Small Airways Disease in Cystic Fibrosis:

Improving Efficacy of Treatment

AUKJE BOS

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Aukje Bos

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Small Airways Disease in Cystic Fibrosis: Improving efficacy of treatment

Afwijkingen van de kleine luchtwegen in cystic fibrosis:
Verbetering van de effectiviteit van behandeling

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CONTENTS

Chapter 1	General introduction	7
Chapter 2	Inhaled antibiotics: dry or wet?	15
Chapter 3	The fate of inhaled antibiotics after deposition in patients with cystic fibrosis: How to get drug to the bug?	31
Chapter 4	Patient-specific modeling of regional antibiotic concentration levels in airways of patients with cystic fibrosis: Are we dosing high enough?	77
Chapter 5	Patient-specific modeling of regional tobramycin concentration levels in airways of patients with cystic fibrosis: Can we dose once daily?	101
Chapter 6	Patient-specific evaluation of regional airway dornase alfa concentration levels in a cohort of patients with cystic fibrosis using Computational Fluid Dynamics	119
Chapter 7	Daily observations of nebuliser use and technique (DONUT) in children with cystic fibrosis	137
Chapter 8	Pharmacokinetics and tolerability of once daily double dose tobramycin inhalation in cystic fibrosis using controlled and conventional nebulization	155
Chapter 9	General discussion	169
Chapter 10	Summary / Samenvatting	197
Appendices	List of abbreviations	209
	About the author	211
	List of publications	213
	PhD portfolio	215
	Dankwoord	217

CHAPTER 1

General introduction

INTRODUCTION, AIMS & OUTLINE

Cystic fibrosis

Cystic fibrosis (CF) is a severe hereditary and life-threatening disease in the Caucasian population, affecting 70,000 patients worldwide.[1] In the 1950s, a child with CF would rarely live long enough to attend elementary school. Luckily, life expectancy has dramatically improved due to the development of new treatments and treatment approaches. The current median predicted survival is close to 40.[2]

The main cause of mortality and also morbidity is progressive lung disease.[3] Patients with CF have an increased susceptibility to airway infections due to the defects in the CF transmembrane conductance regulator (CFTR). Due to a dysfunction in the CFTR channel, ion transfer over the membrane of epithelial cells is abnormal what leads to dehydration of the airway surface liquid in the lungs. This results in thick, viscous mucus that impairs mucociliary clearance and obstructs the airways. Because of these alterations, it is difficult for CF patients to eliminate inhaled bacteria from the lungs. A persistent infection arises, which damages the airways and eventually leads to respiratory failure.[4]

The most common mutation causing CFTR dysfunction is the dF508 mutation, but over 2000 mutations have been identified.[1] The disease is complex and the rate of progression of CF lung disease varies widely from person to person, even among patients carrying identical CFTR mutations.[4] Despite the significant improvements in the treatment of the disease, there is still no cure and lung infections remain a serious problem for patients living with CF.

Small airways disease

An important component in the pathophysiology of CF lung disease is small airways disease,[5-7] which starts early in life. Most infants with CF diagnosed through newborn screening have evidence of small airways disease already in the first year of life.[8] Differences in infant lung function are seen between children with CF and healthy control subjects and areas of bronchiectasis, mucus plugging and air trapping are observed on chest computed tomography (CT) scans.[4] In addition, in patients with end stage lung disease 10-75% of the lung volume is dysfunctional due to small airways disease.[9] Despite this large body of evidence that small airways disease is an important component of CF lung disease, current aerosol therapy is probably inefficient in targeting these small airways.

Treatment of CF lung disease

The mainstay of the management of lung disease in patients with CF is aerosol therapy. Inhaled drugs are used to thin the mucus or fight lung infections. Multiple microorganisms play a role in the pulmonary infections in CF patients, but the most important contributor to progression of CF lung disease is *Pseudomonas aeruginosa (Pa)*.[10] Inhaled antibiot-

ics play a key role in the eradication and chronic suppressive therapy of *Pa* infections. Currently, 3 inhaled antibiotics are registered for use in patients with CF: Tobramycin, Aztreonam Lysine and Colistin.

Inhaled antibiotics

First attempts of inhaling antibiotics, 50 years ago, were done by off label use of parenteral preparations in conventional nebulizers. However, due to the osmolality and preservatives in the preparations their use was not tolerated very well.[1]

Tobramycin

In 1997 tobramycin inhalation solution (TIS) came to market, especially designed for the lung as a preservative free formulation that had an osmolality and pH closer to that of the airway surface liquid.[1,11] In large clinical studies it was shown that maintenance with TIS significantly increased lung function up to 12%, reduced bacterial density in sputum and reduced pulmonary exacerbations.[1] Since its FDA approval it is the first choice of therapy for lung infections caused by *Pa*. Tobramycin is an aminoglycoside antibiotic and works by inhibiting protein synthesis of the bacteria. For the treatment of *Pa* lung infections twice daily nebulization of 300 mg tobramycin during 28 days is recommended in a month on month off regimen. However, various dosage regimens were investigated, with dosages ranging from 80 mg twice daily to 600 mg thrice daily.[12,13] For a first infection with *Pa,* a one month treatment with TIS is sufficient to eradicate Pa in two thirds of the patients. Unfortunately, this still means that in around one third of patients eradication therapy of *Pa* fails and the infection becomes chronic.[14] One of the possibilities for failure of TIS to eradicate *Pa* is that its bactericidal effect is concentration dependent. Thus insufficient peak concentrations throughout the lung will result in incomplete killing of *Pa*.

Aztreonam lysine for inhalation

In 2010, aztreonam lysine for inhalation (AZLI) was approved by the FDA for treatment of *Pa* infections. AZLI is a monobactam antibiotic that has been proven to improve lung function and reduce respiratory symptoms, bacterial density and exacerbations. It inhibits cell wall biosynthesis of gram-negative bacteria. Instead of aminoglycosides, AZLI demonstrates time-dependent killing. Thus, the degree of bacterial killing does not primarily depend on the peak concentration of the antibiotic, but is more dependent on the total time that AZLI concentrations remain above the susceptibility breakpoint of bacteria.[15] The recommended dosing regimen for AZLI is three times daily 75 mg, like TIS in a month on month off regimen.[1]

Colistin

Nebulized colistin is frequently used for treatment of *Pa* lung infections in patients with CF despite very few controlled trials evaluated its efficacy. For eradication of early *Pa* infections, only the combination of nebulized colistin with oral ciprofloxacin has been investigated. Compared to no treatment, eradication of *Pa* was seen more often in the colistin group (odds ratio 0.12) (26 children). Two trials compared nebulized colistin with oral ciprofloxacin to either TIS alone (58 children) or TIS with oral ciprofloxacin (223 patients >1 year) and showed comparable efficacy to TIS.[16] For chronic *Pa* infections, 2 trials compared nebulized colistin to placebo (54 patients in total) and 1 trial compared colistin to TIS (115 patients). No advantage was seen in patients treated with colistin compared to placebo. In the comparison to TIS, patients treated with colistin did not show any improvement in lung function while an increase of 6.7% in lung function was seen in patients receiving TIS.[3] Colistin is a polymyxin antibiotic and works by binding to and disrupting the outer cell membrane of bacteria. One million units colistin (=80 mg) are administered twice daily.

Dry powder inhalers

More recently, dry powder formulations of antibiotics have been developed as a time efficient alternative for nebulized antibiotics. Administration is much quicker, for example for tobramycin the podhaler was developed which allows inhaling the required dose in 5 min instead of approximately 20 minutes when inhaled by nebulizer. Clearly, this can greatly improve adherence to therapy. Colobreathe is a dry powder inhaler for colistin. Both dry powders showed noninferiority relative to nebulized tobramycin.[17,18] A disadvantage of dry powder inhalers is that the aerosol characteristics of the inhaled drug are dependent on the inhalation maneuver by the patient. In this thesis we focus on inhaled antibiotics administered by nebulizers.

Other inhaled drugs in CF

Other inhaled drugs used by patients with CF are mucolytics/mucous mobilizers, anti-inflammatory drugs and bronchodilators. Apart from dornase alfa, these inhaled drugs are outside the scope of this thesis.

Dornase alfa

Dornase alfa (Pulmozyme) is a highly purified solution of recombinant human deoxyribonuclease (rhDNase). It reduces viscoelasticity of CF sputum by cleaving extracellular DNA,[19] which improves clearance of the sputum from the lungs. Dornase alfa significantly improves lung function, reduces pulmonary exacerbations and is used in patients with CF since 1992. The recommended dosing regimen is once daily inhalation of 2.5 mg dornase alfa, although some patients benefit from twice daily inhalation.[20]

Despite the use of inhaled antibiotics and dornase alfa in CF, lung disease still progresses. It has been recognized that inhalation therapy using one of the above described drugs results in high concentrations of the drugs in sputum.[15,21] However, concentrations in the small airways after nebulization are largely unknown. For antibiotics we know that concentrations above a certain threshold are required before they become effective. This threshold is called the minimal inhibitory concentration (MIC). Hence, it is well possible that in partly or completely obstructed airways concentrations remain below this threshold resulting in sub optimal treatment of these diseased lung areas. This is likely to occur especially in the small airways. Suboptimal concentrations might thus contribute to the progression of CF lung disease.

Nebulizers and deposition

Several factors are of influence on the amount of drug that is able to bypass the upper airways and can be deposited after a long journey in the small airways. Particle related factors are the shape, size and density of the particles. Smaller particles are more likely to bypass the upper airways and of being transported to and deposited in the small airways. Patient related factors include the diameter of the airways, the breathing pattern of the patient and structural abnormalities of the airways due to the disease. Children have smaller airways and higher inspiratory airflows relative to adults. These two factors lead to more central airway deposition. Clearly there are many factors that determine whether sufficient drug reaches the diseased areas of the lung where we most want it.

Also the type of nebulizer is of great influence on the lung deposition. There are many different types of nebulizers, which have different mechanisms of aerosol generation. There are jet, ultrasonic and vibrating mesh nebulizers, with or without smart nebulizer technology.

Traditionally, conventional jet nebulizers are used for nebulization of antibiotics. Tobramycin and colistin are registered for use with the Pari LC Plus nebulizer (Pari GmbH, Starnberg, Germany).[22] The Pari LC Plus nebulizer is a reusable breath-enhanced jet nebulizer without smart technology. A compressor provides continuous aerosol delivery. As aerosol is wasted during exhalation, this type of nebulizer is relatively inefficient. AZLI is registered for use with an electronic nebulizer, the eFlow (PARI Innovative Manufacturers; Midlothian, USA). This is a vibrating mesh nebulizer. Mesh nebulizers are more efficient than jet nebulizers because there is virtually no loss of drug during exhalation and they have only a small residue after nebulization.[23]

With smart nebulizers, aerosol is only delivered to the patient during a pre-set fraction of the inspiration. The Akita is an example of a smart electronic system in combination with a jet nebulizer. This is a controlled-inhalation device that directs the flow and depth of each inhalation by coaching the patient in correct inhalation technique. This nebulizer

is known to increase deposition of aerosol in the small airways.[24] Moreover, the Akita has shown to improve efficacy of inhaled dornase alpha by 70% compared with 10-20% for the standard jet-nebulizer.[25] The I-neb is another example of a smart electronic system in combination with a mesh nebulizer. It works in a similar way as the Akita. Both smart nebulizers allow monitoring of patient adherence to treatment.[26] Overall smart nebulizers are more efficient and can potentially achieve higher lung doses compared to traditional jet nebulizers.

Adherence and inhalation technique

For correct use and for the inhaled drugs to be effective, the patient needs to be adherent to the treatment regimen. The treatment of CF lung disease is complex and takes one to two hours per day. Most of this time is spent on nebulizer therapy. Adherence to treatment decreases with the duration and complexity of treatment. Only 32% of patients with CF is fully adherent to a twice or trice daily treatment regimen of nebulized antibiotics.[27,28] However, even if patients take medication daily, the delivery of drug into the lungs may fail due to an incorrect inhalation technique.[29,30] A poor inhalation technique reduces the amount of deposited drug at the site of action and thus reduces the effectiveness of medication. For this reason, much attention is given to instructions on inhalation technique by the CF nurses. Surprisingly little research has been done to evaluate the efficacy of these instructions in CF patients. For asthmatic children, it is known that technique related to inhalation therapy is poor. An incorrect inhalation technique was observed in 22-79% of these patients.[31-33] For CF this has never been studied. What happens in the home situation or what mistakes are made is not known.

Aims of the study

General aim

We aimed to improve treatment of small airways disease in CF by improving the efficacy of current inhaled drugs.

Specific aims

Estimating drug concentrations

- To develop a patient-specific airway model to predict concentrations of inhaled drugs throughout the bronchial tree in patients with CF.
- To study the impact of structural lung changes and breathing profile on local drug concentrations in the airways of patients with CF.

Improving efficacy of current inhaled drugs

- To review what happens to inhaled antibiotics after deposition in the airways of patients with CF.
- To investigate the pharmacokinetics of a double dose of tobramycin inhaled with a smart and conventional nebulizer in patients with CF.
- To study the inhalation technique of children with CF while they are nebulizing at home.

Outline of this thesis

Chapter 1 contains the introduction to the studies that were performed in this thesis.

Chapter 2 reviews the key issues related to inhaled antibiotic therapy, both nebulized and dry powder formulations of antibiotics.

Chapter 3 provides a review on what happens to antibiotic aerosol particles after deposition in the airways of patients with CF and how local conditions affect its clinical efficacy.

Chapter 4 describes the results of a study in which patient-specific airway models were used to estimate AZLI concentrations in both central and small airways of patients with CF and to study the relation between structural lung disease and deposition of AZLI.

Chapter 5 reports the results of a study predicting aerosol deposition patterns of inhaled tobramycin after once and twice daily dosing in patients with CF delivered with the Akita or Pari LC Plus nebulizer.

Chapter 6 shows the results of a study comparing airway concentrations throughout the bronchial tree of inhaled dornase alfa, delivered with the Akita with that of the eFlow rapid and Pari LC Plus nebulizer.

Chapter 7 describes the results of an observational study evaluating the day-to-day inhalation technique of children with CF in the home situation.

Chapter 8 reports the results of a study investigating the pharmacokinetics and tolerability of once daily double dose tobramycin inhalation in patients with CF using the controlled-inhalation Akita and conventional Pari-LC Plus nebulizer.

Chapter 9 provides a general discussion on the results of the studies performed in this thesis.

CHAPTER 2

Inhaled antibiotics: dry or wet?

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ABSTRACT

Dry powder inhalers (DPI) delivering antibiotics for the suppressive treatment of *Pseudomonas aeruginosa* in cystic fibrosis patients were developed recently and are now increasingly replacing time-consuming nebuliser therapy. Noninferiority studies have shown that the efficacy of inhaled tobramycin delivered by DPI was similar to that of wet nebulisation. However, there are many differences between inhaled antibiotic therapy delivered by DPI and by nebuliser. The question is whether and to what extent inhalation technique and other patient-related factors affect the efficacy of antibiotics delivered by DPI compared with nebulisers. Health professionals should be aware of the differences between dry and wet aerosols, and of patient-related factors that can influence efficacy, in order to personalise treatment, to give appropriate instructions to patients and to better understand the response to the treatment after switching.

In this review, key issues of aerosol therapy are discussed in relation to inhaled antibiotic therapy with the aim of optimising the use of both nebulised and DPI antibiotics by the patients. An example of these issues is the relationship between airway generation, structural lung changes and local concentrations of the inhaled antibiotics. The pros and cons of dry and wet modes of delivery for inhaled antibiotics are discussed.

INTRODUCTION

Cystic fibrosis (CF) lung disease results in abnormal secretions in the lung that foster infection and inflammation, even early in life.[34,35] The vicious cycle of infection, inflammation and thick pulmonary secretions leads to early structural lung damage and to abnormal pulmonary function tests. In addition to bronchiectasis, small airways play an important role in early CF lung disease.[5] In advanced lung disease, the geometric changes related to small airways disease are significantly more severe relative to large airway changes. Progressive bronchiectasis and small airways disease eventually lead to end-stage lung disease.[36] It has long been recognised that pulmonary infection, particularly by *Pseudomonas aeruginosa,* is associated with progressive structural lung damage.[35] For this reason, after showing that eradication therapy using nebulised antibiotics was effective in preventing chronic infection by *P. aeruginosa,*[14,37,38] this has become standard of treatment.[39,40] For those patients who develop chronic *P. aeruginosa* infection, maintenance treatment using nebulised antibiotics has become the standard of treatment to suppress this microorganism chronically.[39-42]

Nebulised antibiotics against *P. aeruginosa* were developed as an alternative for intravenous therapy to deliver high concentrations of the antimicrobial agent directly to the site of infection, with the dual aims of improving efficacy and reducing toxicity. Nebulised tobramycin, colistin, and aztreonam lysine are the most commonly used nebulised antibiotics for this indication. Of these inhaled antibiotics tobramycin inhalation solution (TIS) has been studied most extensively. TIS should be used in a "1 month on and 1 month off" treatment cycle. Maintenance treatment with TIS has been shown to reduce exacerbations, improve lung function and improve quality of life.[41,43] In addition, early treatment using inhaled nebulised tobramycin against *P. aeruginosa* given at the time of first isolation prevents chronic infection in about two-thirds of patients.[14,44,45] Similar efficacy was recently observed in a study with nebulised aztreonam lysine.[46] Furthermore, treatment with nebulised aztreonam lysine has been shown to be effective in delaying the need for inhaled or intravenous anti-*P. aeruginosa* antibiotics for pulmonary exacerbations in CF patients, and in an improved quality of life.[47] Nebulised colistin is generally used as continuous treatment to suppress *P. aeruginosa* growth.[18,48]

Until recently antibiotic maintenance treatment for chronic *P. aeruginosa* infection could only be delivered by nebulisers. Unfortunately, nebulisers have many disadvantages such as the need for rigorous cleaning after each use to reduce the risk for contamination, they are relatively bulky to carry around and nebulisation time, particularly for older systems can be lengthy. More recently, a tobramycin inhalation powder (TIP) inhaler was developed as a more patient-friendly alternative to TIS.[49] In general, dry powder inhalers (DPIs) allow fast delivery, are more portable, require minimal cleaning and are disposable, reducing the risk of contamination. Similarly, a DPI for colistin (Colobreathe;

Forest Laboratories Inc., New York, NY, USA) has also recently been developed.[18,50] Other antibiotics that are in development as DPI formulations are ciprofloxacin, levofloxacin, vancomycin and clarithromycin.[51-55] Regulatory studies of TIP and Colobreathe have shown non-inferiority relative to the nebulised solutions. However, even though these regulatory studies of TIP and Colobreathe showed equal efficacy of the DPI compared with the nebulised inhaled formulation, one should take into consideration that there are many patient- and device-related differences between the two inhalation modalities that might affect the efficacy of treatment. It is unlikely that the efficacy of antibiotics delivered by DPI is equivalent to that of wet nebulisation for all patients.

Health professionals prescribing inhaled antibiotics for the treatment of chronic *P. aeruginosa* in CF or in other patients groups such as non-CF bronchiectasis[56,57] should be well aware of differences in administration between DPIs and nebulisers to allow them to identify patients who might benefit from switching from nebuliser to DPI treatment, and to enable them to give appropriate instructions to patients. The aim of this review is to discuss key issues related to inhaled antibiotic therapy to optimise the effectiveness of both nebulised and DPI antibiotics.

AEROSOL PARTICLES AND DEPOSITION

For efficient aerosol treatment of both central and small airways, it is important to consider a number of factors that determine whether a sufficient fraction of the inhaled particles are able to bypass the upper airways and to be deposited onto the target area, namely the large and small airways. These factors can be divided into particle-related factors and patient-related factors.

The aerodynamic behaviour of a particle depends on shape, size and density of the particles. The size distribution of an aerosol is usually described as the mass median aerodynamic diameter (MMAD), which refers to the droplet diameter above and below which 50% of the mass of drug is contained. In general aerosol particles smaller than 5 μm are thought to be respirable. However, particles with a MMAD between 2 and 5 μm have a lower probability of bypassing the upper airways and of being transported to and deposited in the small airways relative to 1-2 μm particles (fig. 1). Unfortunately, small particles carry little drug. In addition to the geometric size of the particle, the particle density determines transport velocity and deposition probability. Spheres that have the same transport velocity exhibit the same aerodynamic behaviour and have similar deposition patterns in the lung. This means that particles that are large geometrically and are porous (*i.e.* have a low density) will behave aerodynamically like particles that are small geometrically and are nonporous (*i.e.* have a high density). This effect of density

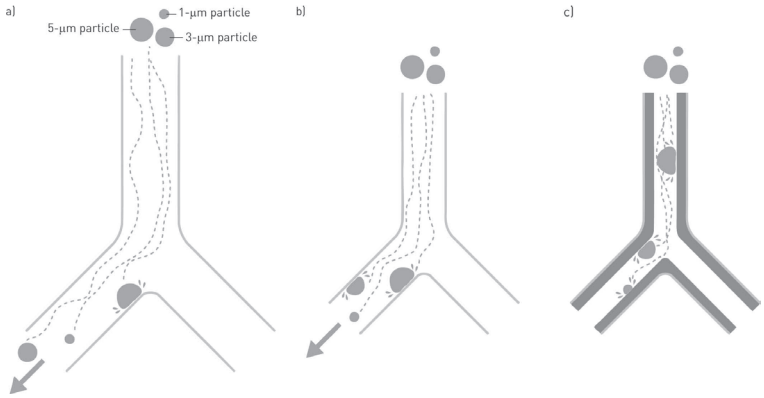

Figure 1. Schematic view of the bronchial tree showing the relation between airway size, flow velocity, and deposition of three differently sized aerosol particles (1, 3 and 5 µm). a) In the healthy lung, the 5-µm particle has the highest probability of being deposited onto the mucosa of the central airways due to inertial impaction. b) In the healthy lung of a child, the airways are narrower and flow velocities of the inhaled particles are higher. As a result, the 3- and 5-µm particle are deposited on the central airway mucosa. c) In the diseased lung of a child, the airways are thickened due to mucosal swelling by inflammation and mucus. As a result the cross-sectional diameter of the central airways is even smaller relative to the healthy lung (b); in addition, mucus depositions cause more turbulence of the inhaled air. As a result the 1-, 3- and 5-µm particles are deposited on the central airway mucosa. Reproduced and modified from *Tiddens. Ital J. Pediatr 2003 (vol 29:39-43)* with permission from the publisher.

on aerodynamic diameter is being used in the development of DPI drug formulations containing dry porous particles.

Patient-related determinants of lung deposition and distribution within the airways include, firstly, the diameter of the large airways. Children have smaller airways and higher inspiratory airflows relative to adults, both of which facilitate central airway deposition (fig. 1).[58] The second patient-related factor determining particle deposition is the quality of the inhalation manoeuvre. This quality depends on age, physical capability, disease severity and the cognitive ability of the patient to perform specific inhalation manoeuvres. It is well recognised that even well-trained and capable patients might vary their inhalation technique considerably from day to day. A high inspiratory flow rate will result in more turbulence in the central airways and, therefore, result in an increased deposition of drug in the upper airways.[59] A slow inhalation manoeuvre, however, will result in less turbulence in central airways and, therefore, in a higher probability of aerosol particles bypassing the central large airways. Hence, ideally, an aerosol should be inhaled using a slow and deep inhalation so even large particles containing a high drug mass have a higher probability of bypassing the central large airways and making it all the way down into the diseased small airways. The third patient-related factor that determines particle deposition is the presence of structural abnormalities of the airways and/or mucus in the airways, which both can result in disturbance of the airflow pattern and thus in increased

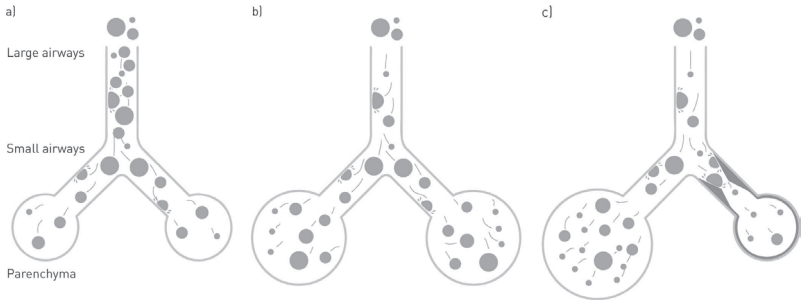

Figure 2. Schematic representation of the distribution of aerosol particles throughout the lung for the normal lung and the diseased lung. a) Distribution of aerosol particles for a patient with normal lung lobes while the patient is inhaling the antibiotic using tidal volume breathing. Note that there is homogeneous distribution of the antibiotic between lung segments, and deposition is higher in the central airways relative to the small airways and parenchyma. b) Same patient as in (a) inhaling deeply. Note that more drug reaches the small airways and parenchyma. Furthermore, there is equal expansion of the two lung lobes. c) The patient has considerable lung disease in one lobe, and is inhaling quickly and deeply. The diseased lobe has a higher airway resistance and reduced compliance relative to the healthier parts of the lung. The expansion of the diseased lobe is slower relative to the healthier parts. As a result, there is preferential flow to the healthier lobe. In addition, the partial obstruction of the airway to the diseased lung lobe causes a turbulent flow pattern and increased aerosol deposition. The overall result is that the healthy lung lobe receives more antibiotic relative to the diseased lobe.

deposition at the sites of obstruction.[59-61] The fourth patient-related factor is the ability of the lung to expand. Recent modelling studies showed that lobes with substantial structural damage received less inhaled antibiotic.[61] It is likely that structural abnormalities such as fibrosis in CF lungs have a negative impact on lung expansion (fig. 2).[62] As a result, there is a preferential airflow to the healthier regions of the lung.

Hence, to select the most appropriate inhalation device for a patient, we should not only take aerosol characteristics of the inhaled drug into account but also age, inhalation flow pattern related to the device and the severity of CF lung disease.

LOCAL CONCENTRATIONS OF ANTIBIOTICS

It is generally believed that inhaled antibiotics are so effective because of the high sputum concentrations that were observed in the pivotal studies.[41,42] However, this concept is probably overly simplistic for a number of reasons. Firstly, the high concentrations measured in sputum are most likely to reflect drug primarily deposited in the large airways. As discussed, high central airway deposition can be the result of high turbulent flows leading to upper and central airway deposition, especially of larger inhaled particles, due to inertial impaction (fig. 3). Secondly, high concentrations in the central airways mean that less drug is available for the remainder of the bronchial tree, especially for the small

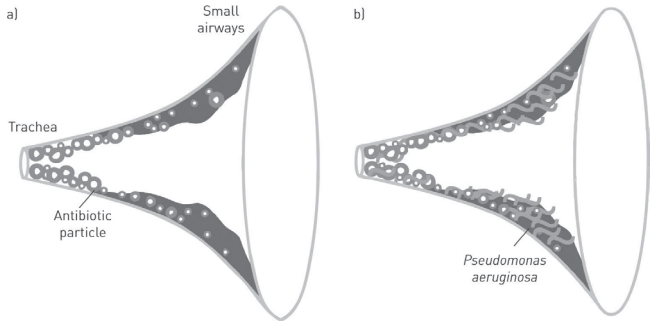

Figure 3. Schematic view of the total cross-sectional area of the bronchial tree. On the left, the narrow diameter of the trachea is shown. The total cross-sectional diameter and surface area of all small airways is substantially larger relative to the trachea. Note that the inflammatory thickening of the airway wall in the small airways is more severe in the small airways relative to the large airways. Furthermore, note that the density of deposited particles is larger and, thus, antibiotic concentration is higher in the central airways relative to the small airways. In addition, large and small particles are primarily deposited in the central large airway. In the small airways, more small particles can be observed and only few large particles. B) In the same bronchial tree as in (a), *Pseudomonas aeruginosa* are distributed throughout the bronchial tree, assuming equal density. Note that antibiotic concentration in the small airways is lower than in central airways; as a result, concentrations might fall below the minimal inhibitory concentration and be insufficient to adequately treat *P. aeruginosa.*

airways. It should be kept in mind that drug bypassing the central airways is distributed over the vast surface area of the small airways. It is estimated that the total surface area of the small airways in adolescents is in the order of 1.2-1.3 m^2. For each consecutive airway generation, concentrations will be lower as the total surface area of the airway surface increases exponentially. In addition, the velocity of airflow drops from central airways towards the small airways. This changes the principal mechanism of deposition by inertial impaction in more central large airways to sedimentation by gravitational forces in the smaller airways.[63] Thirdly, as discussed in the previous paragraph, diseased areas will receive a lower dose of the drug.[61] Downstream to the site of obstruction, less drug will be available for deposition. Fourthly, airflow is preferentially directed towards the healthier regions of the lung (fig. 2). Taking all these factors into account, it is highly likely that a wide range of sputum concentrations exist throughout the lung, and that concentrations in the small airways are likely to be low and might even fall below therapeutic levels depending of the mass of drug inhaled and on the aerosol characteristics.[61] Whether the use of a DPI or nebuliser for the inhalation of a specific antibiotic results in differences in the surface areas with very high and subinhibitory levels is unknown but should be considered.

What could the consequences of the aforementioned issues be for clinical practice? Firstly, it is unclear whether high antibiotic concentrations in central airways well above the minimal inhibitory concentration (MIC) translate to more effective killing of *P. aeruginosa*. If not, this should be considered a waste of drug that could better be delivered to

the small airways and to more diseased regions with possibly subinhibitory concentrations. Secondly, low/subinhibitory concentrations in the more diseased and obstructed areas are not effective, and can lead to on-going infection in these regions. Hence, even when spirometry outcome measures improve during a cycle of inhaled antibiotic treatment, it is likely that the diseased areas of the lung remain undertreated. This could explain the effectiveness of treatment using intravenous rather than inhaled antibiotics, which may result in more effective antibiotic concentrations in diseased areas. Thirdly, subinhibitory concentrations can lead to the development of resistance. The development of resistance in patients on chronic treatment with inhaled antibiotics has been well studied. Indeed, for TIS, a small increase in resistance has been observed. However, this development of resistance did not correlate with any efficacy parameter,[64] but it is doubtful that the resistance of expectorated sputum gives an accurate reflection of the distribution of resistance throughout the lung. It has been well recognised that the distribution of microorganisms in the CF lung is inhomogeneous. Hence, it is not surprising that, even for a single species, a wide distribution of MICs can exist.[65,66]

Clearly, for inhaled antibiotics, it is important to obtain sufficiently high concentrations throughout the bronchial tree. In the next sections, the basics and pro and cons of nebulisers and DPIs for the delivery of inhaled antibiotics are discussed for a better understanding of the relationship between the delivery device and airway concentrations of inhaled antibiotics.

WET NEBULISERS: THE BASICS

Nebulisers convert liquid medication into a mist and can be used to deliver a wide range of drug formulations for inhalation. There are different types of nebulisers, which have different mechanisms of aerosol generation. The differences in delivered aerosol between nebuliser systems currently available are significant.[67] There are jet, ultra-sonic and vibrating mesh nebulisers, with or without smart nebuliser technology. The most frequently used systems to nebulise antibiotics are jet nebulisers. Jet nebulisers consist of a compressor that sucks air from the environment through an air filter and generates airflow through the nebuliser containing a Venturi tube. In the Venturi, the airflow is mixed with the fluid and a primary aerosol is formed. A baffle in the nebuliser further disintegrates the droplets into smaller aerosol particles. Most of the generated aerosol particles will fall back in the medication cup and will be re-nebulised. The patient inhales the aerosol while tidal breathing from a reservoir through a mouthpiece or face-mask. For children, a good face mask design is important to maximise efficiency.[68,69] A child old enough to inhale through a mouthpiece should do so, as the efficiency of aerosol delivery can be doubled relative to inhalation by face mask.[70]

The recently introduced mesh nebulisers use either a vibrating or fixed membrane with a piezoelectric element with microscopic holes to generate an aerosol. Vibrating mesh devices have a number of advantages over jet nebuliser systems. They are very efficient because there is almost no loss during exhalation and the residue after nebulisation is usually <0.3 mL, whereas in a jet nebuliser, 1-1.5 mL is left. Mesh nebulisers are silent and are generally portable, as they operate as effectively when using batteries as when using mains power. Lung deposition of mesh nebulisers is more efficient than conventional nebulisers, varying between 30% and 80% of the loading dose, depending on the device.[71]

For smart nebulisers, the breathing pattern can be set in such a way that aerosol is only delivered during a pre-set fraction of the inspiration. In addition, for some nebulisers, the depth of the inhalation manoeuvre and the flow rate of the inhalation can be set. The deeper the inhalation, the shorter the nebulisation time. Almost no drug is lost during exhalation. Smart nebulisation systems can incorporate either jet or mesh nebulisers but the release of drug is controlled electronically, rather than allowing continuous release of drug. The I-neb (Philips Respironics, Parsippany, NJ, USA) is an example of such a system using a mesh nebuliser, while the Akita (Activaero, Munich, Germany) is an example of a smart system using a jet nebuliser.

Smart nebulisers are substantially more efficient and may achieve lung deposition of 60-80% of the loading dose (using mesh technology),[71] compared to 5-15% with traditional jet nebulisers.[22] Furthermore, more efficient deposition of aerosol in the small airways can be achieved, which may result in more effective treatment of small airway obstruction.[25]

Although there are many advantages, vibrating mesh and smart nebulisers are still not extensively tested in children and there is little clinical information available. Lung deposition is improved but evidence for dosage recommendations is either lacking or based on *in vitro* or adult data. New-generation nebulisers for inhaled antibiotics can be used as long as efficacy and toxicity data are available, especially when using potentially toxic antibiotics such as inhaled tobramycin.

NEBULISERS FOR INHALED ANTIBIOTICS; PROS AND CONS

Inhaled antibiotics are registered for use with a specific nebuliser-compressor combination. Phase III studies with TIS were performed with the LC Plus nebuliser (Pari GmbH, Munich, Germany) with either the Pari Turbo Boy or PulmoAide compressor (DeVilbiss, Mannheim, Germany).[41] The nebulisation of colistin is registered for use with the Pari LC Plus nebuliser and with the I-neb nebuliser in the UK. However, no proper Phase III registration studies were conducted for nebulised colistin.

For the nebulisation of aztreonam for treatment of chronic *P. aeruginosa* airway infection, the Pari eFlow mesh nebuliser has been registered.[47]

The use of nebulisers has some well-recognised advantages. 1) They are a platform to deliver drugs that are only available as fluids. 2) They can be used for all ages from infancy into adulthood. 3) The nebulised drug can be inhaled while the patient is breathing tidally. Hence, no specific inhalation manoeuvre is required. 4) Over the last few decades, smart nebulisers such as the I-neb and the Akita have been developed that allow electronic data logging. This supplies objective information for patient and CF team on treatment adherence.[25,28] 5) Smart nebulisers such as the Akita allow the control of inhalation competence. Hence, the patient has to follow a pre-set inhalation profile, optimising treatment efficacy and efficiency. 6) Smart nebulisers can be set to efficiently target the small airways, which play an important role in CF lung disease.[5,72] To do so, a personalised inhalation profile can be programmed onto a smart card in the nebuliser. Improved delivery of dornase alfa to the small airways using the Akita nebuliser resulted in substantial improvement of small airways patency. Similar approaches could theoretically be of benefit to improve efficiency and efficacy of other nebulised drugs such as hypertonic saline and antibiotics.

There are some important disadvantages related to nebuliser therapy. 1) It is time-consuming. For patients on maintenance treatment with dornase alpha and inhaled antibiotics, nebuliser therapy can take up 2 h per day.[73] This time is needed for preparation of the nebuliser, nebulisation of the drug and cleaning the nebuliser after its use. For colistin, up to 10 minutes extra time is needed to dilute the appropriate dose in water for injection to obtain an isotonic solution. This is not the case for TIS or aztreonam lysine, which are readily available in a unit-dose vial. 2) The use of nebulisers includes the risk of contamination when not properly cleaned.[74] 2) Nebulisers are bulky and less portable than other devices. 4) Nebulisers require regular maintenance. Over time, the air filter of jet nebulisers gets polluted with dust particles and, thus, should be replaced at regular intervals. Similarly, 'lifelong' nebulisers suffer from wear and tear, and thus require replacement once to twice a year. Furthermore, the compressor output of jet nebulisers should be periodically examined as per the manufacturer's instructions. For the Pari eFlow mesh nebuliser used for aztreonam for inhalation solution (AZLI; Cayston (Gilead Sciences Inc., Forest City, CA, USA)) therapy, maintenance issues are different. Occlusion of the holes can occur,[75] which prolongs treatment time. Hence, they require careful cleaning after each use and frequent replacement of the mesh to prevent build-up of deposit and blockage of the apertures.[75] Therefore, a new mesh is delivered with each monthly package of aztreonam. In addition, the mesh should be replaced if nebulisation time exceeds 5 min. Overall, a great need was felt by the CF community and the pharmaceutical industry to develop more time-efficient, less cumbersome alternatives for nebulized antibiotic therapy.

DPIS: THE BASICS

In DPIs for antibiotics, the drug is present as a dry powder formulation in a capsule. The loading dose of the capsules for antibiotics is in the 20-150-mg range and, therefore, substantially higher than that of antiasthma drugs, which is mostly in the 50-200-µg range. For this reason, multiple capsules can be needed to inhale a sufficient mass of the antibiotic into the lungs. The technical properties of the dry powder formulation combined with the properties of the inhaler determine how the powder can best be inhaled. Both the mass and the aerosol characteristics of the released aerosol depend on the inhaled volume and on the inspiratory flow profile generated by the patient.

TIP is formulated using PulmoSphere technology (Novartis AG, Basel, Switzerland). This is a spray-drying technique, which generates relatively large porous particles that disperse very easily. For each treatment, four capsules of 28 mg TIP need to be inhaled through a low-resistance inhaler to get a lung dose equivalent to that of 300 mg nebulised TIS. A low flow of 30 L/min is sufficient to release the drug from the capsule and to disperse the aerosol particles. TIP has an MMAD <4 µm and a grain size distribution of 1.7-2.7 µm.[76] The low-resistance inhaler allows the patient to generate a wide range of inspiratory flows.[77] In a controlled laboratory setting, it was shown that almost all CF-patients of 6 years and older were able to generate flows of ≥30 L/min. However, some patients obtained inspiratory flows as high as 170 L/min through a low-resistance inhaler. Unfortunately, as discussed, high inspiratory flows increase oropharyngeal deposition and can reduce lung deposition, especially in the small airways. To empty all drug from the TIP capsule, an inspiratory volume of 1 L is sufficient to release all dry powder.[77] Most patients of 6 years and older were able to inhale a volume of ≥1 L.[77] However, to ensure that all drug is released from the capsule, it is recommended to repeat the inhalation manoeuvre twice for each capsule.

Colobreathe is formulated as micronised particles that, in general, do not disperse very easily. For each administration, one 125-mg capsule needs to be inhaled through a low-resistance inhaler to get a lung dose equivalent to that of 160 mg nebulised colistin. It is claimed that an inspiratory flow of 30 L/min through the inhaler is required to disperse the micronised drug optimally into respirable aerosol particles.[47] However, currently, there are no published data available describing the aerosol characteristics of the colistin DPI. To ensure that all drug is released from the capsule, it is recommended to repeat the inhalation manoeuvre twice for each capsule.

Clearly, the inspiratory flow and volume when inhaling an antibiotic from a DPI are important determinants of the deposition pattern and efficacy. Surprisingly, the optimal inhalation profiles of TIP and Colobreathe® have not been clearly defined to date; this should be further investigated to optimise this form of inhaled antibiotic treatment. Next, patients should be trained to use the optimal inhalation technique with training aids and this technique should be regularly evaluated.

DPIS: PROS AND CONS

DPIs have major advantages over nebulisers. 1) Administration is quick. For TIP, four capsules can be easily inhaled in 5 min. 2) DPIs do not require extensive cleaning after use. 3) Maintenance of the DPI is not required. 4) They are easy to carry around. 5) The capsules are packaged in sealed blisters and do not require refrigeration. Overall, this method of delivery is more convenient and sterile, which is an important consideration for CF patients who are highly susceptible to lung infections.

The most important disadvantage of a DPI inhaler is that the aerosol characteristics of the inhaled drug and, therefore, of the lung deposition can be highly dependent on the inhalation profile generated by the patient through the DPI. To our knowledge, no field studies have been published that observed how CF patients operate these DPIs in daily life. The DPIs for TIP and colistin are low-resistance inhalers. Hence, when a patient inhales forcefully, very high inspiratory flows can be obtained, resulting in a high oropharyngeal and central airway deposition.[59] In addition, this can result in cough. In the TIP *versus* TIS study, cough was reported as adverse event in 48% of the subjects on TIP *versus* 31% in the patients on TIS.[49] The optimal inhalation profile is formulation dependent. Hence, for the spray-dried, hollow porous particles of TIP, a slow and deep inhalation might be sufficient to disperse the drug while reducing upper airway deposition and improving deposition into the small airways. The optimal inhalation for the micronised colistin formulation is difficult to predict. It is likely that the dispersion of this formulation is highly flow dependent. A very high inspiratory flow might be needed to generate a

Table 1. Inhaler devices for currently available inhaled medication in cystic fibrosis

Inhaled drug	Nebuliser	DPI	pMDI
Hypertonic saline (7%)	+	-	-
Mannitol	-	+	-
Dornase alpha	+	-	-
Bronchodilators	+	+	+
Inhaled corticosteroids	+	+	+
Tobramycin	+	+	-
Colistin	+	+	-
Aztreonam	+	-	-
AmBisome	+	-	-
Liposomal Amikacine	+	-	-
Ciprofloxacin	-	+	-
Vancomycin	-	+	-
Clarithromycin	-	+	-

AmBisome is manufactured by Gilead Sciences, Uxbridge, UK. DPI: dry powder inhaler; pMDI: pressurised, metered-dose inhaler.

sufficiently large fraction of small particles to treat the small airways effectively. For dry powder antibiotic formulations that are still in development, it will be important to educate prescribing physicians and patients to understand the key characteristics of each formulation (and device) and to be aware of the optimal inhalation profile required for that formulation. Another factor to consider is that when DPIs are prescribed to a patient for antibiotics and other medications that require different inhalation patterns (table 1), this is likely to result in confusion and erroneous use. Finally, we should investigate whether patients can be trained consistently "not inhale too fast" or if the devices can be modified to ensure that patients inhale within the correct range of inspiratory flows (*e.g.* by increasing the device resistance or the use of visual/auditory aids).

DRY OR WET?

Taking into account the pros and cons of nebulisers and DPIs for maintenance antibiotic treatment, it is clear that DPIs are more convenient for patients and less conspicuous to use, and from this perspective, the device of preference for patients. However, the most important reason for the physician to select one or the other should be primarily based on effectiveness. When selecting the potentially most effective inhalation device for/with the patient, several considerations should be taken into account (table 2). In the development program of TIP, it was designed to be equally effective as TIS. To accomplish

Table 2. Considerations when prescribing inhaled antibiotics

The concentration gradient of an inhaled antibiotic goes progressively down from central airways towards the small airways
There is a preferential flow of inhaled antibiotic towards the more healthy regions of the lung
The more diseased the lung, the more inhomogeneous the deposition pattern and the more regions will be suboptimally treated
Subinhibitory concentrations for inhaled antibiotics are likely to occur in advanced disease
Inhalation of an antibiotic by DPI is faster and cleaner relative to nebulised antibiotics
The aerosol characteristics of an inhaled antibiotic by DPI depend on formulation, device and inhalation manoeuvre
Each antibiotic inhaled by DPI has a device- and formulation-specific optimal inhalation profile
The patient (and parents in the case of children) should both be aware of the optimal inhalation profile
The efficacy of inhaled antibiotic therapy is determined by adherence and inhalation competence
Inhalation technique should be repeatedly evaluated and patients (parents) repeatedly trained
In case of a suboptimal therapeutic treatment result, check and recheck inhalation competence
For patients using a DPI but who cannot reproducibly generate the optimal inhalation profile, consider switching back to a nebuliser or to a smart nebuliser that guides the patient in optimising the inhalation manoeuvre

DPI: dry powder inhaler.

this, TIP was aimed to match the aerosol and pharmacokinetic characteristics of TIS as closely as possible. Hence, in the phase I and II of the TIP development programme, a dose of TIP was selected to match the deposition characteristics and resulting pharmacokinetic profile of 300 mg TIS. Eventually, four capsules each containing 28 mg of the spray-dried tobramycin powder formulation equalled the pharmacokinetic profile of 300 mg TIS. Next, the efficacy of the TIP formulation was tested against nebulised TIS in phase III trials including 553 patients in a noninferiority design with a 6% noninferiority margin using change in forced expiratory volume of 1 s (FEV_1) % predicted relative to baseline as the primary end-point. The response profile of TIP and TIS for FEV_1 and colony-forming units were identical.[41,49] However, despite the statistically proven noninferiority response pattern of TIP and TIS in the phase III regulatory trial, it is likely that differences in efficacy between TIP and TIS performance exist, taking into account the many differences between DPI and nebuliser platforms for delivery of tobramycin as discussed in the previous paragraphs. More subtle effects on efficacy might easily have remained unnoticed because of the noninferiority study design. For colistin, the comparison between the DPI and the nebuliser therapy is less clear as no comparator Phase III trials have been conducted and the characteristics of the micronised dry powder formulation have not been described in detail in the literature. Better understanding of the impact of factors such as age, severity of disease and deposition variability on regular use of these devices are all of key importance in optimising the use of DPI antibiotic formulations.

For antibiotic treatment of newly acquired *P. aeruginosa,* nebulised antibiotics are considered the standard of treatment.[40] For patients without elevated *P. aeruginosa* antibodies, 1 month of nebulised TIS or AZLI has been shown to be effective in eradicating *P. aeruginosa* in up to ~90% of patients.[14,46] However, positive *P. aeruginosa* antibodies or history of recurrent *P. aeruginosa* infection reduced the chance of successful eradication in 36-48% of patients. *P. aeruginosa* infection is associated with the development of structural lung abnormalities.[78] In the light of the issues discussed in previous paragraphs, it is quite possible that failure might be related to subinhibitory levels of inhaled antibiotics in areas of the lungs with structural disease. Taking into account the differences that can exist in the aerosol distribution pattern between TIS and TIP, it cannot automatically be assumed that TIP is equally effective as TIS in eradicating *P. aeruginosa.* Hence, until eradication studies with TIP or Colobreathe have been undertaken, the treatment options for first *P. aeruginosa* acquisition is TIS, AZLI or nebulised colistin.[14,46]

FURTHER OPTIMISATION

The aforementioned issues offer considerable opportunities for further improvement of inhaled antibiotics. Current therapy is "one size fits all". Child or adult, early or advanced

disease; are all treated with the same regimen. It might well be that for more advanced disease, a higher dose is needed to cover all airway generations with antibiotic concentrations above MIC. For TIP and TIS, a once daily double dose might result in larger areas with concentrations above MIC and may therefore be more efficacious than current twice-daily administration. In addition, because of the difference in mechanism of action of various antibiotic classes, the frequency of administration should be optimised for each class. Advanced mathematical modelling can help us to determine this relationship and to design a specific dose relative to disease severity. Furthermore, it might be possible to improve the effectiveness of eradication therapy by increasing the dose in those patients with more advanced disease and in whom primary eradication therapy fails. Current DPI devices do not control the inhalation flow by the patient. Hence, there is an opportunity to optimise the inhalation manoeuvre of recently developed DPIs based on their characteristics. Training aids will be needed to facilitate this training. In the future, smart DPIs might be developed that guide the patient through the optimal inhalation manoeuvre.

CONCLUSION

Inhaled antibiotics are of key importance in the treatment of CF-related lung disease. Care should be taken to ensure that the small airways are efficiently targeted, even in diseased regions of the lung. Whether this is the case depends on many factors such as age, inhalation manoeuvre, severity of structural lung disease and other factors. Nebulisers are important especially for those inhaled antibiotics that are only available as a fluid. The use of nebulisers requires that technical maintenance is well organised. When possible, DPIs should be used to reduce the treatment burden. CF caregivers and patients should be aware that there are major differences between the inhalation manoeuvre of a nebulised antibiotic and a DPI. Aerosol deposition by DPIs can vary widely in relation to the inhalation manoeuvre. Hence, switching a patient from a nebuliser to a DPI requires careful instruction of the optimal inhalation manoeuvre for that specific antibiotic. The optimal inhalation manoeuvre should be clearly defined by the pharmaceutical industry.

All aspects of inhaled antibiotic therapy should be carefully and frequently evaluated with the patient in the starting phase and, when used routinely, at least once a year. Alternative dose regimens for inhaled antibiotics need to be further investigated.

CHAPTER 3

The fate of inhaled antibiotics after deposition in patients with cystic fibrosis: How to get drug to the bug?

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ABSTRACT

Background: Chronic airway infections are an important factor in progressive lung disease in patients with cystic fibrosis (CF). These infections are most often treated with inhaled antibiotics of which deposition patterns have been extensively studied. However, the journey of aerosol particles does not end after deposition within the bronchial tree, but continues through thick mucus layers and biofilm generated by bacteria.

