using MRMs for the

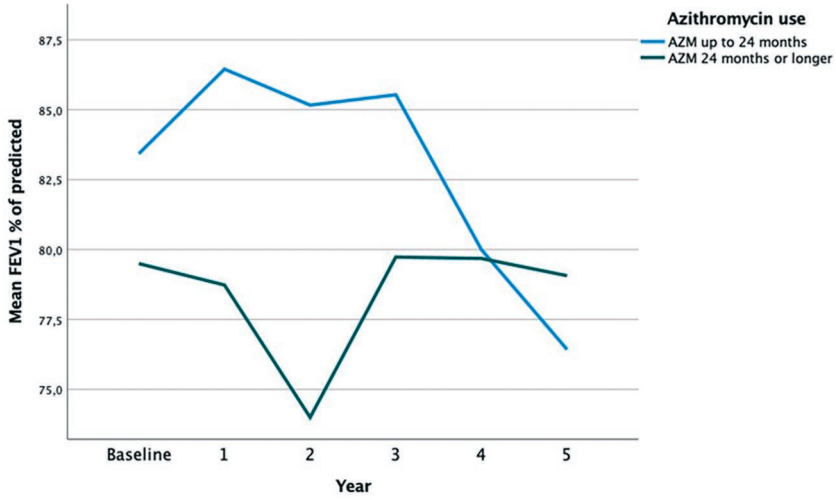
preselected analytics. Chromatographic separation was chieved using an Acquity HSS T3, 200*2.1, 1.7uM analytical column and a linear gradient between solution B (methanol) and solution A (0.05M formic acid) with a flow rate of 0.5mL/min. The gradient was programmed: 0-3 min 95% A and 5% B, 3 – 3.1 min 95% A and 0% A and 5% B 100% B, 3.1 5 min 0% A and 100% B, 5 – 5.1 min 0% A 95% A and 100% B 5% B; all steps were linear. Data processing was performed using Masslynx 4.2 sofware.

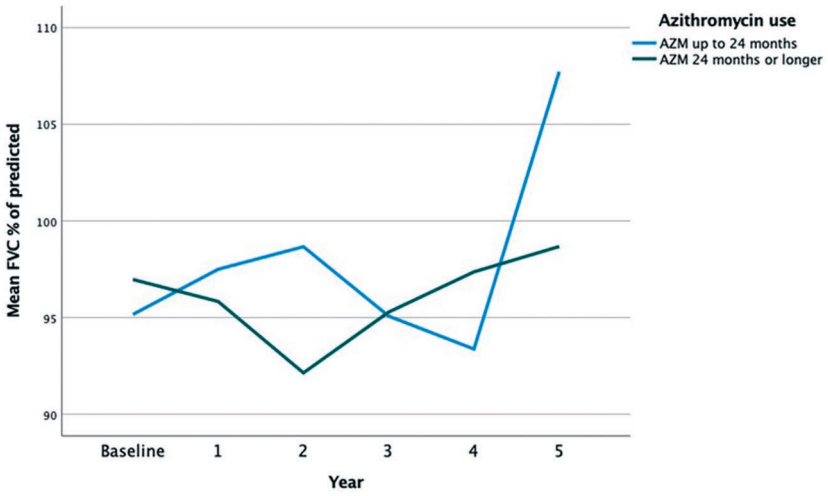
Chapter 5

Efficacy and safety of long-term azithromycin in bronchiectasis, analysis up to 5 years of maintenance treatment

Submitted

Supplemental 1: Spirometry measurements during 5 years of AZM maintenance treatment





Supplemental 1; Spirometry measurements during 5 years of AZM maintenance treatment. All values are expressed as mean (SD); *X*-axis: years; *Y*-axis mean FVC or FEV_1 % of predicted; AZM; azithromycin.

CHAPTER 7

Effects of longterm tobramycin inhalation solution (TIS) once daily on exacerbation rate in patients with non-cystic fibrosis bronchiectasis. the battle randomized controlled trial

Terpstra, Lotte C., et al. "Effects of long-term tobramycin inhalation solution (TIS) once daily on exacerbation rate in patients with non-cystic fibrosis bronchiectasis." Respiratory Research 23.1 (2022): 330.

Supplemental 1: Overview of the in- and exclusion criteria of the BATTLE study

Inclusion criteria	Exclusion criteria
1. Age ≥ 18 years	1. Any exacerbation within the month prior to the start of the study
2. The presence of chronic respiratory symptoms such as cough, dyspnea, expectoration of sputum	2. Diagnosis of Cystic Fibrosis
3. Confirmed bronchiectasis by (HR)CT	3. Diagnosis of Active Allergic Bronchopulmonary Aspergillosis
4. Documented history of at least 2 pulmonary exacerbations treated with courses of antibiotics and/or prednisolone within 12 months before inclusion	4. Any oral, IV or inhaled antibiotics (except for macrolides) within 1 month prior to the start of the study
5. No course of antibiotics or maintenance antibiotics (except for macrolides) 1 month prior to the start of the study	5. Any IV or IM corticosteroids or change in oral corticosteroids (> 10mg) within 1 month prior to the start of the study
6. Minimal one documented sputum or BAL-fluid culture with gram-negative bacteria or S. aureus within 12 months	6. Any change/start treatment regimens macrolides, hypertonic saline, inhaled mannitol or other mucolytics, corticosteroids within 1 month prior to the start of the study
7. Growth of protocol defined pathogens (gram-negative bacteria or *S. aureus*) in sputum at randomization	7. Severe immunosuppression or active malignancy
	8. Active tuberculosis or non-tuberculous mycobacterial infection

