General introduction and aims of study

General introduction

Respiratory disease is frequently present in childhood. It can be divided into upper airway disease and lower airway or lung disease. Two important lung diseases in childhood are cystic fibrosis (CF) and asthma. They are important because CF is the most common lethal genetic disease in the Caucasian population and asthma is one of the most frequent chronic diseases of childhood, with a prevalence of approximately 10% of children in the Western world. In both lung diseases the small airways, located in the periphery of the lung, are frequently affected. To provide optimal care for patients with lung disease it is important to accurately measure the severity of lung disease and to effectively treat the affected airways.

The most frequent test employed to monitor lung disease is spirometry. Spirometry is a lung function test that was developed over 100 years ago; it is well standardized and validated, widely distributed, and relatively easy to perform for children of 6 years and older. However, most parameters derived from spirometry are more sensitive to obstruction of the large than the small airways. Additionally, spirometry can only be performed reproducibly in children over the age of 6 years since it requires active cooperation. Spirometry is successful in only 45 – 75% of children aged three to five years, while children below the age of three years are unable to perform spirometry. Consequently, there is a great need for alternative non-invasive monitoring instruments for young children.

Treatment of lung disease preferably involves inhaled medications because aerosol therapy has the advantage that the drug is directly delivered to the site of action. This results in a more rapid effect, a lower dose needed for treatment and fewer systemic side effects. However, the specific inhalation technique required to deliver drug efficiently to the lungs is complicated and further research is needed to improve aerosol therapy.

These considerations apply to dornase alfa (Pulmozyme®, recombinant human DNase) which, among other inhaled therapies, is important in the treatment of CF lung disease and is used off-label in some other pediatric lung diseases such as asthma. Official registration studies for dornase alfa were performed two decades ago using conventional nebulizers that deposited a relatively low concentration in the lung and could not be adjusted to individual patient needs. Using more modern technology, treatment with dornase alfa may possibly be optimized to generate a maximal benefit for patients with chronic lung disease.

The studies described in this thesis aim to contribute to improvements in the care of children with chronic lung diseases, by identifying the best methods to monitor lung disease and improving the effectiveness of aerosol therapy.

To this end, we aimed to answer the following questions:

- Which parameter derived from spirometry is the most sensitive to early lung disease?
- Which alternative measurements can provide information on the condition of the lungs in young children who are not yet able to perform spirometry?
- What is known from current literature on the use of dornase alfa in CF?
- Can we improve the response to dornase alfa by using a new nebulizer that more efficiently deposits medication in the small airways in CF patients?
- Can dornase alfa improve lung function in children with asthma and persistent small airways obstruction?

Aims of study

To answer the research questions mentioned above, a number of studies were performed. The aims of the studies presented in this thesis were:

- To assess the differences in spirometry parameters between healthy children and children with CF, and to evaluate which parameter is most suitable to monitor small airway disease in CF.
- To evaluate the feasibility of
Lung clearance index (LCI), calculated from multiple breath washout (MBW), and
Home nocturnal pulse oximetry and home nocturnal cough recording as alternative efficacy outcome
measures in young children with CF.

- To review the present literature on the use of dornase alfa in CF lung disease.
- To evaluate the efficacy of preferential deposition of dornase alfa in the small versus large airways, both in
  stable CF patients and in patients admitted to the hospital because of a respiratory tract exacerbation (RTE).
- To evaluate the efficacy of dornase alfa in children with asthma and persistent small airways obstruction.

Outline of this thesis

Chapter 1 presents a general introduction and the aims of the studies described in this thesis.

Chapter 2 provides background on the lung diseases CF and asthma, routine monitoring of lung disease, and aerosol
therapy.

Chapter 3 describes the results of a retrospective comparison of longitudinal spirometry data from CF patients with
data from healthy subjects.

Chapter 4 describes the results of a prospective cross-sectional study on LCI, nocturnal oxygen saturation and
nocturnal cough in a group of CF patients and healthy children aged 0-4 years.

Chapter 5 presents a review of the literature on the use of dornase alfa in CF patients.

Chapter 6 reports the results of a multi-center, double-blind, randomized controlled clinical trial on the efficacy of small
versus large airways deposition of inhaled dornase alfa in stable CF patients.

Chapter 7 reports the results of a multi-center, double-blind, randomized controlled clinical trial on the efficacy of small
versus large airways deposition of inhaled dornase alfa in CF patients admitted to the hospital for a pulmonary
exacerbation.

Chapter 8 describes the results of a randomized double-blind placebo controlled clinical trial on the efficacy of
dornase alfa in children with clinically stable asthma and persistent small airways obstruction.

Chapter 9 provides a summary and general discussion of the results of the studies described in this thesis.

Chapter 10 presents a summary, in Dutch, of the study results discussed in this thesis.
Background

The lung and the airways

The primary function of the human lung is to absorb oxygen into the blood and to discard carbon dioxide from the blood. As a result of breathing movements, air moves in and out of the lung via the bronchial tree.

The bronchial tree is a sequentially bifurcating system of airways. As a consequence of this branching anatomy, the surface area expands greatly from the trachea, with a cross-sectional area of 2.5 cm² in adults, through the proximal airways (e.g., third generation; A± 50 cm²) and distal airways (20th to 25th generation; A± 2 m²) to the large surface area of the alveoli (A± 130 m²). The epithelial surface of the respiratory tract is continuously exposed to inhaled potentially dangerous substances such as pollutants, dust, viruses and bacteria but the mucociliary apparatus protects the tracheobronchial tree against these substances.

Mucociliary clearance is the process of routine removal of inhaled particles from the airways. It depends on a complex interaction between the epithelial cells of the airways and the liquid layer on top of these cells. Cells on the epithelial surface of the bronchial tree have cilia that continuously beat. These ciliated cells are found from the trachea to the respiratory bronchioles. The airway surface liquid (ASL) lies above the epithelial surface and contains a mixture of different substances such as glycoproteins, lipids, secretory IgA immunoglobulins, lysozyme, peroxidase, DNA, and actin. The ASL consists of two layers: a mucus layer and a periciliary liquid layer (PCL). The mucus layer contains high molecular weight macromolecules, such as glycoproteins, which contribute to the binding capacities of the mucus. The PCL is a mucus-free zone at the cell surface, and approximates the height of the outstretched cilia. The PCL is crucial because it provides a low-viscosity environment in which the cilia can beat and prevents the mucus layer from sticking directly to the epithelial cell surface. Inhaled particles and micro-organisms are trapped in the mucus layer and are removed from the airways in two ways: either slowly but continuously by the beating cilia before being swallowed (mucociliary clearance), or by cough (cough clearance).

Cystic fibrosis

Cystic fibrosis (CF) is the most common lethal genetic disease of the Caucasian population and is characterized by abnormal mucociliary clearance. It has an autosomal-recessive mode of inheritance and an estimated incidence of one in 3500 ± 4500 newborns. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CFTR is an apical membrane protein that regulates chloride transport in secretory epithelial cells. CF mutations result in dysfunction of the CFTR protein, causing impaired chloride secretion and sodium hyperabsorption. CF is a multisystem disease in which chronic respiratory disease is the major cause of morbidity and mortality and is therefore the main focus of clinical care and research.

In the lung, CFTR dysfunction results in depletion of the ASL and abnormal mucociliary clearance. This results in chronic lower airway infection and inflammation, which start early in life and cause progressive structural lung damage eventually leading to respiratory failure and early death (figure 1). The small airways are damaged early in the disease process, as indicated by trapped air on expiratory CT scans and by lung function tests in young children.
Monitoring CF lung disease

Tests to evaluate CF lung disease can be divided into two groups: those that evaluate the structure of the lung and those that evaluate lung function.

Lung structure can be visualized by radiological techniques such as chest radiography and chest computed tomography (CT) scanning. The use of these imaging modalities is restricted because they expose the patient to radiation and therefore increase the lifetime probability of cancer. Chest magnetic resonance imaging (MRI) does not expose the patient to ionising radiation but is still at an early stage of development and requires further validation before it can be used as an outcome parameter on a large scale.

Lung function in CF can be measured by an array of tests, such as spirometry, infant lung function testing, multiple breath wash out, etc. Of these tests, spirometry parameters are the most widely used and are currently the best validated outcome measures. Multiple breath wash out (MBW) is a promising test that can be performed in infants and older children. In the next paragraphs we will discuss spirometry and MBW in more detail.

Spirometry

Spirometry plays an important role in the monitoring and management of lung diseases in children. Spirometry was developed over 100 years ago, is well validated and standardized, widely distributed, and relatively easy to perform for children aged 6 years and older. Patients are asked to inhale as deeply as possible and then blow out as fast and as deeply as possible into the spirometer. A number of parameters can be derived from these spirometry tests, which are considered important outcome parameters:

- Forced vital capacity (FVC) is the maximal volume (in litres) of air exhaled after a maximal inspiration. A low FVC can indicate loss of lung volume.
- Forced expiratory volume in 1 second (FEV₁) is the maximal volume (in litres) of air exhaled in the first second of a forced expiration after a maximal inspiration. FEV₁ is a parameter especially sensitive to obstruction in the large airways.
Forced Expiratory Flows after exhalation of 25, 50 and 75% of FVC (FEF25, FEF50, and FEF75, respectively) are flows measured at different cut-off points during the forced exhalation (in litres per second). These parameters are considered to be sensitive to obstruction of the smaller airways.

Although spirometry is widely used as an outcome parameter in CF lung disease, it has a number of limitations. Firstly, the most widely used endpoint for clinical trials in CF patients is FEV1. With improvements in CF care and survival over the past two decades, FEV1 in childhood has steadily improved and now is in the normal range in a large proportion of children and teenagers with CF. Consequently, FEV1 is no longer sufficiently sensitive to detect and monitor disease progression in these age groups. In addition, it is clear that considerable lung pathology can be present despite normal FEV1. Studies using infant pulmonary function testing, bronchoalveolar lavage and chest CT scans show that airway inflammation, small airways disease and bronchiectasis can be present early in life even in children with normal FEV1. Thus, more sensitive outcome parameters to detect early CF lung disease are urgently needed.

Secondly, routine spirometry can be performed reproducibly only in children from the age of 6 years because it requires active cooperation. Nevertheless, pre-school spirometry has been developed in paediatric lung function laboratories using computer-incentive games. Its success rates vary between 45% and 75% of children aged three to five years. Children below the age of three years are unable to perform spirometry. For these children infant pulmonary function testing (ipFT) can be used. However, ipFT is time consuming, technically difficult, and requires sedation. Consequently, there is a great need for alternative sensitive non-invasive monitoring tests that can be used throughout childhood to replace spirometry.

Multiple breath wash out

A promising lung function test to monitor lung disease in children is multiple breath wash out (MBW). It is claimed that MBW does not require active cooperation and can be performed even in young children without sedation. For this test, subjects need to breathe regularly through a facemask or mouthpiece while an inert tracer gas is washed in. Tracer gases that can be used are helium or sulphur hexafluoride (SF6). The wash-in phase continues until equilibrium is reached, defined as a visually stable plateau of the molar mass signal. Next, supply of tracer gas is stopped and the patient breathes room air to wash out the tracer gas. This wash-out phase is completed when the tracer gas concentration falls below 1/40 (2.5%) of the plateau concentration. An alternative approach is to use nitrogen (N2) as tracer gas. In this case no wash-in phase is needed since room air already contains N2. 100% oxygen is used to wash out N2.

Lung clearance index (LCI) is a key parameter derived from the MBW test. LCI is calculated as the cumulative expired volume needed to lower the tracer gas concentration to 1/40 (2.5%) of the plateau concentration divided by the functional residual capacity. An elevated LCI indicates inhomogeneity of ventilation and small airways disease. Elevated LCI has been observed in young children, suggesting it may be an early marker of small airways disease. Since it has been claimed that the same reference values for LCI can be used for young children and adults and LCI is associated with the presence and extent of structural lung damage on CT scans, LCI may be a suitable tool for long-term follow-up of children and adults. This has been challenged by a recent study in infants with CF which showed that in children younger than two years of age there are only weak associations between LCI and lung damage as determined by chest CT. In addition, it is not clear whether LCI is as sensitive as chest CT to detect disease progression. Disadvantages of the MBW test are that it requires expensive equipment and is to date technically demanding. Hence, the MBW test is an interesting test but further validation studies are needed to determine its role as a method to detect and monitor CF lung disease.