Objectives: To review what happens to antibiotic aerosol particles after deposition in the airways of patients with CF and how local conditions affect its clinical efficacy.

Methods: We searched Embase, Medline, Web-of-Science, Scopus, Cochrane, PubMed publisher and Google Scholar databases from inception to September 2015. Original studies describing the effect of CF sputum or bacterial factors on antibiotic efficacy and liposomal formulations or co-medication to increase efficacy were included. Two authors independently assessed the study eligibility of the selected publications.

Results & conclusions: 2669 articles were screened of which 35 met the inclusion criteria for this review. Based on these articles, which mainly consisted of *in vitro* studies, we conclude that the clinical efficacy of inhaled antibiotics can be reduced by many factors after deposition in the airways. Aminoglycosides were the most extensively studied antibiotic and are adversely affected by molecules within CF mucus and the alginate layer surrounding *Pseudomonas aeruginosa*.

INTRODUCTION

Patients with cystic fibrosis (CF) have difficulties clearing inhaled bacteria from the lungs due to the presence of thick, viscous mucus that obstructs the airways and impairs mucociliary clearance. This causes a relentless cycle of chronic infections and inflammation; leading to progressive lung disease, which is the primary cause of morbidity and mortality in patients with CF.

Chronic lung infections in CF are mainly treated with inhaled antibiotics, most commonly tobramycin, aztreonam and colistin. Deposition patterns of inhaled antibiotics have been extensively studied. However, after deposition in the airways, the antibiotic must first overcome several challenges before it can perform its activity against the bacteria. Firstly, the aerosol particles need to dissolve in the ELF and in mucus.

Secondly, the antibiotic has to diffuse towards the bacteria of which the location of bacteria in the lungs of patients with CF varies. In case of chronic infections with *Pseudomonas aeruginosa (Pa),* the location is most likely intraluminal within the mucus and not directly at the airway epithelium or at the surface of the mucus.[79] As antibiotics diffuse through the mucus layer, they may bind to mucus particles and this is thought to impair the antibiotic bioavailability at the site of infection.

Thirdly, the antibiotic has to overcome barriers generated by the microorganisms. Multiple microorganisms play a role in CF-related pulmonary infections, although *Pa* has been studied most extensively. *Pa* produces a protective slimy layer called alginate[80] and can grow in a biofilm within the mucus in CF airways. A biofilm is a microcolony of bacteria surrounded by a self-produced polymer matrix, which confers greater resistance against antibiotics.[81]

Hence, many factors influence the clinical efficacy of inhaled antibiotics after deposition and prior to reaching the bacteria. The aim of this systematic review is to provide a critical appraisal of the literature to answer the question, 'what determines the effect of inhaled antibiotics after deposition into the lungs in patients with CF?'

METHODS

An extensive electronic literature search was conducted to identify as many relevant articles as possible. These articles were published from inception of the databases to September 25 2015, as indexed by Embase, Medline, Web-of-Science, Scopus, Cochrane, PubMed publisher and Google Scholar (Table 1).

Two independent reviewers (ACB and KP) screened the titles and abstracts for initial eligibility. Every article, considered useful by at least one of the authors was included.

Table 1 – Search terms

Database	Searches
Embase	('cystic fibrosis'/de OR 'lung fibrosis'/exp OR (((cyst* OR lung OR pulmonar*) NEAR/3 fibro*) OR fibrocyst* OR mucoviscid* OR cf)) **AND** ((((('antibiotic agent'/exp OR 'antibiotic therapy'/de OR 'antiinfective agent'/de OR 'antibiotic sensitivity'/exp OR 'antimicrobial activity'/exp OR levofloxacin/de OR (antibiotic* OR antimicrob* OR antibact* OR (anti NEXT/1 (biotic* OR microb* OR bact*)) OR tobramycin* OR colisti* OR colisiti* OR colomycin* OR colymycin* OR (coly NEXT/1 mycin*) OR tadim OR aztreonam OR aminoglycoside* OR amikacin* OR levofloxacin* OR 'mp 376' OR azithromycin* OR vancomycin* OR gentamicin OR bactericid*):ab,ti) **AND** (inhalation/de OR 'oral spray'/de OR 'inhalational drug administration'/de OR 'nebulization'/de OR inhaler/exp OR nebulizer/exp OR aerosol/de OR powder/exp OR (inhal* OR vapor* OR vapour* OR aerosol* OR spray* OR mist OR atomi* OR nebuli* OR compressor* OR powder* OR dry OR dried OR jet OR ultraso*):ab,ti)) OR (gernebcin OR tobi OR tsi OR bramitob OR cayston OR azli OR (liposom* NEAR/3 amikacin*)):ab,ti) **AND** (pharmacodynamics/exp OR pharmacokinetics/exp OR 'drug efficacy'/de OR 'concentration (parameters)'/exp OR 'concentration response'/de OR 'drug sputum level'/de OR 'sputum analysis'/de OR clearance/exp OR (pharmacodynam* OR pharmacokinet* OR effectiv* OR efficien* OR efficac* OR concentrat* OR sputum* OR mucocilliar* OR mucus OR ((lining OR surface) NEAR/3 (fluid* OR liquid*)) OR clearance* OR 'half life'):ab,ti))
Medline	Modelled search strategy designed for Embase
Web-of-Science	Modelled search strategy designed for Embase
Scopus	Modelled search strategy designed for Embase
Cochrane	Modelled search strategy designed for Embase
PubMed Publisher	Modelled search strategy designed for Embase
Google Scholar	Modelled search strategy designed for Embase

Articles were selected based on the following inclusion criteria: (i) effect of CF sputum or (ii) bacterial factors on antibiotic efficacy; (iii) local efficacy determined by antibiotic concentration or number of molecules; (iv) liposomal formulations or co-medication to increase efficacy; (v) original research. The exclusion criteria are as follows: (i) article not in English or Dutch; (ii) data solely on clinical efficacy of inhaled antibiotics; (iii) no abstract or full text available; (iv) inhaled non-antibiotic drugs; (v) antibiotic combinations; (vi) nanoparticles to increase efficacy (vii) review/overview.

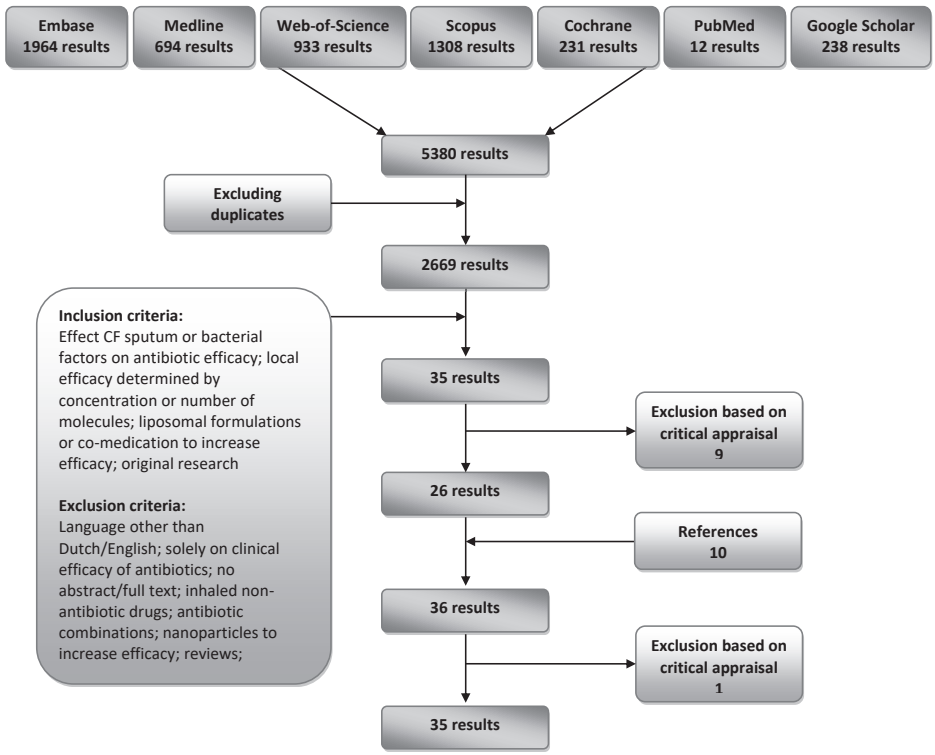
Both reviewers assessed the full text of each selected article to ensure that they met the eligibility criteria, developed to critically appraise the selected publications. These criteria were based on the Grading Recommendations Assessment Development and Evaluation (GRADE) criteria by the GRADE working group and the scoring methods of descriptive studies by Slim *et al.*[82,83] The set of eligibility criteria was more extensive for comparative studies than for non-comparative studies. Using these criteria the relevance and validity of the selected articles were scored independently by the two reviewers. Each criterion received 0, 1 or 2 points (online supplementary Table S1) and the total

score was made up by the sum of all criteria. This score was used as a measure of data quality and a total score of 20 and 38 could be obtained for non-comparative and comparative studies, respectively. Studies with a total score on critical appraisal of ≥11 were included in this review. For the completed critical appraisal table, see Supplement S2. Discrepancies between reviewers during the review process were resolved by discussion until a consensus was obtained. Reference lists of the included articles were searched for additional potentially relevant articles, assessed using the same set of criteria.

RESULTS

Overview of the included studies

Out of 2669 articles, 35 articles were included in this review (Figure 1), of which most articles described *in vitro* experiments (n=33) and two articles described animal studies. The main factors having an impact on each antibiotic class are described in Table 2. An overview of the main results per step in the pathway of the inhaled antibiotic is described in Table S3 and details of the 35 studies are shown in the online supplementary Table S4.



The following antibiotic classes were studied: aminoglycosides (n=28 studies), β-lactam antibiotics (n=12), fluoroquinolones (n=2), tetracyclines (n=1) and other antibiotics (n=9).

Table 2 – Impact per antibiotic class

Factor	Antibiotic class	Impact
CF mucus in general	Aminoglycosides	• Reduces diffusion[89] • Strong binding[96,98,100]
	β-lactam AB	• Reduces diffusion[88] • Negligible binding[94,100]
	Fluoroquinolones	• Reduces diffusion[90,92]
	Other (Polymyxin B)	• Strong binding[100]
Mucin	Aminoglycosides	• Strong binding[94,97] • Reduces efficacy of liposomal forms more strongly than free forms[87]
	β-lactam AB	• Reduces diffusion[88] • No binding[94]
DNA	Aminoglycosides	• Strong binding[93, 94, 97, 101]
	β-lactam AB	• Reduces diffusion[88] • No binding[94]
	Other (Polymyxin B)	• No binding[101]
Nucleic acids	Aminoglycosides	• Strong binding[94, 97]
Bacterial endotoxins (LPS,LTA)	Aminoglycosides	• Strong binding[101]
	Other (Polymyxin B)	• Binding[101]
Anaerobic conditions in mucus	Aminoglycosides	• Reduces efficacy[80, 107, 108]
	β-lactam AB	• Reduces efficacy[80, 107, 108]
	Macrolides	• Reduces efficacy[80]
	Fluoroquinolones	• Reduces efficacy,[80, 108] remain bactericidal[107]
	Tetracyclines	• Reduces efficacy[108]
	Other	• Cotrimoxazol: Reduces efficacy[80] • Chloramphenicol: Reduces efficacy[108] • Colistin: Reduces efficacy,[80] Increases efficacy[109]
Salt content in mucus	Aminoglycosides	• Reduces efficacy (magnesium, calcium, nitrate)[108, 110] • Decreases binding to alginate (sodium chloride, potassium, calcium, phosphate)[112, 113]
	β-lactam AB	• Carbenicillin: nitrate increases efficacy if aerobic, but effect abolished if anaerobic[108] • Ceftazidime: nitrate no effect[108]
	Fluoroquinolones	• Nitrate decreases susceptibility of organisms to AB[108]
	Tetracyclines	• Nitrate no effect[108]

Table 2 – Impact per antibiotic class (continued)

Factor	Antibiotic class	Impact
	Other (Chloramphenicol)	• Nitrate no effect[108]
Alginate	Aminoglycosides	• Reduces diffusion[81, 111, 112, 114] • Binding[81, 112, 113, 114]
	β-lactam AB	• Reduces diffusion[88]
	Tetracyclines	• Reduces diffusion[88]
	Other (Polymyxin B)	• Reduces diffusion[114] • Binding[114]
Liposomal formulation	Aminoglycosides	• Increases efficacy,[101-104] decreases efficacy[87] • Reduces binding[101]
	Other (Polymyxin B)	• Increases efficacy[101] • Reduces binding[101]
Dornase alfa	Aminoglycosides	• Increases binding,[95] decreases binding[94] • Enhances bactericidal efficacy,[87] reduces bactericidal efficacy[95]
	Fluoroquinolones	• Enhances bactericidal efficacy[92] • Enhances diffusion[92]
Mannitol	Aminoglycosides	• Enhances bactericidal efficacy[106]
	Fluoroquinolones	• Enhances bactericidal efficacy[91] • Enhances diffusion[90]
Alginate lyase	Aminoglycosides	• Improves diffusion[114] • Tobramycin and amikacin: Enhances bactericidal efficacy[87, 114] • Gentamicin: Enhances bactericidal efficacy,[114] no effect on bactericidal efficacy[87]
NaCl	Aminoglycosides	• Enhances bactericidal efficacy of tobramycin,[106] antagonistic effect on tobramycin[105]
	Fluoroquinolones	• Enhances bactericidal efficacy[90] • No effect on diffusion[90]
	Other (Colistin)	• Synergistic effect[105]
Glucose	Aminoglycosides	• Enhances bactericidal efficacy[106]
ALX-109&ALX-009	Aminoglycosides	• Enhances bactericidal efficacy[116-118]
	β-lactam AB	• Enhances bactericidal efficacy[116-118]
Cationic amphiphiles	Aminoglycosides	• Reduces binding with DNA[93]
Citrate	Aminoglycosides	• Synergistic action with amikacin[115] • Reduces bactericidal efficacy of tobramycin[115]
	Macrolides	• Synergistic action with erythromycin[115]
	Other	• Synergistic action with colistin[115] • Reduces bactericidal efficacy of polymyxin B[115]
Succinic acid	Aminoglycosides	• Reduces bactericidal efficacy of tobramycin[115]
	Other	• Synergistic action with colistin[115] • Reduces bactericidal efficacy of polymyxin B[115]

AB = antibiotic; CF = cystic fibrosis; LPS = lipopolysaccharides; LTA = lipoteichoic acid.

1. Dissolution of antibiotic in mucus layer (Table S4, part 1)

After deposition on the mucus layer, local bioavailability of the inhaled antibiotic first depends on the solubility of the drug in the mucus. Within CF patients, the secretor and non-secretor phenotype are described: exocrine secretions between these patients differ due to the presence or absence of ABH glyconjugates.[84] Tobramycin was shown to dissolve faster in mucus of secretors relative to non-secretors. However, the question is if this is clinically relevant as this difference was only by 5 minutes.[84,85]

2. Diffusion through mucus layer (Table S4, part 2)

Mucus primarily consists of mucin (glycoprotein) but also contains proteins, DNA, lipids and cellular debris. After dissolution, the drug needs to diffuse through the mucus layer to reach the bacteria, whereby the viscosity and elasticity of CF mucus is increased due to various factors. Mucin molecules in CF mucus are very long, extensively branched and have been shown to interact with other macromolecules in mucus secretions, including: albumin which increased its viscosity, and DNA which increased its elasticity.[86] Additionally, elevated concentrations of actin and alginate contributed to the increased viscoelasticity of CF mucus.[87] Due to the long length of the mucin glycoprotein backbone and its branching, they have a high tendency to interact and obstruct drug transport *in vitro,*[86] in particular liposomal antibiotics.[87]

For β-lactam antibiotics, the presence of mucin alone caused a 2-fold delay in the diffusion rate (1.1 to 0.5 μm/mm^2/h) compared to the baseline rate determined in buffer. The addition of DNA resulted in a 10-fold delay in the rate of diffusion (0.1 μm/mm^2/h).[88]

Aminoglycoside diffusion was also delayed by mucus of CF patients compared to buffer, but no specific numbers were reported. Furthermore, diffusion was not improved by combining gentamicin powder with the amino acid L-leucine.[89]

Combinations of ciprofloxacin dry powder with mannitol showed enhanced diffusion and significantly higher antibacterial activity against *Pa* than ciprofloxacin-NaCl or ciprofloxacin-lactose particles[90] (also against *Pa* growing in biofilm).[91] Ciprofloxacin-NaCl particles also showed higher antibacterial activity against *Pa* (albeit to a lesser extent), although NaCl alone had no effect on drug diffusion.[90] Finally, ciprofloxacin-dornase alfa powder showed greater antibacterial activity than ciprofloxacin dry powder due to the better dissolution and diffusion abilities of ciprofloxacin.[92] Ciprofloxacin-dornase alfa powder completely diffused after 30 minutes while ciprofloxacin powder alone was not completely dissolved even after 2 hours.[92]

In summary, the diffusion rate of aminoglycosides, β-lactam antibiotics and fluoroquinolones through CF mucus is reduced but may be increased by coadministration with mannitol or dornase alfa.

3. Mucus binding (Table S4, part 3)

Apart from delaying the diffusion of antibiotics, macromolecules present in mucus can bind to certain antibiotics and drastically reduce their efficacy, as only free drug can be active against bacteria. In particular, the efficacy of aminoglycosides (cations) is reduced,[93] due to their electrostatic interactions with anionic electrolytes.[94]

Tobramycin,[94-96] amikacin[97] and gentamicin[96,98] have been shown to bind to mucin and free DNA. Their affinity for mucin was higher in the presence of free DNA.[87,95] The latter is released from lysed leukocytes and bacteria during infections.[95] In mucus collected from CF patients during exacerbations, a substantial fraction of inhaled tobramycin was bound to mucus,[94] which reduced its activity by a factor of approximately 30.[93] Binding was dependent on tobramycin concentration[96] and the concentration of macromolecules,[84,94] in which higher tobramycin concentrations resulted in higher concentrations of free drug. Thirty percent of tobramycin was bound at tobramycin concentrations of 5-15 μg/ml while 15% was bound at concentrations of 25-50 μg/ml.[96]

Tobramycin (15-95%) exhibited strongest binding to mucins and DNA.[93] Up to 60% (range 1-60%) of amikacin[97] and 52% (range not reported) of gentamicin was bound to mucus of patients with CF.[98]

Aminoglycosides show lower affinity to negatively charged components in mucus in an alkalotic compared to an acidic environment.[98] The pH in CF airways (not measured in mucus) varies from 6.5-7.5 and seems to be constant from central to peripheral airways. In patients with pneumonia, the pH in infected bronchi was significantly lower than that in non-infected bronchi (6.48 versus 6.69).[99]

Polymyxin B and neomycin show strongly elevated minimal bactericidal concentrations (MBC) due to binding to CF mucus.[100,101] Polymyxin B appeared to bind to bacterial endotoxins within CF mucus, but not to DNA or actin filaments.[101] No binding was observed between mucus and β-lactam antibiotics[94,100] and the role of mucus binding in fluoroquinolones or macrolides was not studied in the selected articles.

3.1 Methods to reduce drug-mucus interaction

3.1.1 Liposome-entrapment

Most studies show that liposome-entrapment reduces antibiotic inhibition by macromolecules and enhances the bactericidal activity of antibiotics. Inhibition of aminoglycosides by DNA and actin filaments, and by bacterial endotoxins was reduced by 4-fold and 100-fold, respectively, when entrapped in liposomes.[101] Additionally, liposomal formulations were significantly more efficacious in reducing the *Pa* load in rats[102] and reduced the minimal inhibitory concentrations (MICs) for *Pa* strains *in vitro* and *Burkholderia cenocepacia* strains in rats.[103,104] However, one study showed that liposomal aminoglycoside

activity was inhibited to a greater extent by mucins than free aminoglycosides (up to 32-fold vs up to 8-fold).[87]

3.1.2 Co-treatment with dornase alfa

Conflicting results were described for the co-treatment of aminoglycosides with dornase alfa. Dornase alfa was shown to increase free tobramycin in sputum by approximately 30%[94] and enhance the bactericidal activity of free and liposomal aminoglycosides. Specifically, the higher the concentration of dornase alfa, the stronger the bactericidal activity.[87]

Conversely, another study reported the decline in free tobramycin and its activity following dornase alfa treatment.[95] A possible explanation given by the authors was that while dornase alfa did indeed cut the DNA into smaller strands, the charge of the strands remained unchanged. Therefore, the smaller strands were still able to bind positively charged antibiotics.

3.1.3 Cationic amphiphiles and N-acetylcysteine

Cationic amphiphiles are positively charged lipid solutions that have the potential to decrease binding between DNA and tobramycin and thereby enhance its antibacterial activity. By matching the cationic amphiphiles in charge and shape, tobramycin was competitively displaced from DNA complexes by these agents, resulting in a 15-fold increase in tobramycin activity.[93]

Co-treatment with 1 mg/ml of the mucolytic *N*-acetylcysteine (NAC) did not influence the bactericidal activity of free and liposomal aminoglycosides.[87]

3.1.4 Co-treatment with Nacl

Co-administration of NaCl (tested NaCl concentrations: 0.3%, 0.9%, 2.3% and 4.05%) had a synergistic effect with colistin at a concentration of 4.05% for the treatment of *Pa* and at all concentrations for *Escherichia coli.*[105] Studies investigating the coadministration of NaCl and tobramycin are inconclusive. Both an antagonistic effect on tobramycin,[105] as well as improved tobramycin efficacy against young *Pa* biofilms[106] were shown when NaCl was added. Nevertheless, in patients with CF who use one of these antibiotic formulations and inhaled saline, the timing of inhaled saline in relation to tobramycin inhalation will need to be taken into account.[105]

In summary, mucus binding appears to reduce the efficacy of aminoglycosides but not β-lactam antibiotics, and may be reduced by liposome-entrapment or coadministration of amphiphilic molecules.

4. Influence of oxygen level in mucus (Table S4, part 4)

Thickened mucus layers in the lungs of CF patients contain areas of low oxygen tension,[107] with an anaerobic environment near the epithelial surface and higher oxygen levels at the top of the mucus layer.[79]

Aminoglycosides,[80,107] β-lactam antibiotics,[107,108] chloramphenicol and tetracycline[108] showed reduced efficacies under anaerobic conditions, whereas tobramycin and ciprofloxacin were approximately twice less effective. For tobramycin, 50% of *Pa* isolates were killed under aerobic conditions, 30% under anaerobic conditions[80] and the log reduction dropped from 5.67±0.00 to 2.14±0.42.[108] For ciprofloxacin, the log reduction dropped from 5.05±0.31 to 2.61±0.13 under anaerobic conditions.[108] On the contrary, levofloxacin maintained its bactericidal effect under anaerobic conditions.[107] Likewise, colistin may even be more effective under anaerobic conditions. Reductions in minimum biofilm eradication concentrations (MBECs), MIC_{50}, MIC_{90}, MBC (2-fold, 8-fold, 4-fold and 2-fold reductions, respectively) against *Pa* were shown under anaerobic conditions.[109] However, another study found decreased killing of *Pa* isolates by colistin under anaerobic compared to aerobic conditions (75% vs 100%).[80]

In summary, low oxygen tension reduced the efficacy of aminoglycosides, β-lactam antibiotics, tetracyclines and chloramphenicol. Colistin, however, may be more efficacious.

5. Influence of salt content of mucus (Table S4, part 5)

The antibacterial activity of certain antibiotics is highly dependent on the ionic environment.[98] In CF, the ionic environment of mucus changes as a result of cell lysis; as evidenced by higher calcium levels detected in CF mucus compared to non-CF patients.[86] Magnesium and calcium have a stabilizing influence on the cell walls of *Pa,* which is primarily driven by divalent cations, and thereby delay the effect of aminoglycosides.[110] The monovalent salt sodium did not have any measurable effect on gentamicin, while the divalent magnesium salt completely shielded *Pa* from its activity.[110]

For *E. coli*, the protection by salts could be solely attributed to ionic strength and not to the type of salt. When the ionic strength was increased *in vitro* from 0.12 to 0.14 μ with $MgCl_2$, NaCl or Na_2SO_4, gentamicin activity against *E. coli* ranged between 40-50%.

Under anaerobic conditions, nitrate decreased the bactericidal activity of aminoglycosides and fluoroquinolones by half.[80,108] Mucus of CF patients contains 250-350 μmol/L nitrate and concentrations can be as high as 1000 μmol/L.[107] Nitrate had little effect on the efficacies of chloramphenicol, tetracycline and ceftazidime.[108]

In summary, the ionic environment is another important variable that can reduce the effectiveness of inhaled antibiotics against *Pa.*

6. Barriers generated by *Pseudomonas aeruginosa*

6.1 Non-mucoid Pa*, Mucoid* Pa *and alginate formation*

Pa has the ability to grow under aerobic and anaerobic circumstances and exists in both a mucoid and non-mucoid formation. Chronic *Pa* infections are associated with more mucoid variants that produce the polysaccharide alginate and form biofilms within the lungs of patients with CF. Alginate is an important factor in the resistance of *Pa* against antibiotics, as it increases the colonization rate within the respiratory tract. Importantly, alginate seems to act as an ionic trapping agent for positively charged aminoglycosides and polymyxin B, thereby reducing the uptake and early bactericidal effect of antibiotics. Additionally, alginate inhibits the non-opsonic phagocytosis of monocytes and neutrophils, thus allowing the bacteria to avoid the phagocytic immune response.[87] Due to these barriers, achieving an inhibitory concentration at the surface of a mucoid colony is not sufficient to eliminate *Pa.* To kill the bacteria, bactericidal antibiotic concentrations need to be attained at the cell surface for a sufficient period of time.[88]

6.2 Alginate and diffusion of antibiotics (Table S4, part 5)

Alginate reduces the diffusion of antibiotics *in vitro* and is evidenced by the fact that aminoglycosides exhibited diffusion coefficients in alginate, which were approximately 20% of the β-lactam values (0.65 versus 3.7 x 10^{-6} cm^2/s).[81,111,112] This can be attributed to the fact that not only does the alginate itself form a physical barrier to the antibiotic, but also because the positively charged aminoglycosides (in contrast to the β-lactam antibiotics) bind to the negatively charged alginate polymers.[80,81,113] This is further evidenced by the fact that 2% *Pa* alginate suspension completely inhibited the diffusion of gentamicin, tobramycin and polymyxin B, whereas the diffusion of the negatively charged carbenicillin was not impeded by this suspension.[114] Furthermore, diffusion rates for tobramycin were consistently lower than for gentamicin.[112] The binding of alginate to antibiotic appeared to be concentration dependent[81] as aminoglycosides formed precipitates with the alginate at a low alginate to antibiotic ratio. This phenomenon disrupted the gel structure, resulting in diffusion of aminoglycosides at a rate that was even faster than that of the β-lactams.[112]

For β-lactam antibiotics, the diffusion rate was strongly reduced by alginate as the molecular weight of these antibiotics was increased. Like mucin, free DNA further reduced the diffusion of β-lactam antibiotics through alginate gels.[88]

In summary, the diffusion of both aminoglycosides and β-lactam antibiotics are reduced through the alginate layer surrounding *Pa*. Ultimately, this reduced diffusion contributes significantly to the difficulty in eradicating mucoid *Pa* from the airways of CF patients.[112]

6.3 Biofilm formation

Within biofilms, a subpopulation of persistent *Pa* cells is formed and characterised by reduced metabolic activity and tolerance to antibiotics.[106] It was shown that MBECs for three different *Pa* strains increased between 8 and 512 times for free aminoglycosides and 8 to 256 times for liposomal aminoglycosides when growing in biofilm.[87] The efficacy of macrolides to eradicate bacteria within mature biofilms was markedly reduced relative to aminoglycosides.[115]

6.4 Therapies to overcome barriers generated by Pa

Alginate lyase (AlgL) is an enzyme that can degrade alginate and facilitate the diffusion of aminoglycosides to the target bacteria.[114] Co-treatment with AlgL increased bacterial susceptibility to antibiotics and phagocytosis, and reduced alginate levels. Additionally, AlgL was more effective at enhancing the activity of aminoglycosides than dornase alfa.[87] This effect differed per aminoglycoside antibiotic, where AlgL treatment alone increased the bactericidal activity of tobramycin and amikacin (free and liposomal form). Likewise, the combination of dornase alfa-AlgL enhanced the bactericidal activity of tobramycin and that of free but not liposomal amikacin.[87] For gentamicin, one study showed enhanced bactericidal activity,[114] while neither AlgL nor the combination with dornase alfa demonstrated any effect on its activity in another study.[87]

An excess of iron can induce biofilm formation by *Pa* and is evidenced by the fact that the iron concentration in the ELF of CF patients is 400-fold higher than in non-CF patients.[116] Drugs containing different combinations of lactoferrin (iron-binding glycoprotein) and hypothiocyanite (bactericidal agent; ALX-009 and ALX-109) had an additive effect on tobramycin and aztreonam in reducing both biofilm formation and disrupting established *Pa* biofilms. Both lactoferrin and hypothiocyanite are part of the normal innate immune response but their secretion by airway cells is reduced in CF.[116-118]

The addition of mannitol improved tobramycin efficacy by 99.5% against young *Pa* biofilms (pre-grown for 5h) and by 77% against established biofilms (pre-grown for 20h). However, mannitol had no effect on clinical strains with high resistance to tobramycin. The addition of glucose resulted in similar outcomes, albeit to a lesser extent. Similarly, NaCl required 2-fold higher osmolarities than mannitol to obtain a similar effect and had no effect on established biofilms.[106]

Finally, co-treatment with dispersion compounds (citrate and succinic acid) has been investigated to enhance biofilm eradication. Combinations of citrate with amikacin, colistin or erythromycin and succinic acid with colistin resulted in significantly enhanced killing of bacterial populations compared with control populations. However, *increased* bacterial viability was seen when tobramycin and polymyxin B were combined with dispersion compounds.[115]

In summary, co-treatment with AlgL and dornase alfa seems to increase the efficacy of aminoglycosides in the presence of alginate. Treatment of *Pa* growing in biofilms can be improved by co-treatment with iron binding drugs, dispersion compounds or mannitol.

DISCUSSION

To our knowledge, this is the first systematic review on what happens to inhaled antibiotics after deposition in the airways of patients with CF. All results were primarily drawn from *in vitro* studies, from which we can conclude that the clinical efficacy of antibiotics is negatively affected by many factors after deposition in the airways (Figure 2). Aminoglycosides, which were the most intensively studied relative to other inhaled antibiotics, seemed to be most adversely affected by these factors.

Following dissolution, the drug needs to diffuse through the mucus layer to reach the site of the bacteria. The high concentration of macromolecules in mucus of patients with CF increases its viscosity, thereby impeding antibiotic diffusion.[87] Slow diffusion across CF mucus layers may play an important role in reduced pulmonary bioavailability as antibiotic molecules may be cleared by alveolar macrophages before reaching the bacteria. Therefore, coadministration of mannitol and dornase alfa may improve the diffusion of antibiotic molecules through mucus.[90,92]

Figure 2 – Pathway of the inhaled antibiotic after deposition on the mucus layer
After depositing in the airways, the aerosol particle needs to dissolve in the airway surface layer or mucus layer. Next, the antibiotic needs to diffuse to the site where the bacteria are located. During the diffusion process through the mucus layer the aerosol particle can bind to molecules in the mucus. Also, the oxygen level, salt content and pH of the mucus are of influence on the antibiotic efficacy. Finally, the antibiotic has to overcome barriers generated by the microorganisms

During the diffusion process the inhaled antibiotic may bind to mucus, thereby limiting the amount of free drug available to be efficacious against bacteria. Aminoglycosides showed substantial binding to mucus from CF patients[94] while this was not observed in β-lactam antibiotics. This difference in binding can be attributed to the fact that aminoglycosides are positively charged, whereas β-lactam antibiotics are neutrally or negatively charged. As mucus macromolecules are negatively charged, they show a high affinity for positively charged antibiotics. This level of binding may be reduced by the coadministration of drugs such as cationic amphiphiles, which competitively bind to the macromolecules within the mucus. This resulted in saturation of the binding sites and a higher aminoglycoside bioavailability.[93] Conflicting results on the coadministration of dornase alfa have been observed by different authors; one study found a decrease in binding,[94] while another found an increase in binding.[95] To the best of our knowledge, the effect of mucus on the efficacy of fluoroquinolones or macrolides has not been studied.

Low oxygen levels and high salt concentrations within the mucus were shown to reduce the effectiveness of antibiotics. Specifically, aminoglycosides, β-lactam antibiotics, tetracyclines and chloramphenicol were rendered less efficacious against *Pa* under anaerobic conditions. Low oxygen levels within mucus may increase colistin activity.[80,107,109] With regard to salt concentrations, *Pa* was rendered less susceptible to killing by aminoglycosides in the presence of magnesium and calcium.[110] Additionally, nitrate decreased the efficacy of aminoglycosides and fluoroquinolones by half under anaerobic conditions.[108]

Ultimately, when antibiotic molecules make it to the vicinity of the bacteria, they still need to overcome multiple barriers generated by the microorganisms. The alginate layer surrounding *Pa* is an important contributing factor to its resistance of *Pa* against antibiotics and the patient's innate immune response. In general aminoglycosides bind to alginate while β-lactam antibiotics do not. Diffusion through alginate was impaired for all tested antibiotics, but most strongly for aminoglycosides. Coadministration of Algl showed promising results *in vitro*, with improved diffusion rates and enhanced bactericidal activity.[87,114] Another important barrier generated by *Pa* is biofilm formation, which drastically reduced effective killing. Treatment of *Pa* growing in biofilms can be further improved by co-treatment with iron binding glycoproteins,[116-118] mannitol or dispersion compounds.[106,115]

The limitations of this systematic review are the following; firstly, out of the 35 publications selected for analysis, 9 publications were selected by screening the reference lists of the included articles. The search term "INHALED" was obligated in the title or abstract, while not all studies specified the route of administration.

Secondly, a high level of inter-publication variability was observed for the following aspects of the studies; concentrations of antibiotic, types of alginate or exopolysaccha-

ride, types of buffer or medium. These differences prevented an accurate comparison between the results.

Thirdly, the majority of the studies were performed *in vitro* and hence, caution is required when extrapolating these results to *in vivo* conditions. Clearly, *in vivo* studies are needed to investigate the relevance of the *in vitro* observations for the effectiveness of inhaled antibiotics in patients. In addition, most publications did not distinguish between intravenous, nebulised or dry powder antibiotics. This is important as aerosol particles are first required to dissolve before diffusion through the mucus and alginate can occur. Finally, there is a clear disbalance in the antibiotics studied in the literature; aminoglycosides were most extensively studied, followed by β-lactam antibiotics. Although, these antibiotic classes are frequently used to treat pulmonary infections in CF, little is known about other frequently used antibiotics such as colistin.

Based on *in vitro* studies we conclude that aminoglycosides can be strongly affected after deposition in the airways. The composition of CF mucus is an important determinant of the *in vitro* efficacy of aminoglycosides, as well as the alginate layer surrounding *Pa*. In order to eradicate a microorganism, an antibiotic needs to overcome all the aforementioned barriers before reaching the outer membrane of the microorganism and ultimately bind to specific target sites. Importantly, higher concentrations allow more antibiotic molecules to reach these target sites. For future research, both advanced modelling and *in vivo* studies are required to further establish the role of encapsulated antibiotic formulations and coadministration with other drugs in improving the local efficacy of inhaled antibiotics.

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SUPPLEMENTARY TABLES

Table S1 – Critical appraisal table

Criteria	Additional information per criterion
Study population	CF patients (2), non-CF humans (1), animal study (0), in vitro/modelling study (0)
Topics	Direct answer or indirect answer to the research questions: direct (2), indirect (1), no answer (0)
Study design	experimental (2), observational (1), rest (0)*
Clearly stated aim?	
Endpoints appropriate?	Standardisation of outcome; clear definition of outcome measurement used
Unbiased assessment of endpoint?	
Methods reproducible?	
Reporting bias	Selective reporting
Results objective?	
Conclusions justified?	
Additional criteria for comparative studies	
Selection bias	Random sequence generation and allocation concealment: both (2), 1 out of 2 (1), none (0)
Performance bias	Blinding of participants and personnel
Detection bias	Blinding of outcome assessment
Attrition bias	Incomplete outcome data
Adequate control group	
Contemporary groups	
Baseline equivalence	If not: has there been a correction in the analysis?
Follow-up appropriate?	
Loss to follow-up <5%	If more loss to follow up: has selective loss been ruled out?
Adequate statistics?	
Confounders	
Applicable?	Sputum binding, concentration, abnormal CF sputum, resistance
Total	

Score as 2 (reported adequately), 1 (reported inadequately/unclear), 0 (not reported), n/a (not applicable).

** Experimental studies: RCT's, systematic reviews, meta-analysis. Observational studies: cohort studies, case-control studies, case series, case reports. Rest: animal studies, modelling studies, in vitro-studies*

Table S2 – Critical appraisal completed

	Moreau-Marquis 2015[116]	Pompilio 2015[109]	Ong 2014 (abstr)[91]	Barraud 2013[106]	Russo 2013[89]	Ross 2013[115]	Moreau-Marquis 2012 (abstr T)[117]	Moreau-Marquis 2012 (abstr A)[118]	Yang 2011 (Biotech Bio)[90]
Study population[1]	0	0	0	0	0	0	0	0	0
Topics[2]	1	1	1	1	2	1	1	1	1
Study design[3]	0	0	0	0	0	0	0	0	0
Clearly stated aim?	2	2	2	2	2	2	2	2	2
Endpoints appropriate?[4]	2	2	2	2	2	2	2	2	2
Unbiased assessment of endpoint?	2	2	2	2	2	2	0	0	2
Methods reproducible?	2	2	1	2	2	1	1	1	2
Reporting bias[5]	2	2	1	2	2	1	1	1	2
Results objective?	2	2	2	2	2	2	2	2	2
Conclusions justified?	2	2	2	2	2	2	2	2	2
Additional criteria for comparative studies									
Selection bias[6]									
Performance bias[7]									
Detection bias[8]									
Attrition bias[9]									
Adequate control group									
Contemporary groups									
Baseline equivalence[10]									
Follow-up appropriate?									
Loss to follow-up <5%[11]									
Adequate statistics?									
Confounders									
Applicable?[12]									
Total	**15/20**	**15/20**	**13/20**	**15/20**	**16/20**	**13/20**	**11/20**	**11/20**	**15/20**

Score as 2 (reported adequately), 1 (reported inadequately/unclear), 0 (not reported), n/a (not applicable). T = tobramycin, A = aztreonam

[1] Study population: CF patients (2), non-CF humans (1), animal study (0), in vitro/modelling study (0); [2] Direct answer or indirect answer to the research questions: direct (2), indirect (1), no answer (0); [3] Study design: experimental (2), observational (1), rest (0); [4] Standardisation of outcome; clear definition of outcome measurement used; [5] Selective reporting; [6] Random sequence generation and allocation concealment: both (2), 1 out of 2 (1), none (0); [7] Blinding of participants and personnel; [8] Blinding of outcome assessment; [9] Incomplete outcome data; [10] If not: has there been a correction in the analysis?; [11] If more loss to follow up: has selective loss been ruled out?; [12] Sputum binding, concentration, abnormal CF sputum, resistance.*

** Experimental studies: RCT's, systematic reviews, meta-analysis. Observational studies: cohort studies, case-control studies, case series, case reports. Rest: animal studies, modelling studies, in vitro-studies.*

Continuation 1

	Barja 2010[85]	King 2010[107]	Potter 2010 (abstr)[105]	Alipour 2009 (JAC)[87]	Alipour 2009 (Plos)[101]	Purdy Drew 2009[93]	Yang 2010 (Pharm Res)[92]	Meers 2008[102]	Barboza 2008[84]
Study population[1]	0	0	0	0	0	0	0	0	0
Topics[2]	1	1	1	1	2	2	1	1	1
Study design[3]	0	0	0	0	0	0	0	0	0
Clearly stated aim?	2	2	2	2	2	2	2	2	2
Endpoints appropriate?[4]	2	2	2	2	2	2	2	2	2
Unbiased assessment of endpoint?	2	2	0	2	2	2	2	2	2
Methods reproducible?	2	2	1	2	2	2	2	2	2
Reporting bias[5]	2	2	1	2	2	2	2	1	2
Results objective?	2	2	2	2	2	2	2	1	2
Conclusions justified?	2	2	2	2	2	2	2	1	2
Additional criteria for comparative studies									
Selection bias[6]									
Performance bias[7]									
Detection bias[8]									
Attrition bias[9]									
Adequate control group									
Contemporary groups									
Baseline equivalence[10]									
Follow-up appropriate?									
Loss to follow-up <5%[11]									
Adequate statistics?									
Confounders									
Applicable?[12]									
Total	**15/20**	**15/20**	**11/20**	**15/20**	**16/20**	**16/20**	**15/20**	**12/20**	**15/20**

Score as 2 (reported adequately), 1 (reported inadequately/unclear), 0 (not reported), n/a (not applicable). T = tobramycin, A = aztreonam

[1] Study population: CF patients (2), non-CF humans (1), animal study (0), in vitro/modelling study (0); [2] Direct answer or indirect answer to the research questions: direct (2), indirect (1), no answer (0); [3] Study design: experimental (2), observational (1), rest (0); [4] Standardisation of outcome; clear definition of outcome measurement used; [5] Selective reporting; [6] Random sequence generation and allocation concealment: both (2), 1 out of 2 (1), none (0); [7] Blinding of participants and personnel; [8] Blinding of outcome assessment; [9] Incomplete outcome data; [10] If not: has there been a correction in the analysis?; [11] If more loss to follow up: has selective loss been ruled out?; [12] Sputum binding, concentration, abnormal CF sputum, resistance.*

** Experimental studies: RCT's, systematic reviews, meta-analysis. Observational studies: cohort studies, case-control studies, case series, case reports. Rest: animal studies, modelling studies, in vitro-studies.*

Continuation 2

	Halwani 2007[103]	Mugabe 2005[104]	Hill 2005[80]	Borriello 2004[108]	Hatch 1998[114]	Hunt 1995[95]	Bataillon 1992[97]	Bolister 1991[88]	Gordon 1988[112]
Study population[1]	0	0	0	0	0	0	0	0	0
Topics[2]	1	1	1	1	1	2	2	2	2
Study design[3]	0	0	0	0	0	0	0	0	0
Clearly stated aim?	2	2	1	2	1	2	2	2	2
Endpoints appropriate?[4]	2	2	2	2	1	2	2	2	2
Unbiased assessment of endpoint?	2	2	2	2	2	2	2	2	2
Methods reproducible?	2	2	2	2	2	2	2	2	2
Reporting bias[5]	2	2	2	2	2	2	2	1	2
Results objective?	2	2	2	2	2	2	2	2	2
Conclusions justified?	2	2	2	2	2	2	2	2	2
Additional criteria for comparative studies									
Selection bias[6]									
Performance bias[7]									
Detection bias[8]									
Attrition bias[9]									
Adequate control group									
Contemporary groups									
Baseline equivalence[10]									
Follow-up appropriate?									
Loss to follow-up <5%[11]									
Adequate statistics?									
Confounders									
Applicable?[12]									
Total	**15/20**	**15/20**	**14/20**	**15/20**	**13/20**	**16/20**	**16/20**	**15/20**	**16/20**

Score as 2 (reported adequately), 1 (reported inadequately/unclear), 0 (not reported), n/a (not applicable). T = tobramycin, A = aztreonam

[1] Study population: CF patients (2), non-CF humans (1), animal study (0), in vitro/modelling study (0); [2] Direct answer or indirect answer to the research questions: direct (2), indirect (1), no answer (0); [3] Study design: experimental (2), observational (1), rest (0); [4] Standardisation of outcome; clear definition of outcome measurement used; [5] Selective reporting; [6] Random sequence generation and allocation concealment: both (2), 1 out of 2 (1), none (0); [7] Blinding of participants and personnel; [8] Blinding of outcome assessment; [9] Incomplete outcome data; [10] If not: has there been a correction in the analysis?; [11] If more loss to follow up: has selective loss been ruled out?; [12] Sputum binding, concentration, abnormal CF sputum, resistance.*

** Experimental studies: RCT's, systematic reviews, meta-analysis. Observational studies: cohort studies, case-control studies, case series, case reports. Rest: animal studies, modelling studies, in vitro-studies.*

Continuation 3

	Ramphal 1988[94]	Nichols 1988[81]	Mendelman 1985[96]	Tannenbaum 1984[113]	Levy 1983[98]	Slack 1981[111]	Davis 1978[100]	Beggs 1976[110]
Study population[1]	0	0	0	0	0	0	0	0
Topics[2]	2	1	2	2	2	1	2	1
Study design[3]	0	0	0	0	0	0	0	0
Clearly stated aim?	1	2	2	2	2	2	2	2
Endpoints appropriate?[4]	2	2	2	2	2	2	2	2
Unbiased assessment of endpoint?	2	2	2	2	2	2	2	2
Methods reproducible?	2	2	1	2	2	2	1	2
Reporting bias[5]	2	2	2	2	2	2	2	2
Results objective?	2	2	2	2	2	2	2	2
Conclusions justified?	2	1	1	2	2	2	2	2
Additional criteria for comparative studies								
Selection bias[6]					0			
Performance bias[7]					0			
Detection bias[8]					0			
Attrition bias[9]					2			
Adequate control group					1			
Contemporary groups					1			
Baseline equivalence[10]					0			
Follow-up appropriate?					n.a.			
Loss to follow-up <5%[11]					n.a.			
Adequate statistics?					0			
Confounders					0			
Applicable?[12]					Sputum binding			
Total	**15/20**	**14/20**	**14/20**	**16/20**	**20/38**	**15/20**	**15/20**	**15/20**

Score as 2 (reported adequately), 1 (reported inadequately/unclear), 0 (not reported), n/a (not applicable). T = tobramycin, A = aztreonam

[1] Study population: CF patients (2), non-CF humans (1), animal study (0), in vitro/modelling study (0); [2] Direct answer or indirect answer to the research questions: direct (2), indirect (1), no answer (0); [3] Study design: experimental (2), observational (1), rest (0); [4] Standardisation of outcome; clear definition of outcome measurement used; [5] Selective reporting; [6] Random sequence generation and allocation concealment: both (2), 1 out of 2 (1), none (0); [7] Blinding of participants and personnel; [8] Blinding of outcome assessment; [9] Incomplete outcome data; [10] If not: has there been a correction in the analysis?; [11] If more loss to follow up: has selective loss been ruled out?; [12] Sputum binding, concentration, abnormal CF sputum, resistance.*

** Experimental studies: RCT's, systematic reviews, meta-analysis. Observational studies: cohort studies, case-control studies, case series, case reports. Rest: animal studies, modelling studies, in vitro-studies.*

Continuation 4 (Excluded articles)

	Zsembery 2014 (abstr)	Omri 2014 (abstr)	Ruge 2013	Forbes 2011	Bosquillon 2010	Groneberg 2004	Grimwood 2003	Worlitzsch 2002	Bhat 1996	Slack 1982
Study population[1]	0	0	1	1	0	1	1	2	0	0
Topics[2]	1	1	2	0	1	1	1	0	2	1
Study design[3]	0	0	1	1	1	0	1	0	0	0
Clearly stated aim?	2	1	2	2	2	1	1	2	2	0
Endpoints appropriate?[4]	2	0	1	1	2	1	1	2	2	0
Unbiased assessment of endpoint?	1	1	0	1	1	1	1	2	2	0
Methods reproducible?	0	1	1	0	0	0	0	2	2	0
Reporting bias[5]	1	0	1	1	1	1	1	2	2	0
Results objective?	1	1	1	1	1	1	1	2	2	0
Conclusions justified?	1	1	2	2	1	2	2	2	2	0
Additional criteria for comparative studies										
Selection bias[6]								0		
Performance bias[7]								0		
Detection bias[8]								0		
Attrition bias[9]								2		
Adequate control group								2		
Contemporary groups								2		
Baseline equivalence[10]								0		
Follow-up appropriate?								n.a.		
Loss to follow-up <5%[11]								n.a.		
Adequate statistics?								2		
Confounders								2		
Applicable?[12]			Review					Solely Pa		
Total	**9/20**	**6/20**	**12/20**	**10/20**	**10/20**	**9/20**	**10/20**	**26/38**	**16/20**	**1/20**

Score as 2 (reported adequately), 1 (reported inadequately/unclear), 0 (not reported), n/a (not applicable). T = tobramycin, A = aztreonam

[1] Study population: CF patients (2), non-CF humans (1), animal study (0), in vitro/modelling study (0); [2] Direct answer or indirect answer to the research questions: direct (2), indirect (1), no answer (0); [3] Study design: experimental (2), observational (1), rest (0); [4] Standardisation of outcome; clear definition of outcome measurement used; [5] Selective reporting; [6] Random sequence generation and allocation concealment: both (2), 1 out of 2 (1), none (0); [7] Blinding of participants and personnel; [8] Blinding of outcome assessment; [9] Incomplete outcome data; [10] If not: has there been a correction in the analysis?; [11] If more loss to follow up: has selective loss been ruled out?; [12] Sputum binding, concentration, abnormal CF sputum, resistance.*

** Experimental studies: RCT's, systematic reviews, meta-analysis. Observational studies: cohort studies, case-control studies, case series, case reports. Rest: animal studies, modelling studies, in vitro-studies.*

Table S3 – Main results according to step in antibiotic pathway

Step in antibiotic pathway	Number of studies	Main results
1. Dissolution in mucus layer	2 studies describing the same *in vitro* study	• Tobramycin dissolves easier in mucus of secretors → more rapid effect (2 studies)
2. Diffusion through mucus layer	4 *in vitro* studies	• Aminoglycosides (1 study), β-lactam (1 study), Fluoroquinolones (2 studies): CF mucus reduces antibiotic diffusion
3. Binding to molecules in mucus	8 *in vitro* studies	• Aminoglycosides (7 studies): o Strong binding to mucin (2 studies), DNA (4 studies), nucleic acids (2 studies) and bacterial endotoxins (LPS, LTS) (1 study). Strong binding mucus in general (3 studies). o Binding tobramycin 15-95% (2 studies), amikacin 1-60% (1 study), gentamicin 52% (1 study) o Liposomal form: binding reduced 4 to 100-fold (1 study) o Dornase alfa treatment: ▪ Increased binding (1 study) ▪ Decreased binding (1 study) • β-lactam AB: negligible binding to CF mucus (2 studies) • Polymyxin B (2 studies): o Strong binding to mucus in general (1 study). Binding to bacterial endotoxins, but no binding to DNA/F-actin (1 study) o Liposomal form: binding reduced up to 100-fold (1 study)
4. Influence of oxygen level in mucus	4 *in vitro* studies	• Aminoglycosides (3 studies), β-lactam AB (3 studies), Macrolides (1 study), Cotrimoxazol (1 study), Tetracyclines (1 study), Chloramphenicol (1 study): Reduced efficacy in anaerobic conditions. • Fluoroquinolones: o Reduced efficacy in anaerobic conditions (2 studies) o Remain bactericidal in anaerobic conditions (1 study) • Colistin: o Reduced efficacy in anaerobic conditions (1 study) o Increased efficacy in anaerobic conditions (1 study)
5. Influence of salt content in mucus	2 *in vitro* studies	• Aminoglycosides: efficacy reduced by salts (2 studies), high ionic strength (polyanions) (1 study) • Fluoroquinolones: susceptibility of organisms to AB decreased by nitrate (1 study) • β-lactam AB (1 study): o Carbenicillin: nitrate increased efficacy if aerobic, but effect abolished if anaerobic o Ceftazidime: nitrate no effect • Chloramphenicol and tetracycline: nitrate no effect (1 study)

Table S3 – Main results according to step in antibiotic pathway (continued)

Step in antibiotic pathway	Number of studies	Main results
6. Diffusion through *Pa* alginate layer	10 *in vitro* studies	• Aminoglycosides: o Inhibited in diffusion by alginate, stronger inhibition than β-lactam AB (3 studies) o 12-fold reduction in aminoglycoside concentration in 1.5% w/v *Pa* alginate (1 study) o Decrease in aminoglycoside binding to alginate in presence of salt (1 study) • β-lactam AB: inhibited in diffusion by alginate (1 study) • Polymyxin B: inhibited in diffusion by alginate (1 study)
7. Liposomal formulations	3 *in vitro* studies, 2 animal studies	• Aminoglycosides: o Liposomal form more efficacious than free form (4 studies) o Liposomal form less efficacious than free form (1 study) • Polymyxin B: liposomal form more efficacious than free form (1 study)
8. Co-treatment with other medications	15 *in vitro* studies	• Dornase alfa: o Enhanced bactericidal activity of aminoglycosides (2 studies) o Reduced bactericidal activity of aminoglycosides (1 study) o Enhanced bactericidal activity of fluoroquinolones (1 study) • Mannitol: enhanced bactericidal activity of aminoglycosides (1 study) and fluoroquinolones (2 studies) • Alginate lyase: improved diffusion rates and enhanced bactericidal activity of aminoglycosides (2 studies), combination with dornase alfa most effective • N-acetylcysteine: no effect on bactericidal activity of aminoglycosides • NaCl: o Synergistic effect on colistin (1 study) o Enhanced bactericidal activity of tobramycin (1 study) o Antagonistic effect on tobramycin (1 study) • Glucose: enhanced bactericidal activity of aminoglycosides (1 study) • ALX-109&ALX009: enhanced bactericidal activity of aminoglycosides (1 article, 1 abstract) and β-lactam AB (1 article, 1 abstract) • L-leucine: no influence on aminoglycoside permeability through mucus (1 study) • Cationic amphiphiles: reduced binding between aminoglycosides and DNA (1 study) • Dispersion compounds: o Synergistic action of citrate with amikacin, colistin or erythromycin and succinic acid with colistin (1 study) o Reduced bactericidal activity of tobramycin and polymyxin B (1 study)

AB = antibiotic; CF = cystic fibrosis; LPS = lipopolysaccharides; LTA = lipoteichoic acid.