Inclusion criteria	Exclusion criteria
	9. Chronic renal insufficiency (eGFR < 30ml/ min)
	10. Use of loop diuretics, urea or mannitol
	11. Earlier diagnosed hearing impairment, balance disorders or neuromuscular disorders
	12. Serious active haemoptysis
	13. Have received an investigational drug or device within 1 month prior to the start of the study
	14. Serious or active medical or psychiatric illness
	15. Pregnancy and childbearing
	16. History of poor cooperation or non-compliance
	17. Unable to use nebulizers
	18. Allergic for tobramycin (or NaCl 0.9%)

In-and exclusion criteria. *Abbreviations:* Active allergic bronchopulmonary aspergillosis (ABPA); cystic fibrosis (CF); non-tuberculous mycobacterial infection (NTM); High resolution computed tomography (HRCT)

Supplemental 2: Overview of the study assessments for the BATTLE study

Primary assessment	Secondary assessments
1. Reduce in number of exacerbations	1. Time to next exacerbation
	2. Change in lung function (FEV1% and FVC%)
	3. Change in QoL measurements (QoL-B, LRTI-VAS, Leicester cough)
Safety assessments	**Additional assessments**
6. Occurrence of any AE or SAE	1. Development of tobramycin resistance in sputum (if possible, with MIC values)
7. Occurrence of bronchospasm during the tolerance test	2. Bacterial load in sputum and pathogen eradication
8. Occurrence of bronchospasm, dyspnea, cough, or other respiratory symptoms during the study	3. Occurrence of new pathogens
9. Occurrence of hearing impairment/ tinnitus	4. Change in inflammatory markers in serum
10. Change in safety laboratory values (renal and liver function)	5. Analyses of the use of inhaled medication (time consuming, treatment burden)

Abbreviations: Adverse event (AE); Serious adverse event (SAE); non-tuberculous mycobacterial infection (NTM); Forced expiratory volume in one second (FEV_1); Forced vital capacity (FVC); Quality of life (QoL); quality of life bronchiectasis questionnaire (QoL-B); Lower respiratory tract infections – Visual Analogue Scale (LRTI-VAS); Leicester cough questionnaire (Leicester cough); Minimum Inhibitory Concentration (MIC);

Supplemental 3: Results of the longitudinal analysis, exacerbation, lung function and QoL for the PP-population

All randomized patients who received and completed treatment according to the study protocol for at least 9 months were included in the per protocol (PP) analysis. A total of 29 patients were included in the per protocol analysis. 17 (65%) patients were treated with TIS, and 22 (84%) patients were treated with placebo. Longitudinal analysis showed a RR of 0.70 (0.42 – 1.17)

	Difference (TIS-placebo) 26 weeks	***P* value**	**Difference (TIS-placebo) 52 weeks**	***P* value**
QoL_B_physical	-7.73	0.27	-4.44	0.51
QoL_B_role	-13.14	0.01	-0.95	0.85
QoL_B_vitality	-5.60	0.37	6.48	0.29
QoL_B_emotional	-0.83	0.82	-6.04	0.10
QoL_B_social	-9.58	0.07	-2.37	0.65
QoL_B_treatmentburden	10.53	0.22	0.11	0.99
QoL_B_healthperc	-9.17	0.07	2.98	0.55
QoL_B_resp_sympt	-7.46	0.16	-0.53	0.92
Lrti_vas_dyspnoea	0.31	0.68	0.26	0.73
Lrti_vas_tiredness	-0.44	0.54	-0.42	0.55
Lrti_vas_colour_phlegm	-0.82	0.34	-0.89	0.29
Lrti_vas_cough	0.09	0.90	-0.52	0.49
Lrti_vas_pain	0.76	0.23	0.64	0.30
Lrti_vas_total_score	-0.25	0.91	-0.64	0.77
Leic_score_physical	-0.20	0.54	-0.21	0.52
Leic_score_psych	-0.60	0.11	0.36	0.32
Leic_score_social	-0.72	0.05	-0.12	0.72
Leic_score_total	-1.5	0.14	-0.35	0.72
FEV_1%	-2.19	0.35	-3.83	0.13
FVC%	-4.54	0.16	-3.16	0.35

Abbreviations: Forced expiratory volume in one second (FEV_1); Forced vital capacity (FVC).

Supplemental 4: Results of the longitudinal analysis of lung function and QoL for the mITT population

	Difference (TIS-placebo) at 26 weeks	*P* value	Difference (TIS-placebo) at 52 weeks	*P* value
FEV_1%	-0.30	0.89	-1.61	0.488
FVC%	-1.90	0.51	-0.70	0.82

Abbreviations: Forced expiratory volume in one second (FEV_1); Forced vital capacity (FVC).

	Difference (TIS-placebo) at 26 weeks	*P* value	Difference (TIS-placebo) at 52 weeks	*P* value
QoL_B_physical	-6.76	0.70	-3.65	0.53
QoL_B_role	-8.70	0.06	1.60	0.72
QoL_B_vitality	-5.64	0.31	4.07	0.47
QoL_B_emotional	0.07	0.98	-4.17	0.19
QoL_B_social	-8.78	0.07	-4.68	0.33
QoL_B_treatmentburden	4.15	0.17	3.52	0.63
QoL_B_healthperc	-5.89	0.20	1.89	0.68
QoL_B_resp_sympt	-5.14	0.24	0.07	0.99
Lrti_vas_dyspnoea	4.16	0.50	0.19	0.76
Lrti_vas_tiredness	-0.76	0.23	-0.49	0.43
Lrti_vas_colour_phlegm	-0.91	0.21	-1.38	0.06
Lrti_vas_cough	-0.14	0.84	-0.56	0.40
Lrti_vas_pain	0.75	0.18	0.66	0.23
Lrti_vas_total_score	-0.35	0.86	-1.38	0.48
Leic_score_physical	-0.20	0.48	-0.16	0.55
Leic_score_psych	-0.42	0.19	0.27	0.38
Leic_score_social	-0.49	0.11	-0.08	0.79
Leic_score_total	-1.51	0.08	-0.49	0.55

Abbreviations: Quality of life (QoL); quality of life bronchiectasis questionnaire (QoL-B); Lower respiratory tract infections – Visual Analogue Scale (LRTI-VAS); Leicester cough questionnaire (Leicester cough).