Treatment of CF lung disease

Improving mucociliary clearance is an important component of the treatment of CF lung disease. The aim is to mobilize as much sputum as possible from the lung on a daily basis by various chest physiotherapy techniques or physical exercise. In addition, most patients use inhaled mucoactive drugs that help to clear sputum. Two mucoactive drugs have a positive effect on mucociliary clearance in patients with CF: dornase alfa (Pulmozyme®, recombinant human DNase) and hypertonic saline. Dornase alfa reduces sputum viscosity by hydrolysing extracellular DNA in sputum. In CF lung disease maintenance treatment using dornase alfa improves lung function and reduces the number of pulmonary exacerbations. Hypertonic saline (7% saline) is believed to restore airway surface liquid hydration and to improve mucociliary clearance. Maintenance treatment with hypertonic saline improves FEV1 and decreases the number of pulmonary exacerbations in CF. A comparative study showed that hypertonic saline is not as effective as daily dornase alfa.

Antibiotics are the other important component of the treatment of CF lung disease. Oral, inhaled and intravenous antibiotics are used on a regular basis to eradicate or suppress infections in the airways.
Despite intensive treatment of CF lung disease, most patients face a gradual decline in lung function. Hence, it is important to develop new, more effective drugs and to improve responses to currently used therapies. One of the options to improve the response to mucolytics is to target the small airways more efficiently. Functional tests and imaging techniques have shown that the small airways are heavily involved in CF lung disease starting early in life. Unfortunately, the small airways are difficult to treat using conventional aerosol therapy. Current nebulizers, including those used in clinical practice and in trials investigating the efficacy of inhaled mucolytics, are inefficient and do not deposit sufficient drug in the small airways. Therefore, there is good reason to believe that the small airways have been under-treated. Recently, more efficient smart nebulizers have become available that can potentially target more of the inhaled drugs like dornase alfa to the small airways. However, it is unknown whether more efficient delivery of dornase alfa to small airways using a state-of-the-art nebulizer will improve small airway patency in children with CF.

Asthma

Approximately 10% of children in the Western world have asthma. Asthma is a complex multifactorial disease, in which airway wall inflammation and edema, increased bronchomotor tone, increased sputum volume, increased sputum viscoelasticity and decreased mucociliary clearance play a role. Asthma is typically characterized by variable airway obstruction. Symptoms that patients experience include tachypnea and dyspnea, impaired exercise tolerance, cough and disturbed sleep.

Asthma is an inflammatory disease affecting large, intermediate and small airways. A study that assessed small airway function by spirometry, MBW testing, and alveolar and bronchial nitric oxide measurements provided evidence that the small airways are a major site of the disease process in children with allergic asthma.

Monitoring asthma

The most important monitoring tools for asthma are spirometry and fractional concentration of exhaled nitric oxide (FeNO).

Spirometry

FVC, FEV₁, and FEF₂₅₋₇₅ are measured in asthmatic children just as in CF patients, although there are clear differences in the course of these diseases. In contrast to CF patients, most children with asthma do not experience a longitudinal decline in spirometry parameters. Generally, spirometry may be decreased in asthmatic patients when not sufficiently controlled or at the time of an asthma exacerbation.

There are a number of reasons for poor control: a persistent factor in the patient’s environment that triggers asthmatic inflammation (e.g. allergens, tobacco smoke, pollutants, humidity, etc.), a too low dose of inhaled corticosteroids, poor adherence to anti-asthma medication, or incorrect use of medication. Recent international surveys showed that 30–50% of all children with asthma are not well controlled.

Asthma exacerbations are often triggered by viral infections or allergen exposure. Airway inflammation is a key part of the lower airway response in asthma exacerbation, and occurs together with airflow obstruction and increased airway responsiveness. An asthma exacerbation is accompanied by an increase in symptoms such as cough, wheeze, and shortness of breath.

Fractional concentration of exhaled nitric oxide (FeNO)

The fractional concentration of exhaled nitric oxide (FeNO) is a marker of asthma; exhaled nitric oxide is increased in proportion to bronchial wall inflammation, induced-sputum eosinophilia and airway hyperresponsiveness.

FeNO measurements are easy to perform, fast and non-invasive. The ATS and the ERS issued a statement in 2005 on equipment specifications and the standardization of procedures for the measurement of FeNO. In short, a patient should first exhale without the mouthpiece in place, then place the mouthpiece in his mouth and inhale through the mouthpiece up to total lung capacity. With the mouthpiece in place, the patient should exhale steadily while maintaining a steady flow rate for 6 s (rate, 50 mL/s), guided by a feedback animation on the computer.

The finding of elevated FeNO is helpful in establishing the correct diagnosis of asthma. When respiratory symptoms and airflow limitation are present, an elevated FeNO has high sensitivity and specificity for diagnosing asthma. FeNO levels correlate with symptom frequency and bronchodilator use but not with FEV₁. Increases in FeNO are associated with worse asthma controls, and FeNO levels are reduced in a dose-dependent manner with anti-inflammatory treatment.

Although FeNO measurement is a helpful and easy test, there are also some disadvantages: FeNO may be within
normal limits in mild nonatopic asthmatic patients or in patients already receiving ICS therapy. In these situations, a normal level of FeNO does not exclude asthma. Additionally, the level of FeNO does not differentiate between different grades of asthma severity.

**Treatment of asthma**

The aims of the pharmacological management of asthma are to control asthma symptoms and to normalize lung function. Cornerstones of anti-asthma treatment are inhaled corticosteroids and short- and long-acting bronchodilators. In many children with asthma who use maintenance medication, lung function tests return to (near) normal values. However, 30 to 70% of asthma patients do not gain optimal asthma control and continue to have symptoms. In addition, up to one third of patients continue to show abnormal spirometry values. Persistent airflow limitation was observed in 31% and 27% of children with difficult asthma. Although small airway obstruction and inflammation play an important role, current asthma treatment is not primarily focused on the small airways and there are many reasons to believe that these airways are often not treated optimally.

Additionally, it is likely that mucus retention still contributes to airways obstruction in childhood asthma despite current treatment. In children who died from or with asthma, mucus plugs were observed especially in the small airways. Despite the contribution of mucus retention to asthmatic symptoms, very few studies have been done to investigate whether mucolytic therapy is beneficial. Several case reports suggest that dornase alpha may have a role in the treatment of acute severe asthma. It has been used for therapy-resistant atelectasis in asthmatic children and for the treatment of status asthmaticus but it is unknown whether maintenance treatment with dornase alpha can reverse persistent small airway obstruction in stable asthmatic patients.

**Inhalation therapy**

Inhalation of aerosolized drugs is the preferred route for the treatment of most lung diseases. The major advantage of aerosol therapy over other routes, such as oral or intravenous administration, is that the drug is directly delivered to the site of action. This results in a more rapid effect, a lower dose needed for treatment and it reduces systemic side effects. A disadvantage of aerosol therapy is that the technique required to deliver a sufficient amount of drug to the lungs is complicated. Better understanding of the mechanisms of aerosol deposition in the lung and of the factors influencing drug dose and deposition is needed to optimize aerosol treatment. This is especially true for children.

Inhaled aerosols are deposited in the airways due to impaction, sedimentation and diffusion. Impaction is the collision of a particle on an airway surface. The larger the particle and the higher its velocity, the more likely the particle is to impact. Impaction is the main deposition mechanism in the upper and central airways. Sedimentation is deposition of a particle due to gravity. Sedimentation is an important deposition mechanism in the smaller airways where air velocity is low. Because settling due to gravitational forces takes some time, breath-holding after inhalation will increase deposition by sedimentation. Diffusion is the spread of particles through random Brownian motion from regions of higher concentration to regions of lower concentration. Diffusion is an important deposition mechanism in the bronchioles and alveoli. For particles with a diameter smaller than 0.5 μm, the probability of deposition by diffusion is inversely related to particle size.

Important factors that determine the distribution pattern of inhaled aerosol particles in patients are:

- Particle size and distribution
- Anatomy of the airways
- Structural changes of the airway
- Breathing pattern

**Particle size:** aerosol particles vary in size, shape and density. The aerodynamic behaviour of an aerosol can be described by the aerodynamic diameter. Particles with a certain aerodynamic diameter have the same aerodynamic behaviour as a sphere of unit density with that diameter. Medical aerosols usually consist of an aerosol cloud that contains particles with a wide range of aerodynamic diameters. An aerosol cloud can be described by the mass median aerodynamic diameter (MMAD) and the geometrical standard deviation (GSD). The MMAD is the diameter at which 50% of the particles in an aerosol have a larger mass and 50% have a smaller mass. The GSD is a measure of the distribution of particle diameters and is defined as the ratio of the diameter of particles on the 84.2% percentile and the MMAD. Particles with a MMAD > 10 μm are unlikely to bypass the upper airways and penetrate into the tracheobronchial tree, whereas particles with a MMAD < 0.5 μm can penetrate deep into the lung but have a higher probability of being exhaled. In general, smaller particles have a higher probability than larger particles of being deposited in the small airways. An alternative value to characterize an aerosol is the volume median diameter.
(VMD). The VMD does not take into account particle density, but describes the average particle size in an aerosol cloud, such that fifty percent of the volume of the aerosol is composed of particles smaller in diameter than the VMD and fifty percent is larger than the VMD. Since VMD does not take particle density into account, it is not possible to indicate size cut-off points under which a particle will penetrate into the tracheobronchial tree. For example, a relatively large particle with a very low density is more likely to penetrate deep into the lung than a small particle with a very high density. For particles that contain a large amount of water, the VMD and MMAD are more or less interchangeable, because the relative density of water is 1.0.

Anatomy of the airways: the geometry of the bronchial tree is important for the deposition pattern of an inhaled aerosol. The airway diameter is smaller and air flow is higher in children than in adults. As a result, aerosol particles tend to deposit more in the central airways in children than in adults. See figure 2.

![figure 2](image)

**Figure 2.** In the adult, particles of 5, 3 and 1 µm are inhaled. The largest particles have the highest probability of impacting on the airway wall; smaller particles have a higher chance of penetrating more deeply into the lung. In the smaller airways of children air velocity is higher and the distance from the centre of the lumen to the airway wall is smaller. Therefore, even mid-size particles (3 µm) have a high probability of depositing on the airway wall before they can penetrate deeply into the lung. In a child with diseased airways, airway wall thickening and mucus lead to a higher chance of deposition, even for small particles. (Used with permission from Tiddens, Ital. J Pediatr.)

Airway pathology: pathological changes in the bronchial tree result in changes to the deposition pattern of inhaled aerosols. The following changes related to lung disease can affect aerosol deposition: oedema of the airway wall; mucus; bronchoconstriction; impaired growth of the airway; remodelling of the airway wall; and emphysema. These pathological changes in general do not change total lung deposition but result in a more inhomogeneous deposition pattern with even more deposition in the central than the small airways.

Breathing pattern: the breathing pattern used to inhale an aerosol has an important influence on the deposition pattern of particles in the respiratory tract. High inspiratory flow enhances the impaction of particles in the large airways. Low inspiratory flow enables particles to penetrate more deeply into the lung. Additionally, a slow deep breath will enhance deposition in the small airways compared with normal breathing.

Aerosol delivery devices

In general, there are three types of devices that can be used to deliver aerosol to the lung: dry powder inhalers (DPI), pressurized metered dose inhalers (pMDI) and nebulizers.

DPIs contain the drug as a dry powder, which can be contained in a single-dose blister or in a large reservoir from which doses are loaded prior to inhalation. The drug is released by the patient’s inhalation effort. The aerosol characteristics of the inhaled dry powder are in general dependent on the drug formulation, on the design of the DPI and on the inspiratory flow through the DPI that the patient is able to generate.

A pMDI contains a solution or suspension of drug that is pressurized in a canister. When the canister is actuated, one
A nebulizer device generally consists of two parts: a compressor that generates the airflow and a nebulizer where the liquid drug is transformed by the airflow into an aerosol. Thus, a nebulizer device generates an aerosol cloud that contains droplets of aerosolized medication. In conventional nebulizers a continuous flow is used to generate the aerosol. During the inhalation process the patient breathes through a mouthpiece for 10 â€“ 15 minutes, inhaling medication with each breath. These conventional nebulizers are inefficient because medication generated during exhalation is lost to the environment and a relatively large residue of drug remains inside the nebulizer. More recent nebulizers are breath-enhanced or breath-actuated, which ensures that most of the drug is nebulized only during inhalation. Treatment using a nebulizer is generally more time-consuming than with a DPI or pMDI. Administration of a single dose using a DPI or pMDI takes maximally one minute while nebulizer treatment takes 10-15 minutes, excluding the time needed for cleaning the nebulizer after each treatment. Therefore, a pMDI or DPI is preferred when possible. However, when medication is only available as a fluid, a nebulizer device is needed.