Table S4 – Results table

1. Dissolution of antibiotic in mucus layer

First author[ref.]	Study design	Origin material	Drugs studied	Results measured in	Results	Conclusion
Barboza et al. 2008 Barja et al. 2010[84,85]	In vitro	• Sputum of 10 CF patients (CF patients' presence or absence of ABH glycoconjugates: 6 secretor and 4 non-secretor phenotype)	• Tobramycin	• Typical interaction time of solubilisation (t_o = time to reach maximum rate of change in process) and solubilisation interval (Δt = effective time interval corresponding to solubilisation process) of sputum nebulized with tobramycin, measured with photoacoustic analysis	• Mean t_o for non-secretor 13.7 and for secretor 8.8 minutes (p = 0.03) • Mean Δt were similar for non-secretors and secretors (4.9 vs 4.8 minutes)	• The results suggest that tobramycin dissolves easier in the mucus from secretor than non-secretor phenotypes

2. Mucus diffusion

First author[ref.]	Study design	Origin material	Drugs studied	Results measured in	Results	Conclusion
Russo 2013[89]	In vitro	• Artificial mucus model consisting of, amongst others, mucin from porcine stomach	• Gentamicin respirable powder • Gentamicin powder with 15% (w/w) L-leucine content	• Permeation time measured by means of Franz-type vertical diffusion cells	• Gentamicin permeability significantly reduced by artificial mucus • Leucine only had a faint influence on gentamicin permeation properties (from slightly decreasing permeation rate to no effect to minimally increasing permeation rate) • No specific numbers; results presented in graphs	• Permeation rate of gentamicin was decreased in mucus when compared with buffer • Leucine did not influence gentamicin permeability

Yang 2011 (Biotechol Bioeng)[90]	In vitro	• Artificial mucus with and without • *Pa*	• Ciprofloxacin dry powder • Mannitol • NaCl • Lactose	• Coomassie blue added to particles to visualize particle penetration through artificial mucus; gelatin gel placed underneath artificial mucus → amount of Coomassie blue in gelatin gel proportional to extent of particle penetration • Antibacterial effectiveness measured by counting number of active bacterial colonies • Rheological properties (elastic an vicious portions of mucus) measured with rheometer	• Mannitol enhanced trans mucus diffusion of Coomassie blue • Bacterial counts in the artificial mucus after 1h incubation were the least for Cipro-mannitol particles, intermediate for Cipro-NaCl particles and greatest for Cipro-lactose particles • NaCl and mannitol decreased the storage modulus of artificial mucus by respectively 23% and 31%, Cipro-NaCl and Cipro-mannitol decreased the storage modulus with 17% and 19% respectively	• Ciprofloxacin encapsulated in mannitol particles showed significantly higher antibacterial activity of the drug on *Pa* grown in artificial mucus than Cipro-NaCl or Cipro-lactose particles • Mannitol-based particles increased local water content in artificial mucus and enhanced drug penetration into it
Yang 2010 (Pharm Res)[92]	In vitro	• *Pa* (ATCC27853) purchased from the American Type Culture Collection • Artificial sputum	• Ciprofloxacin dry powder • Dornase alfa • Cipro/Dornase alfa powder	• MIC determined by broth microdilution procedures • CFU counted	• Cipro/Dornase alfa powder complete dissolution and diffusion after 30 min • Cipro powder after 2h still Cipro powder visible on surface of artificial sputum • Cipro/Dornase alfa powder significantly lower CFU count after 1h than Cipro powder but this difference disappeared at 2 hours • Both dose-dependent antibacterial activity • Dose-response curves: bactericidal effect of Cipro/Dornase alfa powder consistently and significantly exceeded that of Cipro powder	• Cipro/Dornase alfa powder showed better antibacterial activity than Cipro powder

Bolister 1991[88]	In vitro	• Extracellular alginate produced by a mucoid *Pa* strain from sputum of CF patient • Mucin from sputa of CF patients • DNA from calf thymus	• Amoxicillin • Ampicillin • Benzylpenicillin • Carbenicillin • Cloxacillin • Flucloxacillin • Mezlocillin • Piperacillin • Ticarcillin • Clavulanic acid • 6-aminopenicillanic acid • VX-VC-43	• Diffusion rates measured with spectrophotometry and with three compartment diffusion cells	• Diffusion rate was decreased as the antibiotic molecular weight increased • Normal diffusion rate of ticarcillin: 1.1 nM/mm^2/h • With 1 or 2% w/v mucin 0.9 nM/mm^2/h • With 4% w/v mucin 0.5 nM/mm^2/h (0.5 µm /mm^2/h) • With 1% w/v alginate 0.6 nM/mm^2/h • Addition of DNA 1.5% w/v + mucin 4.0% w/v 0.1 µm /mm^2/h	• Both mucin and alginate formed a barrier to penetration of antibiotics • At equivalent concentrations alginate delayed antibiotic at a greater extent than mucin • However at physiological concentrations of mucin, this became the greater barrier to penetration and this effect was further increased by addition of DNA • DNA also increased viscosity & elasticity of the sputum

Pa = Pseudomonas aeruginosa; MIC = minimal inhibitory concentration; CFU = colony forming units; CF = cystic fibrosis

3. Binding antibiotics to macromolecules in mucus

First author[ref.]	Study design	Origin material	Drugs studied	Results measured in	Results	Conclusion
Purdy Drew 2009[93]	In vitro	• DNA from calf thymus • *Pa* (PAO1) from freshly streaked agar plate	• Tobramycin • Cationic amphiphiles	• MIC_{90} measured by microbroth dilution growth inhibition assays	• MIC_{90} of free tobramycin = 0.4 µg/mL • MIC_{90} of tobramycin + DNA = 12.5 µg/mL • MIC_{90} of tobramycin + DNA + cationic amphiphiles = 0.8 µg/mL	• Adding cationic amphiphiles can limit the binding between DNA and tobramycin • DNA interacted preferentially with cationic lipids for a broad range of tobramycin concentrations as long as the tobramycin concentration was less than that required to neutralize DNA charge (tobramycin/DNA = 1) • Optimized cationic amphiphile solutions have the potential to enhance antimicrobial function in highly infected environments that contain increased concentrations of anionic inflammatory polymers

Alipour 2009 (Plos)[101]	In vitro	• Reference *Pa* strain (ATCC 27853) purchased • Clinical isolate strains (PA-48912-1, PA-48912-2, en PA-48913) obtained from clinical microbiology laboratory • Expectorated sputum from 9 CF patients • Pooled CF sputum (intact and diluted or autoclaved before mixing with cation-adjusted Mueller-Hinton broth)	• Tobramycin (liposomal and free form) • Polymyxin B (liposomal and free form)	• CFU/ml determined • MBC determined by standard microbroth dilution assay • MBC defined as lowest concentration of antibiotic that resulted in less than 30 CFU live bacteria/Petri dish	• Free tobramycin inhibited by • 125-1000 mg/L DNA/F-actin • 1-1000 mg/L LPS/LTA (bacterial endotoxins) • Liposomal tobramycin inhibited by • 500-1000 mg/L DNA/F-actin • 100-1000 mg/L LPS/LTA • Free polymyxin B inhibited by • Not inactivated by DNA/F-actin • 1-1000 mg/L LPS/LTA • Liposomal polymyxin B inhibited by • Not inactivated by DNA/F-actin • 100-1000 mg/L LPS/LTA • Effect DNA/F-actin/LPS/LTA on MBCs in 18 h period: • Free tobramycin: MBC increased 16x • Liposomal tobramycin: MBC increased 4x • Free polymyxin B: MBC increased 64x • Liposomal polymyxin B: MBC increased 64x • Efficacy in CF sputa: • Free tobramycin: 512 mg/L; 5.4±0.2 logs • Liposomal tobramycin: 128 mg/L; 5.3±0.1 logs • Free polymyxin B: 32 mg/L; 3.9±0.1 logs • Liposomal polymyxin B: 8 mg/L; 3.8±0.1 logs	• Liposome-entrapment reduced antibiotic inhibition up to 100-fold and the CFU of endogenous *Pa* in sputum by 4-fold compared to the conventional antibiotic

Hunt 1995[95]	In vitro	• Sputum from children and adults with CF • 6 *Pa* strains from 6 CF patients (4 mucoid and 2 non-mucoid) • Mock sputum: mucin from porcine stomachs and DNA from calf thymus	• Tobramycin • Dornase alfa	• Bioactive tobramycin was measured by making killing curves • Free tobramycin measured with fluorescence polarization immunoassay with TDX apparatus	• Dornase alfa treatment did not significantly change amount of bioactive tobramycin • At 2 and 4 hours, 7 of 8 means had higher bacterial counts with Dornase alfa → more binding of tobramycin was seen with Dornase alfa treatment	• Dornase alfa did not increase the bioactivity of tobramycin in sputum • Sputum binding was increased by Dornase alfa
Bataillon 1992[97]	In vitro	• Sputum from 6 patients with CF, 4 patients with chronic bronchitis and 3 with bronchiectasis during hospitalization for infectious exacerbations	• Amikacin	• Free and total levels of amikacin measured by HPLC • When mixtures were in low quantity, levels were measured by fluorescence polarization immunoassay with TD_x analyser • Total and free amikacin also measured by microbiological assay	• Binding percentages CF patients, 1-60%: • 2 patients: >50% • 3 patients: 13-28% • 1 patient: <10% • Binding percentages chronic bronchitis patients: • 4 patients: <10% • Binding percentages bronchiectasis patients: • 3 patients: <10% • There was binding to mucin glycopeptides, nucleic acids and DNA • Equivalent results measured by microbiological method	• Amikacin bound markedly to DNA, mucin glycopeptides and nucleic acids in sputum of patients with CF • Antibiotic binding to macromolecules of patients with chronic bronchitis and bronchiectasis was weak

Ramphal 1988[94]	In vitro	• Macromolecule portion extracted from sputum of CF patients	• Tobramycin • Ceftazidime • Dornase alfa	• Free ceftazidime measured by HPLC • Free tobramycin measured by fluorescence polarization with the TDX apparatus • Equilibrium dialysis used to verify results	• Tobramycin (100 mg/L): • 95% binding when added to solutions with macromolecule concentration of 10 g/L • 75-83% binding when added to 1.5 g/L of fraction mucin rich fraction of mucus → after dornase alfa treatment increase in free drug of 10 mg/L • 81-91% binding when added to 1.5 g/L of nucleic acid rich fraction of mucus → after dornase alfa treatment increase in free drug of about 30 mg/L • Ceftazidime (25 and 50 mg/L) • 0-19% binding added to solution with macromolecule concentration of 10 g/L	• Significant binding of tobramycin to both the mucin glycoprotein rich fraction and DNA rich fractions of sputum of patients with CF • Amount of binding was dependent on the macromolecule concentration • Ceftazidime binding to sputum was negligible
Mendelman 1985[96]	In vitro	• 6 *Pa* isolates isolated from 6 different patients (3 mucoid, 3 rough) • Sputum of 15 CF outpatients not receiving antibiotics active against *Pa*	• Tobramycin • Gentamicin	• Tobramycin contents of dialysis sac and broth assayed by radioenzymatic method • Measurement of *Pa* density not described	• Tobramycin and gentamicin activity were reduced in the presence of sputum • Tobramycin: • 5-15 µg/ml: 30% was bound to sputum, 70% free drug • 25-50 µg/ml: ~15% was bound, 85±17% free drug	• CF sputum antagonized the bioactivity of aminoglycosides

Levy 1983[98]	In vitro	• 5 strains of *Pa* isolated from sputum of children with CF (3 mucoid, 2 nonmucoid) • Sputum of adults with bronchiectasis	• Gentamicin	• Bactericidal activity determined by quantitation of number of viable bacteria (number of CFU of *Pa* per ml) • Percentage of binding to sputum and serum measured by equilibrium dialysis	• Proportion binding to CF sputum was 52%, binding to bronchiectasis sputum 48%, binding to serum was 18% • Proportion of organisms killed in both sputum dialysates significantly and similarly lower than in the serum dialysate • Serum dialysate was significantly more inhibitory against 3 of 5 *Pa* strains than sputum dialysate • No difference between antagonism of dialysates of sputum from patients with CF and that of patients with bronchiectasis	• Gentamicin bound both to sputum from patients with CF and to sputum from patients with bronchiectasis • Level of bioactivity of gentamicin against *Pa* was lower in purulent sputum from patients with CF or bronchiectasis than in serum
Davis 1978[100]	In vitro	• 12 strains of *Pa* from clinical microbiology laboratory • Sputum from patients with CF	• Polymixin B • Neomycin • Tobramycin • Gentamicin • Carbenicillin	• Mean number of wells in which MBC is increased	• Polymixin B • 4.6 wells (range 1.5-6.5) increased MBC • Addition of 50% sputum completely blocked bactericidal activity of 5 µg/ml • Neomycin 2.7 wells (range 1-4) increased MBC • Tobramycin 1.5 wells (range 0.5-2.5) increased MBC • Gentamicin 1.0 wells (range 0-2) increased MBC • Carbenicillin 0.1 wells (range -1 to +3)	• Sputum from patients with CF strongly increased MBC of polymyxin B and neomycin, lesser effect on MBC of tobramycin and gentamicin, no effect on carbenicillin

Pa = Pseudomonas aeruginosa; MIC_{90} = Minimum inhibitory concentration that inhibits 90% of the isolates; CF = cystic fibrosis; CFU = colony forming units; MBC = Minimal bactericidal concentration; LPS = lipopolysaccharides; LTA = lipoteichoic acid

4. Influence oxygen level mucus on antibiotic

First author[ref.]	Study design	Origin material	Drugs studied	Results measured in	Results	Conclusion
Pompilio 2015[109]	In vitro	• 12 *Pa* strains isolated from sputum of CF patients	• Colistin	• MIC measured by broth microdilution technique • MBC evaluated by plating onto Mueller-Hinton agar 10-µl aliquot from wells • MBEC measured by colony count onto Mueller-Hinton agar	• MIC_{50} and MIC_{90} values significantly lower under CF-like conditions • MBEC values lower under CF-like conditions, though not significant • CF-like conditions (anaerobic atmosphere, pH 6.4) • MIC_{50}: 0.5 µg/ml • MIC_{90}: 1 µg/ml • MBC_{50}: 2 µg/ml • MBC_{90}: 8 µg/ml • Colistin bacteriostatic (mean MBC/MIC 4.8) • $MBEC_{50}$: 128 µg/ml • $MBEC_{90}$: 512 µg/ml • Standard conditions (aerobic atmosphere, pH 7.4) • MIC_{50}: 4 µg/ml • MIC_{90}: 4 µg/ml • MBC_{50}: 4 µg/ml • MBC_{90}: 4 µg/ml • Colistin bactericidal (mean MBC/MIC 1.16) • $MBEC_{50}$: 256 µg/ml • $MBEC_{90}$: 1024 µg/ml	• Colistin activity against both planktonic and sessile *Pa* cells was increased under acidified and anaerobic environment, similar to those found in the CF lung
King 2010[107]	In vitro	• 114 *Pa* isolates obtained from the CF Referral Centre for Susceptibility and Synergy Studies at Columbia University and 2 laboratories (40% mucoid phenotype)	• Tobramycin • Levofloxacin • Amikacin • Aztreonam	• MIC measured by broth microdilution method • Bactericidal activity of levofloxacin determined with aerobic and hypoxic time-kill assays	• Levofloxacin in anaerobic conditions: • MIC_{50} 2x increase • MIC_{90} no change • MIC distribution of nonmucoid and mucoid isolates similar • Tobramycin higher MICs among both nonmucoid and mucoid isolates in anaerobic conditions:	• Levofloxacin remained its bactericidal effect under anaerobic conditions • MICs for amikacin, tobramycin and aztreonam were significantly higher under anaerobic conditions

					• Mean MIC 7x increase • MIC_{50} 4x increase • Amikacin higher MICs among both nonmucoid and mucoid isolates in anaerobic conditions: • Mean MIC 4x increase • Aztreonam higher MICs among both nonmucoid and mucoid isolates in anaerobic conditions: • Mean MIC 6x increase • MIC_{50} 16x increase • Nonmucoid isolates more resistant to all antibiotics than mucoid isolates	
Hill 2005[80]	In vitro	16 multi-drug resistant *Pa* strains from the sputa of 16 patients with CF (10/16 were mucoid)	Tested at levels suitable for nebulized use: • Tobramycin • Colistin Tested at levels suitable for intravenous or oral use: • Amikacin • Tobramycin • Azithromycin • Cefepime • Ceftazidime • Ticarcillin-clavulanate • Ciprofloxacin • Cotrimoxazol • Meropenem	• The percentage of *Pa* isolates for which the single antibiotic or combination was bactericidal • Bactericidal effect for aerobe and anaerobe planktonic cultures was assessed with broth microdilution method according to NCCLS guidelines • Biofilm bactericidal activity was examined for turbidity in the well using a plate reader	• Anaerobically and biofilm-grown bacteria significantly less susceptible to single and combination antibiotics than corresponding aerobic planktonically grown isolates • Bactericidal antibiotic combinations under anaerobic conditions different from those that were bactericidal against the same organisms grown as biofilms • Colistin most effective under all conditions, followed by meropenem • Some combinations antagonistic • No association between mucoidy and bactericidal activity • Over 75% of the isolates were resistant for the following single antibiotics: tobramycin, cefepime, ceftazidime, ticarcillin-clavulanate and cotrimoxazole • None sensitive to amikacin or ciprofloxacin	• All antibiotics were less effective under anaerobic- and biofilm conditions • There was no correlation between class of drug or mode of growth and bactericidal activity • Selection of antibiotic combinations commonly bactericidal against clinical isolates grown under aerobic planktonic, anaerobic planktonic and biofilm conditions may prove to be more effective in reducing bacterial load

Borriello 2004[108]	In vitro • *Pa* PAO1	• Carbenicillin 250 µg/ml • Ceftazidime 10 µg/ml • Chloramphenicol 250 µg/ml • Ciprofloxacin 1 µg/ml • Tetracycline 250 µg/ml • Tobramycin 10 µg/ml	• MICs determined on Mueller-Hinton agar with Etest strips • Viable cell numbers enumerated by plating serially diluted samples on tryptic soy agar plates by the drop plating method • Killing reported as log reduction calculated relative to cell count at time of initiation of antibiotic exposure	• 4-h-old colony biofilms: • Ciprofloxacin: • Aerobic: 5.05±0.31 LR • Anaerobic: 2.61±0.13 LR • Tobramycin: • Aerobic: 5.67±0.00 LR • Anaerobic: 2.14±0.42 LR • Carbenicillin, chloramphenicol, tetracycline and ceftazidime also reduced efficacies under anaerobic growth conditions • Nitrate supplementation, aerobic: • Most antibiotics little effect on efficacy • Carbenicillin: • Without nitrate: 0.34±0.27 LR • With nitrate: 4.04±0.31 LR • Nitrate supplementation, anaerobic: • Ciprofloxacin and tobramycin levels of killing decreased by half • Chloramphenicol, tetracycline and ceftazidime no effect on efficacy • Carbenicillin enhanced effect abolished	• Anaerobic conditions reduced the efficacies of all six antibiotics • Under aerobic conditions nitrate supplementation had little effect on the efficacies of most antibiotics, with the exception of carbenicillin • Under anaerobic conditions, nitrate supplementation decreased the levels of killing by ciprofloxacin and tobramycin and had no effect on the other antibiotics

Pa = Pseudomonas aeruginosa; CF = cystic fibrosis; MIC = minimal inhibitory concentration; MBEC = Minimum biofilm eradication concentration; MIC_{50} and MIC_{90} = Minimum inhibitory concentration that inhibits 50 and 90% of the isolates, respectively; NCCLS = National Committee for Clinical Laboratory Standards; LR = log reduction; MBC = Minimal bactericidal concentration

5. Influence salt content mucus on antibiotic

First author[ref.]	Study design	Origin material	Drugs studied	Results measured in	Results	Conclusion
Beggs 1976[110]	In vitro	• *E. coli* ATC25922 and *Pa* ATCC 27853 obtained from Difco Laboratories	• Dihydrostreptomycin sulfate • Gentamicin	• Ionic strength (μ) determined by conductivity procedure • OD read at 600 nm in spectrophotometer	• *E. coli* • Ionic strength alone accounts for antagonism by salt of the activity of dihydrostreptomycin and gentamicin • *Pa* • Both nonspecific effect of ionic strength and specific, divalent cation-dependent mechanism • Nutrient broth: protection of organism from both antibiotics with sodium salts increased as function of ionic strength • $MgCl_2$ added to nutrient broth → for both antibiotics shift from complete growth inhibition to full protection • Sodium salts in Mueller-Hinton broth → no effect on gentamicin activity • $MgCl_2$ added to Mueller-Hinton broth → complete protection from gentamicin • No specific numbers; results presented in graphs	• Low initial salt content (range of ionic strength 0.02 – 0.14) applied the rule: the higher the ionic strength, the lower the antibacterial efficacy of aminoglycosides • With high initial salt content, ionic strength became much less important
Borriello 2004[108]	→ described at 4. Influence oxygen level mucus on antibiotic					

E. coli = Escherichia coli; Pa = Pseudomonas aeruginosa; $MgCl_2$ = magnesium chloride

6. Diffusion through alginate layer of *Pseudomonas aeruginosa*

First author[ref.]	Study design	Origin material	Drugs studied	Results measured in	Results	Conclusion
Moreau-Marquis 2015[116]	In vitro	• *Pa* strain PAO1 • *6 Pa* clinical strains isolated from sputum of CF patients(3 mucoid) • *Pa* biofilms from CF sputum grown at the apical side of airway epithelium	• Tobramycin • Aztreonam • ALX-109	• Human bronchial epithelial cells monolayers were visually inspected using phase-contrast microscopy • Efficacy in preventing development of biofilms determined by CFU counting by serial dilutions and spot titer	• ALX-109 alone: • Reduced PAO1 biofilm formation by 0.7 log units • No effect on established PAO1 biofilms • Effect on established clinical biofilms strain dependent, reduction in CFU by 1-2 log units for 4 clinical isolates, no effect on other 2 isolates • ALX-109 combined with tobramycin: • Tobramycin alone reduced PAO1 biofilm formation by 3.9 log units, combined with ALX-109 4.4 log units • Tobramycin alone reduced established PAO1 biofilms by 3 log units, combined with ALX-109 by 7 log units • Effect tobramycin alone on established clinical biofilms strain dependent: reduction in CFU by 1-3.5 log units for 4 clinical isolates, no effect on other 2 isolates. Combined reduction by 2-6 log units in all 6 clinical isolates • Mucoid *Pa* isolates most susceptible to combination • ALX-109 combined with aztreonam: • Aztreonam alone reduced PAO1 biofilm formation by 4 log units, combined with ALX-109 5 log units	• ALX-109 had an additive effect on tobramycin and aztreonam in reducing biofilm formation and disrupting *Pa* biofilms

					• Aztreonam alone reduced established PAO1 biofilms by 1.5 log units, combined with ALX-109 by 3 log units • Aztreonam alone reduced all established clinical biofilms by 2-5 log units, combined with ALX-109 reduction of an additional 1-3 log units • Equal effect on mucoid and non-mucoid clinical isolates	
Moreau-Marquis 2012 (2 abstracts: 1 tobramycin, 1 aztreonam)[117,118]	In vitro	• *Pa* strain PAO1 • Clinical *Pa* strains isolated from sputum of CF patients	• Tobramycin • Aztreonam • ALX-009	• CFU determined by serial dilutions and spot titer • *Pa* (PAO1) planktonic growth assessed by measuring OD_{600}	• ALX-009 alone: • Reduced PAO1 biofilm formation by 1 log unit • No effect on established PAO1 biofilms • No effect on established nonmucoid clinical biofilms (n=3) • Reduced established mucoid clinical biofilms by 1 log unit (n=3) • ALX-009 combined with tobramycin: • Tobramycin alone reduced established PAO1 biofilms by 4 log units, combined with ALX-009 by 7 log units • Tobramycin alone no effect on established nonmucoid clinical biofilms (n=3) • Tobramycin alone reduced established mucoid clinical biofilms by 1 log unit (n=3), combined with ALX-009 by 2.5-3 log units • ALX-009 combined with aztreonam	• Combination of ALX-009 with tobramycin disrupts established PAO1 biofilms and mucoid clinical biofilms, but not the established nonmucoid clinical biofilms • Combination of ALX-009 with aztreonam completely inhibits growth of planktonic *Pa*, reduces *Pa* biofilm formation and disrupts established *Pa* biofilms

					• Aztreonam alone reduced PAO1 biofilm formation by 3 log units, combined with ALX-009 by 4 log units • Aztreonam alone reduced established PAO1 biofilms by 3 log units, combined with ALX-009 by 4 log units • Combination completely blocked planktonic growth of *Pa*	
Alipour 2009 (JAC)[87]	In vitro	• Clinical *Pa* strains from lungs of CF patients, one mucoid (PA-489121) and one non-mucoid strain (PA-489122) • Reference non-mucoid *Pa* strain (ATCC 27853) • Mucin from pigs stomach	• Dornase alfa • AlgL • NAC • Gentamicin (liposomal and free form) • Tobramycin (liposomal and free form) • Amikacin (liposomal and free form)	• MIC and MBC measured by microbroth dilution method • Effect of AlgL on biofilm bacterial counts measured by spectrophotometer • Enhancing effects of Dornase alfa or AlgL on aminoglycoside activity measured by bacterial killing assay	• AlgL treatment of biofilms: • No significant reduction in bacterial counts • Reduction MBECs for non-mucoid ATCC 27853 biofilm: 2- to 8-fold for free and no change to 4-fold for liposomal aminoglycosides • Reduction MBECs for mucoid PA-489121 biofilms: 4- to 8-fold for free and liposomal aminoglycosides • No significant effect on non-mucoid PA-489122 biofilms • Dornase alfa and AlgL treatment of sputum: • Presence of Dornase alfa, AlgL or both significantly improved bacterial killing of free and liposomal aminoglycosides • Neither combination completely eradicated growth • (note: they used a sputum dilution of 1/10) • NAC treatment of the 3 *Pa* strains: • No significant improvement in activity of free and liposomal aminoglycosides	• Co-administration of Dornase alfa and AlgL enhanced activity of aminoglycosides in reducing biofilm growth and sputum bacterial counts. NAC did not improve aminoglycosidic activity • Required concentration of AlgL in clinical setting might be higher

					• Mucin inhibited liposomal aminoglycosides more than free aminoglycosides (up to 32-fold vs up to 8-fold)	
Hatch 1998[114]	In vitro	• Alginate isolated from culture supernatants of *Pa* mucoid strains FRD1 and 144M, both originally obtained from sputum of CF patients	• Tobramycin • Gentamicin • Carbenicillin • Polymixin B • Alginate lyase	• Inhibition of bacterial growth (represented in zone sizes) • Enzyme activity of alginate lyase measured by electrophoresis	• Alginate concentration of 2% • Activity of gentamicin, tobramycin and polymyxin B against *Pa* completely blocked • Carbenicillin not inhibited • Addition of alginate lyase: • FRD1 alginate: • Gentamicin activity restored to 79% • Tobramycin activity restored to 92% • 144M alginate • Greater number of alginate lyase required • Gentamicin activity restored to 56% • Tobramycin activity restored to 84%	• Aminoglycoside diffusion is impaired by alginate • Addition of alginate lyase improves the diffusion rates and inhibitory activity of the antibiotics
Bolister 1991[88]	In vitro	• Extracellular alginate produced by a mucoid *Pa* strain from sputum of CF patient • Mucin from sputa of CF patients • DNA from calf thymus	• Amoxicillin • Ampicillin • Benzylpenicillin • Carbenicillin • Cloxacillin • Flucloxacillin • Mezlocillin • Piperacillin • Ticarcillin • Clavulanic acid • 6-aminopenicillanic acid • VX-VC-43	• Diffusion rates measured with spectrophotometry and with three compartment diffusion cells	• Only the results for ticarcillin were displayed • Normal diffusion rate of ticarcillin: 1.1 μm/mm^2/h. With addition of 1% w/v alginate: 0.6 μm/mm^2/h	• Diffusion rate of ticarcillin was decreased in the presence of 1% w/v alginate

Gordon 1988[112]	In vitro	• Alginate extracted from a CF-derived mucoid *Pa* strain • Commercial alginate	• Ceftazidime • Cefsulodin • Piperacillin • Tobramycin • Gentamicin Each 25 mL of 500 mg/L	• Binding measured with equilibrium dialysis • β – lactam diffusion was measured with a spectrophotometer with peristaltic pump and flow-through cuvette • Aminoglycoside diffusion measured with agar-diffusion assays	• Percentage antibiotic in donor cell at equilibrium in HEPES buffer (commercial and *Pa* alginate respectively): • β-lactam antibiotics: 44-60% and 36-62% • Aminoglycosides: 1.0-4.5% and 1.0-4.5% • Extent of aminoglycoside binding reduced in Phosphate Buffered Saline (commercial and *Pa* alginate respectively): • Gentamicin: 34% and 15% • Tobramycin: 28% and 22% • High ratio of alginate to antibiotic: diffusion coefficients of aminoglycosides were approximately 20% of the β-lactam values • Low ratio of alginate to antibiotic: aminoglycosides caused precipitation in alginate after 2 hours with apparent disruption of structure and aminoglycosides diffusion rate became faster than the β-lactam antibiotics • Diffusion of β-lactam antibiotics was twice as fast in *Pa* alginate compared with commercial alginate	• Both alginates showed a strong affinity for aminoglycosides but not for the β – lactam antibiotics • Aminoglycoside binding to alginate was reduced by the presence of physiological concentrations of salts • Ratio of alginate to antibiotic may be of strong influence to penetration because in the latter situation aminoglycosides diffused faster than β – lactam
Nichols 1988[81]	In vitro	• Exopolysaccharide isolated from two mucoid strains of *Pa* from the sputum of CF patients • Sodium alginate from alga *Macrocystis Pyrifera*	• Tobramycin	• Zones of growth inhibition measured • Amount of binding analyzed in terms of Langmuir adsorption isotherm equation • Equilibrium dialysis; alginate 1.0% w/v and exopolysaccharide 1.3% w/v	• Binding of tobramycin to sodium alginate and *Pa* exopolysaccharide were similar both in maximum amount of binding and concentration dependence • Sodium alginate reduced zone sizes of growth inhibition by tobramycin	• Tobramycin binds to sodium alginate and *Pa* exopolysaccharides • Degree of binding of tobramycin to *Pa* exopolysaccharide quantitatively accounted for reduction in zone sizes of growth inhibition

Tannenbaum 1984[113]	In vitro	• Alginate from a clinical isolate of mucoid *Pa* strain from the sputum of a CF patient	• Tobramycin (10 µg/ml) • streptomycin (200 µg/ml) • clindamycin (7.5 µg/ml) • penicillin (7.5 µg/ml)	• Binding measured by equilibrium dialysis • Binding calculated from residual concentrations of antibiotics in free solution by biological assay	• Tobramycin and streptomycin bound to alginate, clindamycin and penicillin did not • Streptomycin 2-fold greater binding to alginate than tobramycin • In presence of physiological concentrations of saline, none of the antibiotics bound to alginate	• Indication that binding of the cationic antibiotics to alginate was of an ionic nature • However, disappearance of binding by physiological salt concentrations indicate that mucoid *Pa* are not more resistant to cationic antibiotics because of this binding
Slack 1981[111]	In vitro	• Sodium alginate from Macrocystis pyrifera • Preparation of exopolysaccharide from a mucoid *Pa* strain	• Gentamicin • Neomycin • Carbenicillin • Netilmicin • Moxalactam • Piperacillin • Cefsulodin	• Diffusion measured in Iso-Sensitest agar by measuring diameters of zones of inhibition	• Diffusion rates of aminoglycosides were much diminished in the presence of sodium alginate • *Pa* exopolysaccharide at concentration of 1.5% w/v caused a 12-fold reduction in netilmicin concentration • Considerably less effect on the diffusion rates of β-lactam antibiotics	• Positively charged aminoglycosides were much more affected than negatively- or neutral β-lactam antibiotics

Pa = Pseudomonas aeruginosa; CF = cystic fibrosis; CFU = colony forming units; AlgL = Alginate lyase; NAC = N-acetylcysteine; MIC = minimal inhibitory concentration; MBC = Minimal bactericidal concentration; MBEC = Minimum biofilm eradication concentration; w/v = weight per volume

7. Liposomal formulations

First author[ref.]	Study design	Origin material	Drugs studied	Results measured in	Results	Conclusion
Meers 2008[102]	Animal study (rats)	• Sputum of CF patients • *Pa* strain PA3064 from a University (mucoid)	• Amikacin (free or liposomal) • Tobramycin (free)	• CFU analyzed from dilutions of the homogenates into Mueller-Hinton broth	• Free amikacin (6 mg/kg): • 3x/week: Reduction in $\log_{10}$ CFU from 5.0±0.5 to 4.6±0.4 • Liposomal amikacin (6 mg/kg): • 3x/week: Reduction in $\log_{10}$ CFU from 5.0±0.5 to 3.2±0.4 • once daily: Reduction in $\log_{10}$ CFU from 6.3±0.9 to 2.7±1.2 • every other day: Reduction in $\log_{10}$ CFU from 6.3±0.9 to 3.9±1.9 • Free tobramycin (3 mg/kg): • Twice daily: Reduction in $\log_{10}$ CFU from 6.3±0.9 to 3.0±1.4	• Inhalation of nebulized liposomal amikacin is significantly more efficacious in the reduction of bacterial load in a chronic *Pa* infection model than an equal dose of free amikacin
Halwani 2007[103]	Animal study (rats)	• *Burkholderia cenocepacia*, non-mucoid (M13637 and M13638) and mucoid (M13642 and M13643) obtained from CF patients	• Amikacin (free and liposomal) • Gentamicin (free and liposomal) • Tobramycin (free and liposomal)	• MICs determined by broth dilution method • CFU determined on Mueller-Hinton agar plates	• MICs of liposomal antibiotics against *Burkholderia* strains significantly lower than those of the free drugs	• Liposomal formulations reduced MICs for highly antibiotic-resistant strains and enhanced the antibiotics penetration into the bacterial cells
Mugabe 2005[104]	In vitro	• Non-mucoid (PA-1, PA-48912-2 and M-57192R) and mucoid (PA-48912-1, PA-48913 and M-26250) strains of *Pa* isolated from sputum of pulmonary infected CF patients • Lab strains of *S. aureus* (ATCC 29213) and *Pa* (ATCC 27853)	• Gentamicin • Liposomal gentamicin	• MICs determined by agar dilution method	• MICs of liposomal gentamicin formulations for 2 highly gentamicin-resistant mucoid and non-mucoid clinical strains of *Pa* were significantly lower than MICs of free gentamicin (1-2 vs 256-512 mg/L) • MICs of liposomal gentamicin for low or moderate gentamicin-resistant strains were at least one-half of the MICs of free gentamicin for the same organisms	• MICs of liposomal gentamicin for all clinical isolates of *Pa* were lower than the MICs of free gentamicin • Liposomal gentamicin altered the susceptibilities of clinical isolates from gentamicin resistant to either intermediate or susceptible

Alipour 2009 (Plos)[101] → described at 3. Binding antibiotics to macromolecules in mucus

Alipour 2009 (JAC)[87] → described at 6. Diffusion through alginate layer

CF = cystic fibrosis; Pa = Pseudomonas aeruginosa; CFU = colony forming units; MIC = minimal inhibitory concentration

8. Co-treatment with other medications

First author[ref.]	Study design	Origin material	Drugs studied	Results measured in	Results	Conclusion
Ong 2014 (abstract)[91]	In vitro	• *Pa* biofilm	• Ciprofloxacin • Mannitol • Combination of ciprofloxacin and mannitol	• Antibacterial effectiveness evaluated using CFU counts and scanning electron microscopy	• Nebulization of mannitol significantly enhanced the antibacterial efficacy of ciprofloxacin	• Co-administration of mannitol and ciprofloxacin could be a potential new strategy to improve antibiotic therapy
Barraud 2013[106]	In vitro	• Laboratory strain *Pa* PAO1 • 2 mucoid clinically relevant strains of *Pa (FRD1 and 18A)* isolated from sputum of a chronically infected patient with CF • A PAO1 mutant strain obtained from the University of Washington	• Tobramycin • Mannitol • NaCl • Glucose	• Biofilm viability determined by drop plate method and CFU counts	• Young *Pa* biofilms (PAO1 and FRD1, pre-grown for 5h) • Tobramycin alone: • 40 mg/L: 3 log decrease in CFU • 80 mg/L: 4 log decrease in CFU • Higher concentrations no further reduction • With mannitol (40 mM): • Increased antibiotic effect by 99.5% • With NaCl: • Same osmolarity as mannitol: 6-fold lower effect • 2-fold higher osmolarity than mannitol: same effect as mannitol • With glucose (40 mM): • Same effect as mannitol (99.4% reduction of persisters) • Established biofilms (PAO1, pre-grown for 20h) • Tobramycin alone: • 80 mg/L: 1 log decrease in CFU • Higher concentrations no further reduction	• Addition of mannitol increased tobramycin sensitivity of persister cells, was able to revert the persister phenotype and improve the efficacy of tobramycin • However, mannitol had no effect on a clinical strain with high resistance to tobramycin • Addition of glucose and NaCl also improved the efficacy of tobramycin although to a lesser extent compared to mannitol

					• With mannitol (40 mM): • Increased antibiotic effect by 77% • With NaCl: • No effect • With glucose (40 mM): • Increased antibiotic effect by 76% • Preventing formation of persister cells (PAO1 and FRD1): • With mannitol (40 mM): • Enhanced antibiotic effect by 99.96% • With NaCl (100 mOsm/L) • 96.5% reduction in persister cells compared to tobramycin alone • With glucose (40 mM) • 99.3% reduction in persister cells compared to tobramycin alone • Mannitol no effect on 18A biofilms	
Ross 2012[115]	In vitro	• Mucoid *Pa* laboratory strain BAA-47	Antibiotics: • Amikacin • Tobramycin • Colistin methanesulphonate • Polymyxin B sulphate • Erythromycin Dispersion compounds: • Citrate • Succinic acid	• Live bacteria quantified from confocal microscopy image stacks • Results in percentage live bacteria remaining	• Untreated controls 78.4% • Dispersion compound alone: • Citrate 89.8% • Succinic acid 91.9% • Amikacin alone 50.4% • With Citrate 8.3% • With succinic acid 42.2% • Tobramycin alone 13.9% • With citrate 35.3% • With succinic acid 20.8% • Colistin alone 29.3% • With citrate 2.8% • With succinic acid 13.9% • Polymyxin B alone 18.7% • With citrate 27.7% • With succinic acid 32.0% • Erythromycin alone 67% • With citrate 6.5% • With succinic acid 38.6%	• Combined treatment of *Pa* biofilms with antibiotic and dispersion compounds resulted in a significant reduction in live bacterial population compared with untreated control in three antibiotics • Tobramycin and Polymyxin B provided increased bacteria viability when combined with dispersion compounds • Four combinations synergistic action: citrate with amikacin, colistin or erythromycin and succinic acid with colistin

Potter 2010 (abstract)[105]	In vitro	• *Pa* • *E.coli* • (Origin not specified)	• Tobramycin (32-0 mg/L) • Colistimethate sodium (128-0 mg/L) • NaCl	Not specified	• Tobramycin: • NaCl had an antagonistic effect on the MIC against *Pa* and *E. coli* at all concentrations • Colistimethate sodium: • NaCl had a synergistic effect on the MIC needed to prevent growth of *Pa* at 4.05% concentration • NaCl had a synergistic effect on the MIC against *E. coli* at all concentrations • Effect concentration dependent for both bacteria	• Addition of NaCl had synergistic effect upon the MIC of colistimethate sodium against *Pa* and *E. coli* growths, in contrast to antagonistic effect upon the MIC of tobramycin • May have implications for timing of inhaled saline-based treatment in patients with CF using certain antibiotic formulations

Moreau-Marquis 2015&2012 (2x)[116-118] → described at 6. Diffusion through alginate layer

Russo 2013[89] → described at 2. Mucus diffusion

Yang 2011 (Biotechnol Bioeng)[90] → described at 2. Mucus diffusion

Yang 2010 (Pharm Res)[92] → described at 2. Mucus diffusion

Purdy Drew 2009[93] → described at 3. Binding antibiotics to macromolecules in mucus

Alipour 2009 (JAC)[87] → described at 6. Diffusion through alginate layer

Hunt 1995[95] → described at 3. Binding antibiotics to macromolecules in mucus

Hatch 1988[114] → described at 6. Diffusion through alginate layer

Ramphal 1988[94] → described at 3. Binding antibiotics to macromolecules in mucus

Pa = Pseudomonas aeruginosa; CFU = colony forming units; CF = cystic fibrosis; E. coli = Escherichia coli; MIC = minimal inhibitory concentration

CHAPTER 4

Patient-specific modeling of regional antibiotic concentration levels in airways of patients with cystic fibrosis: Are we dosing high enough?

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ABSTRACT

Background: *Pseudomonas aeruginosa (Pa)* infection is an important contributor to the progression of cystic fibrosis (CF) lung disease. The cornerstone treatment for *Pa* infection is the use of inhaled antibiotics. However, there is substantial lung disease heterogeneity within and between patients that likely impacts deposition patterns of inhaled antibiotics. Therefore, this may result in airways below the minimal inhibitory concentration of the inhaled agent. Very little is known about antibiotic concentrations in small airways, in particular the effect of structural lung abnormalities. We therefore aimed to develop a patient-specific airway model to predict concentrations of inhaled antibiotics and to study the impact of structural lung changes and breathing profile on local concentrations in airways of patients with CF.

Methods: In- and expiratory CT-scans of children with CF (5-17 years) were scored (CF-CT score), segmented and reconstructed into 3D airway models. Computational fluid dynamic (CFD) simulations were performed on 40 airway models to predict local Aztreonam lysine for inhalation (AZLI) concentrations. Patient-specific lobar flow distribution and nebulization of 75 mg AZLI through a digital Pari eFlow model with mass median aerodynamic diameter range were used at the inlet of the airway model. AZLI concentrations for central and small airways were computed for different breathing patterns and airway surface liquid thicknesses.

Results: In most simulated conditions, concentrations in both central and small airways were well above the minimal inhibitory concentration. However, small airways in more diseased lobes were likely to receive suboptimal AZLI. Structural lung disease and increased tidal volumes, respiratory rates and larger particle sizes greatly reduced small airway concentrations.

Conclusions: CFD modeling showed that concentrations of inhaled antibiotic delivered to the small airways are highly patient specific and vary throughout the bronchial tree. These results suggest that anti-*Pa* treatment of especially the small airways can be improved.

INTRODUCTION

Cystic fibrosis (CF) is a severe hereditary and life-threatening disease in the Caucasian population. Most morbidity and mortality (>90% of deaths) is caused by progressive lung disease.[10] Important components of the pathophysiology of CF lung disease are bronchiectasis, small airways disease[5-7,119] and chronic infection with *Pseudomonas aeruginosa* (*Pa*) as the main pathogen.[10]

Inhaled antibiotics play a central role in eradication and chronic suppressive therapy of *Pa* infections. Unfortunately, despite these interventions, lung disease in CF eventually progresses to end-stage, with substantial small airways disease in most patients.[9] Significant mucus accumulation and wall thickening in the small airways has been found in explant CF lungs with end-stage lung disease[120,121] and this has been associated with the presence of *Pa*.[122] Hence, more effective anti-*Pa* therapies, especially those targeted at the small airways, may offer an opportunity to improve patient outcomes.

The generally held view that inhaled antibiotics result in high concentrations within the airways is largely based on high drug concentrations found in sputum.[123,124] However, it is unlikely that sputum concentrations are representative for the small airway concentrations. The drug reaching the small airways is distributed over a much larger surface area, namely a 30-190-fold greater area compared to central airways.[125] In addition, mucociliary transport clears sputum from the small airways via the central airways, taking up additional drug during transit, before expectoration. Thus, the final sputum concentration is likely to overestimate small airway concentration.