Supplemental 5: Overview of adverse events and serious adverse events

Serious adverse events	**28**
Hospital admission	28
Protocol defined pulmonary exacerbation	24
Known cardiac diseases	2
Near- collapse	1
Anaphylactic reaction on amoxicillin clavulanate	1
Adverse events	**157**
Protocol defined pulmonary exacerbation	99
Non-protocol defined pulmonary exacerbation	20
Antibiotics for other reasons	8
Persistent cough/ dyspnea/ hoarseness	4
Tinnitus	3
Headache	3
Strain muscles	3
Tiredness	2
Rectal bleeding/Anemia	2
Nausea/vomiting	2
Chest pain	2
Radiotherapy (treatment malignancy)	2
Dry mouth	2
Heart failure	1
Anaphylactic reaction	1
Renal dysfunction due to prostate hyperplasia	1
Renal dysfunction due to diuretics	1
Renal dysfunction	1

Supplemental 6: Overview of the inflammatory markers in serum

CRP, mean (SD)	**Tobramycin**	**n (%)**	**Placebo**	**n (%)**	***P* value**
Start study	10.2 (9.7)	26 (100)	7.3 (10.6)	26 (100)	0.02
3 months	16.3 (36.7)	22 (84.6)	8.0 (14.5)	26 (100)	0.26
6 months	5.4 (7.2)	20 (76.9)	9.5 (13.9)	23 (88.5)	0.32
9 months	8.5 (12.0)	14 (53.8)	6.5 (7.7)	20 (76.9)	0.83
End study	7.9 (11.3)	19 (73.1)	11.0 (23.8)	23 (88.5)	0.89
Run out (after 4 weeks)	20.1 (43.8)	14 (53.8)	6.5 (6.0)	18 (69.2)	0.76
Leucocytes, mean (SD)	**Tobramycin**	**n (%)**	**Placebo**	**n (%)**	***P* value**
Start study	10.3 (4.4)	26 (100)	9.0 (3.6)	26 (100)	0.25
3 months	10.6 (4.8)	22 (84.6)	9.0 (3.4)	26 (100)	0.24
6 months	10.6 (4.5)	20 (76.9)	9.1 (3.3)	23 (88.5)	0.25
9 months	9.8 (5.6)	14 (53.8)	9.1 (2.4)	20 (76.9)	0.61
End study	10.3 (5.0)	19 (73.1)	8.8 (2.4)	23 (88.5)	0.52
Run out (after 4 weeks)	10.6 (5.5)	14 (53.8)	9.1 (2.3)	18 (69.2)	0.51
Eosinophils, mean (SD)	**Tobramycin**	**n (%)**	**Placebo**	**n (%)**	***P* value**
Start study	0.2 (0.1)	26 (100)	0.3 (0.4)	26 (100)	0.54
3 months	0.2 (0.1)	22 (84.6)	0.3 (0.2)	26 (100)	0.06
6 months	0.3 (0.3)	20 (76.9)	0.4 (0.4)	23 (88.5)	0.13
9 months	0.2 (0.2)	14 (53.8)	0.4 (0.6)	20 (76.9)	0.23
End study	0.2 (0.1)	19 (73.1)	0.4 (0.4)	23 (88.5)	0.02
Run out (after 4 weeks)	0.2 (0.1)	14 (53.8)	0.3 (0.1)	18 (69.2)	0.12

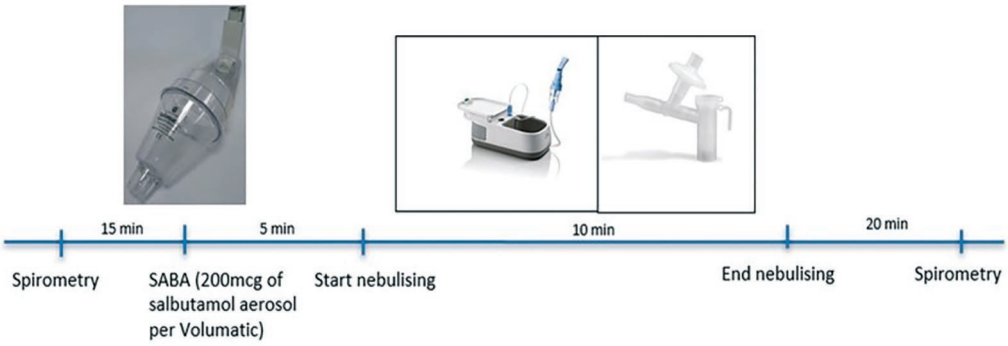
Overview of the inflammatory markers in serum every 3 months during the study visits. During an exacerbation, the inflammatory markers in serum were not measured on regulatory base (not shown).

Chapter 8

Treatment with inhaled antibiotics in bronchiectasis, side effects and evaluation of the tolerance test – analysis from the battle randomized controlled trial

Terpstra, Lotte., et al. Treatment with inhaled antibiotics in bronchiectasis, side effects, and evaluation of the tolerance test; analysis from the BATTLE randomized controlled trial. Clin Respir J. 2023; 1- 6.

Supplemental 1: Time schedule of the tolerance test



Abbreviations: SABA: short-acting beta agonist.