Device competence

Not all inhalation devices are appropriate for all patients, because each type of device requires a specific inhalation technique. For example the use of a DPI, an inhaler commonly used in asthma treatment, requires the patient first to exhale below functional residual capacity, next put the inhaler in his mouth, then inhale through the mouthpiece as deeply and as hard as possible, and finally hold his breath for a few seconds before exhaling. This relatively complex breathing manoeuvre is difficult to perform for some patient groups. Most children younger than 6 years for example, are not able to perform all the steps of the DPI manoeuvre correctly. Additionally, patients with severe lung disease may have difficulty inhaling from a DPI.

Competence in using an inhalation device is of key importance. This was demonstrated in a daily-life study which reported that 76% of patients using a pMDI and 49-54% of those using a breath-actuated inhaler made at least one mistake when using their inhaler. Similarly, it has been reported that between 6 and 96% of patients using DPsIs use their device correctly, depending on the type of inhaler and method of assessment. Incorrect use of an inhaler device leads to a lower lung dose and suboptimal deposition pattern of the inhaled drug and thus suboptimal treatment. It can also lead to more frequent side effects, for example oral candidiasis due to high oral deposition of inhaled corticosteroids.

Similar problems exist for nebulizers. Important factors influencing the total dose delivered to a patientâ€™s airways include the initial fill volume, the efficiency by which the nebulized aerosol is generated, and the residual volume left in the nebulizer on cessation of operation. These factors are prone to errors, such as filling a wrong volume of drug into the nebulizer, mixing multiple drugs together, and stopping the treatment before all medication has been nebulized. A key factor in lung deposition is the breathing pattern of the patient during nebulization. Most frequently, the patient inhales the drug while breathing normally. This breathing pattern results in a more centralized deposition of the aerosol. Slow and deep inhalations will result in a significantly higher lung deposition than normal tidal breathing, especially in the small airways.

Dornase alfa

An important drug for the treatment of CF lung disease that is administered by nebulization is dornase alfa. This drug was developed in the early 1990â€™s. It is a glycoprotein containing 260 amino acids with a molecular weight of approximately 33,000 - 38,000 Daltons. The rationale for developing recombinant human DNase was that sputum of CF patients is rich in leukocyte-derived DNA which contributes to its increased viscoelasticity. Dornase alfa cleaves extracellular DNA through hydrolysis and reduces the viscoelasticity of CF sputum in vitro. In CF it has been shown that dornase alfa improves lung function, reduces antibiotic use, lowers hospitalization rate and slows the rate of decline of FEV₁. Hence, its efficacy in CF is well established and maintenance dornase alfa therapy is widely accepted as the primary treatment to improve macociliary clearance in CF.

Aerosol delivery systems for dornase alfa

Dornase alfa should officially be aerosolized using only a nebulizer system approved by regulatory agencies and the responsible manufacturer. Two jet nebulizers were extensively evaluated in the early clinical trials with dornase alfa, the Marquest Acorn II and Hudson T Updraft II, both used with the PulmoAide compressor. By todayâ€™s standards both nebulizer systems are relatively inefficient and generate a large fraction of large aerosol particles. Deposition
studies with these devices showed that a relatively large proportion of the drug was deposited in the large airways and only little of the drug was deposited in the small airways. Recently, smart nebulizers have become available that have the potential to improve the delivery of dornase alfa. These nebulizers deliver drug more efficiently to the lungs and also coach patients to inhale the drug using the correct inhalation technique. Hence, treatment using these nebulizers has the potential to target more of the inhaled dose to the small airways.

Dornase alfa in this thesis

Dornase alfa is an interesting drug to test the hypothesis that improved deposition in the small airways in CF lung disease will lead to better treatment responses for the following reasons:

Firstly, the effect of dornase alfa on lung function is relatively quick. Improvement of lung function parameters after initiation of dornase alfa treatment in CF patients plateaus after 10-14 days. Hence, the effect of improved delivery of dornase alfa to the small airways should be apparent within a relatively short period of time.

Secondly, most CF patients are on maintenance treatment with dornase alfa, as advised by international guidelines. By switching patients to a smart nebulizer the additional value of improved delivery can be studied.

Another question that we would like to answer is whether dornase alfa is effective in improving persistent small airways obstruction in children with asthma, since case reports have described its successful use off-label in patients with asthma.

The studies addressing these questions will be presented in the next chapters.

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Small airway involvement in cystic fibrosis lung disease. Routine spirometry as an early and sensitive marker

Bakker E.M., Borsboom G.J.J.M., van der Wiel â€“ Kooij E.C., Caudri D., Rosenfeld M., Tiddens H.A.W.M. Submitted to Pediatric Pulmonology

Abstract

In young children with cystic fibrosis (CF) the forced expiratory volume in 1 second (FEV₁) is often normal and a more sensitive measure to detect early obstructive lung disease is needed.

Aim

To evaluate the progression of selected spirometry parameters with age in a cohort of CF patients and healthy children aged 6 to 20 years.

Methods

Retrospective comparison of longitudinal spirometry data from CF patients with data from two cohort studies in healthy subjects. Quantile regression was used to calculate the longitudinal 10th percentile (P₁₀), 50th percentile (P₅₀) and 90th percentile (P₉₀) of forced vital capacity (FVC), FEV₁, and the forced expiratory flow at 75 percent of FVC (FEF₇₅). Sample size estimates were calculated using these three parameters as clinical trial endpoints.

Results

FVC, FEV₁, and FEF₇₅ were all significantly lower in CF patients than healthy children. Abnormalities in FEF₇₅ occurred at younger ages and remained substantially larger than abnormalities in FEV₁ or FVC throughout childhood. Therefore, fewer patients would be required to detect a similar treatment effect if FEF₇₅ is used as a primary endpoint compared with FEV₁ or FVC.

Conclusions

Our data support the use of FEF₇₅ as a more sensitive marker of early CF lung disease than FEV₁ and FVC, because abnormalities in FEF₇₅ occur at younger age and FEF₇₅ is diminished more than other parameters.

Introduction

Cystic fibrosis (CF) is a life-shortening genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. In the lung, abnormal mucociliary transport results in chronic lower airway infection and inflammation, bronchiectasis and chronic progressive obstructive lung disease.

CF lung disease begins in the small airways in infancy in a large proportion of the patients. Forced expiratory volume in 1 second (FEV₁), an important surrogate marker of disease severity, is the most widely used endpoint for clinical trials in CF patients. With improvements in CF care and survival over the past two decades, FEV₁ has also progressively improved and is normal in the majority of young CF patients. Nevertheless, investigations utilizing infant pulmonary function testing, bronchoalveolar lavage and chest CT scans reveal that despite current treatment and earlier diagnosis, airway inflammation and obstruction begin very early in life and are progressive. Thus, a lung function outcome measure more sensitive to early disease than FEV₁ is urgently needed. The forced expiratory flow at 75% of forced vital capacity (FEF₇₅) is thought to be a more sensitive spirometry parameter than FEV₁ for detecting obstruction of the small airways. The contour of the flow-volume curve derived from spirometry is obtained by plotting maximum flows versus expired volume. During expiration, flow increases first rapidly and then decreases in spite of additional effort. Expiratory flows such as FEF₇₅ are largely effort-independent. It is a widely distributed belief...
that FEF, is highly variable and therefore useless as outcome measure. However, this view is contradicted by the results of a number of randomized controlled trials in CF. The usefulness of any parameter depends on the balance between signal and variability. FEF, is more variable than FEV, but this greater variability may well be offset by a larger expected effect size for FEF,. FEF, might therefore be an appropriate endpoint for the evaluation of early stages of CF lung disease, but this concept has not been systematically evaluated. We hypothesized that FEF, might be more abnormal at an earlier age than FEV, or forced vital capacity (FVC) in CF.

The aim of our study was to describe the progression of the spirometry parameters FVC, FEV, and FEF, as a function of age in a group of CF patients aged 6 to 20 years in comparison with longitudinally collected spirometry data from two cohorts of healthy subjects. In addition, we aimed to provide sample size estimates for clinical trials utilizing these parameters as primary endpoints.

**Materials and Methods**

**CF patients**

Serial spirometry data from 1989 to 2008 from 163 CF patients aged 6 to 20 years followed at the Sophia Childrenâ€™s Hospital CF Centre (Rotterdam, The Netherlands) were extracted from the electronic patient dossier. Patients had CF based on standard diagnostic criteria. Data were used only when parents and/or patients had signed informed consent for anonymous retrospective use of clinical data. This retrospective cohort study was approved by the ethical review board of our hospital.

**Healthy controls**

Serial spirometry data were obtained from two previously described cohorts of healthy Dutch children. The first cohort included 610 boys and 178 girls aged 11 to 20 years that performed spirometry at half yearly intervals starting in 1978 until they left school. FVC, FEV, and FEF, were recorded. The second cohort included 206 boys and 214 girls aged 6 to 11 years who performed four spirometry measurements between 1984 and 1987. In this cohort, FVC and FEV, were recorded, but FEF, was not.

**Statistical methods**

Quantile regression was used to describe the developmental patterns of FVC, FEV, and FEF, among CF patients and healthy children, for boys and girls separately. In the primary analysis lung function parameters (in liters or liters/second) were modeled as a function of age. As children with CF are on average shorter than age-matched healthy controls, any observed differences in lung function between CF and healthy control children may in part be due to their differences in height. Thus, the lung function parameters were modeled separately as a function of age and as a function of height. The fitted quantile regression models contained main effects for age or height (the spline), group, and the interaction between these two. The 10th percentile (P), 50th percentile (P) and 90th percentile (P) quantiles were determined; 95% confidence intervals were calculated for the P only (additional details on the statistical methods are available in the supplement at the end of this chapter). A percentile (or centile) is the value of a variable below which a certain percent of observations fall. For example, the 10th percentile is the value below which 10 percent of the observations may be found.

Means and standard deviations of height, weight and lung function raw values and Z-scores of CF patients and healthy children were calculated at the ages of 8, 12 and 16 years. If more than one measurement at the same integer age was available, the one closest to but after the birthday was used for the analyses. The reference equations by Stanojevic et al. were used to calculate z-scores for FVC and FEV, and those by Zapletal et al. for FEF,. Because FEF, data were not available for healthy controls <12 years of age, the predicted value was calculated for each individual based on height using the equations by Zapletal et al. Wilcoxon rank sum tests were used to evaluate differences between healthy children and CF patients. No adjustment was made for multiple comparisons.

Sample size calculations were performed using FEF,, FEV, or FVC as the primary outcome. We assumed that the maximal effect possible is the difference between the average lung function of CF patients and healthy controls. Sample sizes were estimated using a minimal detectable treatment effect equal to 1/3 of this maximal effect for each parameter, with an alpha of 0.05 and a power of 0.80 (for additional details see the supplement provided at the end of this chapter). Statistical analyses were done with SAS version 9.2 TS2M0 (SAS Institute, Cary, North Carolina, USA).

**Results**

There were 7367 spirometry measurements available from 163 CF patients and 8338 measurements from 1371 healthy
children. See table 1 for general characteristics of the CF patients. A histogram of the age distribution of CF patients in this study is given in the supplement at the end of this chapter.

<table>
<thead>
<tr>
<th></th>
<th>CF patients (N = 163)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>79/84</td>
</tr>
<tr>
<td>CFTR mutations (N (%))</td>
<td></td>
</tr>
<tr>
<td>dF508/dF508</td>
<td>96 (59%)</td>
</tr>
<tr>
<td>dF508/Other</td>
<td>49 (30%)</td>
</tr>
<tr>
<td>Other/Other</td>
<td>16 (10%)</td>
</tr>
<tr>
<td>N/A</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>Age (yrs) at start follow up</td>
<td>7.5 (2.5)</td>
</tr>
<tr>
<td>Age (yrs) at end of follow up</td>
<td>19.2 (4.1)</td>
</tr>
<tr>
<td>Years of follow up</td>
<td>7.7 (3.9)</td>
</tr>
</tbody>
</table>

Table 1. General characteristics of CF patients. Data are shown as mean (SD) unless stated otherwise.

Children with CF on average were significantly shorter and lighter than healthy children of the same age. With the exception of FVC z-score among males at age 16, CF patients had significantly lower FVC, FEV\textsubscript{1} and FEF\textsubscript{75}, both expressed as raw values and z-scores at each age tested (Tables 2a and 2b).

Table 2a.

<table>
<thead>
<tr>
<th>N</th>
<th>Age 8 yrs</th>
<th>p</th>
<th>Age 12 yrs</th>
<th>p</th>
<th>Age 16 yrs</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CF (TML)</td>
<td></td>
<td>Healthy (TML)</td>
<td></td>
<td>CF (TML)</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>130.3 (6.7)</td>
<td>0.0004</td>
<td>149.5 (7.5)</td>
<td>&lt;0.0001</td>
<td>171.7 (5.9)</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>133.9 (5.6)</td>
<td></td>
<td>155.1 (7.8)</td>
<td></td>
<td>176.2 (7.4)</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>149.5 (7.5)</td>
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<td>155.1 (7.8)</td>
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<td>171.7 (5.9)</td>
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</tr>
<tr>
<td>298</td>
<td>19.5 (2.1)</td>
<td>0.0038</td>
<td>20.5 (2.5)</td>
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<td>20.5 (2.5)</td>
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</table>

Table 2b.