Very little is known about antibiotic concentrations in the small airways, due to the difficulty of *in vivo* measurement. The progression of small airways disease despite anti-*Pa* treatment suggests that small airway deposition of inhaled antibiotics may be insufficient. To optimize *Pa* eradication and chronic suppression with inhaled antibiotics, it is important to obtain local concentrations equal to or above the minimal inhibitory concentration (MIC). Concentrations below the MIC lead to the development of *Pa* strains with high mutation rates, and hence resistant subpopulations of *Pa* which cannot be eradicated.[126]

Extensive research has been done to understand aerosol deposition mechanisms. It has been well established that aerosol deposition is strongly dependent on particle size,[127] airflow, inhalation technique, lung structural changes and airway obstruction by mucus.[128] CF-patients with more severe lung disease have more central airway deposition compared to healthy individuals.[128] This suggests that dose adjustments and particle size optimization, or inhalation technique, could improve aerosol delivery to the site of infection. However, to maximize drug delivery to the small airways, the impact of age, structural changes and inspiratory flow profile on antibiotic concentrations in different compartments of the bronchial tree needs to be better understood.

Unfortunately, it is difficult to investigate the simultaneous influence of the above-mentioned factors on deposition *in vivo*. An *in silico*, patient-specific model based on computational fluid dynamics (CFD) has been developed to assess the behavior of inhalation medication in airways.[129] This technique has been validated using Single Photon Emission Computed Tomography.[130] To date, this technique has been used to study lung drug deposition in asthma,[63] to assess airflow distribution in both asthma and chronic obstructive pulmonary disease,[129,130] and the bronchodilating effects of β2-agonists.[131-133]

In CF, CFD can allow us to study the relation between airway morphology and local concentrations of inhaled antibiotics. Additionally, by repeating simulations with various model parameters, CFD can provide more information on how to optimize small airway aerosol deposition in CF-patients with structural lung changes.

This is the first study using patient-specific airway models with varying disease severity and CFD to estimate aerosol concentrations in both the central and small airways of patients with CF. We aimed to study the relation between structural lung disease and deposition of an inhaled antibiotic used for suppressive treatment of chronic *Pa* infections, Aztreonam lysine for inhalation (AZLI; Gilead Pharmaceuticals, Foster City, USA). AZLI is a monobactam antibiotic, delivered by the e-Flow electronic nebulizer.[124] We hypothesized that:

a) there is great variation in AZLI concentrations between patients, due to differences in airway geometry and lung disease severity,
b) AZLI concentrations in the small airways would be below the MIC for *Pa* in patients with more severe lung disease, and
c) AZLI concentrations in the small airways could be improved by increasing the dose of AZLI or by modifying the inhalation technique.

MATERIALS AND METHODS

Study population

We included all spirometer controlled volumetric in- and expiratory high resolution CT-scans, with a slice thickness of 1 mm or less, performed as part of the routine annual CF check-up in the CF-centre of Erasmus MC-Sophia Children's Hospital (Rotterdam, the Netherlands) between 2008 and 2012 (aged 5-17 years). Patients were diagnosed with CF by a positive sweat test and/or genotyping for known CF mutations. Demographic data and pulmonary function tests were collected prior to the CT-scan. Pulmonary function test results were expressed as percentages of predictive values, according to Stanojevic for the forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1), and Zapletal for the forced expiratory flow at 75% (FEF_{75}).[134,135] Written informed consent for the use of de-identified data was obtained from the parent/guardian and subjects ≥ 12

years. This retrospective study was approved by the Institutional Review Board of the Erasmus Medical Center in Rotterdam, the Netherlands (MEC-2013-078).

Chest Computed Tomography (CT)

Forty spirometer controlled CT-scans of consecutive CF-patients, acquired as part of routine clinical care, were included. To quantify chest CT abnormalities, we used the validated CF-CT scoring system.[136] The lobar specific CF-CT score per component was used and expressed as a percentage of the maximum possible score per lobe. The component scores for bronchiectasis, airway wall thickening and air trapping were used for analysis. Detailed descriptions of CT scanning protocol and CT evaluation are available in the supporting information of this paper (Text S1).

Reconstruction of three-dimensional airway models

Based on the inspiratory scan, a semi-automatic algorithm was used to reconstruct a patient-specific three-dimensional (3D) model of the intra-thoracic region. This intra-thoracic region was defined arbitrarily as the lower airway. Automatic airway segmentation was performed up to the point where no distinction could be made between the intra-luminal and alveolar air. Following automated segmentation of the bronchial tree, the airways were manually checked. Missing branches were added to the bronchial tree and incorrect branches were deleted when necessary; 3.39±2.51% of the branches needed to be manually altered. The respiratory tract was reconstructed down to the level of airways with a diameter of 1–2mm. The segmented airway tree was converted into a 3D model that was smoothed using a volume compensation algorithm. The smoothed model was trimmed perpendicular to the airway centreline at the trachea (using the middle point of the superior side of the sternum as a landmark) and at each terminal bronchus. Remaining artefacts due to noise in the CTs were then manually removed from the model.

For the upper (extra-thoracic) airways, a generic average adult upper airway model was selected and scaled down in such a way that both the anteroposterior and lateral dimension of the scaled model's trachea, at the location of the sternum, matched the average anteroposterior (1.25cm) and lateral (1.19cm) dimension for the 40 patients. The upper airway model was connected with a reverse engineered mouthpiece of the Pari eFlow. Reverse engineering was done based on a CT-scan of the mouthpiece taken on a GE LightSpeed VCT (80kV, 18.25mAs, 0.311mm slice increment, 0.188mm pixel size, STANDARD reconstruction algorithm). The mouthpiece/upper airway model was trimmed perpendicular to the centreline of the trachea (again using the middle point of the superior side of the sternum as a landmark). This ensured correct positioning of the upper airway model with respect to the patient-specific airway models. Models were then coupled using the freeform hole filling algorithm of 3-Matic.

For each of the 40 CT-scan sets, the patient-specific lower airway model was connected to the selected nebulizer mouthpiece/upper airway model, maximizing the contribution of patient-specific information. All segmentation and 3D model operations were performed in commercially available validated software packages (Mimics 15.0 and 3-Matic 7.0, Materialise N.V., Belgium, Food and Drug Administration, K073468; Conformité Européenne certificate, BE 05/1191.CE.01).

Meshing

The triangulated, mouthpiece/upper/lower airway surface models had a maximum triangle edge length of 0.5 mm and a minimum triangle aspect ratio of 0.4. These models were converted to tetrahedral 3D volume meshes using TGrid 14.0 (Ansys Inc, Canonsburg, PA). A boundary layer with a growth rate of 1.4 was included in the models. Maximal tetrahedral volume was set to 2 mm^3 and maximal equilateral volume-based skewness to 0.9. Grid convergence demonstrated that a mesh size of 2.9±0.7M [1.9–4.6] cells is appropriate for the study, depending on the size of the patient-specific lower airway model. Meshing was done on 1 CPU and meshing time was below 200s.

Reconstruction of three-dimensional lung lobes

From both the inspiratory and expiratory CT-scans, the patient-specific lung lobes were extracted using a semi-automated tool that identifies the fissures separating the lung lobes. The internal lobar flow distribution was calculated based on the lobar volume change from expiration to inspiration. Lung lobe identification has been performed in a commercially available validated software package (Mimics 15.0, Materialise N.V., Belgium, Food and Drug Administration, K073468; Conformité Européenne certificate, BE 05/1191.CE.01).

Inlet of the airway model

Breathing profile. The median age of the patient population (11 years) was used to generate a generic breathing profile based on the following parameters: the median weight of 11 year old Dutch children is 38 kg (boy: 37 kg, girl 38.5 kg)[137]; tidal volume of 10 ml/kg (380 ml); respiration rate (18 breaths per minute).[138] The resulting profile had an inspiration/expiration ratio of 1:2 and a sinusoidal shape, see S1 Fig.

To be able to examine the flow dependency of the simulated results, two additional breathing profiles were generated: (1) a high breathing profile, consisting of a higher tidal volume of 14 ml/kg (532 ml) and the respiratory rate of the youngest age (5 years: 22 breaths per minute), and (2) a low breathing profile, consisting of a lower tidal volume of 6 ml/kg (228 ml) and the respiratory rate of oldest age (17 years: 14 breaths per minute). These additional profiles can also be found in S1 Fig.

Aerosol characteristics. Eleven different trials (Anderson Cascade impactor n=6, next generation impactor n=2, and laser diffraction n=3) studied the diameter distribution of AZLI nebulized via the Pari eFlow (Gilead data on file). The extremes and the median of these unpublished trials were selected for use in the CFD simulations: smallest diameter (2.81±1.47 μm), median diameter (3.18±1.63μm) and largest diameter (4.35±2.05 μm). Furthermore, an *in vivo* characterization of the eFlow showed that 35% of the nominal fill volume is either trapped in the mouthpiece or exhaled.

Flow simulation. Computational fluid dynamics (CFD) flow simulations were performed in Fluent 14.0 (Ansys Inc, Canonsburg, PA). Drug release in the simulated nebulizer was continuous. Therefore, particles were injected during the whole breathing cycle. All simulations were transient using a second order time-stepping algorithm and a time-step of 0.005s. Turbulence was evaluated through large eddy simulations with a turbulent kinetic subgrid model. Aerosol transport was modeled by an implicit Runge-Kutta Lagrangian discrete particle model, with a one-way coupling of the forces from the flow to the particle and taken into account the Saffman lift forces. Transient particle tracking was used and the particle time-step was equal to the flow time-step. Every time-step, 15862 particles were injected. This number is based on particle convergence studies. Particles were considered deposited the moment they hit the airway wall.

The nominal dose of 75 mg AZLI was corrected for the 35% combined inhaler loss and exhaled fraction (Gilead data on file). Due to the incorporation of the exhaled fraction, only the inhalation was modeled. The boundary condition at the inhaler mouthpiece was represented by the inhalation part of the mean breathing profile in S1 Fig. The downstream boundary conditions at the terminal bronchi were set such that the percentage of flow exiting the model towards a lobe did match with the internal lobar flow distribution obtained from the expiratory and inspiratory CT data.

To investigate the influence of the inhalation manoeuvre on local concentrations, additional simulations were performed in a subset of the population (2 tallest, 2 smallest, 2 median sized patients). These additional CFD simulations were performed with the altered breathing profiles described in Section: 'Breathing profiles'.

Calculation of regional AZLI concentrations

Aerosol deposition analyses. To be able to perform regional analyses, the respiratory tract was subdivided into multiple regions. For the airways with a diameter >1–2 mm these regions were obtained from the mouthpiece/upper/lower airway model, see Fig. 1. In this figure the upper airway is divided in two parts: the oral cavity and the pharynx; and the lower airways are divided into central part and distal parts representing the lung segments. Conducting airways with a diameter <1–2 mm could not be distinguished from the CT images and have been added to the patient-specific model by using Phalen's

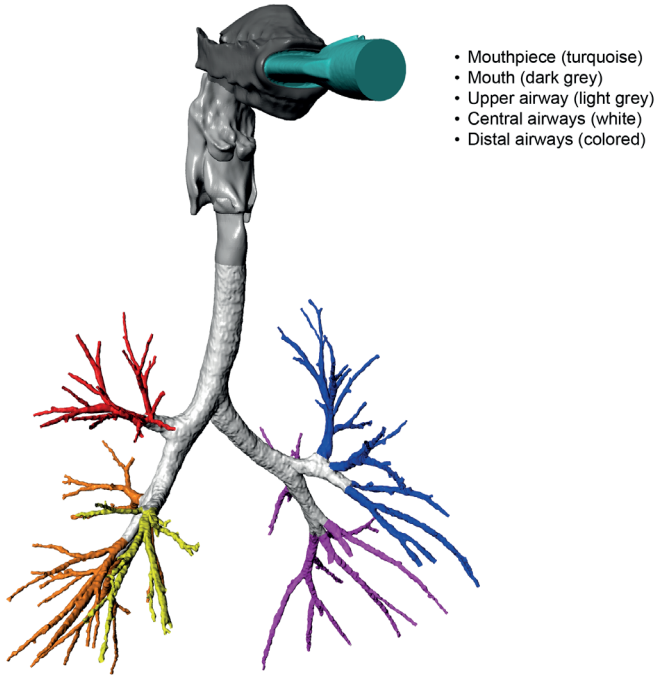


Figure 1 – Coupled mouthpiece/upper/lower airway model
Coupled mouthpiece/upper/lower airway model subdivided in multiple regions. Airways are segmented up to the 5th-9th generation.

description of the airway tree in infants, children and adolescents.[125] For every simulation, a Phalen model was constructed based on the height of the specific patient.

Regional AZLI deposition was evaluated for both the particles depositing inside the model, in every separate zone indicated in Fig. 1; as well as for the particles exiting the model at the terminal bronchi, in the small airways represented by the Phalen model on a lobar basis. Once the aerosol entered the Phalen model of a certain lobe, it was assumed that it was distributed homogeneously.

Airway surface liquid. To compute AZLI concentrations in the airway surface liquid (ASL) throughout the bronchial tree we used a range of thicknesses based on studies in CF. Three different ASL scenarios were considered: thick ASL (7 μm),[139] thin ASL (3 μm)[140] and the mean ASL (5 μm).

AZLI concentrations. For each reconstructed airway and for each lung lobe, the area was calculated and the CFD simulations provided data on the drug deposition in that region. The regional AZLI concentration was computed as follows: the mass of the deposited drug in an airway was divided by the thickness of the lining fluid multiplied by the surface area of that airway.

Since the flow simulations were performed with 3 different sizes for aerosol diameter and the AZLI concentrations were calculated using 3 different thicknesses for ASL, this resulted in 9 different scenarios for which we calculated the concentrations: a scenario with the smallest diameter and smallest ASL thickness, scenario with smallest diameter and median ASL thickness and so on.

Finally, the regional AZLI concentration is expressed relative to the MIC of AZLI for *Pa*. The accurate AZLI concentration for effective killing of *Pa* in an *in vivo* CF lung is not well-defined. Studies on the efficacy of AZLI mostly use a threshold of 10-fold MIC_{90},[15,141] which describes the MIC required to inhibit the growth of 90% of *Pa* strains multiplied by 10. This threshold was used in this study, combined with the highest reported MIC_{90} value in literature: 128 μg/ml.[126] This MIC_{90} value refers to all *Pa* isolates, both non-mucoid and mucoid, as well as strains with and without resistance mechanisms. Thus we expressed the regional AZLI concentration directly after inhalation relative to 10x128 μg/ml = 1280 μg/ml. With this stringent effective AZLI level we took into account the mix of *Pa* populations within one patient with variability in geno- and phenotypes of strains including resistant subpopulations,[126] and clearance of drug starting directly after nebulization.

Statistical analysis

Inter- and intra-observer agreements of CF-CT subscores were calculated using intraclass correlation coefficients (ICC). Although no universally accepted standards are available for what constitutes good reliability, ICC values between 0.4 and 0.6, 0.6 and 0.8, and ≥ 0.8 are generally considered to represent moderate, good and very good agreement, respectively. Systematic errors in component scores were evaluated using Bland-Altman plots, expressing the differences between two observers as a function of their mean.[142]

To establish the correlation between age and disease severity expressed in CF-CT scores and pulmonary function tests, we used Spearman's correlation test. According to Cohen's criteria (1988), correlations between 0.10 and 0.29 are considered weak, between 0.30 and 0.49 moderate and above 0.50 are considered strong.

Differences between multiple groups were investigated using a Kruskal-Wallis test, after which two-by-two comparisons were made using Mann-Whitney tests. The effect size was noted as "r". Correlations between parameters measured in different lobes were studied using a generalized estimating equation with an autoregressive covariance matrix to account for within-subject correlations. All data are presented as median (range). Significance level was set at 0.05 and p-values were corrected for multiple testing using Benjamini and Hochberg correction.[143] All statistical computations were performed using the open-source statistical environment R 2.15.3.

RESULTS

Study population

Forty inspiratory and expiratory chest CT-scans were selected from 31 patients. Baseline characteristics are shown in Table 1. Thirty-nine (98%) CT-scans were spirometer controlled; the remaining scan was performed with technician guidance.

There were no significant differences between the sexes for demographics, pulmonary function tests and CF-CT subscores, therefore the dataset did not have to be split in sex groups.

ICCs for within-observer agreement ranged from 0.85 (air trapping) to 0.93 (bronchiectasis), whereas between-observer agreement ranged from 0.67 (airway wall thickening) to 0.77 (air trapping).

Table 1. Baseline characteristics.

	Value	
N	31	
- Nr of patients with 1 CT	22	
- Nr of patients with 2 CTs	9	
Male	11	35%
Age	11.0	5.8-17.3
Bronchiectasis score (% of max CF-CT score)	2.8	0.0-16.0
Airway wall thickening score (% of max CF-CT score)	3.7	0.0-18.5
Air trapping score (% of max CF-CT score)	22.2	11.1-85.2
FEV_1 %pred	94.2	70.8-115.4
FVC %pred	104.3	78.7-127.9

Data are presented as nr. (%) or median (range), unless otherwise indicated.

Correlations with age

There was a moderate positive correlation between bronchiectasis and age (r_s=0.481, p=0.005). Correlations between age and airway wall thickness (r_s=0.302, p=0.087), air trapping (r_s=0.096, p=0.554) and pulmonary function tests (FVC% pred: r_s=0.053, p=0.885; FEV1% pred: r_s=-0.024, p=0.885) were not significant.

Deposition analyses

The software tool used to identify the boundaries between the lobes in the lungs, i.e. the pulmonary fissures, could not identify the fissure between the right upper and middle lung lobes in 18 patients and between the left upper and lower lung lobes in 1 patient. These lung lobes were excluded for analysis of AZLI deposition.

Significant differences between the lobes were found for all tested CF-CT subscores (bronchiectasis: x^2 =21.70, p<0.001; airway wall thickness: x^2 =25.22, p<0.001; and air trapping: x^2 =20.15, p<0.001). It was found that CF-CT subscores were generally higher in the right upper lobe than in the other lobes (Fig. 2).

There were differences in AZLI deposition between the different lobes (Fig. 3). The highest AZLI concentrations were found in the lower lobes. For the lower lobes, AZLI concentrations were always above 10xMIC_{90} independent of the scenario tested. An inverse correlation between AZLI concentration in a lobe and the CF-CT scores was observed, indicating that more diseased lobes received less drug (Table 2). For example, when assuming small diameters and thin lining fluids, a reduction in AZLI concentration of 439 µg/ml was observed for every 1% of point increase in bronchiectasis score.

AZLI concentrations were calculated with 3 different ASL thicknesses. Because of the formula used for calculations, the ASL thickness was of direct influence on the concentrations: the thicker the ASL the lower the AZLI concentration (S2 Fig.). Therefore, when expressing this regional AZLI concentration relative to 10xMIC_{90}, the thicker the ASL the larger the area of small airways with AZLI concentrations below 10xMIC_{90}.

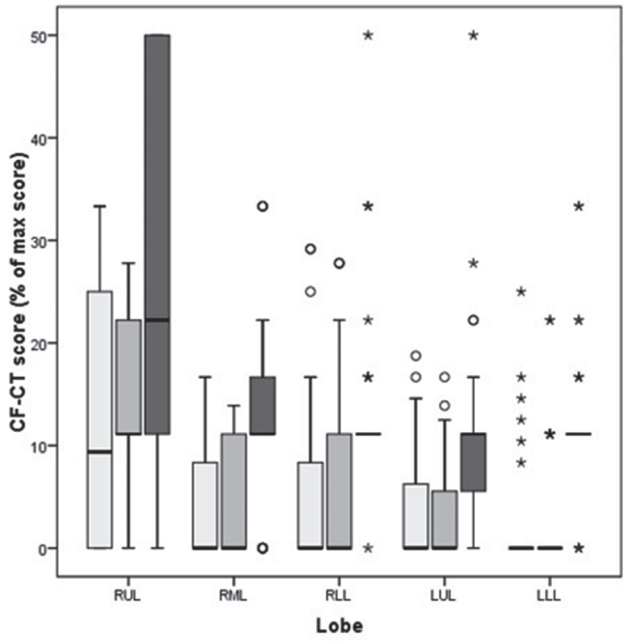


Figure 2 – Comparison of CF-CT subscores per lobe
Comparison of CF-CT subscores per lobe, presented as % of max CF-CT score. Data are presented as median (range), unless otherwise indicated. White bars represent bronchiectasis score, light grey bars represent airway wall thickening score and dark grey bars represent air trapping score. RUL = right upper lobe (n=22), RML = right middle lobe (n=22), RLL = right lower lobe (n=40), LUL = left upper lobe (n=39), LLL = left lower lobe (n=39).

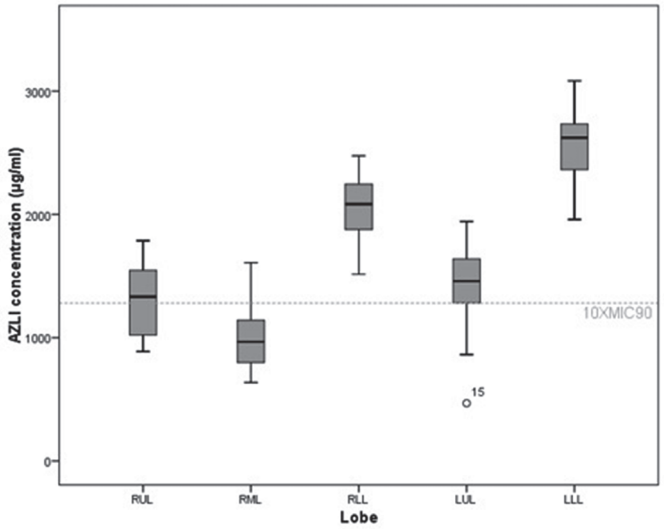


Figure 3 – **Differences between lobes in AZLI concentrations**
Differences between lobes in AZLI concentrations for the scenario of thick airway surface liquid with largest aerosol diameter. Data are presented as median (range), unless otherwise indicated. Significant differences in AZLI concentrations were found between all lobes, except for one pairwise comparison (see Table S1). RUL = right upper lobe, RML = right middle lobe. RLL = right lower lobe, LUL = left upper lobe, LLL = left lower lobe.

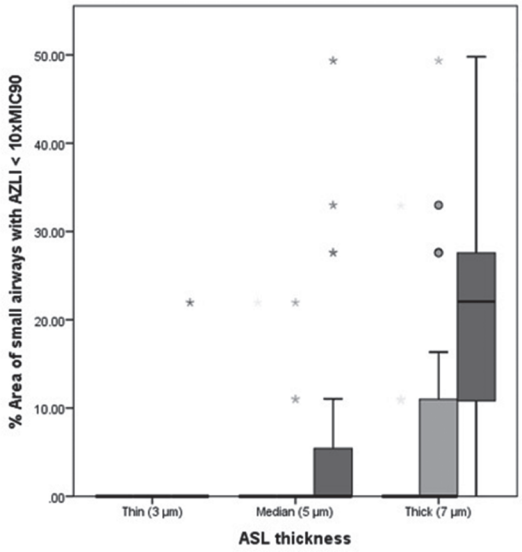


Figure 4 – **Percentage area of small airways with AZLI <10xMIC_{90}**
Percentage area of small airways with AZLI concentrations <10xMIC_{90}. Data are presented as median (range) for the different scenarios. White bars represent the smallest aerosol diameter (2.9 µm), light grey bars represent the median aerosol diameter (3.18 µm) and dark grey bars represent the largest aerosol diameter (4.35 µm). ASL = airway surface liquid.

Table 2. Inverse correlation between AZLI concentration in a lobe and the CF-CT scores

ASL	Diameter (µm)	CF-CT score	Estimate	Std.err	Wald	p-value
		Bronchiectasis	-439	115	14.6	**0.00013**
	Smallest (2.9)	Airway wall thickness	-466	142	10.7	**0.0011**
		Air trapping	-237.5	85.3	7.76	**0.0053**
		Bronchiectasis	-387	108	12.8	**0.00034**
Thin (3 µm)	Median (3.18)	Airway wall thickness	-421	133	10	**0.0016**
		Air trapping	-229.7	78.5	8.56	**0.0034**
		Bronchiectasis	-278.8	86.5	10.4	**0.0013**
	Largest (4.35)	Airway wall thickness	-316.8	106.2	8.9	**0.0029**
		Air trapping	-190.3	61.4	9.6	**0.0019**
		Bronchiectasis	-263.7	69	14.6	**0.00013**
	Smallest (2.9)	Airway wall thickness	-279.6	85.4	10.7	**0.0011**
		Air trapping	-142.5	51.2	7.76	**0.0053**
		Bronchiectasis	-232	64.8	12.8	**0.00034**
Median (5 µm)	Median (3.18)	Airway wall thickness	-252.7	79.9	10	**0.0016**
		Air trapping	-137.8	47.1	8.56	**0.0034**
		Bronchiectasis	-167.3	51.9	10.4	**0.0013**
	Largest (4.35)	Airway wall thickness	-190.1	63.7	8.9	**0.0029**
		Air trapping	-114.2	36.9	9.6	**0.0019**
		Bronchiectasis	-188.3	49.3	14.6	**0.00013**
	Smallest (2.9)	Airway wall thickness	-199.7	61	10.7	**0.0011**
		Air trapping	-101.8	36.5	7.76	**0.0053**
		Bronchiectasis	-165.7	46.3	12.8	**0.00034**
Thick (7 µm)	Median (3.18)	Airway wall thickness	-180.5	57.1	10	**0.0016**
		Air trapping	-98.5	33.7	8.56	**0.0034**
		Bronchiectasis	-119.5	37.1	10.4	**0.0013**
	Largest (4.35)	Airway wall thickness	-135.8	45.5	8.9	**0.0029**
		Air trapping	-81.6	26.3	9.6	**0.0019**

Inverse correlation between AZLI concentration in a lobe and the CF-CT scores, shown for the different scenarios and different aerosol diameters. For example: for every 1% of point increase in bronchiectasis score, a reduction in AZLI concentration of 439 µg/ml is observed assuming small diameters and thin lining fluids. Number of lobes used for analysis: right upper lobe (n=22), right middle lobe (n=22), right lower lobe (n=40), left upper lobe (n=39), left lower lobe (n=39). P-values in bold represent significant differences. ASL = airway surface liquid.

The combination of thin ASL and smallest aerosol diameter resulted in AZLI concentrations above 10xMIC$_{90}$ for both large and small airways. For the combination of thick ASL and largest aerosol diameter, 22% (0-49.79%) of the total area of small airways received AZLI concentrations below 10xMIC$_{90}$. The lowest AZLI value observed in the small airways for the tested population was 468.14 µg/ml or 3.66xMIC$_{90}$. Fig. 4 summarizes the percentage of area of small airways that receive a concentration below 10xMIC$_{90}$ for the different modeling conditions. In Fig. 5, the relative AZLI concentrations in 2 patients are shown for 3 different scenarios. In the central and distal airways, AZLI concentrations 10-100x above the threshold of 1280 µg/ml were observed. In the small airways (visualized per lobe in the images), lower concentrations were seen.

Decreasing the tidal volume and respiratory rates decreased the deposition in the extra-thoracic region (mouth and upper airway), subsequently resulting in significantly less areas with a concentration below 10xMIC$_{90}$ in the lungs (Fig. 6).

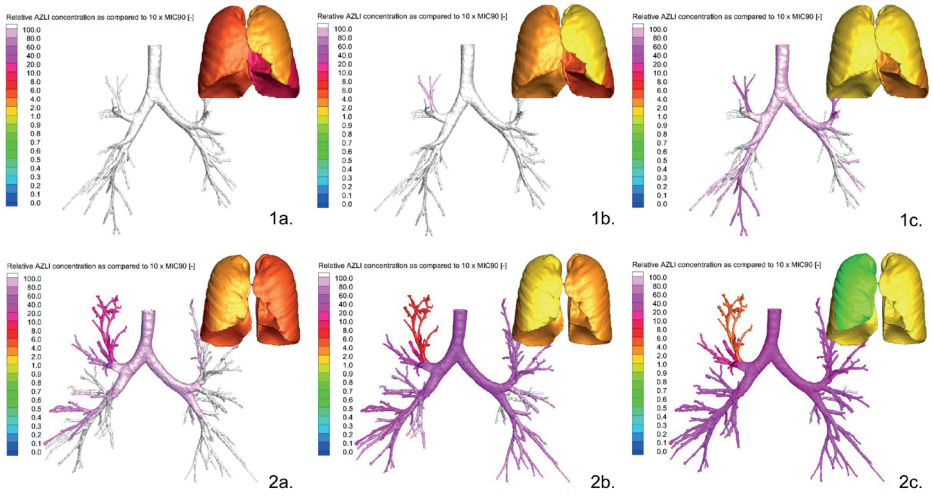


Figure 5 – Relative AZLI concentrations in central and small airways of 2 patients for 3 different scenarios

Simulations of AZLI deposition in 2 patients, representing 3 scenarios of varied airway surface liquid thickness (ASL) and aerosol diameter. Severity of CF lung disease was determined by the CF-CT score (% of total CF-CT score). Scenario a = thin ASL with smallest aerosol diameter; scenario b = median ASL with median aerosol diameter; scenario c = thick ASL with largest aerosol diameter. Part 1a, 1b and 1c: Patient 1, mild CF lung disease: bronchiectasis 0.0%, airway wall thickening 0.0% and air trapping 11.1%. Patient 1 received concentrations > 10xMIC$_{90}$ in the central and small airways independent of ASL thickness and aerosol diameter (Part 1a, 1b, 1c). Part 2a, 2c and 2c: Patient 2, more severe lung disease: bronchiectasis 12.5%, airway wall thickening 11.1% and air trapping 38.9%. Patient 2 received concentrations > 10xMIC$_{90}$ in the central and small airways in scenario a and b (Part 2a and 2b), but AZLI concentrations < 10xMIC$_{90}$ in the small airways in scenario c (right upper and middle lobes) (Part 2c).

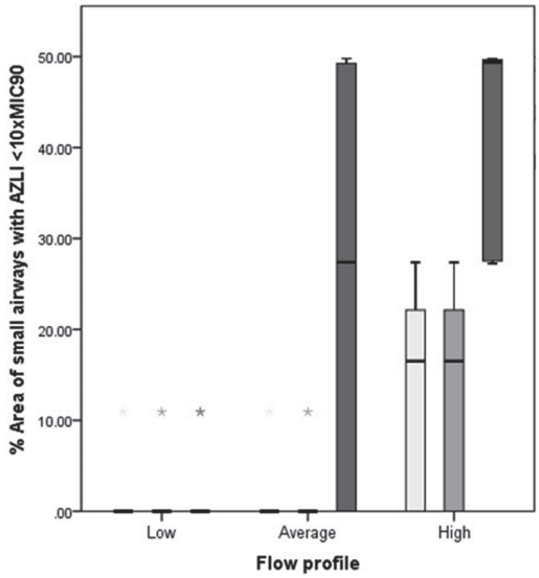


Figure 6 – Influence of inhalation technique on AZLI concentrations
Influence of inhalation technique on AZLI concentrations presented as percentage area of small airways with AZLI <10xMIC_{90}. Low breathing profile: tidal volume of 6 ml/kg (228 ml) and respiration rate of 14 breaths/min. Average breathing profile: tidal volume of 10 ml/kg (380 ml) and respiration rate of 18 breaths/min. High breathing profile: tidal volume of 14 ml/kg (532 ml) and respiration rate of 22 breaths/min. Data are presented as median (range) for the different scenarios. Light grey bars represent the scenario of median ASL (5 μm) with largest aerosol diameter (4.35 μm). The darker grey bars represent the scenario of thick ASL (7 μm) with median aerosol diameter (3.18 μm) and the darkest grey bars represent the scenario of thick ASL (7 μm) with largest aerosol diameter (4.35 μm). The scenarios of thin ASL with all diameters, median ASL with smallest and median diameter and thick ASL with smallest diameter are not represented as all breathing profiles resulted in AZLI concentrations above 10xMIC_{90}. ASL = airway surface liquid.

DISCUSSION

To our knowledge this is the first study that used CFD to estimate patient-specific inhaled antibiotic concentrations throughout the bronchial tree in CF. The most important finding was that the airway concentrations were highly dependent on patient-related factors.

Another important finding of this study was that effective AZLI concentrations above the 10xMIC_{90} threshold for *Pa* were observed throughout the lung in most simulated conditions. However, variables such as particle diameter and ASL thickness had a significant impact on the results. Under certain values of these variables, it was shown that the concentration would drop below 10xMIC_{90} in 22% of the small airways area. The most critical scenario was the combination of a thick ASL and the largest aerosol diameter. However, the lowest observed AZLI concentration in the small airways of the studied population was still above 3xMIC_{90} for *Pa*. For this particular patient, an increase

of 2.7 times the standard nebulized AZLI dose would have resulted in sufficiently high concentrations in the small airways. In the 'best-case' scenario, both large and small airways received AZLI concentrations above 10xMIC_{90}. The observation that regional low concentrations can exist is of great importance, since suboptimal concentrations could result in insufficient killing and are associated with increases in mutation frequencies.[144] These hypermutator strains are resistant against antimicrobials used in CF and hence, rather than elimination of the pathogen, treatment will result in even further selection of these resistant subpopulations.[126]

As in previous studies we observed that the upper lobes were more severely affected by structural disease relative to the other lobes.[119] The reason for this distribution is still largely unknown, however our findings suggest that uneven distribution of inhaled drugs could contribute to this inequality. We observed that even in patients with relatively little structural damage, the upper lobes received lower AZLI concentrations than the lower lobes.

We found an inverse correlation between lobar CF-CT score and AZLI concentration. The population included in this study had early to moderately advanced lung disease on CT, with well-preserved lung function. Patients with more advanced lung disease would be further affected by the uneven distribution of inhaled drugs. These findings match deposition studies in patients with CF, showing that the deposition pattern is more heterogeneous in diseased lungs than in healthy lungs.[127,128] In addition, it supports previous studies showing that penetration of inhaled drugs in deformed or partially obstructed airways is restricted.[128] These results suggest that upper lobes are more vulnerable to under-treatment and that this effect is stronger once structural damage is present.

With our simulations, we showed that lower inhalation flows reduced extra-thoracic deposition, leading to higher AZLI concentrations in the small airways. This finding is consistent with previous studies showing that high flows lead to high extra-thoracic and upper airway drug deposition.[127] Using patient-specific airway modeling, we were able to study the impact of inhalation flow rate and inhaled volume on local airway drug concentrations in the small airways. This information can be used to design smart nebulizers in such a way that adequate small airway concentrations can be obtained,[25] or to define the required medication dose for a patient that, independent of the breathing pattern, results in sufficient drug delivery to critical areas of the lung.

CFD offers a number of advantages that complement available techniques for studying aerosol deposition. Non-imaging techniques, e.g. pharmacokinetic methods, lack the ability to identify dose deposition into different zones of the lungs.[145] Scintigraphic methods do assess the deposition location of inhaled drugs, however, by dividing the lung into several large regions of interests.[146] 3-helium MRI provides structural information and offers a quantification of ventilation down to the alveolar level,[147] however regional deposition of inhaled drugs cannot be derived from this technique. In contrast,

CFD allows detailed information on aerosol deposition at specific anatomical sites to be determined. Another advantage is that CFD allows estimation of AZLI concentrations throughout the bronchial tree, data that are extremely difficult to obtain *in vivo*. To date, great emphasis has been given to sputum concentrations in clinical studies investigating inhaled antibiotics. It is highly likely that these concentrations are primarily reflective of central airway concentrations. The central airway concentrations found in this study were in the range of the fitted sputum concentrations from clinical studies, taking into account that published sputum samples were collected at later time-points compared to this study (data on file). As suggested by our findings, higher concentrations in central airways result in lower concentrations in small airways, challenging the validity of sputum samples as a useful indicator to explain the failure or success of inhaled antibiotics. We utilized CT-scans that were acquired as part of routine clinical care,[148] allowing extraction of extra clinical relevant information without the need for additional radiation. Unlike other deposition study techniques, our model allowed us to study the impact of multiple variables, e.g. differences in particle size, on lung deposition within the same patient. CFD can therefore be used to predict lung deposition and effective dose of newly developed nebulizers. CFD opens up new pathways to further optimize inhalation therapies, even at a personalized level.

Our modeling study has a number of limitations. To allow modeling of AZLI and estimation of concentrations, several assumptions were made. The first assumption was that the antibiotic concentration in the ASL is the most important determinant for effective killing of *Pa*.[149-151] To estimate ASL concentrations we had to consider three different scenarios for ASL thickness. As ASL thickness cannot be measured *in vivo*, we used a number of ASL thicknesses in our model that covered the entire range found previously in *in vitro* data from CF bronchial epithelial cultures.[140] We also did not take into account dissolution of the inhaled antibiotic in sputum that can cover the airway epithelia.[79] Although mucus layer thickness can vary between patients and throughout the bronchial tree, it is reasonable to assume that this ASL layer will be at least 3 μm (thinnest ASL of CF epithelia found *in vitro*). Thus, it is likely that the concentrations we computed are too optimistic. Areas covered by mucus, especially those in regions of the lung with severe disease, may have even lower antibiotic concentrations, potentially decreasing below MIC_{90}.

While ASL concentration is generally considered to be a reliable marker of alveolar antibiotic concentration,[149-151] it is likely only an approximation as it relies on several assumptions. This model does not take into account drug uptake by alveolar macrophages as a measure of intracellular penetration in the lungs.[152] Especially in the chronically infected lung, macrophages may play a substantial role in the pharmacodynamics of anti-infective agents. This model also does not take into account binding of AZLI to sputum.[152] Only unbound drug concentrations are considered to be microbiologically active. In a single study, it was observed that there was little binding of AZLI to CF sputum.[141]

Therefore it is not likely that this effect has a substantial effect on our data. We did not account for mucociliary and cough clearance, which further reduces AZLI concentrations in the airways, and this occurs within minutes after inhalation.[153] Our model assumes that the microbiological effect of a β-lactam antibiotic, such as AZLI, is best predicted using function of time above the MIC (T>MIC).[154,155] Unfortunately, the half-life of AZLI in the airways is not precisely known, but is thought to be approximately two hours in serum. Therefore, a prediction of efficacy of AZLI in the airways based on a single time-point immediately after inhalation is an approximation only. Thus, even though we calculated that the AZLI concentration was well above MIC_{90} for most simulated conditions immediately after nebulization, concentrations may decrease well below MIC_{90}, especially in diseased areas, before a new dose of AZLI is nebulized.

We estimated the concentration of AZLI that could be considered effective for killing *Pa* strains based on previously reported data. However, the ideal AZLI concentration for effective killing of *Pa in vivo* is not well-defined, and varies largely between studies, with MIC_{90} values ranging between 32 and 128 μg/ml.[15,42,141,156,157] Terms indicating the efficacy level of antibiotics, e.g. MIC and MIC_{90}, have been used interchangeably in other studies, making comparison difficult.[154,155] For AZLI, most susceptible bacteria are killed at concentrations 1 to 4-fold their MIC. However, antibiotic concentrations required for killing *Pa* strains in biofilms are substantially higher than for killing *Pa* strains in planktonic growth. In an *in vitro* biofilm model, the time-dependent killing pattern of ceftazidime and imipenem in planktonic bacteria was changed to concentration-dependent killing for biofilm cells. Because of this, higher doses and longer treatment times with ceftazidime were required for the biofilm-growing *Pa* than for planktonic cells. While a concentration of 128xMIC was bactericidal for the wild-type strain (PAO1), a concentration of 2048xMIC was required for its β-lactamase overproducing mutant (PAΔDDh2Dh3).[158] In the registration studies of AZLI, concentrations of more than 2048 μg/ml were required to achieve bactericidal killing of *Pa* in some cases.[42,47,157] This corresponds to an AZLI concentration of more than 16-fold the MIC_{90} referred to in this study. Studies on the efficacy of AZLI mostly use a threshold of 10-fold MIC_{90}, but without clear explanation.[15,141] This threshold was also used for our analyses in this manuscript, but no claims can be made concerning its clinical significance.

Airways with a diameter below 1-2 mm could not be reconstructed from CT data, and were added to the model using Phalen's description of the airway tree in infants, children and adolescents.[125] These model data are derived from subjects without lung disease and were combined with the assumption of homogenous aerosol distribution in these small airways. In CF, it is well recognized that the small airways are progressively involved in early life lung disease.[5,6] Hence, it is likely that our model underestimates the heterogeneity of aerosol deposition, with the assumption of normal structure of the small airways. Moreover, the small airways might be most prone to *Pa* infection[122] and hence an

inhibited AZLI delivery might be most unfavorable for this part of the lung. Even though we could not use real data for the simulation of small airways, the results are still highly relevant for clinical practice. The knowledge that lobes with substantial structural damage receive less inhaled antibiotic has consequences for the current standard treatment regimen. Current therapy is "one size fits all": patients from all ages and with a variety of disease severities are treated with the same regimen. It might well be that a higher dose is needed for patients with more advanced disease to achieve antibiotic concentrations above MIC in all airway generations. Because structural lung disease severity is generally known by the treating physician, personalized therapy could be used, for example, by increasing antibiotic dose, in patients who do not respond to standard treatment.

We did not use patient-specific breathing profiles and upper airway models for this study, since they were not available. Clearly, this would have improved the precision of the simulations. However, as we covered a wide range of breathing patterns in our model, we believe that this represents a significant limitation in our work.

CONCLUSIONS

We demonstrated that inhaled antibiotic concentrations in the small airways are highly patient-specific. A clear relation was found between patient-specific severity of localized lung disease and antibiotic concentrations throughout the lung. This method opens up the possibility of personalizing inhaled antibiotic dose to improve treatment efficacy.

ACKNOWLEDGEMENTS

The authors thank Elizabeth Salamon, department of Pediatric Pulmonology, Rotterdam, for scoring the CT-scans and Tim Rosenow, The University of Western Australia, for checking the grammar and spelling of the manuscript.

S1 TEXT. SUPPLEMENTARY METHODS.

Chest computed tomography

All routine chest CT scan sets were acquired using a 128-slice CT scanner (Somatom Definition Flash; Siemens, Erlangen, Germany). Thirty minutes prior to performing the CT scan, a lung function technician trained the study subjects by practicing the required spirometry manoeuvres in the supine position. Children were trained to obtain a breath-hold at maximal inspiration (total lung capacity, TLC) and maximal expiration (residual volume, RV) for 5–15 seconds. The inspiratory and expiratory slow vital capacities (SVC) achieved during the training were used as the reference values for the spirometric results during the CT scan. The reference SVCs were performed according to the ATS/ERS criteria.[159] Breathing instructions during the CT scan were given by the same lung function technician. During the scan, the lung technician monitored in real time the inspired and expired volumes on the computer screen of the CT-compatible spirometer setup. When the patient reached the correct TLC (inspiratory scan) or RV (expiratory scan) breath hold level, the lung function technician signalled the CT-technician to start scanning. For the technician-guided technique the same breathing instructions were given during the CT scan; however, the inspired and expired volumes were not spirometrically measured.

CT settings

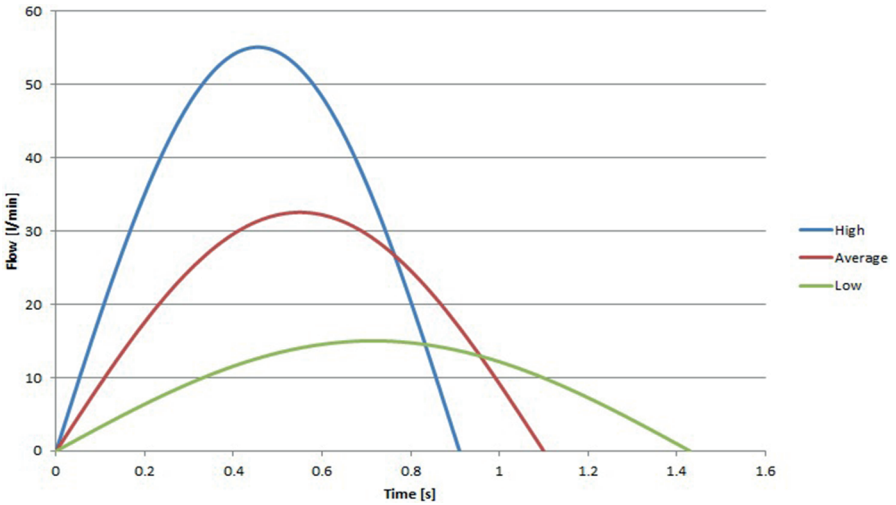
Tube voltages of 80kV (patients < 35kg) or 110kV (patients ≥ 35kg) were used with a 0.6s rotation time. Scanning was done from apex of the lung to base at 1.5 pitch and 6x2mm collimation. Images were reconstructed with a slice thickness ≤ 1.0mm, a slice increment ≤ 0.6mm and kernel B75f. For the inspiratory protocol, a modulating current was used (Siemens) with a reference tube current-time product of 20mAs for optimal image quality. For expiratory CTs, a tube current fixed at 25mA with an effective tube current-time product of 10 mAs (the typical value for a 5-year-old child) was used, producing a lower radiation dose than the inspiratory protocol with sufficient image quality. Total radiation dose was in the order of 0.75 mSv for children below the age of 6 years and 1 mSv in older children.

CT evaluation

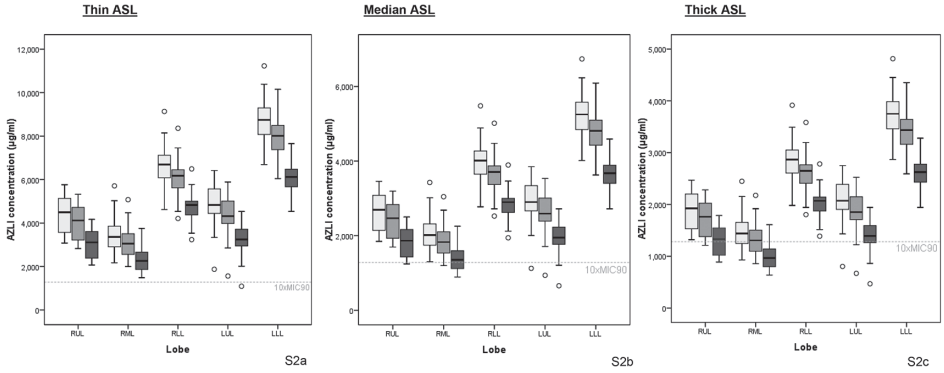
To quantify chest CT abnormalities, we used the validated CF-CT scoring system.[136] This scoring method evaluates the 5 lung lobes and the lingula as a sixth lobe for the following components: 1) severity and extent of central and peripheral bronchiectasis; 2) severity and extent of central and peripheral airway wall thickening; 3) extent of central and peripheral mucus plugging; 4) extent of opacities (atelectasis, consolidation, ground glass pattern); 5) extent of cysts and bullae on inspiratory CTs and 6) the pattern and extent of trapped air on expiratory CTs. The maximal possible composite CT score is 207

points. In the CF-CT scoring method, the CF-CT composite score is calculated by summing the component scores per lobe. Instead of using the CF-CT composite score, the component scores per lobe were used for analysis. The lobar specific component scores were expressed as a percentage of the maximum possible component score per lobe. The component scores for bronchiectasis, airway wall thickening and air trapping were used for analysis.

Prior to scoring, all CT scans were de-identified (Myrian®; Intrasense, Montpelier, France). Next, scans were scored in random order by an experienced observer, with more than 2 years' experience in scoring, who was blinded to clinical background. To assess inter-observer agreement, a second observer with 4 months scoring experience rescored all CT scans. Both observers were initially trained in CF-CT scoring using a standardized instruction module and training sets. Good intra- and inter-observer agreement was established on the training sets before scoring the study CT scans. To establish the intra-observer agreement, observer 1 rescored 25 random selected scans after 3 months. CF-CT scores of observer 1 were used for analysis.



S1 Figure – Breathing profiles (High – Average – Low)
Inhalation part of breathing profiles. Low breathing profile: tidal volume of 6 ml/kg (228 ml) and respiration rate of 14 breaths per minute. Average breathing profile: tidal volume of 10 ml/kg (380 ml) and respiration rate of 18 breaths per minute. High breathing profile: tidal volume of 14 ml/kg (532 ml) and respiration rate of 22 breaths per minute.



S2 Figure – Influence of increases in aerosol diameter and increases in airway surface liquid thickness on AZLI concentrations
AZLI concentrations per lobe showing the influence of increases in aerosol diameter and increases in airway surface liquid (ASL) thickness on the AZLI concentrations. Data are presented as median (range). In each figure the AZLI concentrations are shown per separate lung lobe for the 3 different sizes of aerosol diameter. White bars represent the smallest aerosol diameter (2.9 µm), light grey bars represent the median aerosol diameter (3.18 µm) and dark grey bars represent the largest aerosol diameter (4.35 µm). The separate parts of the figure represent the 3 different thicknesses of ASL. Part S2a shows the AZLI concentrations calculated for the thin ASL (3 µm), part S2b shows the AZLI concentrations calculated for the median ASL (5 µm) and part S2c shows the AZLI concentrations calculated for the thick ASL (7 µm). RUL = right upper lobe, RML = right middle lobe. RLL = right lower lobe, LUL = left upper lobe, LLL = left lower lobe. The larger the aerosol diameter and the thicker the ASL the lower were the AZLI concentrations. Note differences in the scale on the vertical axes between the 3 separate parts of the figure.