Supplemental 2: Analysis of the sub population who withdrawn from the study due to airway hyperresponsiveness

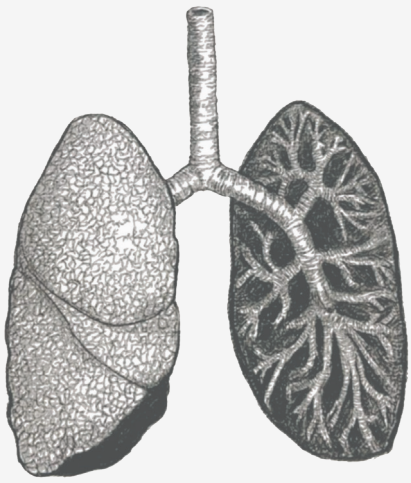
Discontinuation due to airway hyperresponsiveness	**Total n = 6**		
Spirometry measurements	**before**	**after**	***P* value**
FEV_1 Liters	1.7 (0.61)	1.7 (0.56)	0.58
FEV_1% of predicted	79.8 (28.8)	84.0 (31.4)	0.47
FVC Liters	2.8 (0.6)	3.0 (0.5)	0.09
FVC% of predicted	100.2 (15.4)	107.3 (14.2)	0.09
Etiology			
asthma	2 (33.3)		
idiopathic	2 (33.3)		
post-infective	1 (16.7)		
immunodeficiency	1 (16.7)		
Smoking status			
never	5 (83.3)		
actual	1 (16.7)		

Supplemental 3: Overview of the adverse events and serious adverse events in the total population

Serious adverse events	**28**
Hospital admission	28
Protocol defined pulmonary exacerbation	24
Known cardiac diseases	2
Near- collapse	1
Anaphylactic reaction on amoxicillin clavulanate	1
Adverse events	**157**
Protocol defined pulmonary exacerbation	99
Non-protocol defined pulmonary exacerbation	20
Antibiotics for other reasons	8
Persistent cough/ dyspnea/ hoarseness	4
Tinnitus	3
Headache	3
Strain muscles	3
Tiredness	2
Rectal bleeding/Anemia	2
Nausea/vomiting	2
Chest pain	2
Radiotherapy (treatment malignancy)	2
Dry mouth	2
Heart failure	1
Anaphylactic reaction	1
Renal dysfunction due to prostate hyperplasia	1
Renal dysfunction due to diuretics	1
Renal dysfunction	1

Chapter 11

Summary in Dutch



Bronchiëctasieën is een aandoening van de luchtwegen waarbij deze abnormaal verwijd zijn. Hierdoor blijft in de luchtwegen makkelijk slijm achter en is dit een bron van chronische inflammatie en infectie. Deze chronische verwijding van de luchtwegen kan door allerlei oorzaken zijn ontstaan. Hierbij moet men denken aan frequente ontstekingen van de luchtwegen door steeds terugkerende bacteriële of virale infecties. Dit kan zijn in het geval van astma of COPD, maar ook bij chronische aspiratie of een onderliggende afweerstoornis. Ook kunnen bronchiëctasieën ontstaan na een bof of mazelen infectie op de kinderleeftijd, of bijvoorbeeld bij een vroeggeboorte. Bij patiënten met taaislijmziekte, oftewel Cystic Fibrosis (CF), ontstaan ook bronchiëctasieën. In de studies die wij met betrekking tot dit promotieonderzoek hebben verricht wordt deze laatste patiëntengroep niet in het onderzoek betrokken. In de onderhavige studies onderzoeken wij alleen patiënten met de zogenaamde Non-CF bronchiëctasieën.

In hoofdstuk 2 wordt de oorzaak (etiologie) van de bronchiëctasieën onderzocht in relatie tot de kwaliteit van leven, met behulp van een specifieke vragenlijst. Dit betreft de kwaliteit-van-leven-bronchiëctasieën-vragenlijst. We zien in dit onderzoek dat patiënten met een onderliggende COPD naast het ontstaan van de bronchiëctasieën een significant verminderde kwaliteit van leven hebben in vergelijking met de andere etiologieën. We moeten bij deze subgroep derhalve alerter zijn en mogelijk ook eerder een behandeling instellen.

Door de abnormale verwijding van de luchtwegen blijft er slijm in die gebieden achter en ontstaat daar een continue irritatie en chronische ontsteking, waarbij dan ook vaak allerlei bacteriën betrokken zijn. Patiënten ervaren veel klachten van hoesten met slijm, en soms ook bloed ophoesten. Daarbij zijn patiënten erg vermoeid en hebben geen energie voor hun dagelijkse bezigheden. Ook hebben patiënten frequent ontstekingen van de luchtwegen waarvoor antibiotica en soms ook prednison noodzakelijk is. Deze ontstekingen, oftewel exacerbaties, kunnen dan ook leiden tot ziekenhuisopnames. Ook wordt er een afname van de longfunctie gezien en een blijvend verminderde kwaliteit van leven.

Bij patiënten met frequente exacerbaties, meer dan 2 á 3 keer per jaar, kan een onderhoudsbehandeling met een lagere dosering antibiotica voorgeschreven worden. Het gaat dan om het antibioticum azitromycine. In eerdere grote studies is gebleken dat dit zorgt voor een vermindering van het aantal ontstekingen en een verbetering van de kwaliteit van leven.

In hoofdstuk 3 wordt beschreven dat deze onderhoudsbehandeling met azitromycine ook verbetering laat zien van de bekende afwijkingen op CT-scans, passend bij bronchiëctasieën.

Op deze radiologische beelden ziet men een afname van ontstekingen en een vermindering van de ophoping van slijm.

Het werkingsmechanisme van azitromycine is nog niet geheel ontrafeld. Enerzijds zorgt het voor vermindering/verdwijning van de eerdere aangetoonde bacteriën in het slijm (antibacterieel). Maar ook heeft azitromycine een remmend effect op de continue ontstekingsreactie in de luchtwegen, oftewel het immuun-modulerend effect. We denken dat daarbij bepaalde typen ontstekingscellen, zoals bijvoorbeeld de neutrofielen, worden geremd.

In hoofdstuk 4 wordt onderzocht wat het effect van azitromycine is op allerlei typen ontstekingsstoffen (cytokines) in het slijm van de patiënt. In dit onderzoek wordt geen duidelijk verband gezien tussen het gebruik van azitromycine en de afname van deze specifieke cytokines in het slijm van de patiënt. Mogelijk dat andere cytokines hierin een rol spelen; cytokines die niet in deze studies zijn onderzocht. Ook zou dit immuun-modulerende effect mogelijk een minder grote rol spelen dan dat altijd werd gedacht. Aanvullend onderzoek naar het immuun- modulerende effect van azitromycine wordt dan ook geadviseerd.