<table>
<thead>
<tr>
<th>N</th>
<th>Age 8 yrs</th>
<th>p</th>
<th>Age 12 yrs</th>
<th>p</th>
<th>Age 16 yrs</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Healthy (TMLC)</td>
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<td>CF (TMLC)</td>
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<tr>
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<td>1.52 (0.24)</td>
<td>&lt;0.0001</td>
<td>2.04 (0.46)</td>
<td>&lt;0.0001</td>
<td>3.10 (0.79)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>0.41 (0.83)</td>
<td></td>
<td>0.41 (0.84)</td>
<td></td>
<td>0.79 (0.60)</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>2.04 (0.46)</td>
<td></td>
<td>2.55 (0.41)</td>
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<td>3.98 (0.60)</td>
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<tr>
<td>152</td>
<td>3.10 (0.79)</td>
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<td>3.98 (0.60)</td>
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</table>
### Table 2a.

<table>
<thead>
<tr>
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<th>Age 12 yrs</th>
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<th>Age 16 yrs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CF (♂)</td>
<td>Healthy (♂)</td>
<td>p</td>
<td>CF (♂)</td>
<td>Healthy (♂)</td>
<td>p</td>
</tr>
<tr>
<td>57</td>
<td>213</td>
<td>56</td>
<td>298</td>
<td>43</td>
<td>412</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>130.3 (6.7)</td>
<td>149.5 (7.5)</td>
<td>0.0004</td>
<td>155.1 (7.8)</td>
<td>&lt;0.0001</td>
<td>171.7 (5.9)</td>
</tr>
<tr>
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<td>44.0 (8.2)</td>
<td>&lt;0.0001</td>
<td>57.9 (8.9)</td>
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<td>BMI (kg/m²)</td>
<td>16.4 n.a.</td>
<td>17.2 (1.4)</td>
<td>0.0038</td>
<td>18.1 (2.4)</td>
<td>&lt;0.0001</td>
<td>19.5 (2.1)</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>1.87 (0.32)</td>
<td>2.60 (0.54)</td>
<td>&lt;0.0001</td>
<td>3.02 (0.50)</td>
<td>&lt;0.0001</td>
<td>4.17 (0.88)</td>
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<tr>
<td>(z-score)</td>
<td>0.32 (0.28)</td>
<td>0.027 (0.86)</td>
<td>0.043</td>
<td>â¨0.97 (1.42)</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>FVC (z-score)</td>
<td>1.21 (0.73)</td>
<td>1.28 (0.86)</td>
<td>0.0001</td>
<td>â¨1.86 (1.42)</td>
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<tr>
<td>FEV₁ (L)</td>
<td>1.52 (0.26)</td>
<td>2.04 (0.46)</td>
<td>&lt;0.0001</td>
<td>2.55 (0.41)</td>
<td>&lt;0.0001</td>
<td>3.10 (0.79)</td>
</tr>
<tr>
<td>(z-score)</td>
<td>0.24 (0.41)</td>
<td>â¨1.41 (1.28)</td>
<td>0.0001</td>
<td>â¨0.37 (0.84)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>FEV₁ (z-score)</td>
<td>1.17 (0.83)</td>
<td>0.41 (1.42)</td>
<td>&lt;0.0001</td>
<td>â¨0.41 (0.84)</td>
<td>&lt;0.0001</td>
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<tr>
<td>FEF₇₅ (L/s)</td>
<td>0.71 (0.30)</td>
<td>0.81 (0.39)</td>
<td>&lt;0.0001</td>
<td>1.61 (0.46)</td>
<td>&lt;0.0001</td>
<td>1.02 (0.51)</td>
</tr>
<tr>
<td>(z-score)</td>
<td>1.23 (0.37)*</td>
<td>1.42 (1.42)</td>
<td>&lt;0.0001</td>
<td>â¨1.86 (1.42)</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>FEF₇₅ (z-score)</td>
<td>2.43 (1.59)*</td>
<td>â¨3.31 (1.28)</td>
<td>&lt;0.0001</td>
<td>â¨4.33 (1.32)</td>
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</table>

### Table 2b.

<table>
<thead>
<tr>
<th>N</th>
<th>Age 8 yrs</th>
<th></th>
<th>Age 12 yrs</th>
<th></th>
<th>Age 16 yrs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CF (♂)</td>
<td>Healthy (♂)</td>
<td>p</td>
<td>CF (♂)</td>
<td>Healthy (♂)</td>
<td>p</td>
</tr>
<tr>
<td>128.8</td>
<td>133.1</td>
<td>151.8</td>
<td>163.8</td>
<td>0.0026</td>
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</tr>
<tr>
<td>(cm)</td>
<td>(5.3)</td>
<td>(7.1)</td>
<td>(6.4)</td>
<td>(6.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>26.6 n.a.</td>
<td>39.9 (7.7)</td>
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<td>45.3 (8.0)</td>
<td>&lt;0.0001</td>
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<td>19.3 (2.3)</td>
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<tr>
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<td>1.71 (0.32)</td>
<td>2.52 (0.53)</td>
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<td>2.90 (0.53)</td>
<td>&lt;0.0001</td>
<td>3.08 (0.63)</td>
</tr>
<tr>
<td>(z-score)</td>
<td>0.32 (0.26)</td>
<td>1.00 (0.53)</td>
<td>0.0016</td>
<td>0.49 (0.53)</td>
<td>0.0062</td>
<td>1.47 (0.63)</td>
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<tr>
<td>FVC (z-score)</td>
<td>1.10 (0.73)</td>
<td>1.16 (0.99)</td>
<td>0.0001</td>
<td>â¨1.00 (1.16)</td>
<td>0.98</td>
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</tr>
<tr>
<td>FEV₁ (L)</td>
<td>1.39 (0.31)</td>
<td>1.98 (0.52)</td>
<td>&lt;0.0001</td>
<td>2.58 (0.45)</td>
<td>&lt;0.0001</td>
<td>2.23 (0.63)</td>
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<tr>
<td>(z-score)</td>
<td>0.23 (0.45)</td>
<td>1.75 (0.91)</td>
<td>&lt;0.0001</td>
<td>â¨1.35 (1.37)</td>
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<tr>
<td>FEV₁ (z-score)</td>
<td>1.36 (0.77)</td>
<td>1.37 (0.91)</td>
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<td>FEF₇₅ (L/s)</td>
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<td>0.80 (0.48)</td>
<td>&lt;0.0001</td>
<td>1.91 (0.60)</td>
<td>&lt;0.0001</td>
<td>0.68 (0.42)</td>
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<tr>
<td>(z-score)</td>
<td>1.25 (0.48)</td>
<td>4.79 (0.60)</td>
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<td>â¨4.16 (1.37)</td>
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</tr>
<tr>
<td>FEF₇₅ (z-score)</td>
<td>0.31 (0.36)*</td>
<td>0.11 (0.40)</td>
<td>&lt;0.0001</td>
<td>â¨6.36 (1.43)</td>
<td>0.30</td>
<td></td>
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</tbody>
</table>

**Table 2.** Characteristics of CF patients and healthy children at the ages of 8, 12 and 16 years. Table 2a: boys. Table 2b: girls.
<table>
<thead>
<tr>
<th>N</th>
<th>Age 8 yrs</th>
<th></th>
<th>Age 12 yrs</th>
<th></th>
<th>Age 16 yrs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CF (aTM)</td>
<td>Healthy (aTM)</td>
<td>p</td>
<td>CF (aTM)</td>
<td>Healthy (aTM)</td>
<td>p</td>
</tr>
<tr>
<td>57</td>
<td>130.3 (6.7)</td>
<td>133.9 (5.6)</td>
<td>0.0004</td>
<td>149.5 (7.5)</td>
<td>155.1 (7.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>130.3 (6.7)</td>
<td>133.9 (5.6)</td>
<td>0.0004</td>
<td>149.5 (7.5)</td>
<td>155.1 (7.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>213</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>149.5 (7.5)</td>
<td>155.1 (7.8)</td>
<td>&lt;0.0001</td>
<td>171.7 (5.9)</td>
<td>176.2 (7.4)</td>
<td>0.0001</td>
</tr>
<tr>
<td>298</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>171.7 (5.9)</td>
<td>176.2 (7.4)</td>
<td>0.0001</td>
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<td>412</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>28.0 (4.3)</td>
<td>n.a.</td>
<td>n.a.</td>
<td>38.7 (5.8)</td>
<td>44.0 (8.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>16.4 (1.4)</td>
<td>n.a.</td>
<td>n.a.</td>
<td></td>
<td>17.2 (1.4)</td>
<td>18.1 (2.4)</td>
<td>0.0038</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>57.9 (8.9)</td>
<td>63.7 (9.7)</td>
<td>0.0001</td>
<td>19.5 (2.1)</td>
<td>20.5 (2.5)</td>
<td>0.028</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>1.87 (0.32)</td>
<td>2.16 (0.28)</td>
<td>&lt;0.0001</td>
<td>2.60 (0.54)</td>
<td>3.02 (0.50)</td>
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</tr>
<tr>
<td>1.52 (0.26)</td>
<td>1.91 (0.24)</td>
<td>&lt;0.0001</td>
<td>2.04 (0.46)</td>
<td>2.55 (0.41)</td>
<td>&lt;0.0001</td>
<td>3.10 (0.79)</td>
</tr>
<tr>
<td>FEV1 (L)</td>
<td>0.71 (0.30)</td>
<td>1.23 (0.37)*</td>
<td>&lt;0.0001</td>
<td>0.81 (0.39)</td>
<td>1.61 (0.46)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>0.71 (0.30)</td>
<td>1.23 (0.37)*</td>
<td>&lt;0.0001</td>
<td>0.81 (0.39)</td>
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<td>&lt;0.0001</td>
<td>1.02 (0.51)</td>
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<tr>
<td>FEV1 (z-score)</td>
<td>0.71 (0.30)</td>
<td>1.23 (0.37)*</td>
<td>&lt;0.0001</td>
<td>0.81 (0.39)</td>
<td>1.61 (0.46)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>0.71 (0.30)</td>
<td>1.23 (0.37)*</td>
<td>&lt;0.0001</td>
<td>0.81 (0.39)</td>
<td>1.61 (0.46)</td>
<td>&lt;0.0001</td>
<td>1.02 (0.51)</td>
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<tr>
<td>FEF75 (L/s)</td>
<td>0.57 (1.09)</td>
<td>1.31 (2.43)*</td>
<td>&lt;0.0001</td>
<td>0.85 (1.32)</td>
<td>2.82 (1.32)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>0.57 (1.09)</td>
<td>1.31 (2.43)*</td>
<td>&lt;0.0001</td>
<td>0.85 (1.32)</td>
<td>2.82 (1.32)</td>
<td>&lt;0.0001</td>
<td>0.34 (1.46)</td>
</tr>
<tr>
<td>FEF75 (z-score)</td>
<td>0.57 (1.09)</td>
<td>1.31 (2.43)*</td>
<td>&lt;0.0001</td>
<td>0.85 (1.32)</td>
<td>2.82 (1.32)</td>
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</tr>
<tr>
<td>0.57 (1.09)</td>
<td>1.31 (2.43)*</td>
<td>&lt;0.0001</td>
<td>0.85 (1.32)</td>
<td>2.82 (1.32)</td>
<td>&lt;0.0001</td>
<td>0.34 (1.46)</td>
</tr>
</tbody>
</table>

Quantile regression analyses

In quantile regression models using age as predictor, age, group and the interaction of age and group were significant for each lung function parameter (p< 0.0001). Thus, for each lung function parameter, the total curves, and the effect of age on lung function with age differed significantly between CF and healthy controls. Similar patterns were observed in boys and girls.

The different distributions of FVC, FEV1, and FEF75, in CF patients compared to healthy children are shown in Figure 1. The difference between CF patients and healthy children increases with advancing age, and the pattern of abnormality in the CF patients is different for the three lung function parameters.
Figure 1, panel A-F. Panel A, B, C show forced vital capacity (FVC), forced expiratory volume in 1 second (FEV₁) and forced expiratory flow at 75 percent of FVC (FEF₇₅) for boys. Panel D, E, F show FVC, FEV₁, and FEF₇₅ for girls. Data for healthy children are shown in grey, data for male CF patients in blue and data for female CF patients in red. On the X-axis is age in years; on the Y-axis are spirometry values (FVC, FEV₁, and FEF₇₅) in liters or liters/sec.
Dots indicate individual spirometry measurements. The grey lines and colored lines represent the fitted percentile lines for healthy children and CF patients respectively. For each group the lower line represents the P_{10}, the middle line represents the P_{50} and the upper line represents the P_{90}. The grey and colored bands represent the 95% CI of the fitted P_{50} splines.