Table S1. P-values of pairwise comparison of AZLI concentrations between lobes, accompanying figure 3

Lobe	**Comparison with other lobes (p-values)**				
	RUL	**RML**	**RLL**	**LUL**	**LLL**
RUL	-	**0.002**	**1.4E-09**	0.156	**2.7E-10**
RML	**0.002**	-	**2.7E-10**	**5.6E-06**	**2.7E-10**
RLL	**1.4E-09**	**2.7E-10**	-	**6.8E-11**	**5.3E-10**
LUL	0.156	**5.6E-06**	**6.8E-11**	-	**3.3E-13**
LLL	**2.7E-10**	**2.7E-10**	**5.3E-10**	**3.3E-13**	-

P-values of comparison between lobes in AZLI concentrations for the scenario of thick lining fluid with largest aerosol diameter. P-values in bold represent significant differences. RUL = right upper lobe, RML = right middle lobe. RLL = right lower lobe, LUL = left upper lobe, LLL = left lower lobe.

CHAPTER 5

Patient-specific modeling of regional tobramycin concentration levels in airways of patients with cystic fibrosis: Can we dose once daily?

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Submitted

ABSTRACT

Background: Inhaled tobramycin is important in the treatment of *Pseudomonas aeruginosa (Pa)* infections in cystic fibrosis (CF). However, despite its use it fails to attenuate clinical progression of CF-lung disease. The bactericidal efficacy of tobramycin is known to be concentration-dependent and hence, changing the dosing regimen from a twice-daily inhalation (BID) to a once-daily (OD) inhaled double dose could improve treatment outcomes.

Objectives: To predict local concentrations of nebulized tobramycin in the airways of patients with CF, delivered with the small airways-targeting Akita® or standard PARI-LC® Plus system, with different inspiratory flow profiles.

Methods: Computational fluid dynamic (CFD) methods were applied to patient-specific airway models reconstructed from chest computed tomography (CT) scans. The following BID and OD dosing regimens were evaluated: Akita® (150 and 300 mg) and PARI-LC® Plus (300 and 600 mg). Site-specific concentrations were calculated.

Results: Twelve CT-scans from patients aged 12-17 years (median=15.7) were selected. Small airways concentrations were 762-2999 µg/ml for BID and 1523-5997 µg/ml for the OD dosing regimen, which is well above the minimal inhibitory concentration (MIC) of wild type *Pa* strains. Importantly, the OD regimen appeared to be more suitable than the BID regimen against more resistant *Pa* strains and the inhibitory effects of sputum on tobramycin activity.

Conclusions: CFD modeling showed that high concentrations of inhaled tobramycin are indeed delivered to the airways, with the Akita® being twice as efficient as the PARI-LC® system. Ultimately, the OD dosing regimen appears more effective against subpopulations with high MIC (i.e. more resistant strains).

INTRODUCTION

Inhaled tobramycin is important in the treatment of *Pseudomonas aeruginosa (Pa)* infections in cystic fibrosis (CF) and is used to both eradicate early, and suppress chronic *Pa* infections.[22] As tobramycin exhibits concentration-dependent bactericidal activity, the highest possible concentrations above the minimal inhibitory concentration (MIC) are required in order to be optimally efficacious against *Pa*.[160] Furthermore, as sputum binding reduces its biological activity, inhaled concentrations in sputum must be at least 10-fold higher than the MIC for planktonic *Pa* and as high as 100-1000-fold greater for *Pa* growing in biofilm.[161] Patients with CF are typically infected with multiple *Pa* morphotypes, each of which may vary in their susceptibility to antibiotics.[162,163]

Despite the use of current therapies to manage *Pa* infections, small airways disease in patients with CF continues to progress.[5] This may be due to the insufficient deposition of inhaled antibiotics in the small airways, leading to local concentrations that are too low to be effective. Although high concentrations of tobramycin can be obtained in the central airways,[164] concentrations in the small airways are as yet unknown and difficult to measure *in vivo*.

Chronic treatment of *Pa* lung infection is currently defined as twice-daily tobramycin inhalation with the standard PARI-LC® Plus nebulizer. Although this treatment regimen is used in all clinical trials, dosing once daily with a higher dose is likely to improve drug efficacy and safety. Specifically, higher peak levels could be obtained if the complete daily dose is delivered in a single inhalation. Recently, the pharmacokinetics of the once-daily inhaled double tobramycin dose were studied for the Akita® and PARI-LC® Plus nebulizers, where the Akita® is a smart nebulizer that allows for highly efficient targeting of the small airways. Similar pharmacokinetic profiles were found for both nebulizers and when compared to data on standard twice-daily inhalation, higher peak and lower trough levels were observed with the once-daily treatment regimen.[165] Although promising, it is still unknown whether specific targeting of tobramycin to the small airways using a smart nebulizer results in sufficiently high antibiotic concentrations in the small airways.

By using airway models derived from computed tomography (CT) scans of CF patients who differ in disease severity, in combination with computational fluid dynamics (CFD) simulations, aerosol concentrations in the central and more distal airways can be computed. Furthermore, the relationship between airway morphology and airway concentrations can be assessed. This technique also yields similar information to SPECT CT (Single Photon Emission Computed Tomography)[130] and has recently been used to study the association between structural lung disease in CF and the deposition of Aztreonam lysine for inhalation (AZLI; inhaled antibiotic for *Pa* treatment).[166] Unlike tobramycin, using concentrations above the MIC does not improve the bactericidal activity of AZLI (i.e. not concentration-dependent killing) and simulations were run for a different nebulizer (i.e.

eFlow). Therefore, the results of this study cannot be simply extrapolated to tobramycin nebulization.

Our study aimed to predict aerosol deposition patterns of inhaled tobramycin after once- and twice-daily dosing in CF lungs. We used patient-specific airway models and CFD simulations to determine the local concentrations per generation of the bronchial tree of inhaled tobramycin, delivered with the Akita® or PARI-LC® Plus nebulizer.

METHODS

In a previous study, 40 patient-specific 3D models of the airways and lung lobes were reconstructed and used for computational fluid dynamic (CFD) simulations.[166] These airway models were reconstructed from spirometry-controlled inspiratory and expiratory CT scans of patients with CF, acquired as part of routine clinical care. A total of 15 models were from children with CF aged ≥12 years, of which we selected the 6 youngest and 6 oldest airway models for this study. All scans were anonymized and scored in random order by 2 experienced observers, whereby the validated CF-CT scoring system[136] was used to quantify chest CT abnormalities. Component scores for bronchiectasis, airway wall thickening and mucus plugging were combined to compute the total airway disease (TAD) score and are expressed as a percentage of the maximum score (0-100%).

Reconstruction of the airway models and simulations were performed by FLUIDDA nv (Kontich, Belgium) and have been previously reported.[166] Briefly, the deposition of inhaled tobramycin was simulated for the Akita-Jet® compressor with the PARI-LC® Sprint nebulizer set to target peripheral airways, and for the Portaneb compressor with the PARI-LC® Plus nebulizer.

Approval for this retrospective study was obtained from the Institutional Review Board of the Erasmus Medical Center in Rotterdam, the Netherlands (MEC-2014-077). Written informed consent for the use of de-identified data was obtained from the parent/guardian and participants prior to inclusion in the study.

Reconstruction of three-dimensional airway models

Automatic segmentation of the inspiratory scan was used to reconstruct a 3D model of the intra-thoracic region and could be performed down to the level of airways with a diameter of 1-2 mm. This was followed by a manual check of the airways, involving the addition of missing branches and the deletion of incorrect branches, as necessary.

For the CFD simulation, an upper airway model from an average adult was scaled down to match the average tracheal diameter at the location of the sternum for the pediatric

population. The upper airway model was scaled twice, once for the 6 youngest patients and once for the 6 oldest patients. These models were connected to the mouthpiece of the PARI-LC® Sprint nebulizer, which was further connected to the patient-specific lower airway model (Figure S1).

Reconstruction of three-dimensional lung lobes

To extract the patient-specific lung lobes from inspiratory and expiratory CT scans, a semi-automated tool that identifies the fissures separating the lobes was used. For each of these lobes the volume change from expiration to inspiration was used to calculate the distribution of inhaled aerosol to that specific lobe.

Inlet of the airway model

Breathing profile and aerosol characteristics: Akita® nebulizer

The breathing profile for the simulations of tobramycin deposition with the Akita® was based on the predicted FEV_1 value at time of the CT scan for each particular patient. For the 12 airway models, the inhalation time and volume per breath varied between 5-8 seconds and 1.0-1.6L, respectively. The breathing profile had an inspiration-expiration ratio of 1:1.5 and was sinusoidal in shape. Tobramycin nebulization commenced immediately after inspiration began and continued up to the last second of the inhalation, after which a bolus of air was inhaled. The inhalation flow rate was fixed at 200 mL/second and a mass median aerosol diameter (MMAD) of 3.6 and geometric standard deviation (GSD) of 2.0 μm were used for inhalation of Bramitob by the Akita®.[167]

Loading doses of 150 mg and 300 mg tobramycin were used in simulations for the Akita® system. Delivered doses are shown in Table 1 and were based on an *in vitro* study

Table 1 – Doses used for simulations

	Akita®	PARI-LC® Plus
Loading dose BID	150	300
Loading dose OD	300	600
Delivered dose BID	26.95	96.2
Delivered dose OD	53.9	192.4

Loading doses and delivered doses used for the simulations. Doses are in milligrams. These doses are based on an in vitro *study which aimed to calculate the required delivered dose with the Akita® nebulizer to obtain an equivalent lung dose as the PARI-LC® Plus nebulizer. Due to the efficiency of the Akita®, the delivered dose for this system was set to be much lower to obtain an equivalent lung dose to the PARI-LC® Plus. Both nebulizers in this study were filled with 300 mg tobramycin.[12, 13] Thus for the Akita® these doses belong to the once-daily regimen in our study and for the PARI-LC® Plus these doses belong to the twice-daily regimen. BID = twice-daily; OD = once-daily.*

investigating the required delivered doses of different nebulizers to obtain an equivalent lung dose to the PARI-LC® Plus nebulizer.[167,168] Due to the efficiency of the Akita®, the delivered dose for this system was set to be much lower. However, the reduced loss of drug in the central airways meant that concentrations of drug in the small airways would be similar or perhaps even higher than the PARI-LC® Plus system. Additionally, both inspiration and expiration were modelled.

Breathing profile and aerosol characteristics: PARI-LC® Plus nebulizer

The sinusoidal breathing profiles for the simulations of tobramycin deposition with the PARI-LC® Plus had an inspiration-expiration ratio of 1:1.5, where tobramycin nebulization was continuous during both inspiration and expiration. Furthermore, these profiles were generated using the age and length of each patient at time of the CT scan, and the reference formula developed by Zapletal *et al.*[169] Specifically, the mean, upper and lower limits of this formula were used to generate a mean, high and low tidal volume, respectively. Several trials have studied the diameter distribution of tobramycin nebulized with the PARI-LC® Plus system in combination with different compressors. From these studies the smallest and largest reported MMAD were used for the CFD simulations: smallest MMAD (3.4 μm; TOBI® nebulized with PARI-LC® Plus combined with Turboboy SX compressor)[170] and largest MMAD (4.93 μm; TOBI® nebulized with PARI-LC® Plus combined with DevilBiss PulmoAide compressor).[171] Additionally, a GSD of 2.3 was used in these simulations.[172]

Loading doses of 300 mg and 600 mg tobramycin were used in simulations for the PARI-LC® Plus system. Delivered doses are shown in Table 1 and both inspiration and expiration were modelled. Unlike the Akita®, circulating particles that were neither deposited nor exhaled were present following the first expiration with the PARI-LC® Plus. Therefore, the respiratory cycle was simulated twice, from which the results of the second cycle were used for computation of the concentrations.

Computation of tobramycin concentrations

To compute regional tobramycin deposition, the airway surface area of the respiratory tract was subdivided into two regions. For airways with a diameter exceeding 1–2 mm (i.e. large airways), tobramycin concentrations were computed using the combined mouthpiece/upper-/lower airway model derived from chest CT scans.

Airways with a diameter smaller than 1-2 mm (i.e. small airways) were generally not visible on the CT images and hence, were added to the model using Phalen's description of the airway tree in infants, children and adolescents.[125] Phalen's data were obtained from subjects without lung disease and describes airway dimensions up to the sixteenth generation. Once the aerosol entered a lobe in the Phalen section of the model, it was assumed that it would be distributed homogeneously. Regional tobramycin concentrations

in the epithelial lining fluid (ELF) were computed as follows; the fraction of deposited inhaled aerosol in an airway multiplied by the delivered dose, was divided by, the surface area of that airway multiplied by the ELF thickness. A range of ELF thickness was used and based on studies in CF (3, 5, 7 μm).[140] Tobramycin concentrations were calculated for the following variables: the Akita® (1 MMAD, 1 breathing profile) or Pari LC Plus® (2 MMADs, 3 tidal volumes) systems, ELF thickness (3, 5, 7 μm) and the dosing regimen (once- and twice-daily dose). Results are described for the median ELF thickness of 5 μm unless otherwise indicated.

Statistical analysis

Patient characteristics and tobramycin concentrations were summarized using descriptive statistics and all data are presented as the median (interquartile range).

The mixed-effects model was used to assess differences in concentrations between the simulated situations and the significance level was set to 0.05. Specifically, the concentrations calculated for the median ELF were used as outcomes and a separate analysis was performed for the once- and twice- daily dosing regimens. The following variables were included in the model as predictors: TAD score, FEV_1 and FEF_{75} % pred. The advantage of using mixed-effects models is that they account for repeated measurements on the same patients.

Descriptive statistics and intraclass correlation coefficients (ICC) were calculated using SPSS/PC Statistics 21.0 (SPSS Inc. Chicago, IL, USA). Mixed-effects modelling was performed with the statistical software package R (free download from www.rproject.org) version 3.2.2.

RESULTS

Study population

Twelve spirometry-controlled inspiratory and expiratory chest CT scans (n=8 female) were selected for this study. However, two airway models from the same patient were included from two different time points, two years apart. Hence in total the airway models were derived from 11 patients. Baseline characteristics are shown in Table 2 and disease severity, as indicated by the TAD score and FEV_1 % predicted, was highly variable.

Deposition analyses

High concentrations of inhaled tobramycin were delivered to all regions of the lungs by both nebulizers. For the Akita®, the median tobramycin concentration in the *large* airways was 77255 μg/ml when dosed twice daily and 154511 μg/ml when dosed once daily. For the PARI-LC® Plus, median tobramycin concentrations in the large airways were

Table 2 – Baseline characteristics

	Value	IQ Range
Male	33.3%	
Age	15.7	(13.9-17.1)
Total airway disease score (% of max score)	7.3	(4.1-11.1)
FEV_1%pred	92.6	(86.4-115.9)
FEF_{75}%pred	54.7	(35.2-71.5)

Median (interquartile (IQ) range). FEV_1%pred = percent predicted forced expiratory volume in one second; FEF_{75}%pred = percent predicted forced expiratory flow at 75% of forced vital capacity.

84316-94957 µg/ml when dosed twice daily and 168633-189916 µg/ml when dosed once daily.

Figure 1 shows tobramycin concentrations in the *small* airways calculated for the median ELF. Simulations with the Akita® resulted in tobramycin concentrations of 1770 µg/ml when dosed twice daily and 3541 µg/ml when dosed once daily. For the PARI-LC® Plus, tobramycin concentrations were 1066-1800 µg/ml when dosed twice daily and 2133-3598 µg/ml when dosed once daily. Figure 1 also shows how tidal volume

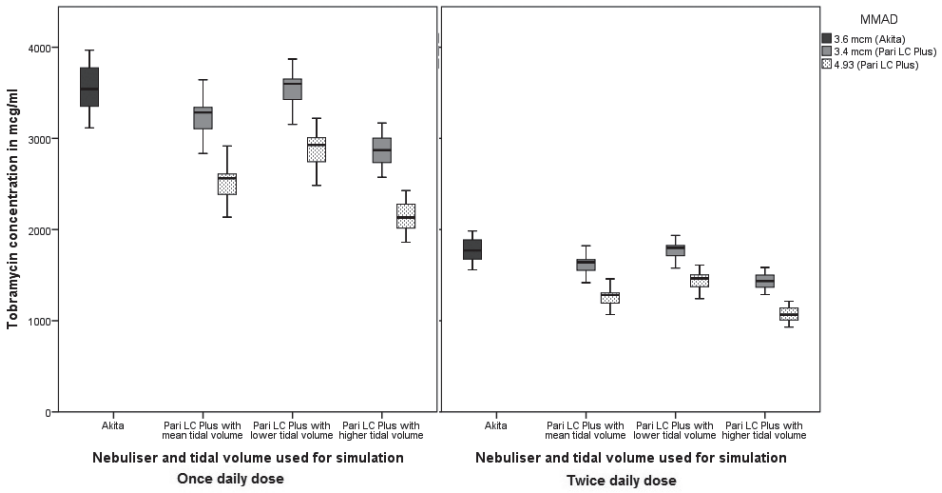


Figure 1 – **Small airways tobramycin concentrations**
Small airways tobramycin concentrations calculated for the median epithelial lining fluid of 5 µm. Concentrations after nebulization of the once-daily dose (boxplot on the left) are higher than concentrations after nebulization of the twice-daily dose (boxplot on the right). Concentrations for the once-daily dose range between approximately 2000 and 4000 µg/ml. Concentrations for the twice-daily dose range between approximately 1000 and 2000 µg/ml. Highest concentrations are obtained with the Akita® and PARI-LC® Plus nebulized with a lower tidal volume and small MMAD of 3.4 µm. For the PARI-LC® Plus, higher concentrations are obtained with the small MMAD of 3.4 µm than the large MMAD of 4.93 µm. MMAD = mass median aerosol diameter.

Table 3. Efficiency of delivering small airways concentrations with the Pari LC Plus® in comparison to the Akita® nebulizer and associations with disease characteristics

Characteristic/Nebulizer	MMAD (µm)	Concentrations (µg/ml)		Estimate		Std. err of estimate		p-value
		OD	BID	OD	BID	OD	BID	
FEV_1 % pred	-	-	-	3.1	1.6	1.7	0.8	0.062
FEF_{75} % pred	-	-	-	4.3	2.1	0.9	0.4	**<0.001**
TAD score	-	-	-	12.8	6.4	3.8	1.9	**<0.001**
Akita®	3.6	3540.94 (3327.21-3784.47)	1770.47 (1663.61-1892.23)					
PARI-LC® Plus (low tidal volume)	3.4	3598.48 (3367.05-3651.84)	1799.79 (1683.52-1825.92)	28.3	14.1	74.5	37.3	0.704
	4.93	2928.16 (2718.61-3008.46)	1464.08 (1359.31-1504.23)	-651.9	-326.0	72.0	36.0	**<0.001**
PARI-LC® Plus (mean tidal volume)	3.4	3284.25 (3093.91-3351.16)	1642.13 (1546.95-1675.58)	-288.5	-144.3	70.0	35.0	**<0.001**
	4.93	2562.62 (2372.88-2615.50)	1281.31 (1186.44-1307.75)	-1013.3	-506.6	66.9	33.4	**<0.001**
PARI-LC® Plus (high tidal volume)	3.4	2871.36 (2725.62-3003.25)	1435.68 (1362.81-1501.62)	-659.2	-329.6	66.3	33.2	**<0.001**
	4.93	2132.66 (2008.99-2288.59)	1066.33 (1004.49-1144.29)	-1393.7	-696.8	64.2	32.1	**<0.001**

Associations between small airways tobramycin concentrations (median, interquartile range) and disease characteristics or nebulizer with breathing pattern for the median epithelial lining fluid and different aerosol diameters. Comparison is made with the Akita® nebulizer. For example: if two patients with the same TAD score, FEV_1 % pred, and, FEF_{75} % pred, were compared, the patient using the PARI-LC® Plus with a low tidal volume and an aerosol diameter of 4.93 µm will have a mean reduction in the peripheral tobramycin concentration of 652 and 326 µg/ml (once- and twice-daily, respectively) compared to the Akita®. P-values in bold represent significant differences. MMAD = mass median aerosol diameter; OD = once-daily; BID = twice-daily.

and MMAD influence tobramycin concentrations in the small airways for the PARI-LC® Plus. Specifically, lower tobramycin concentrations in the small airways were observed with higher tidal volumes and the largest MMAD. Small airways concentrations for all ELF thicknesses are shown in Figure S2 of the Supplementary data.

Significantly lower tobramycin correlations were observed with the PARI-LC® Plus nebulizer than with the Akita® nebulizer, with the exception of inhalation at a low breathing tidal volume and the smallest MMAD (3.4 μm) (Table 3). For example, if two patients with the same TAD score, FEV_1 % predicted and FEF_{75} % predicted were compared, the patient using the PARI-LC® Plus with a low tidal volume and an aerosol diameter of 4.93 μm will have a mean reduction in the peripheral tobramycin concentration of 652 and 326 μg/ml (once- and twice-daily, respectively) compared to the Akita®. Much smaller and reverse associations were seen for FEF_{75} % predicted and the TAD score.

DISCUSSION

In this study, CFD was used to compute local inhaled tobramycin concentrations throughout the bronchial tree in CF after once- and twice-daily dosing. High concentrations of inhaled tobramycin were delivered to all lung regions, with the Akita® nebulizer being twice as efficient as the PARI-LC® Plus. The once-daily dose resulted in higher tobramycin concentrations in the small airways compared to the twice-daily dose (1523-5997 μg/ml versus 762-2999 μg/ml, respectively). This result is promising due to the concentration-dependent bactericidal efficacy of tobramycin; where the higher the concentration in a specific region, the greater the reduction in bacterial density.[173]

However, whether the computed concentrations are sufficient for effective killing throughout the lung remains questionable, as the concentration at which effective killing is obtained is as yet unknown. Due to the substantial heterogeneity of phenotypes and genotypes of *Pa* within a single patient, a range of MIC values exist throughout the lung.[126] Thus, the *in vitro* MIC value does not reflect the wide ranges of MICs that can be present within the lungs of a single patient. Additionally, the MIC reflects the activity of a drug under specific optimal circumstances, and may be far less for micro-organisms in a semi-dormant state. Furthermore, *in vitro* measurements do not take into account the hostile lung environment for antibiotics, where the activity of inhaled tobramycin in the lungs is reduced due to binding to mucin and DNA fragments within the mucus[93,94] and due to biofilm formation by *Pa*.[174] To overcome the effect of sputum binding on tobramycin, it is generally assumed that local concentrations need to be 10 to 25-fold above the MIC. Wild type organisms refer to the phenotype of the typical form of a species, as they occur in nature. These organisms may have intrinsic resistance mechanisms, but not acquired mechanisms. *Pseudomonas* wild types have a MIC of 2 μg/ml or lower.[175,176]

Hence for wildtype *Pa* ($MIC_{2\ \mu g/ml}$), effective killing can be easily achieved as concentrations 381-1500x $MIC_{2\ \mu g/ml}$ were observed in the small airways for the twice-daily dose and 762-2999x $MIC_{2\ \mu g/ml}$ for the once-daily dose. However, for *Pa* strains with *in vitro* MICs above 2 µg/ml (i.e. acquired resistance mechanisms), the concentrations required for effective killing of these strains remain unknown. Assuming the highest MIC value measured *in vitro,* 512 µg/ml ($MIC_{512\ \mu g/ml}$), only the once-daily dose calculated for a thin lining fluid resulted in concentrations 10-fold greater than the MIC (10x $MIC_{512\ \mu g/ml}$). For these simulations, all patients received small airways concentrations above 10x $MIC_{512\ \mu g/ml}$ for both the Akita® and PARI-LC® Plus systems when nebulized with a low tidal volume and small aerosol diameter. For other simulations, only a proportion of the patients received concentrations above the threshold in all airways for the PARI-LC® Plus (Figures 2 and S3).

The problems associated with high MICs and additional sputum binding may be partly overcome by increasing the tobramycin concentration, which serves as the rationale for administering a double dose of tobramycin in a single inhalation. Higher tobramycin concentrations will ultimately result in increased concentrations of free drug that are able to kill *Pa*.[96] Likewise, higher tobramycin concentrations will allow antibiotic particles to penetrate deeper into bacterial microcolonies, as the diffusion of tobramycin is concentration-dependent. The optimal drug concentration will differ between patients, as there is marked variability between patients in the inhibitory activity of mucus against the killing efficacy of aminoglycosides such as tobramycin. To overcome the antagonistic activity of mucus in all patients, including those with the worst-case sputum composition, peak tobramycin concentrations had to be 100-times the MIC to ensure killing of planktonic *Pa*,[177] or even 100-1000-fold greater for *Pa* growing in biofilm.[161] This means that patients with greater antagonistic mucus activity and highly resistant *Pa* strains would possibly need even higher concentrations than the once-daily dose simulated in our study.

Another reason for once-daily dosing of an increased dose of inhaled tobramycin is that aminoglycosides induce a post-exposure effect and therefore, need to be dosed less frequently than β-lactam antibiotics, for example. Tobramycin exposure induces sublethal damage in *Pa* bacteria, which needs to be repaired before regrowth can commence to allow a new dose of aminoglycosides to be effective. The time it takes to repair this damage in part correlates with the post-exposure effect and continues when the antibiotic concentration falls below the MIC. A post-exposure effect of approximately 2 hours has been described for aminoglycosides against *Pa in vitro* using an enzymatic inactivation method. During a simulation in mice, the *in vivo* post-antibiotic effect was even longer (approximately 5 hours) than the effect in *vitro*,[178] as longer half-lives increase the duration of this effect.[179] The half-life of tobramycin measured in sputum was approximately 2 hours post-nebulization of 80 mg tobramycin.[180]

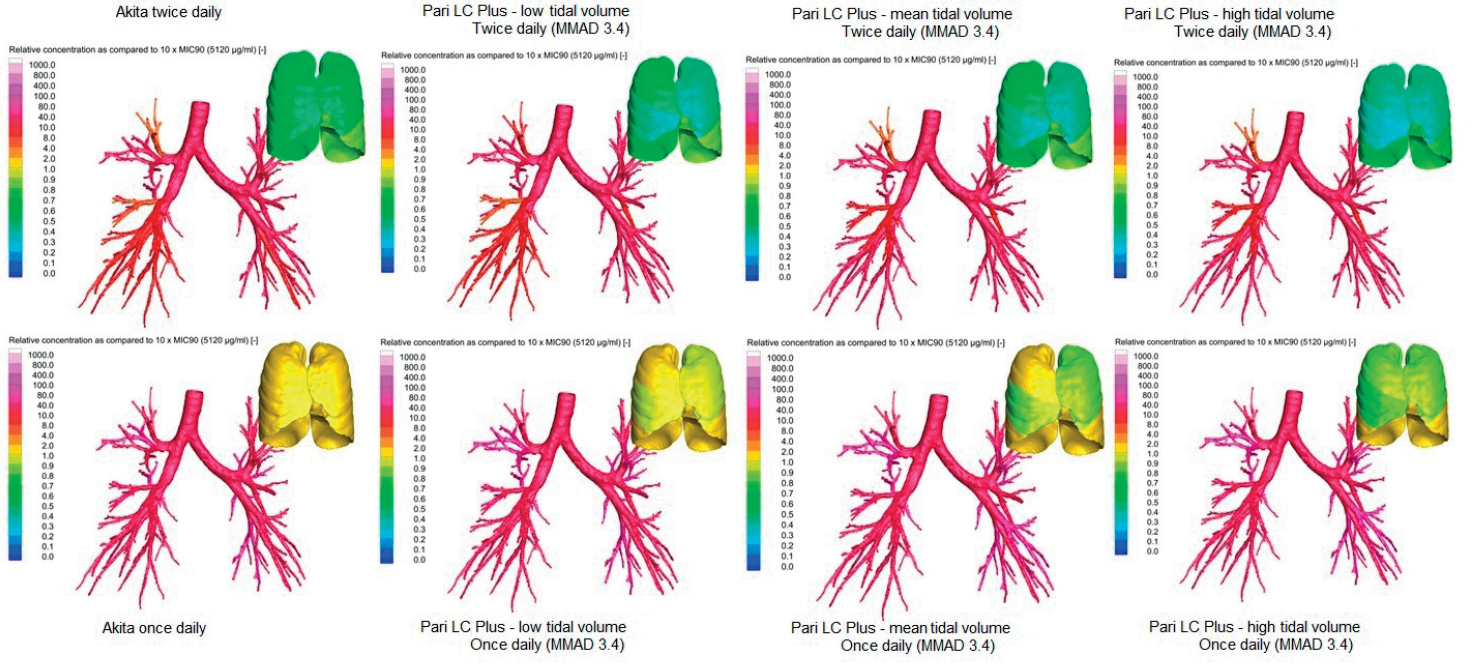


Figure 2 – Relative tobramycin concentrations in large and small airways for 1 patient with 2 different nebulizers

Concentrations in large and small airways of a single patient are represented relative to 10x$MIC_{512\ \mu g/ml}$ for a thin lining fluid (3 µm). In the color map, yellow represents concentrations at the level of 10x$MIC_{512\ \mu g/ml}$, red-purple-white represent concentrations above this level, green-blue represents colors below this level. For the PARI-LC® Plus, images show concentrations for 3 different tidal volumes and the small aerosol diameter of 3.4 µm. The upper panel represents the twice-daily regimen and lower panel the once-daily regimen. For the twice-daily regimen Akita® and PARI-LC Plus showed concentrations below 10x$MIC_{512\ \mu g/ml}$ in the small airways for all scenarios. On a once-daily regimen with the Akita® this patient received concentrations above 10x$MIC_{512\ \mu g/ml}$ in the small and large airways. For the PARI-LC® Plus concentrations were below 10x$MIC_{512\ \mu g/ml}$ in certain lobes for the 3 different tidal volumes.

Although our study supports the use of the once-daily treatment regimen of nebulized tobramycin, drug safety issues need to be considered when increasing nebulized doses of aminoglycosides. A pharmacokinetic study in patients with CF showed that inhalation of a double tobramycin dose with either the Akita® or Pari LC Plus® was well tolerated and resulted in higher peak levels (i.e. likely improved antibiotic effect), though trough levels remained well below the toxic limit. Additionally, studies in intravenous tobramycin showed that a longer clearance period of systemically absorbed tobramycin is associated with a better safety profile,[181,182] suggesting that a once-daily, double dose of inhaled tobramycin is safe.[165] Future clinical studies are needed to examine if once-daily inhalation of a double tobramycin dose is more effective in patients with CF infected with *Pa*, and whether chronic use of this treatment regimen is safe.

A previous CFD study investigating concentrations of AZLI in relation to structural lung disease in CF patients showed that the more diseased lobes received lower levels of the inhaled antibiotic,[166] and this is important for inhaled tobramycin due to its low rate of systemic absorption (9-17.5%).[12] This means that hypoventilated lung areas are undertreated as systemic absorption contributes little to the treatment effect of these areas. For antibiotics with relatively good absorption from the lung (e.g. inhaled levofloxacin),[126] a clinical response in these hypoventilated areas is likely to occur following systemic absorption via the bronchial circulation.

The limitations of CFD modeling have been previously described.[166] Firstly, in order to simulate and predict tobramycin concentrations, assumptions had to be made for ELF thickness and effective tobramycin concentrations. Secondly, the model does not account for sputum binding or mucociliary clearance. Finally, the following parameters were not patient-specific: the upper airway, airways with a diameter below 1-2 mm, and breathing profiles. An exception to this limitation would be the breathing profiles for the Akita®, which were based on the FEV_1 value of that specific patient similar to what would be used in clinics. For the PARI-LC® Plus, a range of breathing profiles was simulated based on the age and height of the specific patient. Future modeling studies could implement patient-specific breathing profiles and upper airways to make an even more reliable estimation of the required tobramycin concentrations for specific patients.

CONCLUSIONS

We demonstrated that high concentrations of inhaled tobramycin are indeed delivered to the lung, with the Akita® being twice as efficient as the PARI-LC® Plus. For effective killing of more resistant *Pa* strains, inhalation of a double tobramycin dose would be required. As inhalation of a double dose is not associated with acute toxicity, we thus recommend a once-daily, double dose of nebulized tobramycin in patients who do not

improve with standard care. The Akita® would be more efficient as only half the dose is required, which reduces treatment time. However, the Pari LC Plus® could also be used when the patient is able to execute a slow and deep inhalation throughout nebulization.

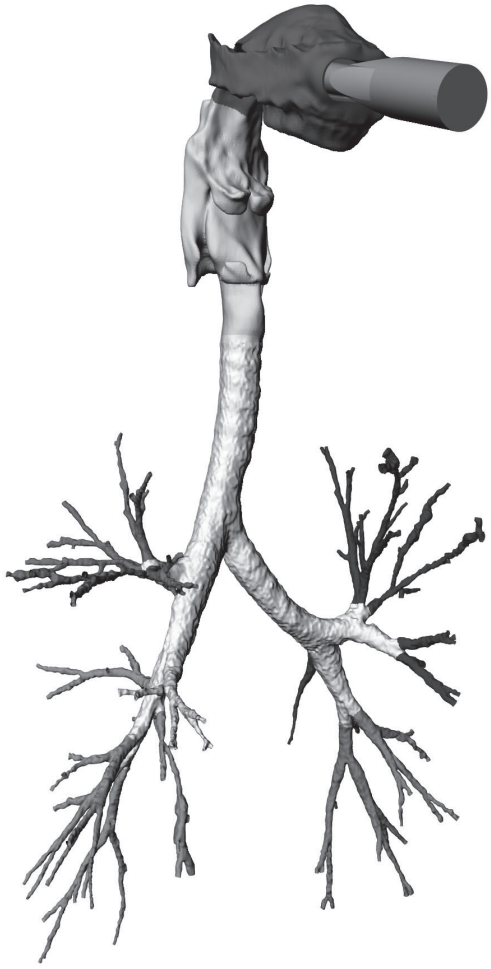
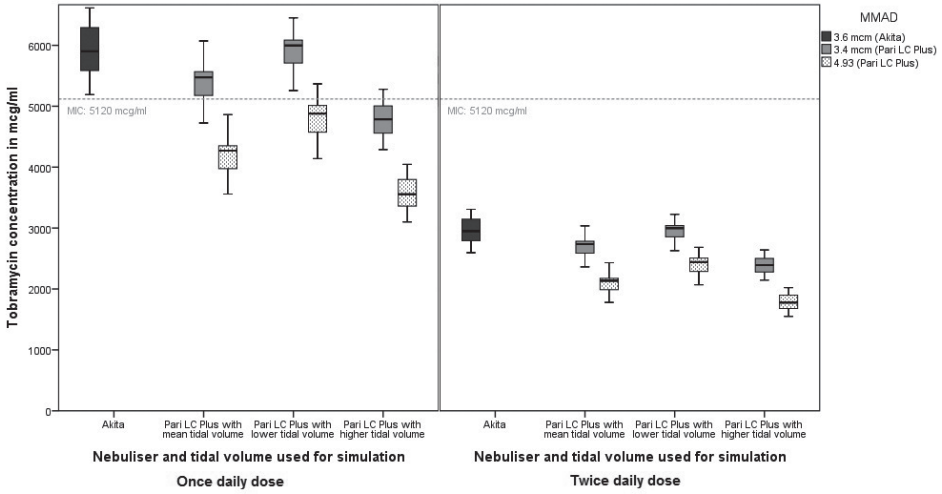


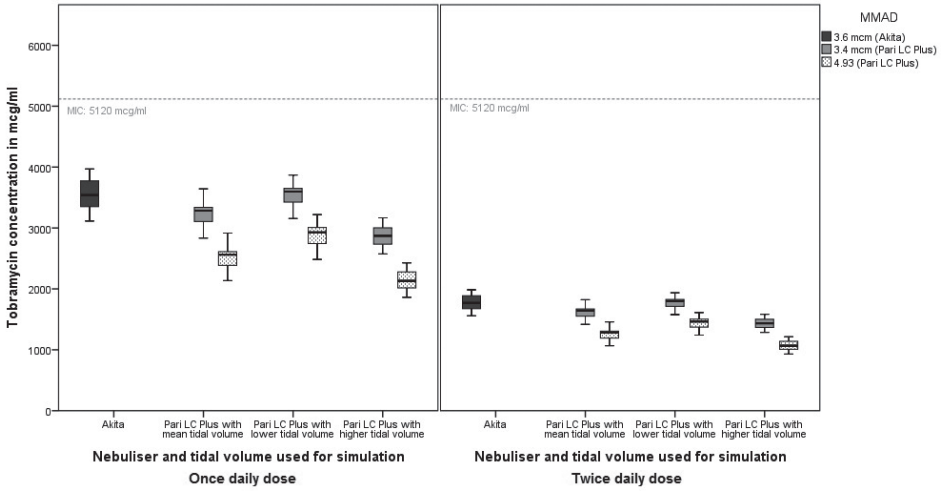
Figure S1 – Coupled mouthpiece/upper/lower airway model
Coupled mouthpiece/upper/lower airway model subdivided in multiple regions. Airways are segmented up to the 5th-9th generation.

Figure S2 – Small airway tobramycin concentrations calculated for different lining fluid thickness



Thin lining fluid (3 µm)

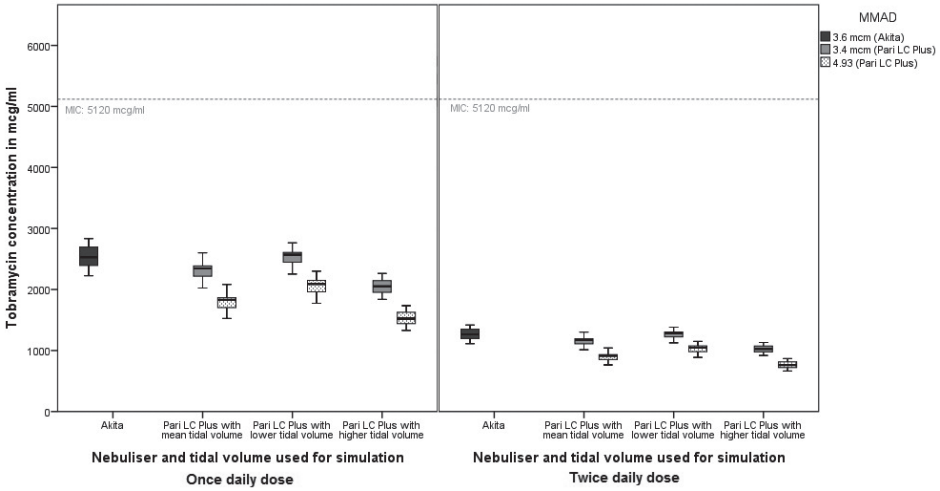
Small airways tobramycin concentrations calculated for the thin epithelial lining fluid of 3 µm. Concentrations after nebulization of the once-daily dose (boxplot on the left) are higher than concentrations after nebulization of the twice-daily dose (boxplot on the right). Concentrations for the once-daily dose range between approximately 3500 and 6000 µg/ml. Concentrations for the twice-daily dose range between approximately 1750 and 3000 µg/ml. Highest concentrations are obtained with the Akita® and PARI-LC® Plus nebulized with a lower tidal volume and small MMAD of 3.4 µm. For the PARI-LC® Plus, higher concentrations are obtained with the small MMAD of 3.4 µm than the large MMAD of 4.93 µm. MMAD = mass median aerosol diameter.



Median lining fluid (5 µm)

Small airways tobramycin concentrations calculated for the median epithelial lining fluid of 5 µm. Concentrations after nebulization of the once-daily dose (boxplot on the left) are higher than concentrations after nebulization of the twice-daily dose (boxplot on the right). Concentrations for the once-daily dose range between approximately 2000 and 4000 µg/ml. Concentrations for the twice-daily dose range between approximately 1000 and 2000 µg/ml. Highest concentrations are ob-

tained with the Akita® and PARI-LC® Plus nebulized with a lower tidal volume and small MMAD of 3.4 µm. For the PARI-LC® Plus, higher concentrations are obtained with the small MMAD of 3.4 µm than the large MMAD of 4.93 µm. MMAD = mass median aerosol diameter.



Thick lining fluid (7 µm)

Small airways tobramycin concentrations calculated for the thick epithelial lining fluid of 7 µm. Concentrations after nebulization of the once-daily dose (boxplot on the left) are higher than concentrations after nebulization of the twice-daily dose (boxplot on the right). Concentrations for the once-daily dose range between approximately 1500 and 2500 µg/ml. Concentrations for the twice-daily dose range between approximately 750 and 1300 µg/ml. Highest concentrations are obtained with the Akita® and PARI-LC® Plus nebulized with a lower tidal volume and small MMAD of 3.4 µm. For the PARI-LC® Plus, higher concentrations are obtained with the small MMAD of 3.4 µm than the large MMAD of 4.93 µm. MMAD = mass median aerosol diameter.

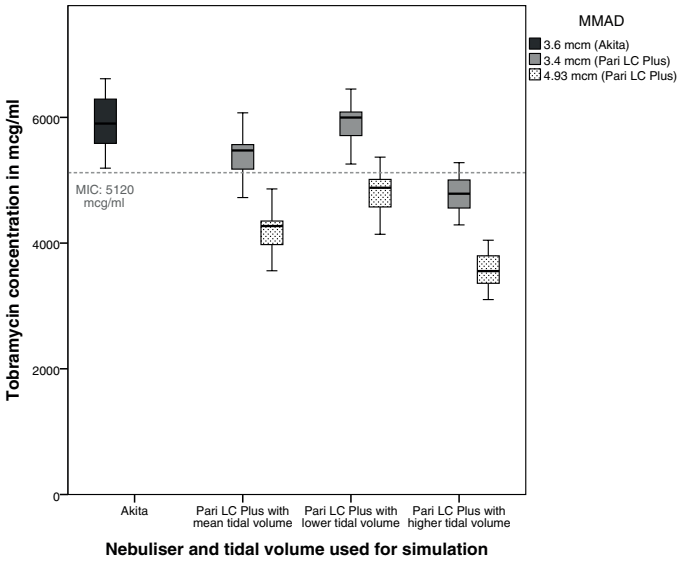


Figure S3 – Boxplot small airways concentrations once-daily dosing calculated for a thin lining fluid related to 10xMIC$_{512\ \mu g/ml}$

Small airways tobramycin concentrations after nebulization of the once-daily dose calculated for the thin epithelial lining fluid of 3 µm. All patients received small airways concentrations above 10xMIC$_{512\mu g/ml}$ with the Akita® and PARI-LC® Plus when nebulized with a low tidal volume and small MMAD of 3.4 µm. None of the patients received concentrations above this level with the Pari LC Plus nebulized with a mean or high tidal volume and large MMAD of 4.93 µm. Only part of the patients received concentrations above the threshold in the other simulated situations for the PARI-LC® Plus. The figure also shows the direct influence of tidal volume and MMAD on the concentrations in the small airways for the PARI-LC® Plus. Lower tobramycin concentrations in the small airways are observed with higher tidal volumes and the largest MMAD. MMAD = mass median aerosol diameter.

CHAPTER 6

Patient-specific evaluation of regional dornase alfa concentration levels in a cohort of patients with cystic fibrosis using Computational Fluid Dynamics

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Submitted

ABSTRACT

Background: Small airways disease (SAD) progresses with age in most cystic fibrosis (CF) patients in spite of maintenance therapy including inhaled dornase alfa. In a clinical study, efficient targeting of dornase alfa to the small airways using a smart nebulizer improved SAD in stable patients with CF on maintenance dornase alfa therapy, but not in patients who were admitted for an exacerbation. We hypothesized that higher than normal doses are required for patients with more severe lung disease and that suboptimal dornase alfa concentrations are obtained in the small airways with conventional nebulizers.

Methods: Computational fluid dynamic (CFD) modeling was applied to patient-specific airway models reconstructed from chest CT-scans (FLUIDDA nv, Kontich, Belgium). Disease severity was scored using CT scoring systems. The following settings were used for simulations: nebulization of 2.5 mg dornase alfa, nebulizer specific aerosol characteristics, and epithelial lining fluid thickness of 5 µm. CFD was performed for the smart Akita® nebulizer targeted to the large airways ($Akita_{large}$) and to the small airways ($Akita_{small}$), and for PARI-LC® Plus or eFlow® nebulizer. Concentrations are expressed relative to an effective dornase alfa concentration of 2.9 µg/ml. Results are reported as median [interquartile range].

Results: 40 CT-scans (35% male) were selected, age 10.9 [8.7-15.2] years, FEV_1 94.3 [81.7-102.5] %pred. Large airways concentrations for the $Akita_{large}$ and $Akita_{small}$ were 15 and 12-13 times higher than for the PARI-LC® Plus and eFlow® (8677, 7225, 588 and 571 µg/ml, respectively). Small airways concentrations were 27-30 and 17-20 times higher ($p < 0.001$) than for the other nebulizers ($Akita_{large}$: 173.4 [166-196], $Akita_{small}$: 111.4 [103-123], PARI-LC® Plus: 6.5 [6-7], eFlow®: 5.6 [5-6] µg/ml). 4.3% and 8.0% of the lung lobes received dornase alfa concentrations below 2.9 µg/ml with the PARI-LC® Plus and eFlow®, respectively.

Conclusions: For a breath actuated nebulizer dornase alfa concentrations in small airways were well above the minimal effective concentration as computed by CFD. For conventional nebulizers, concentrations were around or below the effective concentration especially in diseased areas. CFD simulations confirm observations of a clinical study where higher doses were more effective in reversing small airways obstruction.

INTRODUCTION

Cystic fibrosis (CF) lung disease is characterized by chronic early lung infection and inflammation. As part of this process, deoxyribonucleic acid (DNA) is released from inflammatory cells and bacteria that lyse within the airways. DNA significantly increases the viscoelasticity of mucus causing progressive obstruction due to mucus impaction in the airways, including the small airways.[20,183] This small airways disease (SAD) plays a major role in the pathophysiology of progressive CF lung disease.[5]

Inhaled dornase alfa was developed in the early Nineties to reduce mucus viscosity in CF by enzymatic cutting of free DNA.[19] It was shown that daily nebulization of 2.5 mg dornase alfa reduced obstruction as measured by spirometry and multiple breath washout, reduced the number of exacerbations, and reduced the rate of lung function decline.[19,49] No clear dose response relation was observed between a once daily administration of 2.5 mg dornase alfa and a twice daily administration.[184] For this reason, the recommended dose for maintenance therapy with dornase alfa is once daily 2.5 mg, nebulized with an approved jet nebulizer/compressor combination or the Pari eFlow Rapid® nebulizer system (further referred to as eFlow).[20] Daily treatment of dornase alfa has become standard treatment for CF patients of 6 years and above. However, despite this treatment residual SAD progresses with age in most CF patients.[9] For this reason it was questioned whether high enough doses could ever be deposited in the small airways using the conventional nebulizers which are operated while the patient is using tidal volume breathing.[185] Furthermore, it is well recognized that the nebulizers used in the pivotal trials for dornase alfa are quite inefficient relative to today's state of the art smart nebulizers. In addition, nebulizers like eFlow used in today's clinical practice have different characteristics than those used in the pivotal trials. To investigate whether more efficient delivery of dornase alfa to the small airways would reverse SAD in stable CF patients a randomized controlled study was executed in a small number of patients by Bakker *et al.*[25] In stable CF patients with SAD and who were on maintenance dornase alfa therapy, the use of a conventional nebulizer was switched to a smart Akita® nebulizer (Vectura, UK). Patients were randomized to the Akita set to target the large airways or to the Akita set to target the small airways. This smart nebulizer controls and guides the flow and volume of inhalation of aerosol and reduces loss of aerosol into the environment by limiting nebulization to the inspiratory phase. As a result lung deposition is improved to 70% of the loading dose compared to 10-15% using conventional nebulizers. Therefore, the total lung dose during this study was estimated to be 3-5 times higher compared to conventional nebulizers. The switch to the Akita® nebulizer resulted in substantial improvement of small airways patency.[25] The effect was larger for the small airways group.

In a similar study by Bakker *et al.*,[185] it was investigated whether efficient targeting of dornase alfa to the small airways also would improve SAD in CF patients with a respira-

tory tract exacerbation. CF patients hospitalized for an exacerbation were randomized to small or large airway deposition of dornase alfa using the Akita nebulizer. In this study, for safety reasons, half the dose of dornase alfa was given (1.25 mg) which was estimated to result in a lung dose that would be 1.5-2 times higher than conventional nebulizers. The switch to the Akita nebulizer in these unstable CF patients hospitalized for an exacerbation did not result in more effective treatment of small airways obstruction than other nebulizers in contrast to the study in stable patients. This may have been caused by the lower dose administered in this second trial. As sputum production and central deposition is increased during respiratory tract exacerbations this lower dose may have resulted in under-dosing of the small airways.[185]

Based on the above described two studies, we raised the question whether efficient targeting of dornase alfa to the small airways using a smart nebulizer would result in sufficiently high concentrations in the small airways. We hypothesized that, even in stable patients with SAD, dornase alfa concentrations are not high enough throughout the lung. To answer this question we used computational fluid dynamic (CFD) simulations to estimate aerosol concentrations in large and small airways in relation to disease severity. Using CFD, we computed the percentage of airways that received suboptimal dornase alfa concentrations when dornase alfa was nebulized using either a conventional PARI-LC® Plus nebulizer, an eFlow nebulizer, or the smart Akita nebulizer set to target the large or small airways.