Dit gecombineerde antibacteriële, maar dus ook immuun-modulerende effect heeft ervoor gezorgd dat azitromycine frequent als onderhoudsbehandeling wordt voor-geschreven.

Patiënten gebruiken dit 1x per dag, of om de dag, en gebruiken dit jaren achter elkaar.

In hoofdstuk 5 wordt beschreven wat er gebeurt gedurende dit jarenlange gebruik. Hierin komt naar voren dat gedurende 5 jaar het aantal ontstekingen in de luchtwegen laag blijft, en daarmee het effect van azitromycine blijft bestaan. Wel wordt een toename in resistentie gezien, en zien we ook dat bepaalde typen bacteriën meer aanwezig zijn, dan voor de start van de behandeling met azitromycine. Bij langdurig gebruik van azitromycine zou tussentijds een herbeoordeling en eventueel een proefstop overwogen kunnen worden, met de bedoeling dat hierdoor bijvoorbeeld minder resistentie optreedt.

Daarnaast kan azitromycine ook leiden tot bijwerkingen. Dit zijn met name maag-darmklachten met buikpijn en diarree. Ook worden nierfunctiestoornissen gezien

en hebben sommige patiënten last van oorsuizen. Soms hebben patiënten ondanks het gebruik van azitromycine, toch nog klachten van de luchtwegen en blijven er exacerbaties bestaan. Daarnaast bestaan er interacties met andere medicamenten die patiënten al gebruiken en kan azitromycine derhalve niet altijd voorgeschreven worden. Een alternatief voor het gebruik van azitromycine is een onderhoudsbehandeling met inhalatie van antibiotica. Inhalatie van antibiotica wordt al frequent voorgeschreven bij patiënten met CF bronchiëctasieën. In deze groep patiënten is er ook al veel onderzoek naar gedaan. In de patiëntengroep met Non-CF bronchiëctasieën is dat veel minder het geval.

De onderzoeken die zijn gedaan met verschillende typen inhalatie-antibiotica laten een afname zien van de bacteriën in het slijm. Ook zien we in 1 onderzoek dat mogelijk de tussenliggende tijd tot de volgende exacerbatie langer wordt.

Hoofdstuk 6 beschrijft de studie-opzet van de BATTLE-studie, waarin het effect van verneveling (inhalatie) van tobramycine, een aminoglycosiden antibiotica, wordt onderzocht. In deze studie wordt het effect van tobramycine-verneveling onderzocht bij patiënten met Non-CF bronchiëctasieën met meer dan 2 exacerbaties in het voorgaande jaar.

Het effect van tobramycine op het aantal exacerbaties wordt vergeleken met placebo, wat een zout- verneveling betreft. Patiënten gebruiken de studiemedicatie 1 x per dag gedurende 1 jaar en weten zelf niet welk medicament zij gebruiken. Patiënten houden wekelijks een dagboekje bij waarin wordt gevraagd naar afname van de luchtwegklachten, maar ook wordt gevraagd naar de eventuele bijwerkingen. Bij de start van de studie wordt een proefverneveling verricht in het ziekenhuis, waarbij wordt gekeken of het inhaleren van de medicatie goed gaat en de patiënt geen klachten krijgt van de luchtwegen door de verneveling zelf. Daarna worden de patiënten elke 3 maanden op de polikliniek gezien met voorafgaand bloedonderzoek en een kweek van het slijm. Ook worden specifieke vragenlijsten bijgehouden en wordt gedurende de studie en aan het einde van de studie een longfunctieonderzoek verricht. Vooraf wordt verwacht dat er een afname van 50% in het aantal exacerbaties ontstaat bij het gebruik van tobramycine in vergelijking met de groep placebo gebruikers. De studie wordt uitgevoerd in 6 ziekenhuizen in Nederland.

De resultaten van deze ‘randomized placebo-controlled trial›: The BATTLE study’, worden beschreven in hoofdstuk 7. Door het gebruik van tobramycine-verneveling, 1x

per dag gedurende 1 jaar, wordt een afname in het aantal exacerbaties gezien van ongeveer 30% ten opzichte van de placebogroep. Helaas wordt de vooraf verwachte 50% afname niet behaald, wat mede verklaard zou kunnen worden uit het positieve effect van de zout-verneveling (de placebogroep) op de sputumklaring. Daarnaast zijn er relatief veel patiënten uitgevallen tijdens de studie, zowel in de placebogroep als in de tobramycine groep. Mogelijk komt dit doordat het vernevelen zelf een intensieve behandeling is waar je 20 tot 30 minuten per dag mee bezig bent. Verder worden er de bekende bijwerkingen van tobramycine verneveling geobserveerd, echter komt dit niet bij veel patiënten voor. Concluderend is het vernevelen van tobramycine 1x per dag een veilige manier van behandelen en is dit een potentiële behandelmogelijkheid voor geselecteerde patiënten met bronchiëctasieën.