For FVC, there is a clear difference between healthy children and CF patients, but a substantial portion of CF patients has FVC values within the normal range. At the age of 8 years approximately 30% of CF patients have values of FVC that are below the P_{10} of healthy controls. This percentage remains stable to the age of 16 years, although the magnitude of FVC abnormalities in the CF patients increases (Figure 1, panel a and d). For FEV\textsubscript{1}, the difference between CF and healthy children is larger than for FVC (Figure 1, panel b and e). At the age of 8 years almost 50% of CF patients have FEV\textsubscript{1} values that are below the P_{10} for healthy controls. Around the age of 16 years for boys and 9 years for girls, the P_{50} for CF patients clearly drops below the P_{10} for healthy children. For FEF\textsubscript{75}, the difference between CF and healthy is much larger at all ages than for FVC or FEV\textsubscript{1} (Figure 1, panel c and f). At 8 years, the P_{90} for CF patients is close to the P_{10} of healthy children. While FEF\textsubscript{75} values increase with age in the healthy children, there is almost no increase in FEF\textsubscript{75} from 6 to 18 years in the CF patients, as indicated by the horizontal course of the CF quantiles.

All spirometry measurements from CF patients were included in the analysis, also those obtained during an exacerbation. Since this might have resulted in an overestimation of the difference between CF patients and healthy children, we performed a sensitivity analysis including only the year’s best spirometry values for both groups. Results were similar to the analyses performed on all data, again showing an earlier and more severe decline in FEF\textsubscript{75} than in FEV\textsubscript{1} or FVC in CF patients (data on file).

A second analysis that was performed using height rather than age as predictor in the quantile regression analyses also showed similar results (see Figure E2 in the supplement at the end of this chapter). Differences between CF patients and healthy children were slightly smaller when analyzed using height as a predictor than when using age, indicating that only a small proportion of these differences might be explained by differences in height between CF patients and healthy children.

**Sample size estimates**

To detect a treatment effect equal to 1/3 of the difference between the average lung function of CF patients and healthy controls, fewer patients would be needed in each arm of a clinical trial when the primary endpoint is FEF\textsubscript{75} compared with FEV\textsubscript{1} or FVC (Table 3). This difference is evident at each of the three evaluated ages (8, 12 and 16 years). Depending on age and gender, the number of patients needed in a trial when FEV\textsubscript{1} is used as an endpoint is estimated to be 1.5 to 5 times higher than when FEF\textsubscript{75} is used.

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Treatment Effect</th>
<th>SD</th>
<th>Subjects per arm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys</td>
<td>Girls</td>
<td>Boys</td>
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<tr>
<td>FVC (ml)</td>
<td>8</td>
<td>99</td>
<td>90</td>
</tr>
<tr>
<td>FEV\textsubscript{1} (ml)</td>
<td>8</td>
<td>129</td>
<td>133</td>
</tr>
<tr>
<td>FEF\textsubscript{75} (ml/s)</td>
<td>8</td>
<td>175</td>
<td>212</td>
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<tr>
<td>FVC (ml)</td>
<td>12</td>
<td>140</td>
<td>127</td>
</tr>
<tr>
<td>FEV\textsubscript{1} (ml)</td>
<td>12</td>
<td>171</td>
<td>202</td>
</tr>
<tr>
<td>FEF\textsubscript{75} (ml/s)</td>
<td>12</td>
<td>264</td>
<td>370</td>
</tr>
<tr>
<td>FVC (ml)</td>
<td>16</td>
<td>156</td>
<td>201</td>
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<tr>
<td>FEV\textsubscript{1} (ml)</td>
<td>16</td>
<td>294</td>
<td>352</td>
</tr>
<tr>
<td>FEF\textsubscript{75} (ml/s)</td>
<td>16</td>
<td>567</td>
<td>620</td>
</tr>
</tbody>
</table>

**Table 3.** Sample size estimates for a clinical trial in CF patients that aims to show a treatment effect in FVC, FEV\textsubscript{1} or FEF\textsubscript{75} equal to 1/3 of the difference between the average lung function of healthy children and CF patients found in our study at ages 8, 12 and 16 years. Treatment effects and SD are reported in mL. Numbers of patients needed in one arm of a parallel design clinical trial with two groups for an alpha
In conclusion, our study shows that FEF₂₅₋₇₅ absence of FEF₂₅₋₇₅ average height in Dutch children has increased slightly in the last decades. Secondly, to compute Z-scores we used a different set of reference equations for FVC and FEV₁ given the clear and gradual trend of FEF₂₅₋₇₅ values were estimated using the reference equations by Zapletal, which likely underestimated their variation. However, previous studies have shown the reproducibility of FEF₂₅₋₇₅ to be relatively high in children, with a coefficient of variation less than 10%. Part of the variability in FEF₂₅₋₇₅ will occur due to actual variation in the population, as reflected by larger population standard deviation. Data from the Canadian CF Patient Data Registry showed that the SD of FEF₂₅₋₇₅ was roughly 1.5-times higher than of FEV₁. Our data showed a somewhat larger difference; the SD of FEF₂₅₋₇₅ was about twice as high as for FEV₁. When planning a clinical trial it is important to realize this greater variability may well be offset by a larger expected effect size. Our data clearly show that there is more room for improvement in FEF₂₅₋₇₅ than in FEV₁ in CF patients, especially before adolescence. In line with this observation the Canadian registry reported in CF-patients aged 10-14 yrs a mean FEF₂₅₋₇₅ of 62% predicted, compared to a mean FEV₁ of 77% predicted. Based on our sample size estimations taking into account both SD and expected effect size, FEF₂₅₋₇₅ may be a more sensitive outcome than FEV₁. Importantly, this only holds true if the expected effects of a new intervention on FEF₂₅₋₇₅ are the same or larger than effects on FEV₁. We find this likely, given that small airway disease is a hallmark of CF and many interventions specifically aim to prevent this. Indeed, several clinical trials have given evidence that peripheral flows are sensitive endpoints to detect and monitor CF- lung disease. In the PEIT study (RCT, n=474) dornase alfa was administered for two years to young CF patients with an FVC 48%±85%; the treatment benefit in FEV₁ was 3.2% A±1.2% predicted (p = 0.0060), while the treatment benefit in FEF₂₅₋₇₅ was substantially larger: 7.9% A±2.3% predicted (p = 0.0008). A randomized trial (n=24) on the timing of airway clearance techniques (ACT) found similar results. A significant increase in FEF₂₅₋₇₅ was found in children using dornase alfa before ACT compared with children using dornase alfa after ACT (58.3% vs. 52.5% predicted, p = 0.01)., while in other spirometric parameters no significant difference could be detected. Two more recent intervention studies in CF patients using both FEV₁ and peripheral flows (FEF₂₅₋₇₅ or FEF₅₋₁₅) have reported significant effects on FEF₂₅₋₇₅, while no significant results were found for FEV₁. An important advantage of the use of end expiratory flows such as the FEF₂₅₋₇₅ as outcome parameter in clinical studies is that it can be acquired using routine spirometry which is widely available. Another promising outcome parameter for detecting and monitoring small airways disease in CF is lung clearance index (LCI) using the multiple breath wash out test. An important advantage of the multiple breath wash out test over spirometry is that it can be measured relatively easily in most children below the age of 6 since it requires minimal cooperation and coordination. How LCI compares to FEF₂₅₋₇₅ as outcome parameter to track small airways disease in children with CF of 6 years and above requires further comparative studies.

Strengths of our study are the large sample sizes of both CF patients and healthy controls. Spirometry measurements were acquired longitudinally in the same subjects over multiple years. Additionally, the cohorts of CF patients and healthy controls were Dutch children in similar age ranges. Furthermore, this was a single center study which most likely reduced the noise related to the spirometry measurements. Finally, advanced statistical techniques were used to give insight in the population distribution and development of lung function with increasing age. A number of limitations should be discussed. First, we did not have FEF₂₅₋₇₅ results in the healthy controls <12 years of age. These values were estimated using the reference equations by Zapletal, which likely underestimated their variation. However, given the clear and gradual trend of FEF₂₅₋₇₅ in healthy subjects after the age of 12, we find it unlikely that this was a source of bias. Secondly, to compute Z-scores we used a different set of reference equations for FVC and FEV₁, as for FEF₂₅₋₇₅. Thirdly, there was a difference in the years during which measurements in CF patients and healthy controls were collected; CF patients were measured from 1989 to 2008 and healthy children were measured from 1978 to 1987. Since average height in Dutch children has increased slightly in the last decades, this difference in time of data collection could have led to a modest underestimation of the difference between healthy children and CF patients. Finally, we consider FEF₂₅₋₇₅ and forced expiratory flow at 25-75% of forced vital capacity (FEF₅₋₁₅) to be similar measures that both reflect peripheral flow and are highly correlated since both are volume referenced flow variables. However, in the absence of FEF₅₋₁₅ data for our cohorts we cannot be sure that a similar pattern would be present as for FEF₂₅₋₇₅.

In conclusion, our study shows that FEF₂₅₋₇₅ is a sensitive marker of early obstructive lung disease in CF which can be
used in most children with CF at the age spirometry first becomes feasible. Our findings add further support to the use of FEF<sub>75</sub> as endpoint in clinical trials targeting early stages of CF lung disease, especially when an effect on the small airways can be expected.

**Acknowledgements**

We thank dr. Ph.H. Quanjer and dr. B. Brunekreef for kindly allowing us to use the spirometry data from their studies in healthy children; we thank all CF patients that gave informed consent for anonymous use of their data; we thank the lung function technicians at the Erasmus MC â€“ Sophia Childrenâ€™s Hospital for performing the large number of spirometry measurements.

**References**


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Supplement to Chapter 3

Methods

Data collection cystic fibrosis (CF) patients

Spirometry measurements were performed in the lung function laboratory according to ERS and later ERS/ATS guidelines at each visit to the outpatient clinic and during admissions for an exacerbation. In general patients routinely visited the outpatient clinic every three months and had additional visits in case of symptoms. Hence numbers of spirometry measurements vary from patient to patient (min. 3 to max. 251 measurements per patient). To verify the data in the electronic patient dossier (EPD) a longitudinal plot of all available spirometry data was generated for each patient. Each plot was inspected on the presence of outliers, which were defined as data points that differed 20% or more from the previous spirometry measurement. All outliers were verified against the printed or PDF version of the original spirometry result and were corrected when necessary. Subsequently, verified data on height, weight, gender, age and spirometry variables forced vital capacity (FVC), forced expiratory volume in 1 second (FEV₁), and the forced expiratory flow at 75 percent of FVC (FEF₇₅) of CF patients were extracted from the EPD database into an Excel file for further analyses.

Statistical methods

Quantile regression was used to visualize the developmental patterns of FVC, FEV₁, and FEF₇₅ in the groups of CF patients and healthy children, for boys and girls separately.

Age and group or height and group (CF patients vs. healthy) were used as the only two predictors in the quantile regressions. We used the new experimental Æ“effectÆ” statement with the Æ“splineÆ” option to fit flexible curves for selected quantiles. These splines had five knots defined at 6, 10, 13, 16, and 20 years of age. The same splines were used for the patients and for the healthy controls. The P₁₀, P₅₀, and P₉₀ quantiles were determined, and 95% confidence intervals were calculated for the P₅₀ only. These intervals, the fitted splines and the raw data were then plotted using the Output Delivery System and the Statistical Graphics Procedures in SAS version 9.2. Data points outside the age limits of 6 to 20 years were omitted from the analysis.

The fitted quantile regression models contained main effects for age (the spline), for group, and the interaction between these two. The main effect for age indicated whether the level of the fitted spline changed with age. The main effect for group indicated whether there was an overall difference in level between the groups of CF patients and of healthy children over the complete age range studied. And finally, the interaction between group and age indicated whether the course of the spline over time differed between CF patients and healthy children. Analyses were repeated using height as a predictor instead of age. These quantile regression models contained main effects for height (the spline), for group, and the interaction between these two.

Statistical significance of the included effects was judged from likelihood ratio tests. Only the significance tests of the 50th quantiles were used.

Sample size calculations were performed using FEF₇₅, FEV₁, or FVC as primary outcome.

For FVC, FEV₁, and FEF₇₅, we used the calculated means and SDÆ™s of the CF patients (as shown in table 1), and only the means of the healthy children in these calculations.