METHODS

Reconstruction of three-dimensional airway models and lung lobes

We used 40 patient-specific 3D models of the airways and lung lobes that were reconstructed previously and used for CFD simulations.[166] These airway models were reconstructed from spirometry controlled inhalation- and expiration CT scans of patients with CF aged 6-18 years, acquired as part of routine clinical care. Reconstruction of the airway models was performed by FLUIDDA nv (Kontich, Belgium) as has been described in detail in a previous paper.[166]

Briefly, a 3D model of the intra-thoracic region down to the level of airways with a diameter of 1-2 mm was reconstructed from the inspiratory scan, using automatic segmentation. Missing branches were manually added and incorrect branches manually deleted, as necessary. The upper airways were not included in the CT-scans. Therefore, an upper airway model from an average adult was scaled down to match the average tracheal diameter for the population. This model was connected to the nebulizer specific mouthpieces. For the eFlow the mouthpiece was obtained using reverse engineering, for the

PARI-LC Plus, and Akita - PARI-LC Sprint combination a digital CAD model of the PARI-LC Sprint mouthpiece was used as the mouthpieces of the PARI-LC Plus and PARI-LC Sprint are equivalent. The patient-specific lower airway model was connected to the nebulizer mouthpiece/upper airway model (Supplementary figure S1).

Using a semi-automated image analysis tool, the patient-specific lung lobe volumes were extracted from inspiratory and expiratory CT scans. This tool identifies the fissures separating the lobes. The volume change from expiration to inspiration was used for each lobe to compute the distribution of inhaled aerosol to that specific lobe.

Scoring of Chest CT abnormalities

To quantify chest CT abnormalities, scans were anonymized and scored in random order using the validated CF-CT[136] and PRAGMA-CF scoring systems.[186] With the CF-CT scoring system, on the inspiratory scan, the five lung lobes and lingula were evaluated in the central and peripheral lung regions for severity and extent of the following components: bronchiectasis, airway wall thickening, mucous plugging and opacities (atelectasis, consolidation, ground glass pattern).[187] A total airway disease score (TAD) score was calculated as the sum of bronchiectasis, airway wall thickening and mucous plugging and expressed as a percentage of the maximum score (0-100%) (CFCT-TAD). PRAGMA-CF uses a grid overlaying 10 equally spaced axial CT slices between apex and base. For each grid cell the following CT components are scored on inspiratory scans in hierarchical order (highest to lowest priority): 1. bronchiectasis; 2. mucous plugging; 3. airway wall thickening; 4. atelectasis or 5. normal lung. Trapped air is assessed with a similar methodology on the expiratory scans.[186] For each component the volume is then computed and expressed as a fraction of total lung volume. As for the CF-CT scoring system, a TAD score was calculated as the sum of bronchiectasis, mucous plugging and airway wall thickening (PRAGMA-TAD).[187]

CFD simulations

CFD simulations were performed by FLUIDDA nv (Kontich, Belgium). Deposition of inhaled dornase alfa was simulated for the Portaneb compressor with the PARI-LC Plus nebulizer, for the Akita-Jet with the PARI-LC Sprint nebulizer set to target small airways and set to target large airways, and for the eFlow nebulizer.

Breathing profile and aerosol characteristics for Akita

For dornase alfa deposition with the Akita we used the settings simulating the conditions used in the clinical trial involving stable patients with CF.[25] Inhalation time and volume for both settings (large and small airways) were based on the individual value for Forced Expiratory Volume in 1 second (FEV_1) at the time of the CT-scan for each individual patient. To generate the two different lung deposition patterns, three characteristics of the nebulizer were adjusted: particle size, breathing pattern and timing of aerosol bolus.

For the Akita targeting the small airways ($Akita_{Small}$), the inhalation time and volume per breath varied between 3.8-7.5 seconds and 0.75-1.5 L, respectively. The breathing profile had an inspiration-expiration ratio of 1:1.5. The inspiration part was stepwise and expiration part sinusoidal (Supplementary figure S2). Dornase alfa nebulization commenced immediately after inspiration. The inhalation phase consisted a period of the delivery of the dornase alfa aerosol followed by 1 second of fresh air. This air bolus helps to transport the aerosol deeper down in the small airways. The inhalation flow rate was fixed at 200 ml/second and a mass median aerosol diameter (MMAD) of 4.2 and GSD of 1.6 µm were used.[25]

For the Akita targeting the large airways ($Akita_{large}$) simulating deposition of dornase alfa delivered by a conventional nebulizer, the inhalation time and volume per breath varied between 3.8-5.3 seconds and 0.4-0.7 L, respectively. Again, the inspiration-expiration ratio was 1:1.5, with a stepwise inspiration and sinusoidal expiration (Supplementary figure S3). In contrast to $Akita_{small}$, dornase alfa nebulization did not commence immediately after the start of inspiration. Instead, the inspiration started with a bolus of air without aerosol. Depending on the inhalation time the bolus of fresh air continued for 0.5 to 2.5 seconds after which nebulization started. Nebulization continued for 2.5 seconds regardless of inhalation time, after which, again, a bolus of air without aerosol was inhaled during the last 0.3 seconds of the inhalation. As inhalation time per breath varied between 3.3 and 5.3 seconds, nebulization started 0.5 to 2.5 seconds after start of inspiration. The inspiration cycle was followed by a breath-hold of 3.0 seconds. During the inspiration cycle, inhalation flow rate was 200 ml/second when nebulization was *off* and 60 ml/second when nebulization was *on* (Supplementary figure S3). A MMAD of 6.0 and GSD of 1.6 µm were used.[25]

A loading dose of 2.5 mg dornase alfa was used in simulations for the Akita system. On average 97.18% of the loading dose is delivered to the lungs for the Akita targeting the small airways and 92.33% for the Akita targeting the large airways. This results in an expected delivered dose of 2.43 mg for $Akita_{Small}$ (2.5 mg * 97.18% = 2.43 mg) and expected delivered dose of 2.31 mg for $Akita_{large}$ (2.5 mg * 92.33% = 2.31 mg). These delivered doses were used for the simulations. Both inspiration and expiration were modelled.

Breathing profile and aerosol characteristics PARI-LC Plus and eFlow

The sinusoidal breathing profiles for the simulations of dornase alfa deposition with the PARI-LC Plus and eFlow nebulizers had an inspiration-expiration ratio of 1:1.5, where dornase alfa nebulization was continuous during both inspiration and expiration. Furthermore, these profiles were generated using the specific age and height of each patient at the time of the CT-scan and the reference formula developed by Zapletal *et al.* (mean value).[169] The PARI-LC Plus generates an aerosol with a MMAD of 4.6 and GSD of 2.14 µm.[188] The eFlow generates an aerosol with a MMAD of 4.8 and GSD of 1.8 µm.[188]

A loading dose of 2.5 mg was used in simulations for the PARI-LC Plus and eFlow systems. The delivered dose for PARI-LC Plus has been shown to be 0.570 mg and for eFlow 0.567 mg.[188] Both inspiration and expiration were modelled. Unlike the Akita, circulating particles that were neither deposited nor exhaled were present following the first expiration with the PARI-LC Plus and eFlow. Therefore the respiratory cycle was simulated twice, from which the results of the second cycle were included in the computation of the concentrations.

Computation of dornase alfa concentrations

Regional dornase alfa concentrations were computed by subdividing the respiratory tract into two regions. Concentrations in the large airways (airways with a diameter > 1-2 mm) were calculated using the combined mouthpiece/upper-/lower airway model. Small airways with a diameter < 1-2 mm were generally not visible on the CT images and hence, were added to the model using Phalen's description of the airway tree in infants, children and adolescents.[125] These data describe airway dimensions up to the sixteenth generation and were obtained from subjects without lung disease. Concentrations in the Phalen model were calculated on a lobar basis, as well as for the complete Phalen model (concentrations in all small airways combined). Dornase alfa concentrations were calculated as follows: The deposited fraction in an airway was multiplied by the delivered dose. This number was then divided by the surface area of that airway multiplied by the epithelial lining fluid (ELF) thickness. A range of ELF thicknesses was used based on studies in CF (3 – 5 – 7 μm).[140] Results are presented for the median ELF thickness of 5 μm unless indicated otherwise.

Statistical analysis

Patient characteristics and dornase alfa concentrations were summarized using descriptive statistics and all data are presented as the median [interquartile range].

The mixed-effects model was used to investigate differences in concentrations between the simulated situations. Specifically, the concentrations calculated for the median ELF were used as the outcome. Furthermore, we corrected for disease severity (CFCT-TAD, PRAGMA-TAD, FEV_1 and FEF_{75} % pred.). The advantage of the mixed-effects models is that they can account for the correlation within the same patients.

Significance level was set at 0.05. Descriptive statistics were calculated using SPSS/PC Statistics 21.0 (SPSS Inc. Chicago, IL, USA). Mixed-effects model was performed with the statistical software package R (free download from www.rproject.org) version 3.2.4.

Approval for this retrospective study was obtained from the Institutional Review Board of the Erasmus Medical Center in Rotterdam, the Netherlands (MEC-2013-338). The parent/guardian and participants provided written informed consent for the use of de-identified data prior to inclusion in the study.

RESULTS

Study population

Forty inspiratory and expiratory chest CT-scans were selected from 31 patients, of which 39 CT-scans were spirometry controlled and 1 scan was performed with lung technician guidance. Baseline characteristics are shown in Table 1.

Table 1 – Baseline characteristics

	Value	IQ Range
Male	35%	
Age	10.9	[8.7-15.2]
CFCT-TAD (% of max score)	4.8	[1.2-7.5]
PRAGMA-TAD (% disease per lung)	1.5	[0.4-3.1]
FEV_1%pred	94.3	[81.7-102.5]
FEF_{75}%pred	56.5	[39.4-74.0]

Median [interquartile (IQ) range]. TAD = total airway disease; FEV_1%pred = percent predicted forced expiratory volume in one second; FEF_{75}%pred = percent predicted forced expiratory flow at 75% of FVC.

Deposition analyses

High concentrations of dornase alfa were delivered to the large airways with all nebulizers. For the $Akita_{large}$ and $Akita_{small}$, median dornase alfa concentrations in the *large* airways were 8677 [5964-16616] µg/ml and 7225 [4821-13497] µg/ml. For the PARI-LC Plus and eFlow, *large* airways concentrations were 588 [378-1029] µg/ml and 571 [367-1000] µg/ml, respectively. *Large* airway concentrations for the $Akita_{large}$ and $Akita_{small}$ were around 15 and 12-13 times higher compared to the PARI-LC Plus and eFlow (Figure 1).

Figure 2 shows dornase alfa concentrations in the *small* airways with the different nebulizers calculated for the median ELF. From the mixed-effects, significant differences were found in *small* airways concentrations between all nebulizers, with highest concentrations after simulation with the $Akita_{large}$ and lowest concentrations with the eFlow nebulizer. *Small* airways concentrations for the $Akita_{large}$ and $Akita_{small}$ were 27-30 and 17-20 times higher than for the other two nebulizers ($Akita_{large}$: 173.4 [166-196], $Akita_{small}$: 111.4 [103-123], PARI-LC Plus: 6.5 [6-7], eFlow: 5.7 [5-6] µg/ml). If we compare two patients with the same disease characteristics (same CFCT-TAD score, PRAGMA-TAD score, FEV_1 and FEF_{75} % pred.) the *small* airways concentration after nebulization with the $Akita_{large}$ is 173 µg/ml higher than after nebulization with the eFlow. Similarly, the *small* airways concentrations after nebulization with the $Akita_{small}$ and PARI-LC Plus are, respectively, 109 and 0.85 µg/ml higher than after nebulization with the eFlow (Table 2).

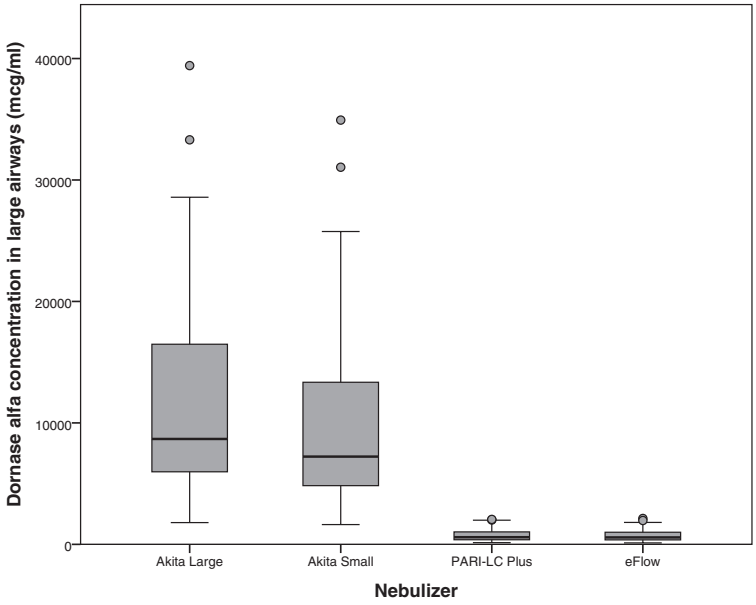


Figure 1 – **Dornase alfa concentrations in the large airways for the different nebulizers**
Dornase alfa concentrations in the large airways calculated for the median epithelial lining fluid (5 µm) after inhalation with the different nebulizers. Higher concentrations in the large airways are obtained with the $Akita_{large}$ and $Akita_{small}$ than with the PARI-LC Plus and eFlow.

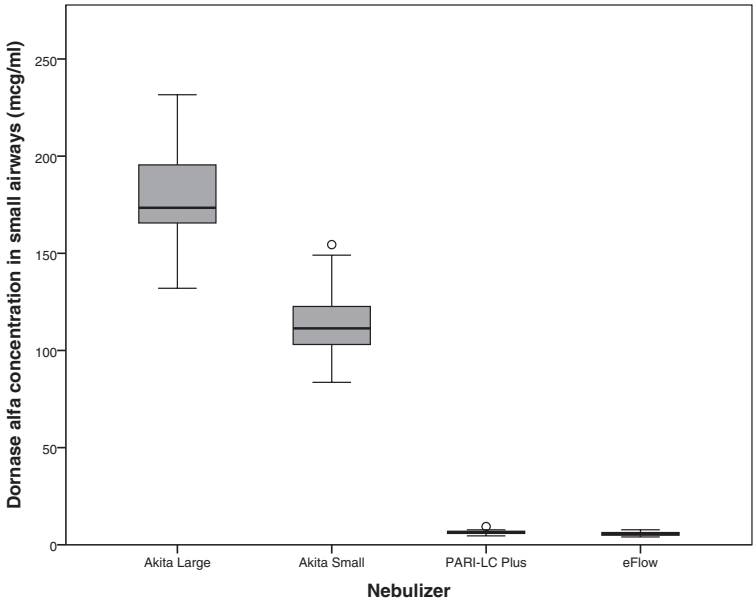


Figure 2 – **Dornase alfa concentrations in the small airways for the different nebulizers**
Dornase alfa concentrations in the small airways calculated for the median epithelial lining fluid (5 µm) after inhalation with the different nebulizers. Much higher concentrations in the small airways are obtained with the $Akita_{large}$ and $Akita_{small}$ than with the PARI-LC Plus and eFlow.

Table 2 – Efficiency of delivering small airways concentrations with the different nebulizers and associations with disease characteristics

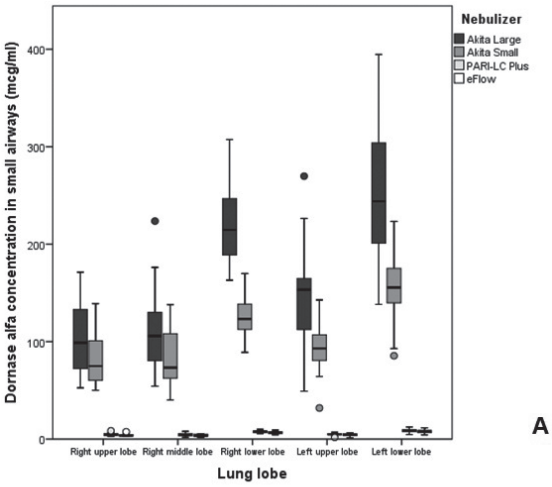
Characteristic/ Nebulizer	MMAD (µm)	Concentrations (µg/ml)	Estimate	Std. err of estimate	p-value
FEV_1 %pred	-	-	-0.13	0.18	0.46
FEF_{75} %pred	-	-	0.11	0.07	0.11
CFCT-TAD	-	-	0.39	0.73	0.59
PRAGMA-TAD	-	-	-0.59	1.28	0.64
$Akita_{large}$	6.0 (GSD 1.6)	173.4 [166-196]	172.72	3.51	**<0.001**
$Akita_{small}$	4.2 (GSD 1.6)	111.4 [103-123]	108.75	2.86	**<0.001**
PARI-LC® Plus	4.6 (GSD 2.14)	6.5 [6-7]	0.85	0.07	**<0.001**
eFlow®	4.8 (GSD 1.8)	5.7 [5-6]			

Comparison of small airways dornase alfa concentrations (median, interquartile range) between the different nebulizers and associations between small airways dornase alfa concentrations and disease characteristics for the median epithelial lining fluid. Comparisons are made with the eFlow nebulizer. For example: if two patients with the same CFCT-TAD, PRAGMA-TAD score, FEV_1 % pred, and, FEF_{75} % pred, were compared, the patient using the $Akita_{large}$ will have a mean higher peripheral dornase alfa concentration of 173 µg/ml compared to the eFlow. P-values in bold represent significant differences. MMAD = mass median aerosol diameter.

Concentrations in all *small* airways combined were 46-80 fold the effective dose of 2.9 µg/ml for the $Akita_{large}$, 29-53 fold 2.9 µg/ml for the $Akita_{small}$, and 1-3 fold 2.9 µg/ml for the PARI-LC Plus and eFlow. When looking at separate lung lobes, the total area of small airways received dornase alfa concentrations well above 2.9 µg/ml using $Akita_{large}$ and $Akita_{small}$ settings while 4.3% and 8.0% of the total area of small airways received dornase alfa concentrations below 2.9 µg/ml with the PARI-LC Plus and eFlow settings, respectively (Figure 3 and 4). When calculating concentrations for each separate lung lobe for a thick ELF of 7 µm, 15.5% and 30.4% of the total area of small airways received dornase alfa concentrations below 2.9 µg/ml with the PARI-LC Plus and eFlow, respectively. Again, all lung lobes received dornase alfa concentrations above 2.9 µg/ml with the $Akita_{large}$ and $Akita_{small}$. When calculating concentrations in the separate lung lobes for a thin ELF of 3 µm, all lung lobes received dornase alfa concentrations above 2.9 µg/ml with the $Akita_{large}$, $Akita_{small}$ and PARI-LC Plus and 1.1% of the total area of small airways received dornase alfa concentrations below 2.9 µg/ml with the eFlow.

Associations with disease severity

No associations were found between small airways dornase alfa concentrations and disease characteristics (CFCT-TAD score, PRAGMA-TAD score, FEV_1 and FEF_{75} % pred) (Table 2).



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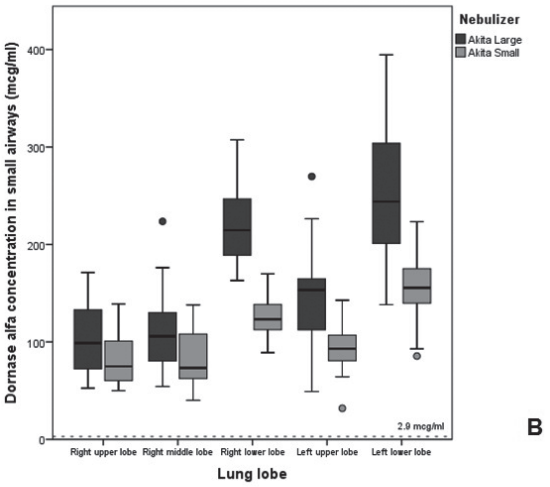

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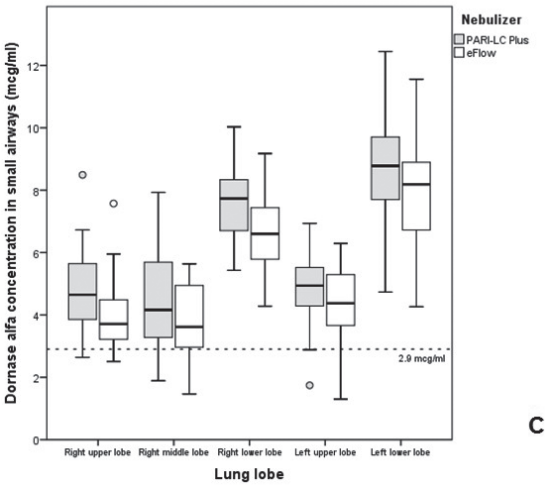

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Figure 3 – Differences between lobes in dornase alfa concentrations for the different nebulizers

Small airways concentrations of dornase alfa in the separate lung lobes, calculated for the median epithelial lining fluid (5 µm). Boxplot a) represents all nebulizers, boxplot b) represents the concentrations for the $Akita_{large}$ (dark grey) and $Akita_{small}$ (grey) and boxplot c) represents the concentrations for the PARI-LC Plus (light grey) and eFlow (white). Note the difference in scale of the y-axis in the boxplot for the PARI-LC Plus and eFlow. The dotted line represents the effective dornase alfa level of 2.9 µg/ml. For the $Akita_{large}$ and $Akita_{small}$, all lung lobes received dornase alfa concentrations well above 2.9 µg/ml (Boxplot b). For the PARI-LC Plus and eFlow some patients received concentrations below 2.9 µg/ml in the right upper lobe, right middle lobe and left upper lobe. All patients received dornase alfa concentrations above 2.9 µg/ml in the lower lobes.

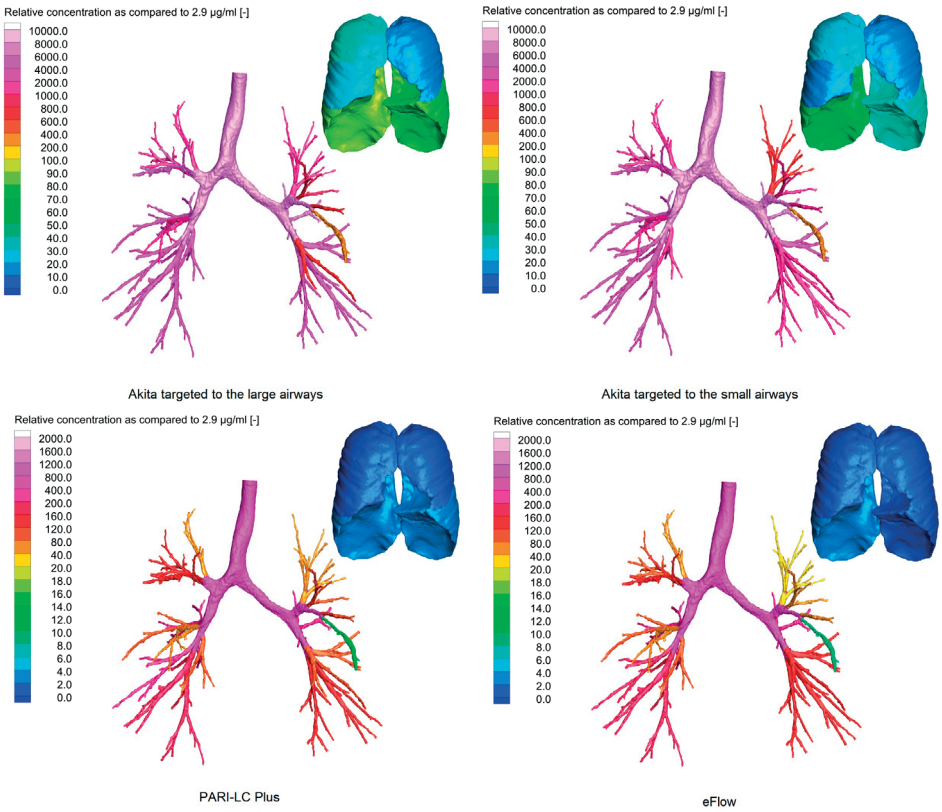

Figure 4 – Relative dornase alfa concentrations in large and small airways for 1 patient with the different nebulizers

Concentrations in large and small airways of a single patient are represented relative to the minimal effective dornase alfa concentration of 2.9 µg/ml for a median epithelial lining fluid (5 µm). Small airways concentrations of the 5 lung lobes are depicted in the figure of the segmented lung. The scale of the color map for the $Akita_{large}$ and $Akita_{small}$ is slightly different from the scale of the color map for the PARI-LC Plus and eFlow, but in all images, the darkest blue color represents concentrations at or below 2.9 µg/ml, concentrations increase above this level from light blue to green-yellow-orange to red-purple-white. From left to right the images show concentrations after nebulization with the $Akita_{large}$, $Akita_{small}$, PARI-LC Plus and eFlow. With the $Akita_{large}$ and $Akita_{small}$, all lung lobes received dornase alfa concentrations above 2.9 µg/ml. However, with the PARI-LC Plus this patient received concentrations below 2.9 µg/ml in the right middle lobe and left upper lobe. With the eFlow nebulizer concentrations were below 2.9 µg/ml in the right upper lobe, right middle lobe and left upper lobe.

DISCUSSION

This study aimed to investigate whether specific targeting of dornase alfa to the small airways using a smart nebulizer would result in sufficiently high concentrations in the small airways in patients differing in disease severity. Using CFD, we determined the per-

centage of airways that received suboptimal dornase alfa concentrations when dornase alfa was nebulized using a conventional PARI-LC Plus nebulizer, an eFlow nebulizer, or the smart Akita nebulizer set to target the large or small airways. The main finding was that all three nebulizers delivered doses to the large airways that were well above doses thought to be needed. However, with the Akita nebulizer set to target the large or small airways, very high dornase alfa concentrations were obtained in the small airways while concentrations for the PARI-LC Plus or eFlow nebulizers were in the critical range. These simulations support a previous clinical study that SAD in CF patients can be improved by increasing the delivered dose of dornase alfa to the small airways by using an efficient smart nebulizer.

Observed doses, especially as delivered by the Akita were well above the concentration of 2.9 μg/ml; a concentration proven to reduce effectively the viscoelasticity of CF sputum.[189] However, for the PARI-LC Plus and eFlow, dornase alfa concentrations were below 2.9 μg/ml in 4% and 8% of the total area of small airways, respectively. In the initial dose finding studies in the dornase alfa development program, doses up to 20 mg were investigated and were well tolerated. However, no clear differences were observed in effectiveness on spirometry parameters between 2.5 and 10 mg.[190] Similarly, in the pivotal trials, a once-daily dose of 2.5 mg was compared to twice-daily 2.5 mg.[184] No difference was observed in FEV_1 between these two dosing regimens, therefore once daily inhalation of 2.5 mg became the recommended dose for use. Later Geller *et al.* investigated, whether small particles of dornase alfa were more effective compared to conventional large particle delivery in a large randomized controlled trial in stable CF patients. Small particles were expected to enhance deposition in the small airways and reduce SAD. A trend was observed in patients treated with the smaller particles to have greater improvement in pulmonary function.[191] Based on these observations, Bakker *et al.* investigated dornase alfa inhalation in stable patients with CF and small airways disease, as diagnosed by reduced end-expiratory flows. These patients were already on maintenance treatment with dornase alfa, but were switched to the smart Akita nebulizer that allowed improved targeting of the small airways. Patients were randomized to receive either preferential large airway deposition or small airway deposition of dornase alfa.[25] In this study it was shown that small airway patency improved substantially and significantly in both study arms; moreover, a trend was observed towards greater improvement in the small airway deposition group. Our CFD findings support the conclusion that with the Akita nebulizer targeted to the large or small airways, high dornase alfa doses were delivered to the small airways compared to more conventional nebulizers. However, based on the clinical study by Bakker *et al.*,[25] we expected highest small airways concentrations with the Akita targeted to the *small* airways. In contrast, the Akita targeted to the large airways resulted in significantly higher dornase alfa concentrations in the large and small airways despite

the larger aerosol particle size. There might be several explanations for this discrepancy. Firstly, CFD computes deposited drug in the segmented airways and airways are segmented up to the 5^{th}-9^{th} generation.[63] Drug that bypasses the segmented airways is assumed to distribute homogeneously throughout the Phalen model. However, in real life it is likely that the $Akita_{small}$ settings result in deeper penetration of aerosols in the more distal small airways than the larger particles generated with the $Akita_{large}$ settings due to the smaller particles, larger inhalation volume and bolus of air at end-inspiration. Thus, we think that in the clinical trial by Bakker *et al.* higher dornase alfa concentrations were obtained in the distal airway generations for patients randomized to $Akita_{small}$ resulting in higher FEF_{75} after 2 and 4 weeks of treatment. Secondly, the patients in the clinical study had more severe lung disease, in particular substantially more SAD as measured with FEF_{75}, but also reduced FEV_1, than the patients from whom CT-scans were used for this CFD study. Reduced FEV_1 is associated with more severe structural changes with higher deposition of inhaled drug at sites of obstruction.[59] Smaller aerosol particles are more likely to bypass these obstructed airways. Thirdly, in the model, the aerosol particles generated by the $Akita_{large}$ are able to bypass the oropharyngeal region due to the extremely low flow rate of 60 ml/second (= 3.6 L/min). The higher flow rate of 200 ml/second (= 12 L/min) during nebulization with the $Akita_{small}$ resulted in higher deposition in the oropharyngeal region due to inertial impaction (Figure S4) relative to ultra-low flow during nebulization for the $Akita_{large}$. It has been shown *in vivo* that an extremely slow inhalation flow of 4.8 L/min was even able to reduce extra thoracic deposition of particles sized 9.5 μm when compared to tidal volume breathing of particles sized 5 μm.[192] It is however questionable whether in our clinical study patients were able to follow the sudden change in flow rate from 200 ml/second to 60 ml/second used for the $Akita_{large}$ settings. Hence, we think that our CFD simulations support our findings that a high dose of dornase alfa is important to reduce small airways obstruction but that it somewhat underestimates oropharyngeal deposition and therefore overestimates large and small airways concentrations compared to real life use for the $Akita_{large}$ settings.

Even though very high doses can be delivered to the small airways using the smart nebulizer it could be the case that, especially in advanced disease or at exacerbation, higher doses are required. In a second study by Bakker *et al.* no difference in efficacy could be observed when the efficacy of $Akita_{large}$ was compared to the $Akita_{small}$ in CF patients admitted for an exacerbation. However, in this study only 1.25 mg was used for safety reasons. According to our CFD results, this would still have resulted in small airways concentrations that were approximately 15-fold or 10-fold the dose obtained with conventional nebulizers with the $Akita_{large}$ and $Akita_{small}$, respectively. However, we feel that this dose was not sufficient in patients with exacerbations as concentrations of DNA were likely to be higher and obstruction more severe relative to the stable patient population as indicated by a FEV_1 of around 58% and FEF_{75} of 25% at the start of the

exacerbation.[94] It is well recognized that during a pulmonary exacerbation, there is a large influx of neutrophils into the airways. DNA is released from necrotic neutrophils resulting in increased DNA concentrations in the airways. Hence, in patients with exacerbations, higher lung dose of dornase alfa might be needed to enzymatically cut all free DNA in the sputum. Despite the negative study by Bakker *et al.* we think it likely that on an individual basis, higher doses of dornase alfa may be relevant. However, this needs to be further investigated in further studies using higher doses of dornase alfa.

Clearly there are limitations to our CFD study.[166] CFD remains our best estimate of the real-life situation but it currently still does not take into account many variable factors such as mucociliary and cough clearance that might play an important role *in vivo*. Secondly, assumptions had to be made regarding ELF thickness and effective dornase alfa concentrations. Finally, breathing profiles, the upper airway and airways with a diameter below 1-2 mm were not patient-specific.

CONCLUSIONS

In this study, using CFD simulations in a CT based airway model, we demonstrated that most airways received effective dornase alfa concentrations using different nebulizers. However, small airways concentrations with the Akita nebulizer were 17-30 times higher than with the PARI-LC Plus and eFlow nebulizer. For the latter two nebulizers, 4.3% and 8.0% of the lung lobes received concentrations below the effective dose of 2.9 µg/ml, respectively. Since the response to dornase alfa between patients is variable, increased doses of dornase alfa or switching to the efficient Akita nebulizer can be considered at an individual basis for patients not responding to standard doses. Our CFD observations support observations of our clinical study and support further studies in larger number of patients to assess safety and efficacy.

ACKNOWLEDGEMENTS

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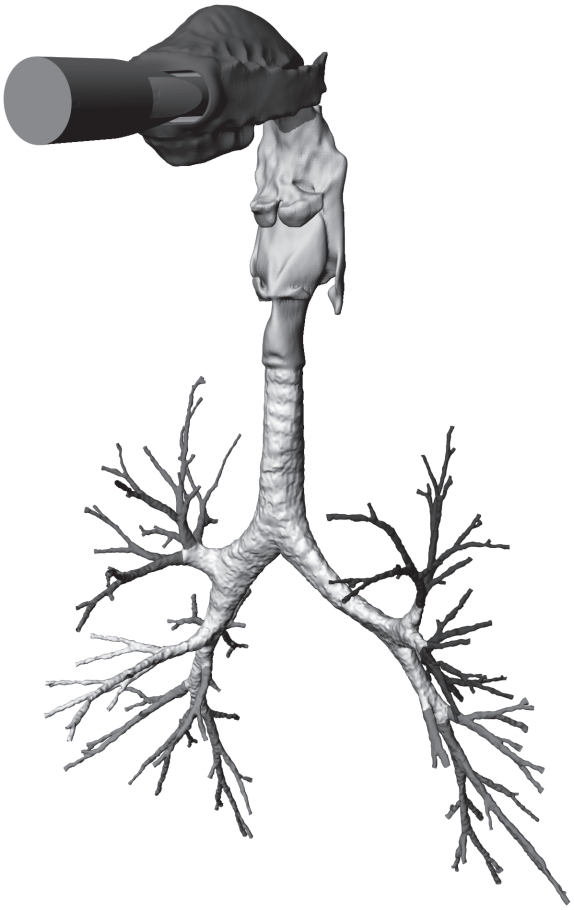

Figure S1 – Coupled mouthpiece/upper/lower airway model
Example of a coupled mouthpiece/upper/lower airway model subdivided in multiple regions. Airways are segmented up to the 5^{th}-9^{th} generation.

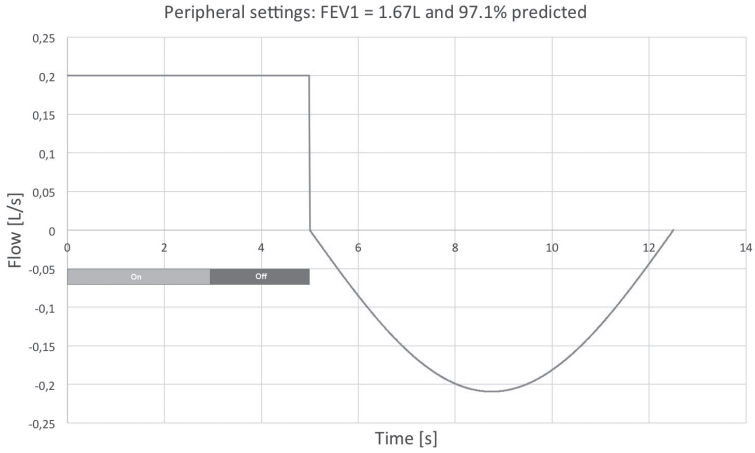

Figure S2 – Breathing profile with the Akita nebulizer targeted on the small airways
Breathing profile used for simulations with the Akita nebulizer targeted to the small airways ($Akita_{small}$). The inspiration part is stepwise and expiration part sinusoidal. Graph shows profile for FEV_1 of 1.67L with an inhalation time per breath of 5.0 seconds. On the x-axis time in seconds is shown and on the y-axis the inhalation flow rate in liters per second (L/s). Inhalation flow rate is fixed at 200 ml/second. Nebulization starts directly after start of inspiration and continues up to the last second of the inspiration (indicated in light grey). During the last second of the inspiration, a bolus of air without aerosol is inhaled (indicated in dark grey).

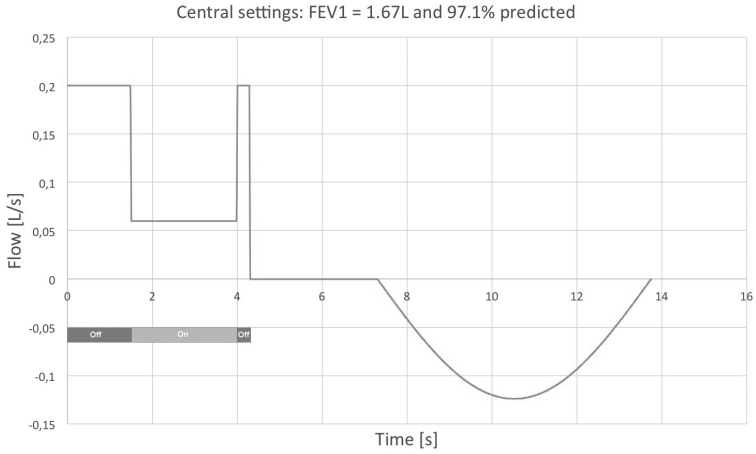

Figure S3 – Breathing profile with the Akita nebulizer targeted on the large airways
Breathing profile used for simulations with the Akita nebulizer targeted to the large airways ($Akita_{large}$). The inspiration part is stepwise and expiration part sinusoidal. Graph shows profile for an FEV_1 of 1.67L with an inhalation time per breath of 4.3 seconds. On the x-axis time in seconds and on the y-axis the inhalation flow rate in liters per second (L/s) are shown. Inhalation flow rate is 200 ml/second when nebulization is *off* and 60 ml/second when nebulization is *on*. Nebulization starts after 1.5 seconds, continues for 2.5 seconds and subsequently, during the last 0.3 seconds a bolus of air without aerosol was inhaled. This is followed by a breath-hold of 3.0 seconds. Parts of the inspiration with just air are indicated in dark grey, parts with aerosol nebulization are indicated in light grey.

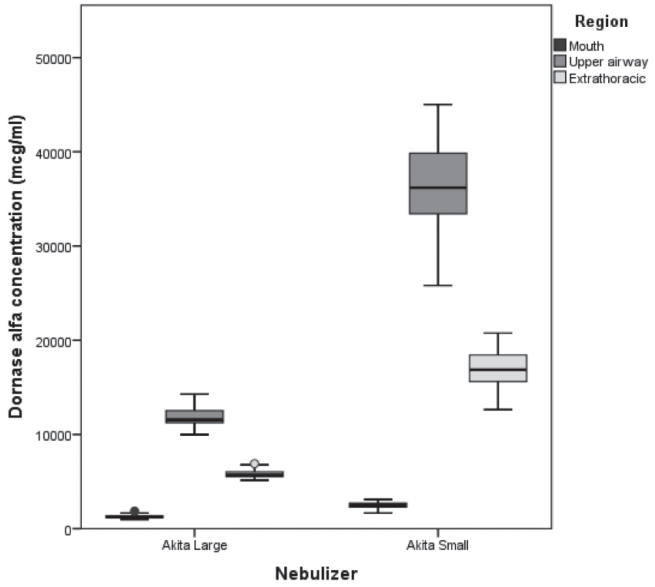

Figure S4 – Deposition of dornase alfa in the extrathoracic region with the Akita nebulizer
Dornase alfa concentrations in the mouth and upper airway calculated for the median epithelial lining fluid (5 µm) after inhalation with the $Akita_{large}$ and $Akita_{small}$. Extrathoracic concentration is a combination from the concentrations in the mouth and upper airway. The higher flow rate of 200 ml/second used with the $Akita_{small}$ results in much higher concentrations in the mouth and upper airway than the lower flow rate of 60 ml/second used with the $Akita_{large}$.

CHAPTER 7

Daily observations of nebuliser use and technique (DONUT) in children with cystic fibrosis

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ABSTRACT

Background: Cystic fibrosis (CF) caregivers focus on correct inhalation technique for nebulisers as this is essential to optimize efficacy of inhaled drugs. However, little is known on this nebuliser technique of patients at home.

Methods: Three "hidden" video registrations were made of 32 children with CF (6-18 years) nebulising at home. Videos were randomly scored on inhalation technique items using nebuliser-specific checklists and a total score was calculated.

Results: Median nebuliser technique was 91.9% of max score. Nebuliser technique was perfect (score 100%) in 23.3% of the patients and incorrect (score 0%) in 13.3%. Most mistakes were made in the required optimal breathing pattern.

Conclusion: Most CF patients had good nebuliser technique on a day-to-day basis. However, errors observed likely resulted in reduced treatment efficacy and, in 13%, no treatment at all. Regular "real life" evaluation by the CF-team can improve inhaled therapy substantially.

Presentations meetings: Data from the manuscript have been presented at the following meetings:

- 20th International Congress on Aerosols in Medicine and Pulmonary Drug Delivery (ISAM), May 30th –June 3rd 2015, Munich, Germany.[193]
- 28th North American Cystic Fibrosis Conference (NACFC), October 9-11 2014, Atlanta, USA.[194]

INTRODUCTION

New treatments lead to improved survival in patients with cystic fibrosis (CF).[195] Unfortunately, this improved survival comes at the cost of a complex treatment regime.[195,196] One to two hours per day are spent on inhalation therapy. This treatment burden poses significant challenges to adherence for patients. It is well recognized that the efficacy of inhalation therapy largely depends on the inhalation technique of patients. A poor inhalation technique reduces the amount of deposited drug at the site of action and thus reduces treatment efficacy. Since inhalation technique is thought to be poor and highly variable in most patients on a day-to-day basis,[25] CF health care workers focus on instructing correct inhalation technique. However, no research has been done to evaluate the efficacy of these instructions in CF patients.

Patient-related determinants and type of device might be important factors in poor inhalation technique. Many CF patients use different devices for inhalation therapy, most often nebulisers but also dry powder inhalers and pressurized metered dose inhalers. The conventional jet-nebuliser is relatively inefficient in delivering aerosols to the lungs, and is time-consuming, bulky and cumbersome. This may have a negative effect on adherence and inhalation technique. Over the last decade, new nebulisers were introduced which are faster, more portable and have improved lung deposition. Moreover, smart nebuliser technology became available, in which a patient is guided in a correct and deep inhalation. Examples of these are the I-neb and Akita nebulisers. Both nebulisers provide positive feedback signals to improve inhaler technique and allow objective monitoring of patient adherence. The Akita has shown to improve efficacy of inhaled dornase alpha by 70% compared with the standard jet-nebuliser.[25] This shows that control of inhalation technique is of clinical benefit and suggests that a lot can be gained by improving technique. However, it is not known what mistakes in technique are made on a daily basis by children with CF. Knowledge about the most common mistakes is needed to improve inhalation instructions.

Besides inhalation technique, correct cleaning, disinfection and maintenance of the nebulisers are part of good nebuliser use. Inadequate or infrequent cleaning can have a negative impact on particle size, increase nebulisation time and increase the infection risk.[197,198] From a study performed in asthmatic children we learned that video observations are a powerful tool to improve our understanding of what happens in the home situation.[199] Based on these observations instructions on inhalation therapy could be improved. To date such studies have never been done in CF.

In this study, our primary aim was to assess the inhalation technique of patients with CF using different nebulisers at home via video registrations. Secondary aims were to compare inhalation technique between different nebulisers and to assess the efficacy of current instructions and compliance with nebuliser cleaning, disinfection and maintenance.

METHODS

Study population

Patients, ages 6-18 years, were included in this prospective, observational study if they had a confirmed diagnosis of CF and used nebulisation therapy. Patients were recruited from the Erasmus MC-Sophia Children's Hospital, Rotterdam, the Netherlands. There were no exclusion criteria. This study was approved by the medical ethics committee of the Erasmus MC (MEC-2013-197) and registered within the Netherlands under number: NL44232.078.13. Written informed consent was obtained from the patients (if ≥ 12 years) and their parents prior to inclusion in the study.

Age, sex, FEV_1, educational level categorized according to the Dutch standard classification[200] and information regarding nebulisation therapy (overall duration, duration use current nebuliser, date last instruction, medication, number of treatments per day) were registered. FEV_1 was expressed as a percentage of predictive values, according to the prediction equations of the Global Lung Function Initiative.[201]

Nebulisers

Three nebulisers were used: I-neb (Philips Respironics, Chichester, UK), Akita-Jet combined with Pari LC Sprint nebuliser (Vectura GmbH, Gemünden, Germany), and Sidestream jet-nebuliser combined with Portaneb™ compressor (Philips Respironics, Chichester, UK). In the I-neb, vibrating mesh technology is incorporated in an Adaptive Aerosol Delivery system. Through continuous adaptation to the patient's breathing pattern, efficient aerosol delivery is achieved.[26] The Akita-Jet is a controlled-inhalation system using smart card technology to coach the patient on correct technique by directing the flow and depth of each inhalation. The I-neb and Akita-Jet are smart nebulisers that only deliver medication during inhalation. The Sidestream is an open vent jet-nebuliser that continuously releases aerosol during both inspiration and expiration.

Hidden video registrations

Inhalation technique was assessed by videotaping children at home while they were nebulising. The camera was hidden in a book (Fig. 1). Study duration was 1 week with home visits at the start and end. At the first home visit, parents were instructed. For patients, aged 6-11 years, parents placed the book in the room where the child would take the nebuliser therapy and made 3 videos while the child was nebulising (parental videos). Parents could choose the days and times during the study week. Children were not told about the videotaping by the investigators. For patients, aged 12-18 years, the same strategy was used. However, the adolescent had to give informed consent and was thus aware of the existence of the camera. We asked the parents to position the camera in a way that it was not directly visible during nebulisations. Patients used their own

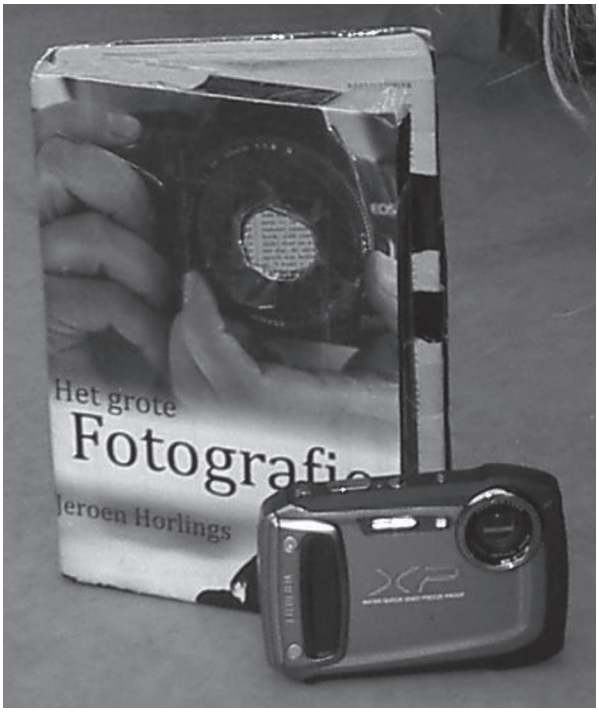

Figure 1 – Camera and book used for hidden video registrations

nebuliser and medication. All parents were instructed to continue the regular routine of nebulisation therapy. Thus, parents did only actively participate in the treatment if this was their regular routine.

At the second home visit the investigator recorded a fourth nebulisation session (investigator video), including the preparation and cleaning procedure. Finally, a survey regarding nebuliser cleaning, disinfection and maintenance routines was completed. Procedures were considered correct if they followed the recommendations of our CF centre (see Table S1, online supplement).

No education was provided until after completion of the study week. At the next scheduled outpatient clinic visit, feedback on inhalation technique was given by showing a video of the involved child to stress positive points as well as technique issues that required more attention.

Checklists

For each nebuliser a checklist was developed, based on items derived from product package inserts, containing all necessary performance steps for optimal inhalation (see Table S2, online supplement). A CF nurse (observer 1), respiratory nurse (observer 2) and pulmonary physician (observer 3) consented in scoring rules during 2 sessions of 2 h. The

videos of 2 patients were used for training and excluded for further analyses of inhalation technique. The surveys regarding cleaning procedure of these 2 patients remained included. Scoring rules were drawn up in a written consensus. The remaining videos were scored by all observers. Observers independently scored three video-recorded nebulisations per patient in random order. Randomisation of the videos was computer-generated with a block size of 10 patients. Each item could be scored as "correct", "doubtful" or "incorrect" (score of 2, 1 or 0, respectively). Time was also considered: an item was scored as correct if the item was performed correctly over two-thirds of the nebulisation time, and scored as doubtful or incorrect if performed correctly respectively over one- to two-thirds and less than one-third of the nebulisation time. In principle, the parental videos were scored. However, if a parental video was of poor quality, observers could score the investigator video instead. If the quality of more than 1 video was considered too poor, inhalation technique was calculated with the available scores. Intra-observer reliability was not evaluated because of the recall risk.