In hoofdstuk 8 wordt nog verder ingegaan op het gebruik van tobramycine-verneveling. In eerdere studies is gezien dat tobramycine-verneveling zelf ook kan zorgen voor klachten van de luchtwegen met hoesten en toename van benauwdheid. Om die reden wordt bij de start van de verneveling altijd een eerste proefverneveling verricht op de polikliniek, waarbij in sommige ziekenhuizen patiënten hier zelfs voor worden opgenomen. In de BATTLE-studie hebben alle patiënten ook deze proef-verneveling, oftewel tolerantietest, ondergaan. Alle patiënten kregen voor het gebruik van de inhalatiemedicatie een luchtwegverwijder en daarna volgde de verneveling met de studiemedicatie. Voor en na het gebruik van de inhalatiemedicatie + studiemedicatie werd een longfunctieonderzoek verricht. Het laatste hoofdstuk, hoofdstuk 8, beschrijft de resultaten van deze analyse. In de studie werden geen luchtwegklachten geobserveerd tijdens deze tolerantietest en werd er ook geen verslechtering in het longfunctieonderzoek gezien. In dit onderzoek werd dus geen meerwaarde gezien van deze tolerantietest. Het is juist wel van belang om de patiënten gedurende de behandeling goed in de gaten te houden omdat wel werd gezien dat er luchtwegklachten ontstonden na ongeveer 2 maanden gebruik van de inhalatiemedicatie. De andere bekende bijwerkingen die werden geconstateerd waren mild, waarbij geen klinisch significant verschil werd gezien tussen de patiënten die werden behandeld met tobramycine of placebo.

Geconcludeerd kan worden dat tobramycine-inhalatie 1x per dag een veilige behandelmogelijkheid is, in een geselecteerde bronchiëctasieën patiëntenpopulatie. En het juist de mogelijkheid biedt voor patiënten met veel exacerbaties en met ook meerdere soorten bacteriën in het slijm.

Uiteindelijk blijft het belangrijk dat goed naar de klachten, maar ook de behoeften van de patiënt wordt gekeken. En daarbij een stapsgewijs behandelplan wordt overwogen en besproken. Als eerste moet gekeken worden naar goede sputumevacuatie, waarbij allerlei specifieke hoest- technieken kunnen helpen. Dit kan ondersteund worden met de zout vernevelingen. En zoals ook eerder al genoemd, is het belangrijk dat een onderhoudsbehandeling met azitromycine wordt overwogen bij bepaalde bronchiectasie-patiënten. Als dat te weinig effect heeft, of er zijn bijwerkingen, dan kan antibiotica-verneveling, zoals dus met tobramycine, worden overwogen.

Het is van belang dat in toekomstige studies hier nog specifieker naar wordt gekeken. Zijn andere soorten antibiotica-onderhoud nog een goede optie? Of kunnen we het gebruik van azitromycine een periode onderbreken, waardoor minder resistentie optreedt, maar het effect wel blijft bestaan.?

En wat is nu het daadwerkelijke effect van de zout-verneveling? De BATTLE-studie suggereert dat alleen zout-verneveling ook het aantal exacerbaties doet dalen, maar is dat werkelijk zo? En welke concentratie zou dat dan moeten zijn?

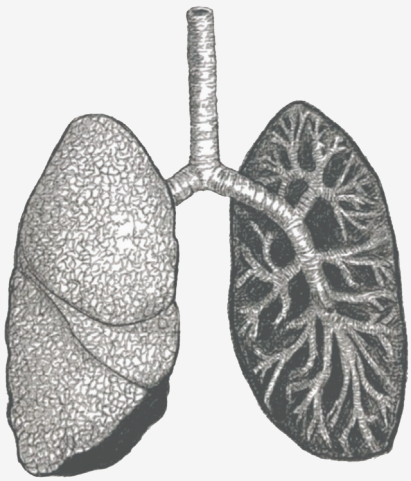
Ook is er steeds meer aandacht voor het remmen van de chronische ontstekingsreactie in de longen bij patiënten met bronchiëctasieën. Bovendien zijn er kortgeleden belangrijke en positieve resultaten gepubliceerd over het medicijn Brensocatib, dat specifiek de eerdergenoemde 'neutrofiele inflammatie' remt. Al met al blijft de groeiende groep bronchiëctasieën-patiënten een interessante populatie voor onderzoek en zijn er veelbelovende studies in opkomst.

Chapter 12

Dankwoord

About the author

List of publications



Dankwoord

1 Januari 2016, de eerste dag van mijn promotietraject.
Een jaar eerder, ik was net in opleiding tot longarts, vroeg Wim Boersma mij of ik interesse had in een promotietraject. Dat leek mij een geweldige kans… Niet wetende, dat ik uiteindelijk pas in 2023 dit traject zou gaan afronden.

En ik in die jaren zo vaak het liedje van Ramses Shaffy in mijn hoofd heb afgespeeld...
'Wij zullen doorgaan…,'

Toch ben ik Wim ontzettend dankbaar voor die vraag en natuurlijk voor alle begeleiding, als mijn copromotor, in de jaren daarna. Want het doen van promotieonderzoek heeft zo ongelofelijk veel toegevoegde waarde. Alle contacten die je opbouwt in den lande, de kansen en mogelijkheden die het mij heeft gebracht, en je op plekken komt waar je anders nooit zou zijn geweest. Het heeft mij wetenschappelijk, maar ook als mens, ontzettend verrijkt.

Want een eigen multicenter placebogecontroleerde randomized controlled trial opzetten is niet iets wat je dagelijks doet. Landelijke goedkeuringen, logistieke puzzels, de productie van zo identiek mogelijke placebo's en dan nog het gekoelde transport van de medicatie. Maar ook de contacten en (financiële) onderhandelingen met de betrokken afdelingen, als apotheek, klinische chemie, en microbiologie. En dat keer op keer per deelnemend centrum.

Alleen was dit mij nooit gelukt.
Velen ben ik, in dit hele traject, dank verschuldigd.

Allereerst natuurlijk Wim.
Je liet mij tijdens mijn promotieonderzoek mijn eigen gang gaan, wat voor mij juist heel goed werkte. Je stimuleerde mij wat betreft nieuwe studie-ideeën en dat bespraken we onder andere in het wekelijks donderdagochtend wetenschapsoverleg.
Dankjewel Wim voor de jarenlange samenwerking en alles wat ik van jou heb mogen leren, met name op het gebied van de long-infectieziekten. Ik ben dan ook vereerd dat ik nu jouw plek heb mogen overnemen in de prachtige opleidingskliniek Longziekten van het NWZ te Alkmaar. En ik heb nu al meer dan eens gehoord tijdens een overdracht... 'Hoor ik daar nu een 2e Wim praten?'