Results

Quantile regression analyses with height as predictor showed similar results to the analyses using age as predictor (see E-Figure 1 of this supplement).
Median FEV1 in girls

Height (cm)

FEV1 (l)
E-Figure 1, panel A-F. Panel A, B, C show FVC, FEV1 and FEF75 for boys. Panel D, E, F show FVC, FEV1 and FEF75 for girls. Data for healthy children are shown in grey, data for male CF patients in blue and data for female CF patients in red. On the X-axis is height in cm; on the Y-axis are spirometry values (FVC, FEV1 and FEF75) in liters or liters/sec. Dots indicate individual spirometry measurements. The grey lines and colored lines represent the fitted splines for healthy children and CF patients respectively.

For each group the lower line represents the P10, the middle line represents the P50 and the upper line represents the P90. The grey and colored bands represent the 95% CI of the fitted P50 splines.
Determining presence of lung disease in young children with cystic fibrosis: lung clearance index, oxygen saturation and cough frequency


Abstract

Background

Accurate assessment of pulmonary status in young children with cystic fibrosis (CF) requires sensitive and objective monitoring techniques.

Objectives

This study aimed to evaluate the feasibility of lung clearance index (LCI) calculated from multiple breath washout (MBW), home nocturnal pulse oximetry and home nocturnal cough recording in young children with CF, and determine whether these tests can distinguish CF patients from healthy controls.

Methods

We performed a prospective cross-sectional study in 20 CF patients and 30 healthy children aged 0-4 years. MBW was performed in awake and unsedated children at the outpatient clinic using a commercially available device. Measurements of nocturnal oxygen saturation and nocturnal cough were done at home using a pulse oximeter and an audiometer.

Results

There was a significant difference in mean LCI between healthy children and CF patients (LCI 7.1 vs. 9.3, p < 0.001). Nocturnal oxygen saturation was normal in both groups and did not significantly differ between the groups. Similarly, cough showed no differences between both groups. Cough varied widely between children and between nights. Success rates for saturation and cough measurements were 90% and were similar for CF patients and healthy children. Success rate for LCI was 75% for CF patients and 50% for healthy children.

Conclusions

Measurements of LCI, nocturnal oxygen saturation and cough were feasible in young children; however LCI was the only variable that showed a significant difference between children with CF and healthy children.

Introduction

Children with cystic fibrosis (CF) develop structural lung disease including bronchiectasis and small airways disease early in life, even in the absence of clinical symptoms or infection. Sensitive and accurate monitoring techniques to assess pulmonary status are required for clinical management and as endpoints in clinical trials. These techniques should be fast, easy to perform and non-invasive, to allow repeated assessment for timely identification and treatment of children at risk of developing irreversible structural lung disease.

For children 6 years and older routine spirometry plays a central role in monitoring and management of CF lung disease. However, pre-school spirometry in children aged 4-6 years is challenging since it requires active cooperation. When measured in a paediatric lung function laboratory using computer-incentive games, pre-school spirometry is successful in 45–75% of the children. Below the age of 4 years spirometry is even more difficult, since the average 3.5-year-old child has a developmental level that allows carrying out three-step commands only.
Therefore, the instructions for a forced expiratory spirometry manoeuvre need to be broken down and taught in a manner that is appropriate to the child's development. Unfortunately, the majority of 2 to 3-year old children are unable to perform pre-school spirometry. Infant pulmonary function testing (iPFT) can be performed in children <3 years using a baby bodybox. However, iPFT is time consuming, technically difficult, and requires sedation. After 2 years, most children have outgrown the equipment for iPFT. Consequently, there is great need for alternative non-invasive monitoring instruments for young children.

A promising lung function test to monitor pulmonary disease in children is multiple breath wash out (MBW), which can be performed without sedation and does not require active cooperation. The lung clearance index (LCI) is calculated from MBW, indicating inhomogeneity of ventilation. Studies have suggested that LCI is more sensitive than spirometry to detect early lung disease in patients with CF. A study in young children showed that lung disease indicated by an elevated LCI starts early in life. However, measurements were performed in children in supine position during sleep after sedation with chloral hydrate. Sedation makes the test even more time consuming and necessitates monitoring of patients during sleep. Therefore we are interested in the feasibility of measuring LCI in young children, while awake and sitting upright. Disadvantages of MBW include the expensive equipment and requirement for a lung function laboratory as it is a technically demanding test.

A second test to monitor pulmonary disease in children is home nocturnal pulse oximetry measuring oxygen saturation (SpO₂) during sleep. A pulse oximeter is relatively cheap, easy to operate, and can be used across all ages. In CF patients with moderate lung disease and normal awake SpO₂, significant nocturnal desaturation can occur. It is unclear whether nocturnal pulse oximetry can be used as a surrogate endpoint for CF lung disease in young children, and whether pulse oximetry is sensitive enough to distinguish between healthy children and children with CF.

A third test to monitor pulmonary disease in children is nocturnal cough. Cough is an important component of mucociliary clearance and is thought to be more frequent in CF compared to healthy control subjects. Cough monitoring has been used in small scale clinical studies, and can be monitored at home using relatively simple audiometry. It is not known whether nocturnal cough monitoring can be used as a surrogate endpoint in young children.

The aim of our study was to evaluate the feasibility of performing MBW, home nocturnal pulse oximetry, and home nocturnal cough recording in young children. In addition we compared the sensitivity of these tests to discriminate CF patients from healthy controls.

Methods

Enrolment

We performed a prospective cross-sectional study to measure LCI, nocturnal oxygen saturation and nocturnal cough in 20 CF patients and 30 healthy children aged 0-4 years. CF patients were recruited from the Paediatric Pulmonology outpatient clinic at the Erasmus MC â€“ Sophia Childrenâ€™s Hospital Rotterdam. Healthy children were recruited from three day-care centres in Rotterdam, located near the Sophia Childrenâ€™s Hospital.

Inclusion criteria for all children were: age 0-4 yrs and weight >8 kg (weight cut-off was selected to standardise MBW data collection with a single dead space reducer for children 8-20 kg).

Exclusion criteria for all children were: respiratory infection in the week prior to enrolment, antibiotics for pulmonary symptoms or hospital admission in the four weeks prior to enrolment, medical conditions that might affect study measurements (e.g. cardiac problems that can influence saturation), ear-nose-throat related interventions in the last month (e.g. tonsillectomy, tympanostomy tubes, etc.). For healthy children, additional exclusion criteria were: history of chronic pulmonary diseases (such as asthma, broncho pulmonary dysplasia, and airway malacia), history of premature birth (< 36 weeks of gestation), current pulmonary disease and maintenance use of pulmonary medications.

This study was approved by the local ethics committee and informed consent was obtained from all parents. This study was registered in the Dutch trial register (http://www.trialregister.nl).

Lung Clearance Index

MBW tests were performed at the outpatient clinic using a commercially available device (Exhalyzer®D, Ecomedics, Switzerland) that was previously described. Helium was used as tracer gas. For technical details see the supplement at the end of this chapter.

Measurements were performed while children were awake and sitting upright, either on their parents' lap or on a chair. To distract children during measurements we used animations on a portable DVD player, a favourite book or a
toy. In most children we used a close-fitting facemask (Laerdal® silicone mask 2). Dead space of the facemask was 24 ml. Only two children aged 4 years were able to use a mouthpiece and nose clip correctly, all other children were measured using a face mask.

Measurements were performed as follows: at the start of a measurement, subjects breathed normally through the facemask or mouthpiece. After a stabilization period of minimally 6 breaths, the bias flow automatically switched to the premixed tracer gas at end expiration, starting the wash-in. Wash-in continued until equilibrium was reached, defined as a visually stable plateau of the molar mass signal. The bias flow was then switched back to room air to start the washout phase, which was completed when the tracer gas concentration fell below 1/40 (2.5%) of the starting level. Wbreath® software (version 3, 19, 3, ndd Medical Technologies, Zurich, Switzerland) was used for data acquisition, storage and analysis.

For each participant we aimed to perform two technically successful measurements. After completing the full washout phase, the minimal time before the next measurement could be started was three minutes. However, in this young age group many children required a longer break to prepare for the next MBW so often the time between measurements was longer. Data acquisition and analysis were performed according to published quality criteria. Measurements were accepted when the child breathed normally during the measurement, and when no leakage of gas was observed. Additional details on quality control of measurements are available in the supplement at the end of this chapter.

Functional residual capacity (FRC) was calculated from the cumulative volume of expired tracer gas divided by the difference between end-tidal gas concentration at the start and end of the washout. LCI was calculated as the cumulative expired volume (CEV), divided by the FRC. Since there are no generally accepted LCI reference values for young children, we defined an abnormal LCI as a value exceeding 1.96 times the SD above the mean value for healthy children in this study.

When two successful LCI measurements were obtained in a patient, the mean of the two measurements was used for analyses. When only one measurement was successfully obtained (usually due to poor cooperation for a second measurement), this measurement was used in the analyses.

Nocturnal oxygen saturation

Nocturnal SpO$_2$ was measured using a Mars 2001 Pulse Oximeter (Novametrix, USA) during two separate nights of sleep at home. This device makes use of a Motion Artefact Rejection System that filters out artefacts caused by motion or low perfusion. Parents were instructed to attach the sensor to the child’s finger or toe and turn on the pulse oximeter when the child went to bed or shortly after the child fell asleep. The sensor was removed by the parents when the child woke up the following morning. A registration was considered appropriate for further analysis when at least 6 hours of successful recording was available. From the stored saturation data we computed mean SpO$_2$ and two desaturation parameters for each child: D4 desaturations, defined as desaturations of 4% below the mean saturation of the child; and D90 desaturations, defined as desaturations below 90% SpO$_2$. Number of desaturations was calculated as well as the total time of D4 and D90 desaturations. For mean SpO$_2$, a value below 95% was defined as abnormal.

Cough measurements

Measurements of cough were performed according to a previously published procedure. Cough measurements were ideally performed on the same nights as the pulse oximetry, but at least within two days of the pulse oximetry. Briefly, a digital audiometer was placed on a stable surface and positioned close to the child to record all sounds during the night. A sound recording was considered acceptable if at least 6 hours of recording were available.

Sound recordings were transferred from the digital recorder to a personal computer. All recordings were analyzed using free open source audio record & edit software, which provided a graphical display for audio analysis applications (Audacity, Boston, USA).

An investigator (JCvdM) identified by ear the nature of all sounds detected by the software on the digital recordings. The beginning of a cough episode was defined as the moment a clear explosive cough was heard. For each patient the duration of each cough episode was computed in seconds. Multiple cough sounds which occurred in quick succession were counted as one episode. Such cough episodes were defined to start at the onset of the first cough sound and end after the last cough (see Figure 1 of the supplement at the end of this chapter). Finally, from the total duration of cough seconds and the total time of the recording we computed the cough seconds per hour (csec/hr) for each night.
Power calculations

There are few data available on LCI, cough and saturation for children in this age range. Therefore it was not possible to do a formal power calculation prior to the study. We decided to invite all CF patients in our centre aged 0-4 years for this study and estimated that at a 70% response rate the number of inclusions would be approximately 15 patients. For each CF patient we aimed to include two healthy controls and thus aimed to include 30 healthy children. With these numbers, differences as great as 0.9 SD can be detected for an alpha of 0.05 and a power of 80%.

Statistical analysis

Analyses were performed with SPSS (version 15.0) or STATA (version 11.1) software. For normally distributed data, values are presented as mean (SD). Non-normally distributed values are reported as median (range).

Differences between group characteristics were assessed by independent samples T-test or Mann-Whitney test, as appropriate. Confidence intervals for the differences of medians between the two groups were calculated by the bootstrap method with 1000 replications. Intraclass Correlation Coefficients (ICC) were used to assess agreement between repeated measurements. Spearman’s correlation (r) was used to determine correlations between LCI, cough and saturation. For all analyses, p-values of <0.05 were considered statistically significant.

Results

Fifty children were included in this study, 20 CF patients and 30 healthy children aged 0-4 years, all recruited between July 2008 and March 2010. Baseline characteristics of the children did not differ between CF patients and healthy children except for BMI, which was lower in CF patients than in healthy controls (Table 1).

<table>
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<td>Age ranges (yrs)</td>
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<td>0.6 – 4.0</td>
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</tr>
<tr>
<td>Sex (M/F)</td>
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<td>19/11</td>
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<td>â”0.5 (1.1)</td>
<td></td>
</tr>
<tr>
<td>Weight (Z-score)</td>
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<td>0.8 (0.9)</td>
<td></td>
</tr>
<tr>
<td>BMI (Z-score)</td>
<td>0.0 (1.0)</td>
<td>0.9 (1.0)</td>
<td></td>
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<tr>
<td>Pancreatic insufficiency (Y/N)</td>
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<td>N/A</td>
<td></td>
</tr>
<tr>
<td>CF mutation (homozygous Phe508del / heterozygous PheF508del)</td>
<td>13/7</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Demographics of the study population at inclusion.