Inhalation technique score

As observers scored three nebulisations per patient, for each item on the checklist three scores were obtained (see Table S2, online supplement). From these 3 scores a mean score per item was calculated for each observer. Next, these mean scores were combined and divided by the number of observers to calculate the final mean score per item. A total score for inhalation technique was calculated with all items that directly affect lung deposition (see steps shown in Table 2). Critical items were given a higher weight. One critical item was defined for all nebulisers: "Place mouthpiece between teeth". A second critical item was defined for the jet-nebuliser: "Seal lips around mouthpiece". Calculation of the total score per device is shown in Fig. 2. Total score was expressed as a percentage of the maximal possible score (4 points) and ranged from incorrect to perfect inhalation technique (0 to 100% of maximal score). If the mean score on the item "Place mouthpiece between teeth" for a patient was ≤1, the patient received a total score of 0%.

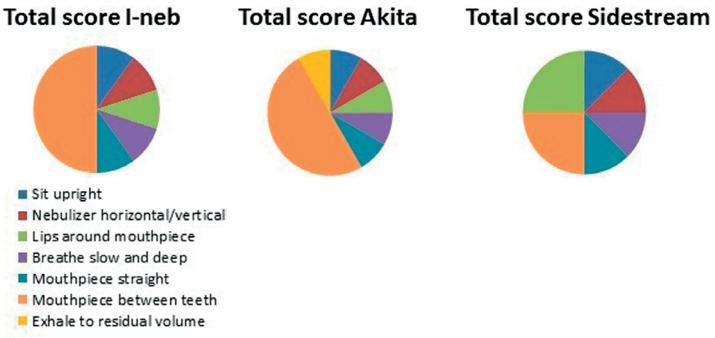

Figure 2 – Formulas used for calculation of total score per device

Statistical analysis

Approximately 80 patients met the inclusion criteria. We aimed to include 30 patients (15 aged 6-11 years and 15 aged 12-18 years). Patients were approached by telephone following the order of an alphabetical list. Patient characteristics were summarized using descriptive statistics. Inter-observer agreement was calculated using intraclass correlation coefficients (ICC), defined as moderate (0.4-0.6), good (0.6-0.8), and very good agreement (≥ 0.8). Scores on parental and investigator videos were compared using Wilcoxon Signed-Rank test.

Multivariable regression analysis was used to evaluate associations between patient characteristics and inhalation technique score. For the I-neb only, correlations between duration of nebulisation and inhalation technique score were investigated using Spearman's correlation coefficients (*r*). According to Cohen's criteria (1988), correlations are considered weak (0.10-0.29), moderate (0.30-0.49) or strong (≥ 0.50). Mixed-effects logistic models were used to investigate differences in mistakes between the nebulisers accounting for the correlation within the same patients.

Data are presented as median (range), unless stated otherwise. Significance level was set at 0.05. Descriptive statistics and Wilcoxon Signed-Rank test were analyzed with SPSS/PC Statistics 21.0 (SPSS Inc., Chicago, IL, USA). All other analyses were performed with the statistical software package R (free download from www.rproject.org) version 3.2.1.

RESULTS

A total of 32 patients were included. Out of 145 patients followed in our CF program, 82 patients were eligible for the study. We attempted to contact all 82 patients by phone until the target number of 32 participants was reached. Of the 50 patients that did not participate, 7 objected to the videotaping, 21 patients could not participate for logistic reasons; the remaining 22 patients could not be reached by phone before reaching the number of 32 participants. Included patients did not differ significantly from other eligible patients within the CF program with respect to disease severity (FEV_1, FVC or total days of intravenous treatment in 1 year). Patient characteristics are presented in Table 1. Most patients used their nebuliser once daily (81.3%) and for 3.0 years (0.0-7.6 years). Overall duration of nebuliser therapy was 7.6 years (3.6-13.0 years). Nebuliser parts were replaced every 3, 6 or 12 months, or as required (18.8%, 18.8%, 31.3% and 15.6%, respectively; 15.6% unknown). The last instruction on inhalation technique was given 1.6 years (0.0-7.6 years) before inclusion and was given by either the nebuliser distributor or CF nurse (53.1% and 46.9%, respectively).

Table 1 – Patient characteristics

	All	**<12 years**	**≥12 years**
N	32	17	15
Male gender (n, %)	15 (47)	9 (53)	6 (40)
Age in years (median, range)	11.5 (6-19)	7.6 (6-12)	14.3 (12-19)
FEV_1 %predicted (median, range)	94.4 (61-121)	107.9 (70-121)	89.5 (61-119)
Nebuliser			
- Akita (n, %)	5 (15.6)	-	5 (33.3)
- I-neb (n, %)	20 (62.5)	10 (58.8)	10 (66.7)
- Sidestream (n, %)	7 (21.9)	7 (41.2)	-
Aerosolized medication[a]			
- Pulmozyme (n, %)	32 (100)	17 (100)	15 (100)
- Hypertonic Saline (n, %)	3 (9.4)	-	3 (20)
- Antibiotic (tobramycin, ambisome or colistin) (n, %)	3 (9.4)	-	3 (20)
Educational level child[b]			
- Low (n, %)	30 (93.8)	17 (100)	13 (86.7)
- Mid-low (n, %)	2 (6.3)	-	2 (13.3)
- Mid-high	-	-	-
- High (n, %)	-	-	-
Educational level parents[b,c]			
- Low	1 (3.1)	1 (5.9)	-
- Mid-low	14 (43.8)	6 (35.3)	8 (53.3)
- Mid-high	8 (25.0)	7 (41.2)	1 (6.7)
- High	9 (28.1)	3 (17.6)	6 (40.0)
Where did you receive instructions regarding cleaning and maintenance?			
- CF nurse (n, %)	15 (46.9)	9 (52.9)	6 (40.0)
- Medical equipment company (n, %)	17 (53.1)	8 (47.1)	9 (60.0)

[a] *In these categories, some respondents checked more than one box.*
[b] *Categorized according to the Dutch standard classification into: (low): no education, primary school, lower vocational training, intermediate general school, ≤ 3 years at general secondary school; (middle-low): > 3 years secondary school, intermediate vocational training, 1st year of higher vocational training; (mid-high): higher vocational training; (high): university degree.*
[c] *Education level for parents based on the highest level of education of one of the parents.*

Two patients were used for the training sessions. Hence, the videos of 30 patients were used for scoring inhalation technique. A very good agreement was found for the total score between observer 1 vs observer 3; ICC 0.81. Observer 2 showed a moderate agreement with observer 1 and 3 (ICC 0.45 and 0.51 respectively). Moreover, observer 2 did not score all items for all patients. Consequently, the total score could not be calculated for 5 patients. For these reasons we excluded observer 2 for further analyses. The

step regarding correct exhalation showed a poor agreement between observer 1 and 3 (ICC 0.38), while the other steps showed a moderate to good agreement (all above 0.47). This item was considered less important for lung deposition than the other items. For this reason we excluded this item from the total score.

Both observers discarded videos because of poor quality. Observer 1 scored 83 videos in total and Observer 3 89 videos. More details on the number of videos reported as poor quality by each observer are shown in Fig. S1 of the online supplement. No significant differences in scores were found between the parental and investigator videos.

Median inhalation technique score was 91.9% of max score. Fig. 3 shows the differences in median inhalation technique score between nebulisers. These were not significantly different. Higher variability in scores on inhalation technique scores was seen for the Sidestream nebuliser than for the other nebulisers, illustrated by a higher coefficient of variation (Sidestream 78.4%; Akita 56.9%; I-neb 29.2%). This difference was significant between the Sidestream and I-neb nebuliser ($p < 0.001$).

Technique scores did not differ significantly between children < 12 years of age and children ≥ 12 years. Inhalation technique was not associated with other patient characteristics, time span since last instruction or duration of nebuliser use. A weak negative

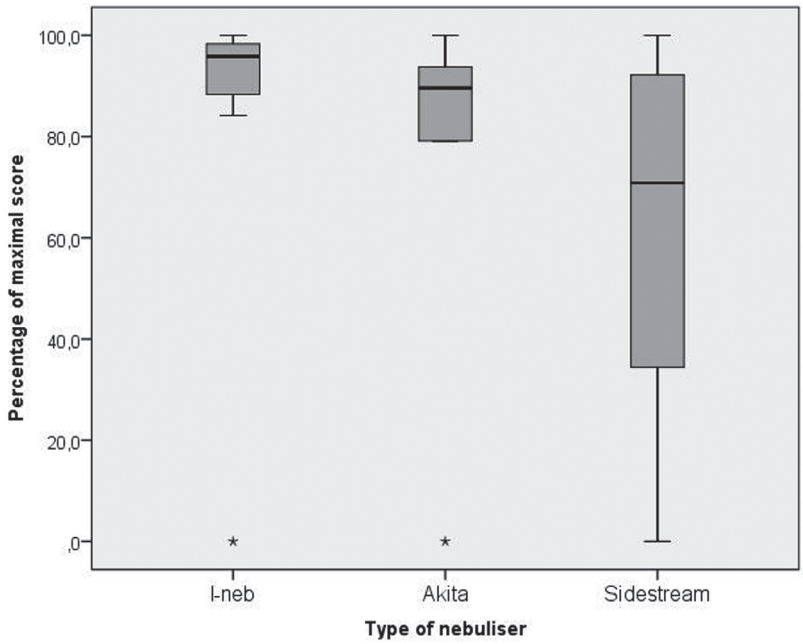

Figure 3 – Median score on inhalation technique per nebuliser

Median score on inhalation technique per nebuliser. Variability in scores was lower for the smart nebulisers than for the Sidestream nebuliser, illustrated by lower coefficients of variation: I-neb 29.2%; Akita 56.9%; Sidestream 78.4%. Number of patients with a score of 0%: I-neb n = 1; Akita n = 1; Sidestream n = 2.

association was found between overall duration of nebuliser therapy and inhalation technique (estimate - 0.382, SE 0.172, p = 0.037). Also, a moderate negative correlation was found for the I-neb between duration of the nebulisation session and inhalation technique (*r* = - 0.531, p = 0.023). However, when correcting for patient characteristics in a multivariable regression analysis, the association did not remain significant. Overall 23.3% of the patients had a perfect inhalation technique (score 100%) and 13.3% of the patients had an incorrect inhalation technique (score 0%). Most mistakes were made in the step regarding the breathing maneuver: "Take slow, deep breaths" (26.7%). Percentages of patients making mistakes per item analyzed per device and in total are presented in Table 2. Significantly more mistakes were seen with the Sidestream nebuliser compared to the I-neb (estimate - 2.329, SE 0.971, p = 0.017). No differences were seen between the Akita and other nebulisers.

Surveys of 32 patients could be used to assess compliance with our recommendations for nebuliser cleaning, disinfection and maintenance. Cleaning, disinfection and maintenance procedures were performed correctly by respectively 68.8%, 71.9% and 35.0% of the patients. More details on mistakes in these procedures are shown in table S3 of the online supplement.

Table 2 - Percentage of mistakes per step.

	I-neb n = 18	**Akita n = 5**	**Sidestream n = 7**	**Total n = 30**
Sit upright or stand	0.0	0.0	28.6	6.7
Hold nebuliser vertically/horizontally	0.0	20.0	28.6	10.0
Exhale to residual volume	NA	20.0	NA	NA
Place mouthpiece between teeth	11.1	20.0	42.9	20.0
Seal lips around mouthpiece	5.6	20.0	28.6	13.3
Take slow, deep breaths	27.8	40.0	14.3	26.7
Keep mouthpiece straight in the mouth	5.6	20.0	71.4	23.3
Exhalation correct: • I-neb and Sidestream: Breath out through the mouthpiece • Akita: Breath out next to mouthpiece	11.1	40.0	14.3	16.7

Mistake is defined as a mean score on a specific step ≤ 1. Abbreviations: NA = not applicable.

DISCUSSION

In this study, the average inhalation technique of CF nebuliser therapy in the home situation was very good. However, still many mistakes in inhalation technique were made, regardless of age and duration of nebuliser use. The video observations were of great value to evaluate daily practice and changed the policy within our CF centre. Now, an-

nually, a nebulisation session is shown within the outpatient clinic followed by direct positive feedback.

The good inhalation technique in this study shows that our instructions on nebuliser use were effective for most patients. Unfortunately, still one in seven children had an inhalation technique score of 0% on a day-to-day basis. The main mistake was that children often placed the mouthpiece in front instead of between the teeth which most likely resulted in low to absent lung deposition and thus in ineffective treatment.

Although mistakes differed between nebulisers, comparison of inhalation technique scores between nebulisers did not reach significance. We expected fewer mistakes with smart than conventional nebulisers. This was statistically shown for the I-neb but not for the Akita nebuliser. Also, there was a significant difference in the variability in scores for the I-neb compared to the jet-nebuliser, indicating that the inhalation technique was more consistent in the I-neb. The difference between the Akita and jet-nebuliser was not significant. This might be due to the small number of included patients for both of these nebulisers. So we cannot draw firm conclusions on this. Differences in mistakes for the positioning of the mouthpiece might depend on the shape/size of the mouthpieces. All 3 nebulisers have a different shape of the mouthpiece, with the I-neb being the smallest (Fig. S2). Smart nebulisers, when used correctly, have the potential to achieve a higher lung deposition reaching up to 60-80% of the loading dose, compared to 5-15% with conventional nebulisers. Furthermore, they have the ability to target drugs to the small airways more efficiently.[25,71,202,203] However, the most common mistakes like an incorrect breathing maneuver and incorrect position of the mouthpiece in the mouth could be made for the conventional nebulisers as well as for the smart nebulisers. Hence, smart nebulisers require as much supervision as conventional nebulisers as mistakes that reduce the efficiency of aerosol delivery can still be made.

For asthma or chronic obstructive pulmonary disease, it is known that in general technique related to inhalation therapy is poor. A wide range, between 4 and 94%, of incorrect technique has been observed.[204-209] It was shown that repeated instructions were effective to improve inhalation technique.[32,210,211] However, even after instructions, up to 50% of these patients still made mistakes.[32,205,209,210] In these populations inhalation technique differed significantly between devices[205,206,209] and some studies reported associations with patient-related determinants like gender,[212-214] age,[205] educational level[209,215] or disease severity.[216] Hence, compared to these studies our patients seem to perform relatively well reflecting the result of a continuous focus by the CF team on inhalation technique. Whether initial training by the CF team or nebuliser distributor makes a difference could be a subject of future studies.

Apart from inhalation technique, nebuliser cleaning and maintenance routines influence the effect of treatment. Patients who regularly clean their nebulisers are less likely to develop bacterial infections.[217] In addition, regular maintenance is important

to maintain nebuliser function. The compliance with cleaning and disinfection recommendations in the present study was high. However, recommendations regarding weekly maintenance of the I-neb were poorly adhered to. Lack of maintenance can result in clogging of the apertures of the vibrating mesh. This leads to increased nebulisation time which is undesirable for patient compliance.[75]

Limitations

We could not use a validated instrument to assess technique as this was the first study that used video observations to investigate nebuliser inhalation technique of CF patients at home. However, since we developed device-specific checklists, used scoring rules defined by specialists in the field and scored three videos per patient, we think that we have provided a reliable method to assess inhalation technique for nebulisers used by patients with CF. Moreover, a very good agreement was seen between observer 1 and 3.

We decided to exclude observer 2 based on the moderate ICCs with observer 1 and 3 respectively while the ICCs between observer 1 and 3 were good. These lower ICCs could be explained by the fact that observer 2 is specialized in asthma, while the other 2 observers are specialized in CF. Therefore, observer 2 was not well familiar with the smart CF nebulisers used in the study and felt insecure about how to score various items. For this reason she decided not to score all items in 5 out of the 30 patients. Importantly, excluding observer 2 did not impact upon the key conclusions of the article.

Even though parents were carefully instructed in the ideal camera position and room lighting, the quality of 13-17% of the videos was considered to be poor. This could have been prevented if the investigator would have recorded all videos ensuring a well-lit room with the same camera position. However, the aim of this study was to objectively explore the inhalation technique of CF nebuliser therapy in daily life. We expected children to change their daily nebulisation routine if investigators recorded the videos. The hidden camera allowed parents to walk away and continue their daily routine, allowing children to forget about the camera. This made more objective assessment of inhalation technique possible. For future studies training of observers can be improved by developing well standardized, more intensive training sessions that include more videos and training sets.

This was a single-centre study with a small sample size. Also, parents could choose 3 nebulisations in the week they received the camera. Theoretically they could have deleted registrations showing poor technique earlier in the week and keep the recording of a better treatment session. Although some selection bias can have occurred, that is likely to have improved the inhalation technique score. Hence, we might have overestimated to some extent the number of patients with a perfect technique and underestimated the number of patients with poor technique. Our findings emphasize the importance of regularly checking the inhalation technique.

In conclusion, the average inhalation technique of CF patients on a day-to-day basis is very good. However, the poor technique observed in one in seven children likely results in poor to absent aerosol deposition in the lungs and thus reduced treatment efficacy. An incorrect inhalation technique may be a reason for treatment failure. Therefore, we recommend evaluation of inhalation technique shortly after initiating nebulisation treatment and at the annual check-up by recording videos of nebulisation sessions with smart phones at home or by a live nebulisation session at an outpatient clinic visit followed by feedback by the CF team.

ACKNOWLEDGEMENTS

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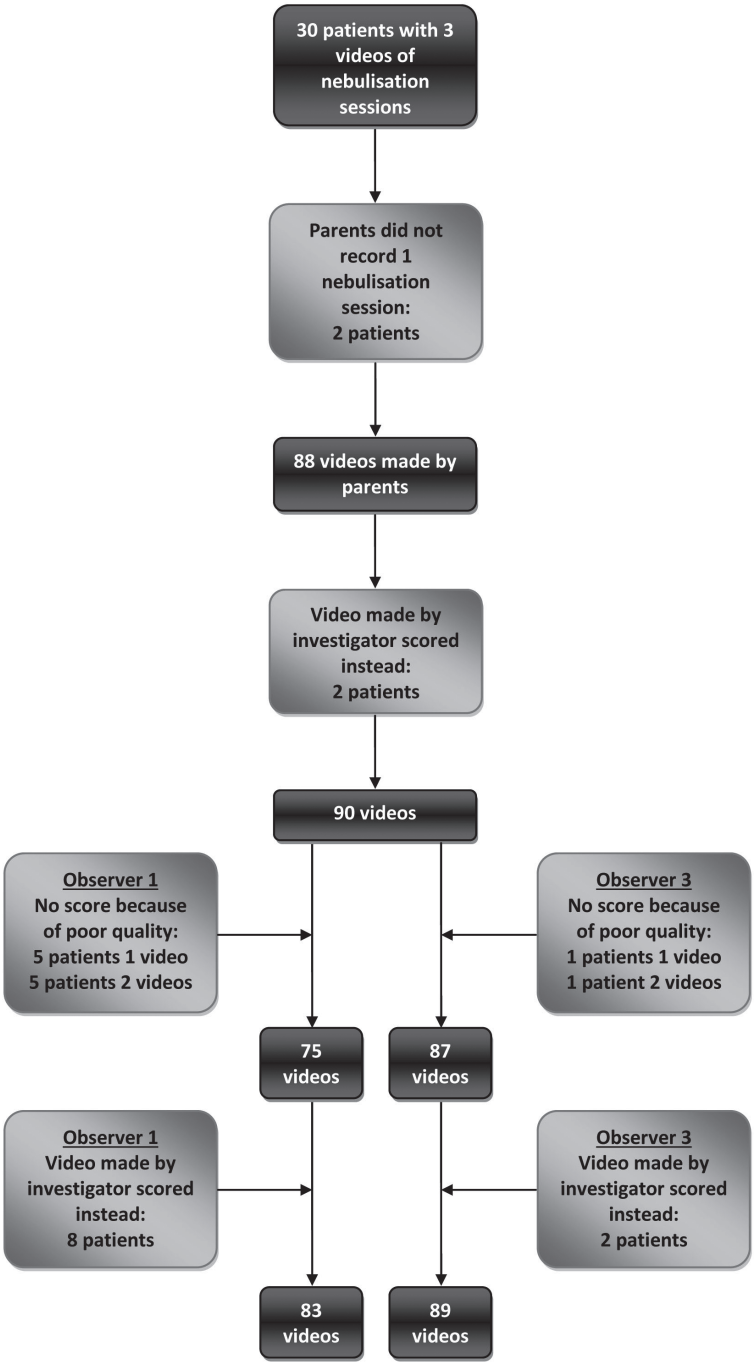

Figure S1 – Flowchart of total number of videos that were scored by each observer

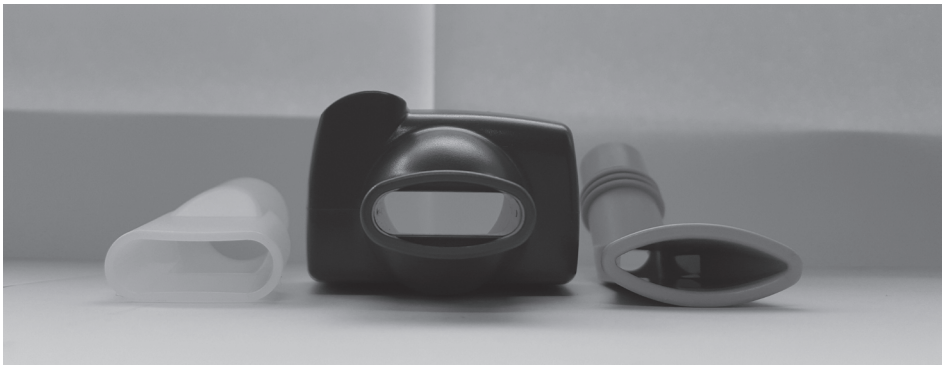

Figure S2 – Shapes of the nebuliser mouthpieces used in the study
From left to right this picture shows the mouthpiece of the Sidestream, I-neb and Akita® nebuliser.

Table S1 - Cleaning recommendations for nebulisers

Procedure	Frequency	Instruction
Cleaning	After every inhalation session	- Having finished the inhalation, pour possible drug residues out of the nebuliser. - Clean directly after use. - Dismantle the nebuliser and wash its different parts. - Use lukewarm tap water and a non-perfumed detergent. - Rinse carefully afterwards. - Shake off as many water droplets as possible. - Air-dry and store dry!
Disinfection	Once daily	- Put the different parts in 70% alcohol for 10 min. - Air-dry. - If necessary rinse in lukewarm water just before the next inhalation session. - Alcohol can be used for 1 week.
Maintenance*	Once weekly	- Put the equipment in boiling water with detergent/vinegar for 10 min. - Air-dry.

Recommendations regarding cleaning, disinfection and maintenance routines for nebulisers of the Erasmus MC-Sophia CF centre.
**Only applicable to I-neb nebuliser.*

Table S2 – Nebuliser checklists for scoring of video registrations

	Score movie 1	Score movie 2	Score movie 3
Items on checklists of all three nebulisers			
1. Transfer medication into drug container			
2. Correctly put nebuliser together			
3. Turn on nebuliser			
4. Sit upright or stand			
5. Hold nebuliser: o I-neb: horizontally o Akita: vertically o Sidestream: vertically			
6. Place mouthpiece between teeth			
7. Seal lips around mouthpiece			
8. Breathing manoeuvre: o I-neb: Take slow, deep breaths o Akita: Take slow, deep breaths o Sidestream: Breath slowly in and out through the mouthpiece			
9. Keep mouthpiece straight in the mouth			
10. Continue with nebulisation until: o I-neb: buzzer sounds o Akita: smart card has counted down to 0 breaths left o Sidestream: nebuliser starts to "sputter"			
Additional item on checklist I-neb			
- Breath in and out through the mouthpiece (After step 8)			
Additional items on checklist Akita			
- Exhale to residual volume (After step 5)			
- Follow instructions shown on display until inhalation is correct (after step 9)			
- Breath out next to mouthpiece (after step above/before step 10)			
Additional items on checklist Sidestream			
- Do not lay Sidestream down on its side, medication will spill out (after step 2)			
- Make sure mist is visibly coming from the mouthpiece/mask (after step 3)			

Nebuliser checklists for scoring of the video registrations containing all necessary performance steps for optimal inhalation. Observers scored three video-recorded nebulisations per patient yielding three scores per item.

Table S3 - Mistakes in cleaning, disinfection and weekly maintenance procedures

	%
Mistakes in cleaning*	**31.3**
- No washing in soapy water	15.6
- No direct cleaning after use	6.3
- Only tap water rinse	6.3
- Dishwasher	3.1
- Dry with kitchen towel	6.3
Mistakes in disinfection	**28.1**
- Alcohol 70% > 10 minutes	21.9
- Alcohol 70% only once a week	6.3
Mistakes in weekly maintenance**	**65.0**
- No maintenance	45.0
- Boiling water once a day	5.0
- Boiling water once in 2 weeks	5.0
- Boiling water once a month	10.0

Mistakes made by patients in the recommended cleaning, disinfection and maintenance routines for nebulisers.

** In this category, some patients made more than one mistake*

*** Only applicable to patients using the I-neb nebuliser (n=20)*

CHAPTER 8

Pharmacokinetics and tolerability of once daily double dose tobramycin inhalation in cystic fibrosis using controlled and conventional nebulization

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ABSTRACT

Background: Better treatment outcomes in cystic fibrosis (CF) may be expected by changing standard twice daily (BID) tobramycin inhalation with the conventional nebulizer to once daily (OD) inhalation at double the standard BID dose with a controlled-inhalation nebulizer. We aimed to determine the pharmacokinetics and tolerability of inhaled double-dose tobramycin with the controlled-inhalation AKITA® and conventional PARI-LC® Plus nebulizer in patients with CF.

Methods: Randomized, open label, crossover study. Pharmacokinetics were assessed in 10 adult CF patients following inhalation of tobramycin (Bramitob®) at double the recommended BID dose with the AKITA (300 mg fill dose) and PARI-LC Plus (600 mg fill dose).

Results: No significant differences were found in pharmacokinetic parameters between the two nebulizers. Median maximum serum levels were 3.44 (2.25-5.49) and 2.84 (0.82-6.63) mg/L for AKITA and PARI-LC Plus, respectively. Trough serum levels were very low for both nebulizers: 0.03 (0.00-0.09) and 0.02 (0.00-0.06) mg/L for AKITA and PARI-LC Plus, respectively. Time to maximum level was comparable: 0.44 (0.08-0.96) and 0.40 (0.08-0.96) hours for AKITA® and PARI-LC® Plus, respectively. Serum levels were well below the toxic limit. Inhalations were well tolerated and no serious adverse events occurred. Nebulization time was 33% shorter with the AKITA®.

Conclusions: OD tobramycin inhalation of the double standard BID dose with a controlled-inhalation and conventional nebulizer resulted in similar pharmacokinetics in the doses given, with serum levels below the toxic limit. Further research demonstrating clinical efficacy and safety of this treatment approach is required.

Dutch trial register number NTR4525.

INTRODUCTION

Small airways disease (SAD), mainly caused by chronic infections with *Pseudomonas aeruginosa (Pa)*, plays an important role in the pathophysiology of cystic fibrosis (CF).[5,25] *Pa* infections are often treated with inhaled tobramycin. Unfortunately, small airways are difficult to target with standard nebulizer therapy. With the controlled-inhalation AKITA nebulizer, lung deposition of 70% of the loaded dose is reached, compared to approximately 10-15% with standard nebulizer therapy.[203,218,219] Moreover, nebulization with the AKITA results in an increased deposition in the small airways,[24] which might lead to improved treatment of SAD.[25]

Current standard therapy of chronic *Pa* lung infection requires twice daily (BID) 300 mg tobramycin inhalation with the conventional PARI-LC Plus nebulizer. Nevertheless, various dosage regimens were investigated, with dosages ranging from 80 mg twice daily to 600 mg thrice daily,[12,13] and other nebulizers than the standard nebulizer are used in daily practice. The bactericidal efficacy of tobramycin is known to be concentration-dependent and there is a post-antibiotic effect.[54] As a consequence, inhalation of the complete daily dose in a single inhalation may have advantages over delivering the dose in two sessions and might also reduce adaptive resistance. For intravenous use, tobramycin once daily (OD) has already been shown to be as effective as thrice daily administration,[181] and to result in less toxicity.[182] Specific targeting of SAD with the AKITA nebulizer in routine CF care may improve bacterial killing even better by delivering a higher fraction of the drug in the targeted lung region. Moreover, OD instead of BID inhalation will most likely improve adherence. Therefore, changing standard BID tobramycin nebulization to OD nebulization with the AKITA nebulizer might lead to better treatment outcomes in patients with CF.

However, tobramycin is potentially toxic with a narrow therapeutic index, and high trough levels can lead to nephro- and ototoxicity. Although this risk is considerably lower for nebulized compared to parenteral administered tobramycin, since only 9%-17.5% of the nominal dose is systemically absorbed after inhalation,[12] doubling the dose can theoretically result in higher serum levels and there are some case reports available of inhaled tobramycin related toxicity.[220-223]

It is not entirely clear if increased deposition in the small airways with the AKITA does have a significant impact on pharmacokinetics, as tobramycin is likely to be absorbed with similar kinetics from the (large) airways and alveoli. Therefore, before initiating a long-term efficacy and safety study investigating OD tobramycin inhalation at double the standard BID dose in patients with CF, it is necessary to determine pharmacokinetics and tolerability of this dosing scheme first. To our knowledge, OD dosing of inhaled tobramycin delivered by the PARI-LC Plus or AKITA nebulizer has never been studied. Two studies investigated the pharmacokinetics of a single inhalation of 600 mg tobramycin in

patients with CF.[224,225] However, these studies used different nebulizers and tobramycin solutions compared to the present study, so data cannot be simply extrapolated.

The aim of this study was to determine the pharmacokinetics and tolerability of OD inhalation of the double recommended tobramycin dose with the controlled-inhalation AKITA and the conventional PARI-LC Plus nebulizer in patients with CF.

MATERIALS AND METHODS

Patients and study design

In a randomized, open-label, crossover study, pharmacokinetics of inhaled tobramycin were assessed in 10 consecutively selected adult CF patients, following a single inhalation of the double recommended dose with the conventional PARI-LC Plus and the controlled-inhalation AKITA nebulizer. The study consisted of two separate visits for tobramycin inhalation in random order, separated by at least a week washout period.

The study was performed in the Centre for Cystic Fibrosis, Haga Teaching Hospital, The Hague, The Netherlands, between March and June 2014. Inclusion criteria were: age 18 years or older, confirmed diagnosis of CF (genetic analysis) and chronic *Pa* infection. Exclusion criteria were: acute pulmonary exacerbation requiring intravenous treatment; intravenous tobramycin; aminoglycoside hypersensitivity; impaired renal function (estimated glomerular filtration rate (eGFR) <60 ml/min); use of loop diuretics; and pregnancy or lactation. Chronic tobramycin inhalation therapy was stopped at least 3 days prior to the first study visit.

This work was approved by the local ethics committee (METC Zuidwest Holland, The Netherlands, METC nr 13-097), the Central Committee on Research Involving Human Subjects (CCMO, The Hague, The Netherlands) and was conducted according to the principles of the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from all patients.

Nebulization

Bramitob (4 mL=300 mg tobramycin, Chiesi Pharmaceuticals B.V., Rijswijk, The Netherlands) was used as investigational drug. Bramitob is licensed for use with the PARI-LC Plus nebulizer, with a recommended fill dose of 300 mg (BID). Dose recommendation for the AKITA is 150 mg tobramycin for inhalation (BID). This is based on *in vivo* study data that showed an equal predicted lung dose when comparing filling doses of 150 mg tobramycin with the AKITA to 300 mg with the PARI-LC Plus. This corresponded to nebulized doses of 60 mg with the AKITA and 240 mg with the PARI-LC® Plus.[226]

In the present study, patients inhaled the double recommended tobramycin dose in one inhalation, corresponding to 300 mg with the AKITA and 600 mg with the PARI-LC

Plus nebulizer. Taking deposition rates of 70% of the nebulized dose for the AKITA and 15% for the PARI-LC Plus nebulizer, we expected this to relate to a deposited dose of 84 mg for the AKITA and 72 mg for the PARI-LC Plus.

The PARI-LC Plus (PARI GmbH, Starnberg, Germany) is a reusable breath-enhanced jet nebulizer that was combined with the Portaneb™ compressor (Philips Respironics, Chichester, UK). Patients operated the compressor and inhaled until "sputtering." The AKITA system (combined with a PARI-LC SPRINT nebulizer, Vectura GmbH, Gemünden, Germany) is a controlled-inhalation device that allows aerosol production during inspiration only. The AKITA directs the flow and depth of each inhalation by coaching the patient in correct inhalation technique. In this study, the device was programmed to preferentially target the small airways. Prior to the actual controlled inhalation, patients were trained on how to inhale with the AKITA nebulizer. The inhalation volume was individualized for each patient and was stored on an individual smart card, which was inserted into the device. Inhalation flow rate was fixed at 200 mL/second. The patient received constant feedback on his/her inhalation performance to maintain a slow and deep inhalation, which allowed improved small airways targeting.

Nebulization time, defined as the time from turning on the compressor until "sputtering" (PARI-LC Plus) or starting and switching off the device (AKITA) was measured.

Spirometry

Spirometry (Jaeger Masterscreen PFT, CareFusion, Hoechberg, Germany) was performed before and 15 minutes after inhalation according to the ATS/ERS guidelines.[159] Measured parameters were: forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC) and forced expiratory flow 25–75% (FEF_{25-75}). Prediction equations of the Global Lung Function Initiative were used (Quanjer GLI-2012 equations).[201] Post dose measurements were compared to baseline values to detect potential bronchospasm following high dose tobramycin inhalation. Clinically significant bronchospasm was defined by a 20% or more reduction in FEV_1.[11,146]

Blood sampling

A venous blood sample was taken before inhalation to measure kidney function. Dried blood spots (DBS) using a finger prick were collected before (t=0) and 30, 60 and 90 minutes after completion of inhalation for tobramycin analysis. Patients collected additional dried blood spots at home, 3 and 24 hours after inhalation.[227] To prevent contamination of tobramycin on the blood spot paper or the patient's fingers, several precautions were taken: 1) patients were not allowed to fill their nebulizers with medication themselves, 2) nebulization was performed in a different room than where the blood spots were collected, 3) before each finger prick, patients had to wash their hands thoroughly with warm water and soap.

Tobramycin analysis

Tobramycin was measured in the blood samples with a DBS assay using high-performance liquid chromatography (HPLC)-tandem mass spectrometry (MS/MS).[228] The lower limit of quantification (LLOQ) was 0.1 mg/L with a coefficient of variation of <2.2%. The DBS assay was previously developed in our laboratory and validated with venous blood samples from CF patients.

Pharmacokinetic analysis

Individual pharmacokinetic parameters were calculated and assimilated with patient tobramycin serum values using a computerized CF-based two-compartment population pharmacokinetic model (MW-Pharm version 3.60, Mediware, Groningen, The Netherlands).[229] Data on the patients' gender, age, height, weight, kidney function, and tobramycin dosages were used to calculate the following parameters: maximum serum level (C_{max}), trough serum level 24 hours after nebulization (C_{trough}), time to C_{max} (T_{max}) and area under the concentration-time curve from 0 to 24 hours ($AUC_{0\text{-}24hr}$).

Systemic absorption can be used as safety signal. Based on once daily intravenous administration, a tobramycin trough level below 1 mg/L is considered to be safe.[181] No clear toxic limits have been defined for tobramycin inhalation yet.

Tolerability and nebulizer satisfaction

Adverse events were monitored. Furthermore, patients were asked to answer questions regarding tolerability of the two inhalations and nebulizer satisfaction.

Statistical analysis

Statistical analysis was performed with SPSS version 17.0 (PASW Statistics, IBM Corporation, Armonk, USA). Paired *t*-tests were used to compare differences between the two nebulizers and study visits. The Wilcoxon Signed Ranks Test was used when data were not normally distributed. *P* values below 0.05 were considered statistically significant.

RESULTS

Patients

Ten patients with CF were included in the study (Table 1). Overall, there were no significant differences in eGFR, FEV_1, FVC and $FEF_{25\text{-}75}$% predicted between the two study visits (respectively, p=0.557, p=0.702, p=0.102 and p=0.414).

Table 1. Patient characteristics

Patient	Sex	Age (year)	Height (cm)	Weight (kg)	eGFR[a] (ml/min)	FEV_1[a] (% predicted)	FEF_{25-75}[a] (% predicted)
1	M	28	175	72	118	86	64
2	M	59	188	93	83	70	56
3	M	36	181	68	126	28	7
4	F	52	163	50	91	53	22
5	M	29	180	65	91	36	10
6	M	23	188	73	137	90	78
7	M	50	179	60	106	52	18
8	F	26	166	55	93	79	43
9	M	22	180	66	94	71	68
10	F	36	157	59	128	61	19
Median		32.5	179.5	65.5	100.0	65.7	32.4
Range		22-59	157-188	50-93	83-137	28-90	7-78

eGFR, estimated glomerular filtration rate based on Modification of Diet in Renal Disease (MDRD) equation; FEV_1, forced expiratory volume in the first second; FEF_{25-75}, forced expiratory flow 25-75%.
[a]Average baseline values of study visit 1 and 2.

Pharmacokinetics

The individual calculated pharmacokinetic parameters are summarized in Table 2. No significant differences were found in C_{max}, C_{trough}, T_{max} and AUC_{0-24h} between the controlled and conventional inhalation. Lower variability in serum concentration was found with the AKITA, illustrated by a lower coefficient of variation (Table 2). C_{trough} was well below the toxic limit for both nebulizers and for all patients. Figure 1 shows the serum concentration-time curves for individual patients after tobramycin nebulization with both nebulizers.

Nebulization time

Mean nebulization time was significantly shorter (p=0.005) with the AKITA (19.20±7.50 min) compared to the PARI-LC Plus (29.50±4.85 min). Except for Patient 2, all patients had a shorter treatment time with the AKITA. During observation, it was noticed that Patient 2 did not actively inhale with the AKITA all the time. This explains the higher treatment time since aerosol is only delivered when the patient is actively inhaling and until the preset dose is attained.

Tolerability

Overall, no clinically relevant declines in FEV_1 values were seen 15 min after completion of nebulization. Individual changes in FEV_1 values from baseline are shown in the Supplementary Table S1.

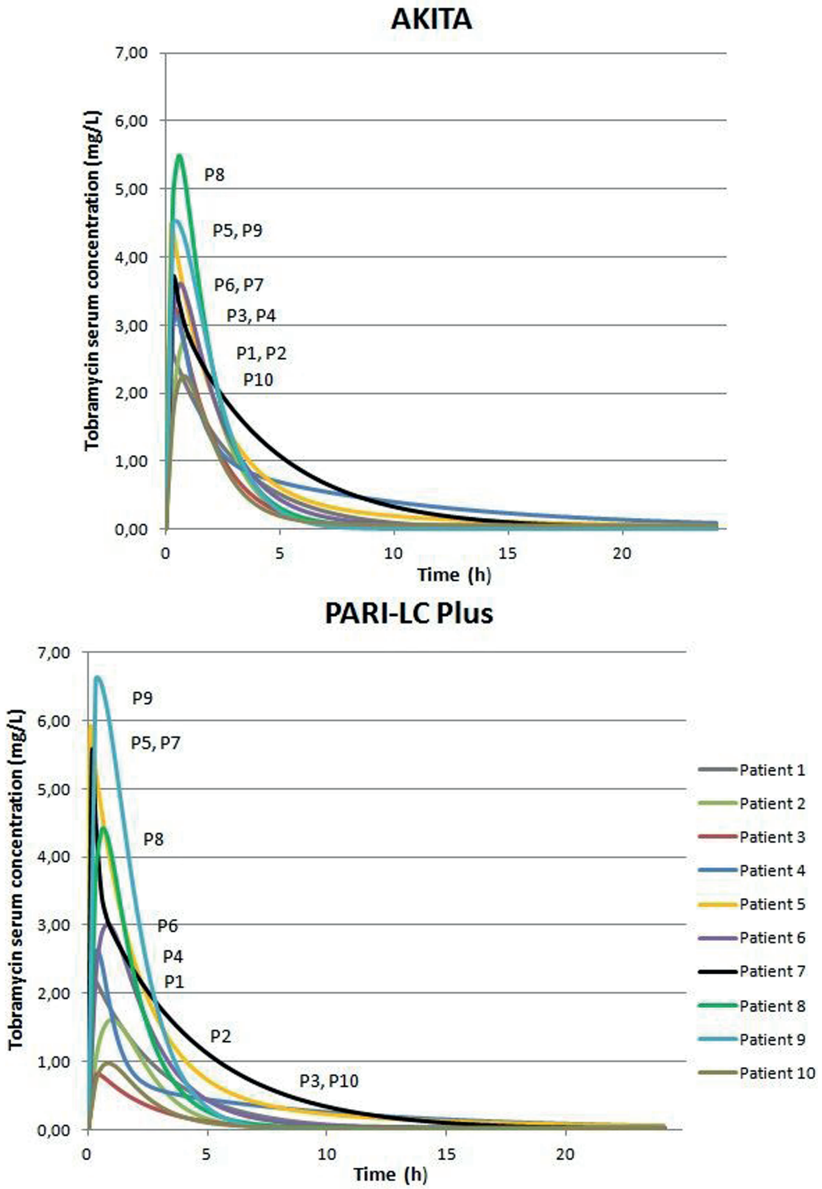

Figure 1. Serum concentration-time curves for all patients after tobramycin inhalation with the two nebulizers. P, patient number; number position in the graph corresponds with maximum serum concentration reached.

Nebulizations of tobramycin were well tolerated by all patients with both nebulizers and no major adverse events were reported (Table 3). Patients reported more side effects during nebulization with the PARI-LC Plus than with the AKITA. Dislike of taste

Table 2. Pharmacokinetic results after controlled inhalation (AKITA®; 300 mg fill dose) and conventional inhalation (PARI-LC® Plus; 600 mg fill dose)

Patient	AKITA®				PARI-LC® Plus			
	C_{max} (mg/L)	C_{trough} (mg/L)	T_{max} (h)	AUC_{0-24h} (h.mg/L)	C_{max} (mg/L)	C_{trough} (mg/L)	T_{max} (h)	AUC_{0-24h} (h.mg/L)
1	2.71	0.00	0.16	9.57	2.19	0.00	0.24	8.17
2	2.85	0.04	0.96	9.08	1.61	0.02	0.96	5.36
3	3.26	0.00	0.40	9.40	0.82	0.00	0.40	3.42
4	3.14	0.09	0.48	14.05	2.65	0.06	0.40	12.04
5	4.48	0.05	0.24	12.90	5.85	0.05	0.08	15.31
6	3.61	0.03	0.64	10.67	3.02	0.03	0.80	9.26
7	3.69	0.01	0.32	14.83	5.46	0.01	0.16	17.65
8	5.49	0.03	0.56	12.76	4.42	0.03	0.64	11.15
9	4.53	0.00	0.40	11.74	6.63	0.00	0.40	16.14
10	2.25	0.05	0.80	7.21	0.97	0.02	0.80	3.62
Median	3.44	0.03	0.44	11.21	2.84	0.02	0.40	10.21
Range	2.25-5.49	0.00-0.09	0.08-0.96	7.21-14.83	0.82-6.63	0.00-0.06	0.08-0.96	3.42-17.65
CV (%)	27.34	96.86	50.32	21.73	62.42	95.35	61.07	50.45
p	0.721	0.102	0.748	0.445				

C_{max}, maximum serum level; C_{trough}, trough serum level, 24h after nebulization; T_{max}, time to maximum serum level; AUC_{0-24h}, area under the concentration-time curve from 0 to 24h.
CV, coefficient of variation. P values were calculated with the Wilcoxon Signed Ranks Test.

was reported most often (60%) for this nebulizer. One patient reported mild dyspnea, however, he did not have a significant change in lung function measurements after nebulization (FEV_1% predicted 78% before vs. 76% after nebulization).

Patient 3 suffered from a viral infection at the second study visit (PARI-LC Plus) with increased cough, sputum and mild deterioration of lung function (FEV_1% predicted 30% at visit 1 vs. 26% at visit 2). He did not require intravenous treatment and thus did not have to be excluded from the study. However, to prevent bronchospasm on top of his cold, salbutamol was administered before start of tobramycin nebulization of the study medication. The nebulization was well tolerated and his post dose FEV_1 measurement remained the same as at baseline.

Nebulizer satisfaction

Responses on the questions regarding nebulizer satisfaction are shown in Table 3. Patients tended to be more positive for the AKITA than for the PARI-LC Plus. However, most patients preferred their own inhalation device for tobramycin over one of the study nebulizers (90%). Inhalation devices used at home were the I-neb (40%), eFlow (40%) and TOBI Podhaler (20%). One patient (10%) preferred the PARI-LC® Plus over his own device for tobramycin inhalation. This patient already used the PARI-LC® Plus for home

Table 3. Questions and responses about tolerability of two inhalations and satisfaction of nebulizers

1. How was the nebulization session tolerated?	▫ Well tolerated		▫ Cough		▫ Bronchospasm		Other:		
	AKITA®	PARI	AKITA®	PARI	AKITA®	PARI		AKITA®	PARI[a]
Patient results	*100%*	*100%*	*0%*	*10%*	*0%*	*0%*	*Dislike of taste*	*0%*	*60%*
							Mild dizziness	*0%*	*20%*
							Mild dyspnea	*0%*	*10%*
							Increased saliva production	*10%*	*10%*
							Irritated throat	*0%*	*10%*
							Hoarseness	*10%*	*0%*
2. Did you think this nebulization was annoying?	▫ Not annoying at all		▫ A little annoying		▫ Moderately annoying		▫ Very annoying		
	AKITA®	PARI	AKITA®	PARI	AKITA®	PARI	AKITA®	PARI	
Patient results	*20%*	*30%*	*80%*	*40%*	*0%*	*20%*	*0%*	*10%*	
3. What did you think of the duration of the nebulization?	▫ Not long at all		▫ A little long		▫ Moderately long		▫ Too long		
	AKITA®	PARI	AKITA®	PARI	AKITA®	PARI	AKITA®	PARI	
Patient results	*10%*	*0%*	*40%*	*20%*	*50%*	*30%*	*0%*	*50%*	
4. What did you think of this nebulizer?	▫ Fine		▫ A little bothersome		▫ Rather bothersome		▫ Did not like it at all		
	AKITA®	PARI	AKITA®	PARI	AKITA®	PARI	AKITA®	PARI	
Patient results	*40%*	*20%*	*40%*	*10%*	*0%*	*40%*	*20%*	*30%*	
5. Do you have a preference for one of the nebulizers?	▫ PARI-LC® Plus			▫ AKITA®			▫ Own nebulizer		
Patient results	*10%*			*0%*			*90%*		
6. What would you think of once daily nebulization of the double dose of tobramycin?	▫ Better than twice daily			▫ I do not care			▫ Rather twice daily shorter nebulization		
Patient results	*90%*			*10%*			*0%*		

Questions 1 to 4 were asked at both study visits. The responses of questions 1 to 4 are divided per study nebulizer: PARI, PARI-LC® Plus. Question 5 and 6 were additional questions after the second inhalation.

[a]*Some patients reported more than one side effect after inhalation with the PARI-LC® Plus nebulizer.*

treatment as a back-up nebulizer. Almost all patients preferred OD inhalation of a double tobramycin dose over BID inhalation of the standard dose (90%).

DISCUSSION

This is the first study investigating the pharmacokinetics and tolerability of once daily inhalation of the double recommended dose of tobramycin with the AKITA and the PARI-LC Plus nebulizer in patients with CF. The main finding is that similar pharmacokinetics were assessed for OD tobramycin inhalation with the AKITA and the PARI-LC Plus nebulizer in the doses given, with serum levels that were not toxic. Furthermore, both inhalations were well tolerated, although patients reported more side effects during nebulization with the PARI-LC Plus. All reported side effects are well-known for inhaled tobramycin and described in the manufacturer product information.

When comparing the pharmacokinetics of OD tobramycin inhalation in this study to pharmacokinetic data from the literature regarding standard BID inhalation with the PARI-LC Plus, we found higher peak levels in our study, while trough levels were low. Mean serum peak levels in literature range from 0.7-1.3 mg/L, compared to 3.4 mg/L and 3.6 mg/L with the PARI-LC Plus and AKITA, respectively, in our study population.[11,146,230,231] Nephrotoxicity is linked to trough levels above 2 mg/L, while levels above 4 mg/L and high cumulative exposure to aminoglycosides are associated with ototoxicity.[232,233] The very low trough levels found in our study population indicate that OD inhalation of a double tobramycin dose has an acceptable safety profile. Hence, considering the concentration-dependent bactericidal efficacy of tobramycin, the OD treatment regimen proposed in our study might result in better efficacy of tobramycin therapy in patients with CF, without the occurrence of acute toxicity.

The mean nebulization time was significantly shorter with the AKITA than with the PARI-LC Plus. This was to be expected, considering the reduced volume of drug needed for nebulization with the AKITA. The results from the questionnaire showed that 30% of patients thought nebulization with the PARI-LC® Plus took too long, while none of the patients indicated that AKITA nebulization was too long. Interestingly, almost all patients preferred OD nebulization with a longer duration over BID nebulization with a shorter duration.