Dank ook aan alle patiënten, die hebben deelgenomen aan het onderzoek. Mij in vertrouwen hebben genomen en ik bij meerdere van hen een kijkje in hun leven heb kunnen nemen. Ik heb zoveel bijzondere momenten met hen gedeeld en vele kopjes koffie bij hen thuis gedronken bij het brengen van de studiemedicatie.

Ook de ondersteuning van de klinische chemie, medisch microbiologie en apotheek van het NWZ was bewonderenswaardig. Dankjewel Wouter Rozemeijer voor het, al direct, meedenken met de eerste opzet van het protocol en meerdere van mijn uiteindelijke publicaties. Waarbij de gevatte opmerkingen vanuit een microbiologische blik mij zeker hebben geholpen.

Maar ook Kitty Molenaar vanuit de apotheek. Wat hebben wij veel contact gehad over de logistieke planning, vervoer en opslag van de medicatie. Alles moest gekoeld. Jouw betrokkenheid bij de studie had een grote rol in het geheel. En ons wekelijks contact als ik weer de medicatie kwam ophalen, liet mij zien hoe betrokken jij was bij de studie, maar ook bij mij. We komen elkaar vast weer snel bij een NWZ-run tegen.

En, niet te vergeten, Casper de Graaff, oud Longarts, die als independent expert, mijn studie controleerde op veiligheid en uitvoerbaarheid. We hadden ten tijde van de BATTLE-studie een aantal keer per jaar een meeting, waarbij wij ook zoveel andere zaken bespraken, dat we soms maar kort, maar secuur, de studie onder de loep namen. Dankjewel voor jouw bijdrage, hetgeen geleid heeft tot een prachtige publicatie.

Daarnaast wil ik de longartsen bedanken van de deelnemende ziekenhuizen die mij ondersteund hebben en mij poli lieten doen in hun werkkamers zodat ik de patiënten kon includeren. Yvonne Berk, Longarts Catharina Wilhelmina Ziekenhuis, Martijn de Kruif, Longarts Zuyderland MC, Dominic Snijders, Longarts Spaarne Gasthuis en natuurlijk Inez Bronsveld Longarts UMC Utrecht, die tevens mijn copromotor is.

Utrecht is de stad waar ik mijn proefschrift zal verdedigen. Na een meeting met Professor Harry Heijerman, hoogleraar Longziekten UMCU, was het direct meer dan logisch dat ik onder zijn leiding zou gaan promoveren. Dank voor het delen van je brede kennis op het gebied van infectie en natuurlijk ook Cystic Fibrosis (CF). Maar ook de betrokkenheid bij mij als persoon. Waarbij je meer dan eens zei... “die promotie komt er echt wel, maar geniet ook van je gezin en neem daar ook de tijd voor”.

Onderzoek in de periferie is anders dan in de academie, maar het is mij ontzettend goed bevallen. Ook was ons cardio-long research-hok op 117 echt niet anders dan in een academische setting. Er werd vooral veel koffiegedronken en samen met Victor, Marthe, Ruud en later ook Nienke heb ik daar een bijzondere tijd gehad. Het was altijd erg fijn om samen te sparren over SPSS, Castor, opzetten van studies, congresdeadlines en subsidieaanvragen.

Het NWZ heeft veel aandacht voor de wetenschap die lokaal wordt uitgevoerd. De Noordwest Academie onder leiding van Betsy van Soelen, biedt daarin veel ondersteuning. Voor mij in het bijzonder heeft Jeroen Doodeman een belangrijke rol gespeeld. Dankjewel voor jouw hulp bij alle statistische en epidemiologische vraagstukken die telkens maar weer op kwamen.

Lieve Evelien, Anke (en eerder ook Lida). Ons researchteam, ons aanspreekpunt. Ik ben elke keer weer zo blij dat jullie er zijn en zo betrokken zijn bij alle farmaceutische, maar ook investigator intiated studies. Het wordt ook meer dan eens bevestigd hoe belangrijk en onmisbaar jullie ondersteuning is. Dankjewel daarvoor!

Lieve Josje, mijn bronchiectasie-voorganger. Na jouw promotietraject met als onderwerp bronchiëctasieën, voelde ik mij vereerd om in jouw voetsporen te treden. We hebben tijdens mijn promotietraject een intensieve samenwerking gehad, je had daarnaast een belangrijke rol bij mijn manuscripten en dat leidde tot een aantal prachtige publicaties. Op naar nog vele congresbezoeken samen, presentaties en organisatie van symposia. En hopelijk kunnen wij een prachtige wetenschappelijke samenwerking tussen onze ziekenhuizen opbouwen.

Lieve longartsen in NWZ. 2020 Het eerste jaar van COVID, wat was het een spannende tijd, vooral die eerste periode. Niet wetende wat ons te wachten stond en hoelang dit allemaal zou duren. Voor mij persoonlijk ook onwerkelijk, 28 weken zwanger en dan de eerste COVID patiënt voor je neus. Door jullie zorg en daadkrachtige beslissing was ik al snel niet meer op de werkvloer en had ik daardoor weer meer ruimte voor mijn promotieonderzoek. Wat heeft dat veel zorgen bij mij weggenomen.
Het geeft aan jullie betrokkenheid en zorgzaamheid voor elkaar. Ik ben ontzettend blij dat ik binnen jullie maatschap ben toegetreden en ik mijn werkzaamheden op het gebied van infectie en wetenschap mag voortzetten. Op naar een jarenlange en fijne samenwerking met oog voor elkaar.