Data are reported as mean (SD) or as number of subjects. N/A = not applicable.

Lung clearance index

A successful LCI measurement was obtained in 15/20 CF patients (75%) and in 15/30 healthy children (50%). There was a trend towards higher success rate in children with CF (p=0.08). Reasons for failure included: rejection of the facemask, crying and/or talking during the test, irregular breathing that did not allow further analysis to compute LCI, or the test did not comply with published quality criteria (see Table 1 in the supplement at the end of this chapter).

Mean LCI was significantly higher in CF patients (LCI=9.3±1.5) compared to healthy children (LCI=7.1±0.7), with a difference between the groups of 2.2 (95% CI 1.3 - 3.1) (p=0.001) (Table 2 and Figure 1). LCI showed a good discriminating value between CF patients and healthy children: the Area Under the Curve (AUC) of the Receiver Operator Characteristic (ROC) curve was 0.90 (see Figure 2 in the supplement at the end of this chapter).
Table 2. LCI, oxygen saturation and cough in CF patients and healthy children.

All data are reported as median (range), except for LCI which is reported as mean (SD).

Figure 1. Lung clearance index (LCI) in healthy children and cystic fibrosis (CF) patients. Circles depict healthy children, diamonds depict CF patients. Horizontal bars indicate mean LCI for each group.

p<0.001 for the difference in mean LCI between CF patients and healthy children.

In CF patients we obtained two technically successful measurements in 8 patients and one successful measurement in 7 patients. For healthy children we obtained two technically successful measurements in 7 children and one successful measurement in 8 children. Since recent guidelines advise to obtain two LCI measurements per patient, we repeated the analysis including only the children who performed two measurements. Results were very similar to the primary analysis: mean LCI was significantly higher in CF patients (LCI = 9.1 Å± 1.7) compared to healthy children (LCI=7.2 Å± 0.8), with a difference between the groups of 1.9 (95% CI 0.4 â€“ 3.4) (p<0.02). Agreement between LCI measurements in children from who two measurements could be acquired was good: Intraclass Correlation Coefficient (ICC)=0.71 (Table 3).
Table 3. Saturation and cough for 2 nights, LCI for 2 measurements.

Data are reported as median (range) except for LCI which is reported as mean (SD). ICC = intraclass correlation coefficient.

The upper limit of normality for LCI calculated from our data as (mean+1.96 SD) was 8.4. Using this cut-off, 11/15 CF patients (73%) had an elevated LCI.

FRC results derived from MBW are available in the supplement at the end of this chapter.

**Nocturnal oxygen saturation**

Success rate for saturation measurements was 90%; successful measurements were obtained in 17/20 CF patients and 28/30 healthy children. Reasons for failure included: technical failure of the device, children not accepting the sensor despite multiple attempts by the parents, lively movements of a child during sleep causing a large number of artefacts in the measurement (see Table 1 in the supplement at the end of this chapter).

Mean nocturnal SpO₂ values had a median of 97.0% for CF patients and 97.7% for healthy children (Table 2). There was a trend towards lower SpO₂ in children with CF (p=0.08). Desaturations were infrequent in both groups and there were no differences in D4 and D90 desaturations between the two groups (Table 2).

Agreement between SpO₂ measurements on two separate nights was fair: ICC=0.41 and Spearmanâ€™s rank correlation coefficient (r) =0.5 (Figure 2). Mean nocturnal SpO₂ for the two nights is shown in Table 3.
Figure 2. Mean saturation measured on two nights. On the Y-axis mean saturation for the 1st night, on the X-axis the 2nd night. Spearman’s rank correlation coefficient ($r_s$) = 0.5, $p=0.001$. Open squares depict healthy children; closed circles depict cystic fibrosis patients. The dotted line shows the line of identity.

Five children had an abnormal nocturnal SpO$_2$ of <95%, including one CF patient and three healthy children who had a mean SpO$_2$ below 95% on one night; and one CF patient who had a mean SpO$_2$ below 95% on both nights.

Cough

Success rate for cough measurements was 90%; cough registration was successful in 27/30 healthy children and in 18/20 CF patients. Reasons for failure included: incorrect handling of the device by the parents, recording time <6 hours, technical failure, or multiple children sleeping in one room, making individual cough registration impossible (see Table 1 in the supplement at the end of this chapter).

Median cough seconds per hour (csec/hr) was 0.4 for CF patients and 0.8 for healthy children (Table 2). There was no significant difference in csec/hr between CF patients and healthy children ($p=0.50$). Ranges for nocturnal cough were relatively wide (Table 3). There was poor agreement between csec/hr measured on two separate nights: ICC=0.25.

Correlations between measurements

LCI did not correlate with mean nocturnal oxygen saturation or with mean cough, in either healthy children or CF
patients. LCI and mean saturation did not significantly correlate: $r_s = -0.28$ (p=0.33) for healthy children and $r_s = 0.35$ (p=0.25) for CF patients. For the correlation between LCI and csec/hr $r_s = -0.23$ (p=0.45) for healthy children and 0.06 (p=0.86) for CF patients.

## Discussion

In this study we evaluated the feasibility and sensitivity of LCI, home nocturnal oxygen saturation and home nocturnal cough measurements to distinguish young CF patients from healthy controls, and found that all three tests were feasible in young children; however LCI was the only variable that distinguished between children with CF and healthy children.

### LCI

Similar to other studies in older children or adults, we observed that LCI was elevated in 73% of young CF patients relative to healthy controls. It is generally thought that abnormal LCI reflects small airways disease. The importance of small airways disease is confirmed by recent papers. A recent study in young children with CF diagnosed by newborn screening showed that CT-detected trapped air was present in 50-60% of infants, indicating early small airways disease. A cross-sectional study in older children showed that elevated LCI was associated with CT-detected structural lung abnormalities. The elevated LCI observed in our study is therefore likely to indicate early CF lung disease. However, the clinical significance of elevated LCI in young children requires further validation. Lum et al. performed MBW and raised lung volume rapid thoracoabdominal compression measurements in infants with CF and healthy controls with a mean age of 41 weeks. In their cohort, lung function abnormalities were detected in 72% of infants with CF, which is very similar to the elevated LCI in 73% of CF patients in our study. However, Lum et al. demonstrated abnormalities in 41% by both techniques performed and in a further 15% by each of the two separate tests. This suggests that complementary information can be obtained by including both MBW and raised lung volume rapid thoracoabdominal compression measurements.

Previous studies of LCI in healthy children report upper limits of normality for LCI between 7.2 and 7.8. Our calculated upper limit of normality of 8.4 is relatively high. These differences in observed values may be partially explained by our younger population. A second explanation is the current lack of standardization for MBW. To date, there is no clear international consensus on equipment or procedures for MBW. For this reason it was suggested that each centre should produce its own reference values. We therefore included a group of healthy children in this study. Recent ATS/ERS guidelines on pulmonary function testing in preschool children include a section on LCI. This document only gives general recommendations on how to perform MBW. It is stressed that because only a few centres have experience with MBW, proposals regarding equipment, procedures, analysis, and interpretation must be considered tentative.

An important observation in our study is that we completed at least one MBW successfully in 75% of the CF patients at their first visit. We observed a trend towards a higher success rate for CF patients compared to healthy children, which is not surprising as 80% of the CF patients were treated with some form of aerosol therapy, and thus were accustomed to a facemask. All children were naive to MBW and therefore it is likely that the success rate can be further increased when children become accustomed to the test if it is routinely performed.

In conclusion, our cross sectional findings support the use of LCI as a sensitive and feasible monitoring tool to detect CF lung disease in young CF patients.

### Nocturnal oxygen saturation

Nocturnal oxygen saturation showed a trend towards a slightly reduced mean saturation in CF patients compared with healthy children in our study. The relatively small number of subjects in this study may have been insufficient to detect a significant difference between SpO$_2$ values in CF patients and healthy controls. In addition, agreement between measurements performed on two separate nights was only fair. A previous study by van der Gießen et al. using nocturnal pulse oximetry in older children with CF showed moderate agreement in SpO$_2$ between nights (ICC = 0.70). This might be explained by the fact that the study by van der Gießen was performed in older children with more advanced disease.

In our study, 2/17 CF patients (12%) and 3/28 healthy children (11%) were found to have an abnormal nocturnal SpO$_2$ on at least one night, with mean SpO$_2$ below 95%. The origin of these abnormal saturation profiles is not clear. It may demonstrate normal biological variation, or it might reflect disease. Desaturations in young CF children can occur due to mucus impaction in airways. Younger children are more vulnerable to develop mucus impaction, since their airway diameter is smaller and central airways can become obstructed more easily. It is less likely that the observed difference between our study and that by van der Gießen is of a technical nature, since the same equipment was used in both
studies.

Even though we observed only a small difference in mean nocturnal \( \text{SpO}_2 \) between CF and healthy subjects we still feel that further exploration of nightly pulse oximetry is warranted. In previous studies episodes of desaturation were observed when infants with CF had symptoms of mild airways inflammation (rhinitis, cough, red throat). Another study in young children with CF admitted to the hospital for an infective exacerbation showed that nocturnal \( \text{SpO}_2 \) was decreased at admission and improved with treatment. Based on these studies and our study we feel that nocturnal pulse oximetry might be an interesting home monitoring tool to detect a change in pulmonary condition. A different monitoring regimen might increase the sensitivity to detect relevant disease. For example measure \( \text{SpO}_2 \) once every week for a longer period of time, or measure multiple nights in a row. A previous study by Kirk et al. demonstrated good agreement between home pulse oximetry and pulse oximetry performed during laboratory polysomnography in children. This indicates that home pulse oximetry is an accurate method. Overall, pulse oximetry remains an interesting, feasible tool for home monitoring in young children with CF to detect changes in pulmonary condition or monitor treatment effects, but further validation studies are required.

Cough

Nocturnal cough registration did not show a difference between CF patients and healthy children in our study. Nocturnal cough was relatively infrequent in both groups. This low cough frequency questions whether cough can be a reliable outcome measure in CF research, as many young patients in our study show nocturnal cough frequency within normal limits, and thus cannot improve. Additionally, cough during the first night did not correlate with cough during the second night, suggesting that cough varies considerably over separate nights. There are two possible reasons for this poor correlation. First, average cough frequency was relatively low and therefore insensitive to measure correlation. Second, different types of cough present in this study might have obscured a possible correlation since cough might be caused by a number of medical conditions such as respiratory infections, gastro-oesophageal reflux or allergies.

A major disadvantage of the method we used for cough analysis is that it is time consuming. In conclusion, cough monitoring in its current form does not seem to be useful to monitor young CF patients. Cough monitoring might be more useful in older CF patients, who generally have more respiratory symptoms and might therefore cough more as well. A study on nocturnal cough in older children with CF showed that cough increased with age.

Conclusions

In conclusion, measurements of LCI, nocturnal oxygen saturation and cough were feasible in young children aged 0-4 years. Mean LCI showed a significant difference between children with CF and healthy children with good agreement between repeated measurements in individual children. Nocturnal \( \text{SpO}_2 \) was normal for both groups, but home pulse oximetry did detect low saturations in 10% of all children. There was no difference in cough between CF patients and healthy children, with poor agreement between cough measured on separate nights, making cough monitoring in its current form unsuitable for monitoring young children with CF.

Based on the results of this study, LCI seems to be a sensitive parameter to detect CF lung disease in young children in the laboratory setting. Further validation of LCI is needed to determine whether it can be used as a surrogate endpoint for CF lung disease. In addition further standardization of the methodology is needed. Studies on home monitoring of pulse oximetry are required to further explore the use of this tool to monitor CF lung disease in young children.

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Supplement to Chapter 4

Methods

MBW measurements

The system used in this study consists of a computer, a constant bypass flow (1000 ml/s) and an ultrasonic flow meter. The ultrasonic flow meter measures molar mass (MMss) and air flow. A pressure driven blender is used to switch from room air to a gas mixture containing room air and gas from a container filled with 21% oxygen and 79% Helium. A dead space reducer type 2 (for children with bodyweight between 8 and 20 kg) was used. A disposable bacterial filter (Spiretteâ“¢) surrounded the dead space reducer. During MBW, flow is determined by measuring transit time of a pulsed ultrasound travelling through the streaming medium (1). Inspiratory and expiratory gas flows were measured by the ultrasonic flow meter and integrated to calculate inspired and expired volumes.

The equipment was set up in accordance with the manufacturerâ€™s instructions. Volume was calibrated before the measurements, with temperature and humidity recorded and entered into the calibration settings. The flow meter was calibrated with a high precision calibration syringe (CareFusion/Jaeger, USA) and considered to be calibrated adequately if integrated volume was within 2% of 1.0 L.