The most commonly reported side effect, 'a dislike of taste', was only reported for nebulization with the PARI-LC Plus. This can probably be explained by increased deposition of tobramycin in the oropharyngeal region when nebulizing with the PARI-LC Plus due to continuous aerosol production during inspiration and expiration.[234] Even though dislike of taste is not severe, it does increase the treatment burden for patients.

When asking the patients' impression, most patients were more satisfied with the AKITA than with the PARI-LC Plus, but preferred their own inhalation device for tobramycin over one of the study nebulizers. Shorter treatment times with their own nebulizer might be the explanation for this. Especially tobramycin inhalation powder requires significantly less time than nebulized tobramycin.[235] However, the AKITA is known to have an increased deposition in the small airways.[24] The deposition pattern for tobramycin is less well described for other nebulizers nor the powder inhaler.[23]

Limitations

The fact that patients preferred their own inhalation device over one of the study nebulizers can be seen as a limitation of the study design. As seen in our cohort, the PARI-LC Plus nebulizer is not commonly used in routine CF care anymore, as it is considered less effective compared to newer nebulizers. However, as tobramycin solution for inhalation is licensed with the PARI-LC Plus nebulizer, we have chosen to compare the controlled-inhalation to the standard nebulizer. Our study design did not include the gold standard for *Pa* infections, which is BID nebulization of 300 mg tobramycin. However, it was not our aim to compare the new dosing regimen to the gold standard, but to determine the pharmacokinetics of a higher dose.

From the results of this study, no concrete statements about efficacy and safety can be made, since it was a single dose study with a small sample size. Although a sample size of 6 to 16 patients is common in this type of pharmacokinetic studies,[146,224-226,236,237] it should be kept in mind when applying the OD treatment regimen in clinical practice and also warrants the need for further research.

The exact inhaled dose of tobramycin was not measured in this study. The smartcard inserted in the AKITA nebulizer was set to deliver a specified dose of 120 mg to the lungs to achieve bioequivalence to the PARI-LC Plus. Unfortunately, the lung dose for the PARI-LC Plus was unknown. To make the most reliable comparison, we have used the filling doses of both nebulizers for calculation of the results.

Finally, one patient (#3) received salbutamol before start of tobramycin nebulization with the PARI-LC Plus to prevent bronchospasm on top of his cold. This may have influenced his post dose spirometry measurements. Also, due to the bronchodilator effects of salbutamol, deposition beyond the central airways might have been increased, which could affect the pharmacokinetic results and result in higher serum levels. However, serum concentrations were very low following the patient's PARI-LC Plus inhalation, thus salbutamol administration did not play a significant role.

CONCLUSION

OD tobramycin inhalation of the double standard BID dose with the controlled-inhalation AKITA and conventional PARI-LC Plus nebulizer resulted in similar pharmacokinetics in the doses given. Nebulization time was significantly shortened, less side effects were reported, and a higher degree of satisfaction was attained with the AKITA nebulizer. OD inhalation of tobramycin might reduce the treatment burden and contribute to better treatment adherence. Since OD inhalation treatment was well tolerated and serum levels were below the toxic limit, further research demonstrating clinical efficacy and long-term safety of this dosing regimen is in place.

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Table S1. Influence of double dosed tobramycin inhalation on FEV_1 in L (% predicted)

	AKITA®		PARI-LC® Plus	
Patient	**Baseline value**	**15 min after stop nebulizationa**	**Baseline value**	**15 min after stop nebulizationa**
1	3.63 (83)	-1.10%	3.89 (89)	-2.06%
2	2.69 (66)	0.00%	2.92 (71)	-10.96%[b]
3[c]	1.34 (30)	-2.99%	1.17 (26)	0.00%
4	1.45 (53)	-5.52%	1.42 (52)	-3.52%
5	1.65 (36)	1.21%	1.72 (37)	-2.33%
6	4.80 (91)	-1.46%	4.65 (88)	-3.44%
7	2.06 (52)	-1.46%	2.10 (53)	-4.76%
8	2.74 (80)	-2.92%	2.67 (78)	-2.62%
9	3.45 (72)	-4.64%	3.36 (70)	-4.46%
10	1.82 (63)	2.20%	1.71 (59)	0.58%

FEV_1, forced expiratory volume in the first second. Quanjer GLI-2012 reference equations were used for predicted values.

[a]Relative to baseline values; [b]This patient experienced difficulty with inspiration during the lung function measurements; dyspnoea at rest was not experienced by the patient or observed by the investigators. [c]This patient was suffering from a cold (viral infection) during the second study visit (PARI-LC® Plus).

CHAPTER 9

General discussion

DISCUSSION

The studies described in this thesis are focused on the efficacy of inhaled drugs in patients with cystic fibrosis (CF). Firstly, we executed a systematic review focused on factors that can affect the clinical efficacy of inhaled antibiotics after deposition in the airways of patients with CF. Secondly, we used computational fluid dynamics (CFD) to estimate concentrations of inhaled antibiotics and dornase alfa throughout the bronchial tree. Thirdly, we performed an observational study investigating the daily inhalation technique of children with CF while nebulizing at home. Fourthly, we performed two studies with the aim to improve the efficiency of current inhaled drugs by varying nebulizer device, dose, treatment regimen and inhalation profile. In addition, we estimated concentrations of inhaled tobramycin using CFD in the small airways and we performed a study on its pharmacokinetics in patients with CF. In this discussion, the main findings of this thesis are discussed in the light of current clinical practice and evidence, and recommendations for future research are given.

Computational Fluid Dynamic Modeling for personalized medicine

Over the last two decades, inhaled antibiotics and mucolytics have become increasingly important for the treatment of CF lung disease. To date, treatments still largely follow a "one size fits all" strategy as all patients receive the same dose and treatment regimen of inhaled drugs. It has been widely recognized that a more personalized approach is needed. CFD is a very promising development that could contribute to a more personalized aerosol treatment. With CFD, inhalation of a drug is simulated on a 3D model of the bronchial tree derived from a patient's chest CT-scan. CFD provides insight in the patient-specific concentrations of inhaled drugs, which opens the possibility of adapting the drug dose on a patient-specific basis.

Concentrations of inhaled drugs with the current treatment regimens in CF

It is well recognized that inhaled antibiotics are effective in reducing the loss in lung function and reducing exacerbations. The efficacy has been linked to the high sputum concentrations found in randomized controlled trials investigating inhaled antibiotics.[11,124] However, it is unlikely that sputum concentrations adequately reflect airway deposition throughout the bronchial tree. It is especially unknown whether high enough concentrations are obtained in the small airways where substantial morphological changes can be observed in CF. Therefore, we developed CFD models to study the distribution of inhaled medications. CFD modeling confirmed that, overall, high concentrations of both inhaled antibiotics as well as dornase alfa are delivered to the large and distal airways with the current treatment regimens (Chapters 4-6). However, it appeared that concentrations of Aztreonam Lysine for Inhalation (AZLI) in the peripheral regions of the lung lobes were

highly variable and patient specific. If a lung lobe was more affected by the disease, less of the inhaled antibiotic was deposited in that lobe (Chapter 4). Concentrations could even drop below the minimal inhibitory concentration (MIC). This is an important finding as it suggests that the inhaled dose needs to be adjusted, taking the severity of lung disease into account. This is not current practice as all CF patients receive the same dose of inhaled antibiotics independent of age and disease severity. For those patients with more advanced lung disease, higher doses might be more effective to treat small airways disease. This concept needs to be studied further in prospective clinical studies.

What is the effective antibiotic concentration?

Unfortunately, it is unknown which concentration of an inhaled antibiotic is needed to reach maximal killing throughout the lung. There is substantial heterogeneity of phenotypes and genotypes of *Pseudomonas aeruginosa (Pa)* within the lung of one CF patient and this heterogeneity increases as disease progresses.[126] Therefore, MICs differ throughout the bronchial tree. Antibiotic concentrations need to be high enough to overcome the MICs of the most resistant strains in the lung as concentrations of inhaled antibiotics below the MIC are thought to result in the development of resistance, hypermutator strains and suboptimal killing of *Pa.*[126] For β-lactam antibiotics like AZLI, the resistance is caused by hyperproduction of β-lactamase and biofilm formation. The β-lactamase can lead to the hydrolysis of β-lactam antibiotics before they reach the bacteria.[158] For ceftazidime, the hydrolysis by β-lactamase changes the mechanism of killing from time-dependent killing in single *Pa* cells (planktonic *Pa*) to a concentration-dependent killing of *Pa* in biofilms.[158] If β-lactamase also changes the mechanism of killing for AZLI, concentration could be a limiting factor in addition to treatment time and this should be kept in mind when treating biofilm infections. Strangely enough, for intravenously administered aztreonam, we know that, even if the sputum concentration does not exceed the lowest reported MIC value, it is still as efficacious as inhaled AZLI.[126] This would suggest that concentrations above MIC would not be more effective than concentrations below MIC. However, it is questionable whether conventional pharmacokinetic/pharmacodynamic MIC targets, which are derived from serum concentrations after intravenous administration and that are correlated with clinical efficacy, apply to inhaled antibiotics. Aerosolized therapy in CF has important advantages over intravenous administration as it minimizes systemic exposure, reduces the risk of systemic adverse reactions and reduces exacerbations. For these reasons, inhalation of antibiotics have become the preferred administration route in CF. CF-specific pharmacokinetic/pharmacodynamics targets for inhaled antibiotics are not well defined. Thus investigating whether changes in doses and treatment regimens for chronic infections in specific patients can be more effective compared to current regimens may be worthwhile.

Influence of mucus and alginate layers

Although local concentrations of inhaled antibiotics are highly relevant for therapeutic efficacy, one should keep in mind that the journey of aerosol particles does not end after deposition within the lung. After deposition in the airways, the clinical efficacy of inhaled antibiotics can be impaired by many local factors. This has been investigated best for aminoglycosides, such as tobramycin. A wide range of molecules within CF mucus and the alginate layer surrounding *Pa* can have a major impact on the efficacy of aminoglycosides (Chapter 3). To compensate for inactivation by these molecules within the sputum, it is generally assumed that tobramycin concentrations need to be 10- to 25 times higher than the MIC.[238] For some patients even higher concentrations, of 100 times MIC, are required to ensure killing of *Pa* due to the antagonistic activity of their sputum.[177] When considering wildtype *Pa* strains which are related to a MIC of 2 µg/ml or lower, the currently used treatment regimen of 300 mg nebulized tobramycin would result in concentrations 381-1500 times the MIC of 2 µg/ml, even in the small airways. Thus, maximal killing should easily be obtained. However, *Pa* strains with MICs above 2 µg/ml have most likely developed resistance mechanisms. The required concentrations for effective bacterial killing are unknown, but the higher the tobramycin concentration, the more killing can be expected. The highest MIC value that has been measured *in vitro* is 512 µg/ml ($MIC_{512\ \mu g/ml}$). Hence, a concentration of 10 x $MIC_{512\ \mu g/ml}$ or even higher is probably required for effective killing of *Pa*. Our CFD modeling showed that we could only obtain concentrations 1.5-6 times higher than this $MIC_{512\ \mu g/ml}$ (Chapter 5). An important clinical implication of our study is that for patients with insufficient response to inhaled tobramycin, a higher dose could be beneficial, at least when it can be administered safely.

Mucociliary clearance

In addition to concentration, the local efficacy of antibiotics depends on the duration of drug availability at the site of action. Mucociliary clearance influences the rate of drug elimination of inhaled antibiotics from the lungs. How it affects the efficacy of inhaled drugs is unclear. Mucociliary clearance is the dominant clearance mechanism in the central airways.[239] Beating cilia are needed for the ascending mucus transport of approximately 3-10 mm per min.[239,240] Along with the mucus, aerosol particles deposited on and trapped in the mucus are transported. Most studies describe a reduced clearance rate in patients with CF compared to healthy persons,[241-245] although more rapid clearance[246,247] and normal clearance rates have also been reported.[241,244,248,249] On the one hand, a reduced clearance rate would be positive for the local efficacy of inhaled antibiotics as the antibiotic particles would have more time to diffuse to the bacteria and execute their function. On the other hand, *Pa* bacteria will not be cleared either and are exposed to sub-MIC levels for longer periods. This could lead to the development of resistant subpopulations of *Pa* and would make reduced mucociliary clearance

unfavorable. Thus, how mucociliary clearance fits into the persistence or clearance of *Pa* infections in patients with CF remains to be established.

Overall, the current inhaled antibiotic treatment regimens appear to result in high concentrations throughout the bronchial tree. However in daily practice, many patients do not improve even when compliant, while receiving current aerosol treatment regimens. The results from this thesis suggest that this can partly be explained by suboptimal concentrations of inhaled drugs in diseased areas of the lungs. CF clinicians need to recognize that there is large genotypic and phenotypic variation between patients. Efficacy of inhaled antibiotics differs from patient to patient, depending on the severity of structural lung disease and on adaptation by bacteria. Hence, not all patients will benefit equally from the same nebulized dose of antibiotics. The landscape of CF should move to more personalized treatment regimens. Adjusting dose, particle size or breathing patterns in these patients is likely to result in more efficient aerosol delivery to the site of infection. It is unlikely that such dose adaptations will be investigated in randomized trials for all currently available inhaled antibiotics. Thus N-of-1 trials should be considered. These trials can be integrated into normal practice and can consist of a random sequence of different treatments with regular and standardized measurements of relevant effects. In this case, an increased dose could be randomized with the regular dose and lung function measurements could be taken as an outcome. Ideally, the treatments are administered double-blind; however, in case of increased doses this will be difficult as the patient might notice that he is nebulizing an increased amount of drug. It is, therefore, important that at least the lung function technician is blinded, and preferably the physician as well. An advantage of N-of-1 trials is that they provide reliable identification of the individual response, non-response or harm. This avoids the costs of ineffective treatment as well as sparing the patient from adverse effects.

First step towards improving efficacy of current inhaled drugs in CF

Given the issues described with current treatment regimens, the question is how to increase concentrations of inhaled drugs in the diseased areas of the lung. In this thesis we studied how we can improve the aerosol therapy: namely by studying the inhalation technique employed by the patient, the device, the drug dose and the treatment regimen.

The patient

A correct inhalation technique is the cornerstone for effective aerosol therapy. Little effect can be expected from inhaled drugs if the patient's inhalation technique is suboptimal. In our modeling studies, an idealized inhalation maneuver was assumed. Clearly, in daily life the inhalation maneuvers vary widely which can greatly affect the concentrations of the inhaled antibiotic throughout the lungs. A slow and deep inhalation is known to

result in a more efficient deposition in the small airways compared to fast inhalation.[128] The influence of breathing profile on airway concentrations of inhaled antibiotics was investigated in this thesis. A slow inhalation using a smart nebulizer resulted in the highest small airway concentrations due to reduced deposition in the extrathoracic region (Chapters 4-6). Therefore, CF teams focus on instructing patients to use correct inhalation technique including a slow inhalation. We showed that, for our CF center, these instructions on nebulizer use were effective for most patients (Chapter 7). Our video observations revealed that the average inhalation technique in the home situation was good. However, we also showed that many errors in inhalation technique were still made; in one in seven children this likely resulted in ineffective treatment due to low or absent deposition of medication in the lungs (Chapter 7). For asthma it was shown that approximately half of the patients who initially learned how to use their inhalers properly, did not maintain this correct technique over time.[250] A proper instruction at the start of inhalation therapy is crucial, but new errors become a habit quickly. This is an important issue as inhalation therapy is vital to the health of the patient, takes considerable time and effort from the patient and is expensive. Therefore, optimizing inhalation technique is an important step in further optimization of currently available therapy. Regular evaluation of a correct inhalation technique should be incorporated in a well structured program of CF-care. The mistakes in inhalation technique differ from patient to patient. Therefore, general instructions are not suitable for all patients. Future studies should focus on whether ineffective inhalation technique can be improved by providing personalized instructions.

Changing the device

Slow, controlled, long inhalation with aerosol boluses in the first part of inhalation can significantly increase aerosol deposition in the small airways. There are currently two smart nebulizers that possess these characteristics.[25,26] One of these smart nebulizers is the Akita jet. The Akita has already been shown to reduce small airways obstruction (FEF_{75}) when delivering dornase alpha.[25] In this study patients were estimated to receive five times the regular lung dose when switched from a conventional nebulizer to the Akita. Our CFD study confirmed that the improvement in small airways obstruction observed in the study by Bakker *et al.* could be attributed to the increased concentrations of dornase alfa in the small airways (Chapter 6). Small airway concentrations were 20-30 times higher when 2.5 mg dornase alfa was nebulized with the Akita compared to the Pari LC Plus or Pari eFlow nebulizers. The same applies to inhaled tobramycin; the concentration in the small airways after nebulization of 300 mg was much higher with the Akita than with the Pari LC Plus (Chapter 5). Tobramycin concentrations similar to the Akita could be obtained with the Pari LC Plus, but only if double the dose was inhaled with a low tidal volume and not when inhaled with an average or high tidal volume. This,

again, stresses the importance of a slow and deep inhalation technique. Unfortunately, it is likely that only few patients will be able to execute such an inhalation regimen on a daily basis using a conventional nebulizer. The smart nebulizer technology in the Akita guides the patient in a correct and deep inhalation. Therefore, smart nebulizers such as the Akita should be recommended for inhalation of drugs in patients with CF instead of the Pari LC Plus or Pari eFlow nebulizers. It is also important to investigate the efficiency of other smart nebulizers such as the I-neb nebulizer or dry powder inhalers (DPI) for the inhalation of antibiotics such as the Twincer.[236]

Drugs are registered with a device. Hence, for the development of new drugs, smart nebulizers or other smart aerosol delivery devices that control inhalation maneuvers should be used. Moreover, even though currently used smart nebulizers guide patients in executing slow and deep inhalation, further developments of even smarter nebulizers are needed as patients can still make mistakes in inhalation technique without noticing. Bridging studies are required for registered drugs inhaled with conventional nebulizers to establish safety with smart delivery devices. Especially for drugs with high systemic absorption, increased serum concentrations might result in toxicity. CFD could play a role in designing these bridging studies. By simulating deposition of a drug with the old and new nebulizer, concentrations throughout the bronchial tree can be compared. If total lung concentrations are similar, safety of the new device can be expected. However, if significantly higher concentrations are obtained throughout the bronchial tree the clinical safety of the new drug-device combination should be investigated further.

Changing drug dose

We showed that inhaling a double dose of tobramycin once in 24 hours does not increase the systemic trough levels (Chapter 8). Serum levels remained well below the toxic limit of tobramycin. This means there is room for increasing the lung dose without increasing the risk for toxicity.

CFD modeling opens the possibility to adapt the drug dose on a patient-specific basis. As a first step, CFD was used to study a double tobramycin dose inhaled with the Akita and Pari LC Plus nebulizer. A double tobramycin dose inhaled with the Akita resulted in concentrations 5-12 times that of the highest MIC value as measured *in vivo* (MIC = 512 µg/ml), while concentrations after inhalation of the standard recommended dose were only 3-6 times as high. For the Pari LC Plus, the concentrations for the double tobramycin dose ranged between 3-12 times this $MIC_{512\ \mu g/ml}$ and for the standard recommended dose the concentrations ranged between 2-6 times this $MIC_{512\ \mu g/ml}$ (Chapter 5). Considering that concentrations of at least 10xMIC are desired, the double tobramycin dose seems promising. However, our findings suggest that perhaps even higher doses are needed to adequately cover all *Pa* bacteria throughout the bronchial tree, including the less susceptible strains in diseased areas of the lung. Especially considering that the antagonistic

activity of the sputum of some patients is so high, it might be that concentrations of 100xMIC are required to ensure killing of *Pa*.[177] This is where CFD could be useful in clinical care. For specific patients not improving with current treatment regimens, CFD can be used to determine the most appropriate dose in relation to those patients' lung disease severity. Subsequently, this dose could be tested in an N-of-1 randomized controlled trial in which the efficacy of the dose based on CFD is compared to the recommended dose.

Changing the treatment regimen

Changing the twice-daily treatment regimen for inhaled tobramycin to a once-daily regimen will reduce the treatment burden for patients significantly. Because inhalation therapy is time consuming and often does not give direct positive feedback because of the lack of immediate clinical effect, it is considered a burden by most patients. Therefore, nonadherence to inhaled therapy is a major problem in patients with CF. Only 32% of patients with CF is fully adherent to a twice- or thrice-daily regimen of nebulized antibiotics.[27] Evening adherence is consistently better than morning adherence, due to the often hectic morning schedules and time-consuming nature of nebulized treatment.[28,251] Once-daily dosing simplifies the treatment plan and makes it easier to incorporate inhaled antibiotics into the daily routine of families.

Moreover, once-daily dosing also offers pharmacological advantages in case of aminoglycoside treatment. Inhalation of one double dose resulted in higher peak levels (Chapter 8) and a longer period of time available for clearance of systemically absorbed tobramycin which reduced toxicity. This has already been shown for intravenous tobramycin, in which once-daily dosing was less toxic and equally effective compared to a thrice-daily regimen.[181,182] Furthermore, aminoglycosides show a post-antibiotic effect. Tobramycin exposure induces sublethal damage in *Pa* bacteria. This needs to be repaired before regrowth can start and a new dose of aminoglycosides can be effective. The time it takes to repair this damage correlates with the post-antibiotic effect and continues when the antibiotic concentration falls below MIC. Because of the post-antibiotic effect, aminoglycosides need to be dosed less frequently than for example β-lactam antibiotics.

Future research perspectives

The new options of inhaled drugs in CF developed recently have resulted in major improvements in prognosis. Although these drugs are effective in a large proportion of patients, many patients with CF still cannot be treated successfully. The key is to find the right treatment and inhalation device for an individual patient in order to prevent progression of structural lung damage and to improve prognosis.

First, future research should focus on the development of a more personalized treatment plan. CFD can play an important role in this development. With CFD multiple changes in treatment approaches can be tested in silico for a single patient. CFD can be

used to define the optimal drug dose and inhalation maneuver for a specific patient that results in adequate drug delivery to diseased areas of the lung. To improve the precision of the CFD simulations, more patient-specific input data like patient-specific breathing profiles and patient-specific upper airway models should be incorporated and validated.

Second, it should be investigated in clinical studies whether the optimal dose as defined by CFD is more effective than the standard dose. A study investigating the efficacy of a double tobramycin dose inhaled once-daily with the Akita nebulizer is currently running. This study is needed to validate our hypothesis that a once-daily treatment regimen can be more effective than a the twice-daily treatment regimen, and that higher lung doses are more effective in treatment of chronic *Pa* infections. Future studies should be performed with efficient and patient friendly delivery devices such as a smart nebulizer or smart DPIs to reduce treatment time and improve adherence. Also, these studies should keep safety in mind. Although no acute toxicity of the double tobramycin dose was seen, long-term safety of this treatment approach remains to be investigated as well as safety of even higher doses.

Third, it would be interesting to investigate whether other smart nebulizers such as the I-neb nebulizer or recently developed DPIs also deposit more of the inhaled drug in the small airways. The increased cough reported for tobramycin DPI compared to nebulization suggests high deposition in the throat and large airways and consequently low deposition in the small airways.[17,23] Whether this is truly the case should be investigated, for example by using CFD.

Fourth, a follow-up study should explore if an ineffective inhalation technique can be improved by providing personalized instructions instead of general instructions. Ideally, this should be a multicenter study including both children and adults with CF to increase the generalizability of the results. Also, inhalation technique in patients using DPIs should be investigated. The question is whether, and to what extent, inhalation technique affects the efficacy of antibiotics delivered by DPI compared with nebulizers. As administration is much quicker with DPIs, it is likely that fewer mistakes will be made out of boredom. However, the amount of drug that is inhaled with a DPI and distribution of drug throughout the lung is dependent on the inhalation volume and technique of the patient.[252] This technique can vary on a day to day basis. Thus, aside from appropriate initial instruction, repetitive personalized instructions seem to be essential for DPIs.

Finally, the ability of mannitol to increase the antibacterial activity of various antibiotics in patients with CF offers an opportunity for future studies. Mannitol is a hyperosmotic drug that changes the viscoelasticity of sputum. It is available as a dry powder for inhalation and has been shown to improve the treatment of *Pa in vitro* when co-administered with ciprofloxacin and tobramycin (Chapter 3).[90,91,106] It might also be able to improve the efficacy of AZLI as the diffusion of β-lactam antibiotics through CF mucus has already been shown to increase when mannitol is added. Future research

should clarify if administration of mannitol prior to inhalation of tobramycin is able to improve treatment of *Pa in vivo*.

CONCLUSION

Current therapy of inhaled drugs in CF is "one size fits all". Patients of all ages and with a wide variety of disease severities are treated with the same regimen. However, distribution of drug throughout the lungs and sensitivity to antibiotics differs between and within patients. Based on our findings, it is likely that some patients require a higher dose to achieve effective concentrations of inhaled drugs in all airway generations because of more advanced disease. If standard treatment fails to improve these patients' clinical status, a more personalized treatment based on key features of the lung and microbiology should be considered to prevent progression of structural lung damage and to improve prognosis.

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CHAPTER 10

Summary / Samenvatting

SUMMARY

The studies described in this thesis are focused on the efficiency of inhaled drugs in patients with cystic fibrosis (CF). We mainly aimed to improve treatment of small airway disease.

Chapter 1 contains a general introduction to cystic fibrosis (CF) and describes the aims of the studies presented in this thesis.

In the first part of this thesis (Chapter 2-3) we provide an overview on inhaled antibiotics in CF: from inhalation of the drug to deposition in the airways to the pathway after deposition.

Chapter 2 provides an overview of the basic technology of aerosol deposition and advantages and disadvantages of inhaled antibiotics administered by nebulizer and dry powder inhaler (DPI). It discusses the patient- and device-related differences between the two aerosol delivery modalities that might affect the efficacy of treatment. Nebulizers are required for inhaled antibiotics that are only available as a fluid. Nebulizers require regular maintenance, and long treatment time. If possible, DPIs should be used to reduce treatment burden. However, CF caregivers and patients should realize that there are major differences between the inhalation technique for a nebulizer and a DPI. Aerosol deposition by DPIs can vary due to differences in inhalation technique. Therefore, careful regular instruction of the optimal inhalation technique is required when a patient is switched from a nebulizer to a DPI.

Chapter 3 is a systematic review on the fate of antibiotic aerosol particles after deposition in the airways of patients with CF. Available literature was reviewed for local conditions that may affect clinical efficacy of inhaled antibiotics. We observed in *in vitro* studies that the clinical efficacy of inhaled antibiotics can be reduced by many factors after deposition in the airways. Aminoglycosides were more intensely studied relative to other inhaled antibiotics and can be adversely affected by these factors. Molecules within CF mucus and the alginate layer surrounding *Pseudomonas aeruginosa* strongly reduce the efficacy of aminoglycosides. Increasing the dose and co-administration of mannitol might compensate for this.

In the second part of this thesis (Chapter 4-6) computational fluid dynamics (CFD) studies are described in which the relations between concentrations of inhaled drugs in the airways and lung damage, breathing patterns for different nebulizers and different dosages were studied. CFD uses patient-specific airway models to predict local airway concentrations of inhaled drugs in CF lungs.

In **Chapter 4** we demonstrated for the antibiotic, aztreonam lysine for inhalation (AZLI), that concentrations in the small airways were highly patient-specific and varied throughout the bronchial tree. An inverse relation was found between AZLI concentration in a lung lobe and lung damage. Thus, more diseased lung lobes are more likely to receive a lower concentration of AZLI. Antibiotics need to reach certain concentrations to be effective; this threshold is defined as the minimal inhibitory concentration. Up to 22% of the total surface area of small airways received AZLI concentrations below the minimal inhibitory concentration for AZLI for the most common micro-organism (*Pseudomonas aeruginosa*) in CF airways.

Chapter 5 reports a CFD study estimating local airway concentrations of the antibiotic, inhaled tobramycin, throughout the bronchial tree in CF after standard and once daily double dosing. We observed high concentrations of inhaled tobramycin in all lung regions. Concentrations with a smart nebulizer (Akita) were two times as high as the conventional nebulizer (PARI-LC Plus). When looking at the required concentration of tobramycin to kill *Pseudomonas aeruginosa* without acquired resistance mechanisms, maximal killing should easily be obtained, both with the standard twice daily dose as well as for the double once daily dose. However to kill subpopulations of more resistant *Pseudomonas aeruginosa* bacteria, once daily inhalation of the double dose will be more effective.

In **Chapter 6**, another CFD study compared airway concentrations of a mucolytic (dornase alfa) throughout the bronchial tree when delivered with the smart Akita nebulizer, PARI-LC Plus or Pari eFlow nebulizer. CFD showed that most airways received sufficient dornase alfa concentrations. However, several lung lobes received suboptimal dornase alfa concentrations with the PARI-LC Plus and Pari eFlow. The Akita resulted in significantly higher dornase alfa concentrations in the small airways compared to the other two nebulizers. More efficient delivery of dornase alfa using a smart nebulizer is a promising strategy to improve treatment of small airways disease.

In the third part of this thesis (Chapter 7-8) we describe two clinical studies that investigated inhalation technique of patients at home and the pharmacokinetics of a double dose of inhaled tobramycin.

Chapter 7 describes an observational study evaluating the day-to-day inhalation technique of children with CF in the home situation. Video registrations with a hidden camera were made of 32 children with CF nebulizing at home. The video registrations were scored by experts in the field. Most patients had a good nebulizer technique on a day-to-day basis. However, still many mistakes in inhalation technique were made, regardless of age and duration of nebulizer use. Most common mistakes were made in the breathing manoeuvre during nebulization. The most crucial mistake was that children often placed the mouthpiece in front of their teeth instead of between their teeth. This

was observed in 13% of the patients and likely resulted in absent lung deposition and thus ineffective treatment. These mistakes can be detected in time by regular real life video registrations of inhalation technique by parents at home and evaluation of these videos by the CF-team.

Chapter 8 reports a pharmacokinetic study of a double dose of tobramycin in patients with CF, inhaled with the smart Akita nebulizer and conventional PARI-LC Plus nebulizer. As discussed in Chapter 5, once-daily inhalation of a double dose instead of twice-daily inhalation of the standard dose might result in more effective killing of *Pseudomonas aeruginosa* in the lungs. Before efficacy of this regimen can be tested, safety of this double dose needs to be assured. Similar pharmacokinetics was observed with both nebulizers for the doses given, with serum levels well below the toxic limit. Inhalation of a double dose of tobramycin was well tolerated and no major adverse events were reported. Furthermore, higher peak and lower trough levels were found with once-daily double dose inhalation compared to literature on standard twice-daily treatment of *Pseudomonas aeruginosa* in CF. Thus, once-daily inhalation of a double dose of tobramycin might lead to better efficacy than standard twice daily inhalation, without acute toxicity.

The last section of this thesis (**Chapter 9**) comprises an overview of the most important findings of our studies, together with a discussion on their clinical implications.

SAMENVATTING

De studies in dit proefschrift zijn gericht op de verbetering van inhalatiemedicatie die gebruikt wordt door patiënten met cystic fibrosis (CF). Hierbij werd voornamelijk gezocht naar een betere behandeling van de afwijkingen van de kleine luchtwegen.

Hoofdstuk 1 geeft een algemene introductie van de ziekte CF en beschrijft de doelen van de studies die in dit proefschrift worden besproken.

Het eerste deel (Hoofdstuk 2-3) van dit proefschrift geeft een overzicht van de weg van inhalatie antibiotica in CF: van inhalatie van de medicatie, depositie in de luchtwegen tot de weg na depositie.

Hoofdstuk 2 beschrijft de mechanismes van aerosol depositie en de voor- en nadelen van inhalatie antibiotica toegediend met een vernevelaar of een droge poeder inhalator (DPI). De patiënt- en apparaat gerelateerde verschillen tussen de twee manieren van inhalatie worden besproken en hoe deze invloed kunnen hebben op de effectiviteit van de behandeling. Vernevelaars worden gebruikt voor inhalatie antibiotica die alleen beschikbaar zijn als vloeistof. Echter, vernevelaars hebben regelmatig onderhoud nodig. Wanneer mogelijk moeten daarom DPI's gebruikt worden om de behandellast te verminderen. Zorgverleners en patiënten met CF moeten zich realiseren dat er grote verschillen zijn tussen de inhalatie techniek van een vernevelaar en een DPI. Aangezien bij DPI's de aerosol depositie afhankelijk kan zijn van de inhalatietechniek, moeten patiënten die worden overgezet van een vernevelaar naar een DPI zorgvuldig geïnstrueerd worden in de optimale inhalatietechniek.

Hoofdstuk 3 is een systematische literatuurstudie naar het lot van antibiotica deeltjes na depositie in de luchtwegen van patiënten met CF. In de literatuur werd gezocht naar lokale omstandigheden die invloed kunnen hebben op de klinische effectiviteit. *In vitro* studies toonden dat vele factoren de klinische effectiviteit van inhalatie antibiotica kunnen verminderen na depositie in de luchtwegen. Voorbeelden van deze factoren zijn moleculen in CF mucus en de slijmlaag om bacteriën. Aminoglycosiden zijn uitgebreider onderzocht dan andere inhalatie antibiotica en worden sterk beïnvloed door deze factoren waardoor de effectiviteit van aminoglycosiden sterk wordt verminderd. Hiervoor kan mogelijk gecompenseerd worden door de dosering te verhogen of gelijktijdig mannitol toe te dienen.

In het tweede deel van het proefschrift (Hoofdstuk 4-6) worden computer simulatie studies (computational fluid dynamics (CFD)) beschreven waarbij de relatie tussen de concentratie van inhalatie medicatie in de luchtwegen en longschade, adempatronen voor verschillende vernevelaars en doseringen werd onderzocht. CFD maakt gebruik

van patiënt-specifieke luchtweg modellen om de lokale concentratie van geïnhaleerde medicijnen te voorspellen in CF longen.

In **Hoofdstuk 4** laten we zien dat de concentraties van het antibioticum, aztreonam lysine voor inhalatie (AZLI), in de kleine luchtwegen zeer patiënt-specifiek zijn en varieren door de bronchiale boom. Een omgekeerd verband werd gevonden tussen de AZLI concentratie in een longkwab en mate van schade in die kwab. Dus in een meer beschadigde longkwab werd een lagere AZLI concentratie gezien. Het is bekend dat antibiotica bepaalde concentraties moeten bereiken om effectief te zijn. Deze drempel wordt de minimale remmende concentratie genoemd. Tot 22% van het totale gebied van kleine luchtwegen ontving AZLI concentraties die onder de minimale remmende concentratie lagen van AZLI tegen de meest voorkomende bacterie (*Pseudomonas aeruginosa*) in CF luchtwegen.

Hoofdstuk 5 toont de resultaten van een studie waarin de lokale concentraties van het vernevelde antibioticum, tobramycine, geschat worden in de bronchiale boom bij CF na een één- en tweemaal daagse dosering. Er werden hoge concentraties van vernevelde tobramycine gezien in alle regio's van de long, waarbij een slimme vernevelaar (Akita) tweemaal zo efficiënt was als meer traditionele vernevelaars (PARI-LC Plus). Voor een drempelwaarde van tobramycine voor *Pseudomonas aeruginosa* zonder verworven resistente mechanismes zou maximale doding makkelijk bereikt kunnen worden, zowel met de twee als met de eenmaal daagse dosering. Echter om de meer resistente *Pseudomonas aeruginosa* bacteriën te doden zou een eenmaal daagse inhalatie met dubbele dosering nodig zijn.

In **Hoofdstuk 6** werden de concentraties van een sputum verdunner (dornase alfa) in de bronchiale boom berekend wanneer verneveld werd met de slimme Akita vernevelaar, of met de Pari eFlow of PARI-LC Plus vernevelaar. De computer simulaties toonden aan dat de meeste luchtwegen voldoende hoge concentraties dornase alfa kregen. Echter, met de PARI-LC Plus en Pari eFlow ontvingen bepaalde longkwabben suboptimale dornase alfa concentraties. Ook werden substantieel hogere dornase alfa concentraties in de kleine luchtwegen bereikt met de slimme vernevelaar vergeleken met de twee andere vernevelaars. Hogere concentraties kunnen veelbelovend zijn voor de behandeling van de afwijkingen van de kleine luchtwegen.

In het derde deel van dit proefschrift (Hoofdstuk 7-8) beschrijven we twee klinische studies waarbij we de inhalatietechniek van patiënten in de thuissituatie onderzochten en de farmacokinetiek van een dubbele dosering geïnhaleerde tobramycine.

Hoofdstuk 7 beschrijft een observationele studie die de dagelijkse inhalatietechniek van kinderen met CF in de thuissituatie onderzocht. Van 32 kinderen met CF die thuis vernevelden werden video opnames gemaakt met een verborgen camera. Deze opnamen werden door een panel van experts beoordeeld. De meeste patiënten bleken gemiddeld

genomen een goede verneveltechniek te hebben. Echter, er werden nog steeds veel fouten in inhalatietechniek gemaakt, onafhankelijk van de leeftijd of van hoe lang de vernevelaar gebruikt werd. De meeste fouten werden gemaakt in ademhalingstechniek tijdens het vernevelen. De meest cruciale fout was dat kinderen het mondstuk vaak vóór in plaats van tussen de tanden plaatsten. Dit werd gezien bij 13% van de patiënten en resulteerde waarschijnlijk in een lage longdepositie en dus in een ineffectieve behandeling. Deze fouten zouden tijdig opgespoord kunnen worden door de inhalatietechniek regelmatig via een videoverbinding live te laten beoordelen door het CF-team.

Hoofdstuk 8 toont de resultaten van een farmacokinetische studie naar een dubbele dosering tobramycine in patiënten met CF, verneveld met de slimme Akita vernevelaar en de conventionele PARI-LC Plus vernevelaar. Zoals in Hoofdstuk 5 besproken, zou het eenmaal daags inhaleren van een dubbele dosering in plaats van tweemaal daags inhaleren van de standaard dosering effectiever kunnen zijn om *Pseudomonas aeruginosa* in de longen te doden. Voordat dit behandelregime getest kan worden, moest vastgesteld worden of deze dubbele dosering veilig is. Er werd gelijke farmacokinetiek gezien met beide vernevelaars in de gegeven doseringen, met serum spiegels die ver onder de toxische grens waren. Inhalatie van een dubbele dosering tobramycine werd (verder) goed verdragen en er werden geen noemenswaardige bijwerkingen gezien. Daarnaast werden hogere piek- en lagere dalspiegels gevonden met deze eenmaal daagse inhalatie, vergeleken met de literatuur over de standaard tweemaal daagse behandeling van *Pseudomonas aeruginosa* in CF. De eenmaal daagse inhalatie van een dubbele dosering tobramycine zou dus mogelijk tot een betere effectiviteit kunnen leiden dan standaard tweemaal daags inhalatie, zonder acute toxiciteit.

Hoofdstuk 9: In dit deel van dit proefschrift wordt een overzicht gegeven van de belangrijkste bevindingen uit het proefschrift en wordt de betekenis van deze bevindingen voor de behandeling van patiënten met CF bediscussieerd.

Appendices

LIST OF ABBREVIATIONS

AB	Antibiotic
AlgL	Alginate lyase
ASL	Airway surface liquid
$AUC_{0\text{-}24hr}$	Area under the concentration-time curve from 0 to 24 hours
AZLI	Aztreonam lysine for inhalation
BID	Twice daily
CCMO	Central Committee on Research Involving Human Subjects
CF	Cystic Fibrosis
CFD	Computational fluid dynamics
CFU	Colony forming units
C_{max}	Maximum serum level
CT	Computed tomography
CFTR	Cystic fibrosis transmembrane conductance regulator
C_{trough}	Trough serum level
DBS	Dried blood spots
DNA	Deoxyribonucleaic acid
DPI	Dry powder inhaler
eGFR	Estimated glomerular filtration rate
ELF	Epithelial lining fluid
FDA	Food and drug administration
FEF_{75}	Forced expiratory flow at 75% of FVC
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GRADE	Grading Recommendations Assessment Development and Evaluation
GSD	Geometric standard deviation
HPLC	High-performance liquid chromatography
ICC	Intraclass correlation coefficients
IQ range	Interquartile range
LLL	Left lower lobe
LLOQ	Lower limit of quantification
LPS	Lipopolysaccharides
LTA	Lipoteichoic acid
LUL	Left upper lobe
MBC	Minimal bactericidal concentration
MBEC	Minimum biofilm eradication concentration
METC	Medisch Etische Toetsings Commissie
$MgCl_2$	Magnesium chloride

MIC	Minimal inhibitory concentration
MIC_{50}	Minimum inhibitory concentration that inhibits 50% of the isolates
MIC_{90}	Minimum inhibitory concentration that inhibits 90% of the isolates
MMAD	Mass median aerosol diameter
MS	Mass spectrometry
NAC	*N*-acetylcysteine
NaCl	Sodium chloride
Na_2SO_4	Sodium sulfate
OD	Once daily
Pa	*Pseudomonas aeruginosa*
PRAGMA-CF	Perth-Rotterdam Annotated Grid Morphometric Analysis for CF
rhDNase	Recombinant human deoxyribonuclease
RLL	Right lower lobe
RML	Right middle lobe
RUL	Right upper lobe
RV	Residual volume
SAD	Small airways disease
SPECT CT	Single Photon Emission Computed Tomography
SVC	Slow vital capacity
TAD score	Total airway disease score
TIS	Tobramycin inhalation solution
TIP	Tobramycin inhalation powder
TLC	Total lung capacity
T_{max}	Time to maximum serum level

ABOUT THE AUTHOR

Aukje Bos was born on the 23rd of April 1986 in Leiden, The Netherlands. She completed her high school at the 'Praedinius Gymnasium, Groningen' in 2004. She preferred to improve her English before starting the Dutch student life and studied biology at Elizabethtown College in the USA for a year. In 2005 she moved to Utrecht to start her medical training at the Faculty of Medicine of Utrecht University. Her enthusiasm for research and cystic fibrosis started in 2010 when she performed a literature review on *Bordetella* species in cystic fibrosis, supervised by dr. H.G.M. Arets and dr. T.F.W. Wolfs at the department of Paediatric Pulmonology, Wilhelmina Children's Hospital in Utrecht. The complexity of cystic fibrosis fascinated her and in 2011 she performed a research project 'Impact of trapped air on quality of life in patients with cystic fibrosis', supervised by Prof. dr. H.A.W.M. Tiddens at the department of Paediatric Pulmonology, Erasmus MC-Sophia Children's Hospital in Rotterdam. Her final rotation of 3 months was spent at the paediatric department of the Gelre Ziekenhuizen in Apeldoorn. She obtained her medical degree in 2011 and continued working at the Gelre Ziekenhuizen as a paediatric resident.

In September 2012 she started her dissertation focused on improving the efficacy of current inhaled drugs in cystic fibrosis at the Paediatric Pulmonology department of Erasmus MC – Sophia Children's Hospital (promotor prof. dr. H.A.W.M. Tiddens, co-promotor dr. H.M. Janssens). During this period she was a board member of the Sophia Researchers Association and organised the Theme Sophia Research Days in 2014.

In January 2017 Aukje will start her paediatric residency at the VU University Medical Center in Amsterdam. She is looking forward to combine clinical work with science in her future career as a paediatrician. Aukje currently lives in Rotterdam, together with David Zitter.

LIST OF PUBLICATIONS

1. **Bos AC**, Beemsterboer P, Wolfs TFW, Versteegh FGA, Arets HGM. Bordetella species in children with cystic fibrosis: What do we know? The role in acute exacerbations and chronic course. J Cyst Fibros. 2011 Sep;10(5):307-12.
2. Tepper LA, Utens EMWJ, Caudri D, **Bos AC**, Gonzalez-Graniel K, Duivenvoorden HJ, van der Wiel ECW, Quittner AL, Tiddens HAWM. Impact of bronchiectasis and trapped air on quality of life and exacerbations in CF. Eur Respir J. 2013 Aug;42(2):371-9.
3. Tiddens HAWM, **Bos AC**, Mouton JW, Devadason S, Janssens HM. Inhaled antibiotics: Dry or Wet? Eur Respir J. 2014 Nov;44(5):1308-18.
4. Van Velzen A, **Bos AC**, Janssens HM. Slimme vernevelaars voor inhalatie-antibiotica bij CF. Pharm Weekbl. 2015 feb 6;150-6.
5. **Bos AC**, van Holsbeke C, de Backer JW, van Westreenen M, Janssens HM, Vos WG, Tiddens HAWM. Patient-specific modeling of regional antibiotic concentration levels in patients with cystic fibrosis: Are we dosing high enough? PLoS ONE. 2015 10(3): e0118454. doi: 10.1371/journal. Pone.0118454.
6. **Bos AC**, Engelkes M, Janssens HM. Assuring adherence and compliance with aerosol therapy. (2015), II. Topic Sessions. Pediatr. Pulmonol., 50: S14-S15. doi:10.1002/ppul.23208.
7. **Bos AC**, van Velzen AJ, Touw DJ, Tiddens HAWM, Heijerman HGM, Janssens HM. Pharmacokinetics and tolerability of once daily double dose tobramycin inhalation in cystic fibrosis using controlled and conventional nebulization. J of Aerosol Med Pulm Drug Deliv. 2016 Jun;29(3):273-80. doi: 10.1089/jamp.2015.1259.
8. **Bos AC**, Tiddens HAWM , Tong Minh K, Heeres I, Overweel-Uijterlinde JL, Kok NE, Andrinopoulou ER, Janssens HM. Daily observations of nebuliser use and technique (DONUT) in children with cystic fibrosis. J Cyst Fibros, 2016 Sep;15(5):645-51. doi: 10.1016/j.jcf.2016.03.005..
9. **Bos AC**, Passé KM, Mouton JW, Janssens HM, Tiddens HAWM. The fate of inhaled antibiotics after deposition in patients with cystic fibrosis: How to get drug to the bug? Accepted for publication in Jounal of Cystic Fibrosis.
10. **Bos AC**, Mouton JW, van Westreenen M, Andrinopoulou ER, Janssens HM, Tiddens HAWM. Patient-specific modeling of regional tobramycin concentration levels in airways of patients with cystic fibrosis: Can we dose once daily?
11. **Bos AC**, Bakker EM, Andrinopoulou ER, Tiddens HAWM. Patient-specific evaluation of Regional Airway Dornase Alfa concentration levels in a cohort of patients with cystic fibrosis.

PhD PORTFOLIO

Summary of PhD training and teaching

Name PhD student: A.C.Bos
Erasmus MC Department: Paediatric pulmonology and Radiology

PhD period: September 2012 – November 2016
Promotor: Prof. dr. H.A.W.M. Tiddens
Co-promotor: dr. H.M. Janssens

1. PhD training

	Year	Workload (Hours/ECTS)
General courses		
- Grant writing course Prof. Quittner	2012	5.0
- Research Integrity	2014	0.3
- Nihes – Biostatistical Methods 1: basic principles	2013	5.7
- BROK ('Basiscursus Regelgeving Klinisch Onderzoek')	2013	1.0
Specific courses (e.g. Research school, Medical Training)		
-		
Seminars and workshops		
- Department research meetings	2012-2016	2.0
- PhD-day, Sophia Children's Hospital and Erasmus MC	2013-2016	1.5
- Young Investigators Day, Paediatric Association of the Netherlands	2013-2015	1.2
- Symposium Dutch Respiratory Society	2014	0.3
- Pediatric Symposium Amsterdam	2014-2015	0.5
- Endnote course	2014	0.2
Presentations		
- Presentations at Grand Round, MAD meetings, CF-centrale, ELIG meeting, Clinical pharmacology meeting (14 presentations until april 2016)	2013-2014	1.5
- Oral presentation Cystic Fibrosis Preceptorship at Erasmus MC	2015	1.0
- Oral presentation Vectura GmbH	2016	1.0
(Inter)national conferences		
- 36th European Cystic Fibrosis Conference [poster presentation]	2013	1.0
- Sophia "Jubileum Congres"	2013	0.3
- Sophia Research Day [poster presentation and oral presentation]	2013&2015	3.0
- Young Investigators Meeting European Cystic Fibrosis Society [oral presentation and poster presentation]	2014	2.0
- North American Cystic Fibrosis Conference [poster presentations + finalist Best Abstract in Clinical Research Award]	2014&2015	3.0
- Conference of International Society for Aerosols in Medicine	2015	2.0
Other		
- Clinical work: new patients at outpatient clinic Department of Pediatric Pulmonology	2012-2016	3.0

2. Teaching

	Year	Workload (Hours/ECTS)
Lecturing		
- Workshop on Nebulisation for Dutch Cystic Fibrosis Society	2016	1.0
Supervising practicals and excursions, Tutoring		
- Inhalation practical for 4th year medical students	2013-2015	1.0
Supervising Master's theses		
- K. Passé, medical student, Erasmus University	2014	3.0
- K. Tong Minh, medical student, Erasmus University	2013	1.5
Other		
- Peer review of articles for international scientific journals	2012-2015	2.0

DANKWOORD

Ik zeg altijd: accepteren kun je leren, maar ook promoveren kun je leren. Dat doe je echter niet alleen. Ik heb vele mensen nodig gehad om te komen waar ik nu sta.

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