Specialistenopleiding, promotieonderzoek en een gezin met 2 jonge kinderen. Het was af en toe echt 'alle ballen hooghouden' en doorgaan. Als de kindjes sliepen, achter de laptop, of 's morgens vroeg in het weekend voordat iedereen wakker werd, nog een aantal uurtjes 'tikken'. Zodat we wel gewoon ook het weekend samen hadden.
En soms toch ook afspraken moeten afzeggen, alles om die deadline van dat tijdschrift te halen of omdat dan eindelijk je thesis naar de leescommissie kan...

Mijn lieve vrienden en vriendinnen. Dankjewel voor jullie betrokkenheid en interesse de afgelopen jaren. En in het bijzonder Jorien, Sanne, Helen, Nicky, Tanja en Cynthia.
Op het moment dat ik mijn dankwoord schrijf gaan we over 4 nachten met elkaar naar Valencia. En wat kijk ik daar naar uit!
Al bijna 30 jaar lief en leed met elkaar gedeeld en elkaar nooit uit het oog verloren. Zo'n bijzondere vriendschap is niet te beschrijven. We zijn vele vakanties samen verder en ik hoop dat wij dat nog jaren blijven doen. Op naar weer die vaste woensdagavond etentjes eens per maand.

Mijn lieve schoonfamilie, af en toe hadden jullie geen besef van waar ik mee bezig was. Is dat promotieonderzoek nu niet eens eindelijk klaar? Met veel belangstelling bleven jullie naar het proces vragen. Dankjewel voor jullie betrokkenheid al die jaren en ook het grote enthousiasme toen eindelijk die datum er was!

Lieve Paul en Maarten, mijn grote broers, maar ik ben vooral kleine zus. Altijd beschermend en zorgzaam naar mij. Betrokken bij elkaar bij alle grote en kleine gebeurtenissen. Trouw de feestdagen samen en met mooi weer onverwacht met z'n allen op hetzelfde strand. En daarnaast niet geheel onbelangrijk, jullie zijn te gekke ooms voor Lauren en Luca.

Lieve Papa en Mama. Dankjewel voor jullie kritische en opbouwende belangstelling ten aanzien van deze thesis.
Ik ben zo blij dat jullie zo betrokken zijn bij ons gezin, we elkaar wekelijks zien en ik bij kleine of grotere problemen altijd bij jullie terecht kan. Op het laatste moment oppas, geen probleem, samen logeren in Heerhugowaard vanwege een weekend vol dienst, geen probleem. Midden in de nacht naar Schiphol voor vakantie of richting een congres, geen probleem. Het zijn heel veel zorgen die worden weggenomen, die zomaar ontstaan met kleine kinderen en allebei een drukke baan. Dankjewel dat jullie er altijd met zoveel liefde voor ons zijn.

Lieve Steef, al 16 jaar mijn grote liefde. Jij hebt mij van geneeskunde-student, dokter zien worden en nu zelfs doctor. We zijn meer dan een team. We gunnen elkaar de wereld en jij begrijpt die gekke medische wereld als geen ander. Weekenden met de kids omdat ik toch weer moest werken, maar ook alle onzekerheden en intense situaties die ik als dokter meemaak. Je bent altijd mijn luisterend oor, mijn rots en hulp. Je bent voor Lauren en Luca een geweldige vader en zij kunnen zich echt geen betere wensen. Altijd feest samen, altijd op pad en genieten samen. Wat heb ik een geluk dat jullie mijn thuis zijn.

En als laatste onze kleine lieve aapies, Lauren Sophie en Luca Monte.
Wat is het een feest om jullie moeder te mogen zijn. Niets is ons meer waard dan gewoon met elkaar zijn. Op naar nog meer momenten, weekenden en vakanties samen. Met lange avonden, pizza, pasta, zwembaden en zee.

About the author

Lotte Carijn Terpstra werd geboren op 4 februari 1986 te Alkmaar. Zij groeide op in Heerhugowaard als jongste van een gezin met 2 grote broers. Zij slaagde voor het Atheneum van de Montessori-stroom op de middelbare school Huygenwaard in Heerhugowaard. Daarna behaalde zij haar propedeuse Biomedische Wetenschappen aan de Universiteit van Amsterdam. Na dit jaar begon zij met de studie geneeskunde aan de Universiteit van Amsterdam. In 2011 behaalde zij haar doctoraal geneeskunde.

Tijdens haar laatste coschappen raakte zij gefascineerd voor de Longgeneeskunde waarna zij in 2012 begon als ANIOS Longgeneeskunde, in destijds het Medisch Centrum Alkmaar. In 2014 zou zij gaan starten met de opleiding tot Longarts en heeft daarom van 2013 tot 2014 gewerkt als ANIOS Interne Geneeskunde. Uiteindelijk is zij in 2014 gestart met de opleiding tot Longarts in het Medisch Centrum Alkmaar onder leiding van Dr. J.G. Van den Aardweg en Drs. C.S. De Graaff. In 2016 heeft zij haar opleiding tot longarts onderbroken voor promotieonderzoek onder leiding van Dr. W.G. Boersma, te Alkmaar en Prof. H.G.M Heijerman, verbonden aan de universiteit van Utrecht. In 2023 heeft zij haar opleiding tot Longarts afgerond onder leiding van Drs. K. Grijm en Dr. N. Barlo.

Tijdens haar opleiding had zij tevens een passie voor organisatie en was zij actief lid van de OR MSNW. Ook had zij een actieve, en met name organisatorische rol, binnen de assistentengroep.

Nog verdere verdieping binnen de infectieziekten heeft zij door middel van een infectiestage in het AUMC te Amsterdam een vervolg gegeven. Daarnaast heeft zij nu landelijk, een actieve rol binnen de sectie infectieziekten van de NVALT, en is betrokken bij de organisatie van het jaarlijkse landelijk infectieziekten symposium. Ook verzorgt zij mede het landelijke CCO-onderwijs infectieziekten.

Per 1 februari 2023 is zij gestart als Longarts in de maatschap Longziekten in Noordwest Ziekenhuisgroep met als aandachtsgebied Infectie en Wetenschap.

List of publications

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