Quality control of measurements

To assess the quality of the breathing pattern we observed the patient and tidal volume traces on the computer screen during the measurement. To detect gas leakage we monitored tidal volumes and marker gas concentration traces on the computer screen (2). Raw data were stored first and then opened in the analysis software. Data quality control was performed according to published quality criteria (3).

An additional check on gas leakage was performed during off line data control after each measurement. A graph of all tidal breathing loops was inspected visually, since a difference in volume between inspiration and expiration can indicate a possible leak.

Results

Successful measurements

Table 1 of the supplement shows reasons for measurement failure of LCI, saturation and cough.

<table>
<thead>
<tr>
<th>LCI</th>
<th>saturation</th>
<th>cough</th>
</tr>
</thead>
<tbody>
<tr>
<td>rejecting the facemask (n = 5)</td>
<td>technical failure (n = 3)</td>
<td>technical failure (n = 4)</td>
</tr>
<tr>
<td>crying and/or talking (n = 12)</td>
<td>not accepting the sensor (n = 1)</td>
<td>incorrect handling of the device (n = 3)</td>
</tr>
<tr>
<td>irregular breathing (n = 2)</td>
<td>registration time too short (n = 6)</td>
<td>registration time too short (n = 6)</td>
</tr>
<tr>
<td>test did not comply with published quality criteria (n = 3)</td>
<td>lively movements during sleep (n = 1)</td>
<td>two children sleeping in one room (n = 1)</td>
</tr>
</tbody>
</table>

Table 1. Reasons for measurement failure per test. Data are shown as numbers per test. Multiple reasons for measurement failure can apply to one single patient, for example when a cough measurement failed the first night because registration time was too short and failed the second night because parents handled the device incorrectly, leaving it out of order.

FRC

FRC was not significantly different between CF patients and healthy children (p=0.25). Agreement between FRC measurements in children from who two measurements could be acquired was good: ICC=0.88. See Table 2 of this supplement.
FRC (ml)        420.2 (203.8)  397.3 (196.5)  0.76
FRC (ml/kg)    29.7 (10.1)    25.7 (8.2)    0.25

Table 2. FRC measurements in CF patients and in healthy children. Data are reported as mean (SD).

LCI

LCI could discriminate very well between CF patients and healthy children, with an Area Under the Curve (AUC) of 0.90. (Figure 1). The optimal cut off value derived from the ROC curve is an LCI of 7.8, which has a specificity of 80% and a sensitivity of 87% for CF.

Figure 1. ROC curve of LCI. The continuous line shows the ROC curve for LCI, the dotted line shows the line of identity.

References


Pharmacology, clinical efficacy and safety of recombinant human DNase in cystic fibrosis


Abstract

Recombinant human DNase (rhDNase) is a mucolytic agent that is primarily used to improve mucociliary clearance in cystic fibrosis (CF). RhDNase is a recombinant human enzyme that is synthesized in a Chinese Hamster Ovary (CHO) cell line. RhDNase enzymatically cleaves extracellular DNA into molecules of shorter length. CF sputum shows high concentrations of DNA released by disintegrating inflammatory cells. Free DNA contributes to the abnormally high viscosity of CF sputum and therefore forms an important target in the treatment of CF lung disease. Clinical studies have shown that daily nebulization of rhDNase is associated with an increase in lung function and a decrease in the frequency of exacerbations in patients with CF.

Introduction

Cystic fibrosis (CF) is the most common inherited disease of the Caucasian population. The birth prevalence of CF is estimated to be one in 3500 – 4500. CF is an autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CFTR is an apical membrane protein that regulates chloride transport in secretory epithelial cells. These mutations result in dysfunction of the CFTR protein, causing impaired chloride secretion and sodium hyper absorption. This process results in the depletion of airway surface liquid and abnormal mucociliary transport. The abnormal mucus then gives rise to chronic lower airway infection and inflammation, typically as early as in infancy.

Chronic respiratory disease is the major cause of morbidity and mortality in CF, and is therefore the main focus of clinical care and research.

Impaired mucociliary clearance is an important problem in most patients with CF. Consequently, treatment is primarily aimed at mobilizing as much sputum as possible from the lung on a daily basis. One means to achieve this is physiotherapy. In addition, most patients use mucoactive drugs that help to clear sputum.

Until now, two mucoactive drugs have shown a positive effect on mucociliary clearance in patients with CF: recombinant human DNase (rhDNase) and hypertonic saline (HS).

RhDNase (Pulmozyme, dornase alpha) was developed in the early 90’s and its efficacy in CF is well established. Maintenance treatment with RhDNase is therefore widely accepted as the primary treatment to improve mucociliary clearance in CF. Many patients with CF benefit from treatment with rhDNase, as it is associated with improved pulmonary function, less antibiotic use, and lower hospitalization rate.

The aim of this review is to discuss what is currently known on rhDNase.

HS is the second drug that improves mucociliary clearance in CF. It is thought that inhalation of HS improves the hydration of the airway surface liquid which in turn improves ciliary motility and thus mucociliary clearance. HS is often used in combination with airway clearance techniques or physiotherapy, to optimize the expectoration of mucus.

Mucoactive drugs

Mucoactive medications can be classified as follows by their mechanism of action: mucolytics (classical and peptide-), expectorants, mucoregulators, and cough clearance promotors (table 1).

<table>
<thead>
<tr>
<th>Mucoactive medication</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expectorants</strong></td>
<td></td>
</tr>
<tr>
<td>Hypertonic saline</td>
<td>Increases secretion volume and increases hydration</td>
</tr>
<tr>
<td>Mannitol</td>
<td>Increases hydration. May increase ciliary beat frequency; break hydrogen bonds between mucins; increase secretion volume.</td>
</tr>
</tbody>
</table>
Classical mucolytics

N-acetylcysteine  
Breaks disulfide bonds linking mucin oligomers
Nacystelyn  
Increases chloride secretion and breaks disulfide bonds
2-Mercaptopethane sulfonate  
Breaks disulfide bonds linking mucin oligomers

Peptide mucolytics

RhDNase (dornase alfa)  
Hydrolyzes DNA polymer with reduction in DNA length
Gelsolin and Thymosin β  
Depolymerize F-actin

Receptor antagonists

Denofusol  
Stimulates ciliary beat frequency and Cl- secretion

Non-destructive mucolytics

Dextran  
Breaks hydrogen bonds and increases secretion hydration
Low molecular weight heparin  
May break both hydrogen and ionic bonds

Mucoregulatory agents

Anticholinergic agents  
Decreases volume of stimulated secretions
Glucocorticoids  
Decreases airway inflammation and mucin secretion
Indomethacin  
Decreases airway inflammation
Macrolide antibiotics  
Decreases airway inflammation and mucin secretion

Cough clearance promotors

Bronchodilators  
Can improve cough clearance by increasing expiratory flow
Surfactants  
Decreases sputum adhesiveness

Table 1. Mucoactive drugs. *modified from Rubin, 2006.

Mucolytics

Examples of mucolytics are 2-mercaptoethane sulphonate (Mesna), N-acetylcysteïne (NAC), and carbocisteine. These were much used in CF before rhDNase became available. NAC disrupts disulfide bonds in mucus. Despite in vitro mucolytic activity and a long history of use, there are no convincing data demonstrating that aerosolized NAC is effective therapy for CF.12,13

RhDNase is a peptide mucolytic. It is an enzyme that breaks down deoxyribonuclease (DNA) strands in airway secretions. RhDNase administered as an aerosol reduces the viscosity and surface adhesivity of CF sputum.14 It is thought that rhDNase is not merely a mucolytic but also a "sputolytic" as it reduces the surface adhesivity of sputum and thereby increases sputum clearability.15

Expectorants

Hypertonic saline (HS) can be classified as an expectorant in CF.16 Recently it was shown that twice-daily inhalation of hypertonic saline solution over 48 week resulted in an modest improvement in lung function and a marked reduction in the frequency of pulmonary exacerbations.8

Only one randomized controlled trial (RCT) comparing rhDNase and HS has been published so far. Over a 12 week period, daily rhDNase treatment resulted in a significantly greater increase in forced expiratory volume in one second (FEV 1) than did HS (16% increase in FEV, for daily rhDNase, 3% increase for HS).17

Development of new mucoactive drugs

New mucoactive drugs for the treatment of CF lung disease are being developed. Thymosin ß4 (Tß4) and gelsolin both reduce the viscoelasticity of CF sputum by changing the polymer structures of proteins (such as filament length, branching and crosslink density). One example is actin, which comprises approximately 10% of the total protein in
leukocytes. It can form long filaments, which together with polymers of DNA and actin contribute to the viscosity of CF sputum. The presence of large amounts (0.1 to 5 mg/ml) of polymerized F-actin in CF sputum\[21\] suggested that Tβ4 and gelsolin could decrease the viscoelasticity of CF sputum. Tβ4 is the major actin-sequestering peptide in eukaryotic cells.\[22\] Gelsolin is an actin-severing peptide that rapidly cleaves non covalent bonds between actin monomers within actin filaments. In vitro studies showed that gelsolin significantly reduced CF sputum viscosity.\[22\]

Interestingly, a recent study investigated in vitro the effect of three different agents on CF sputum viscosity: rhDNase, Tβ4 and gelsolin.\[22\] The sputum cohesivity in the control specimens was 28.4 mm. This decreased significantly after either 30 \(1/\)g/mL of rhDNase (23.4 mm, \(p = 0.003\)) or after 3, 30 or 150 \(1/\)g/mL of Tβ4 (23.7, 23.9, and 22.1 mm respectively - \(p < 0.03\) for each). There was a highly significant dose-dependent fall in cohesivity with gelsolin. Regression analysis of log cohesivity and gelsolin dose demonstrated a log-linear relationship \((r = \hat{a}^{\ast}0.53 \ p = 0.0008)\).

Gelsolin and rhDNase combined (1.5 \(1/\)g/mL each) gave a 77.3% reduction in elasticity \((p = 0.04)\) and an 80.4% decrease in viscosity \((p = 0.01)\) compared with excipient. These changes were greater than those seen with either agent alone.\[22\] This might be explained by the fact that F-actin and DNA form polymers, on which rhDNase cannot exert its action. When these polymers are degraded by gelsolin, rhDNase can again cleave the free DNA. To date, however, clinical efficacy of gelsolin in CF has not been proven yet.

Mannitol is another interesting compound. It is thought to increase hydration of airway secretions. Clinical efficacy in CF has not been shown. Clinical research on the use of this drug is ongoing.

Finally, denofusol tetrasodium is being investigated as a new drug in the treatment of CF. Results of a phase I/phase II multicenter study showed that denofusol is safe to use. Dosages up to 60 mg were inhaled, with good tolerance in most subjects. Percentages of subjects experiencing adverse events did not differ between the denofusol and the placebo group.\[23\] Another trial comparing the efficacy of three different doses of denofusol has recently been completed in CF patients with mild disease (FEV\(_1\) \(\geq 75\%\) predicted). The study was a randomized, double-blind, multi-center, 28-day, phase II clinical trial of denofusol tetrasodium inhalation solution (20, 40, or 60 mg) versus placebo (normal saline). Patients were randomized to receive one of the three doses of denofusol or placebo administered three times daily. Lung function changes from baseline in patients on denofusol (pooling active doses) significantly differed from those in patients on placebo for FEV\(_1\) \((\text{difference } 0.14 L, \ p = 0.006)\), Forced Expiratory Flow from 25\% to 75\% of the FVC (FEF\(_{25-75}\%) \((\text{difference } 0.30 L/s, \ p = 0.008)\), FVC (difference 0.12 L, \(p = 0.022\)) and FEV\(_1\)/FVC (difference 0.02, \(p = 0.047\)). When each of the three dose groups was analyzed separately compared to placebo, the differences were significant for 20 mg and 60 mg of denofusol, but not for 40 mg of denofusol.\[25\]

**Introduction to the compound**

As a result of the inflammatory process in CF, DNA is set free from necrotic leukocytes. CF sputum therefore contains high concentrations of free DNA, which contributes to the high viscosity of the airway secretions.\[26\] Elevated DNA concentrations have been found even in bronchoalveolar lavage fluid from infants with CF.\[27\] This shows that the negative effect of free DNA starts early in life. RhDNase enzymatically cleaves extracellular DNA into molecules of shorter length, thus increasing the “pourability”\[28\] of the CF sputum.\[28\] The liquefied sputum is less adhesive to the airway wall and it can be expectorated more easily.

**Chemistry**

Bovine pancreatic DNase was partially purified more than 60 years ago.\[28\] It was found to significantly reduce the viscosity of purulent lung secretions when incubated with these secretions in vitro.\[28\] Bovine pancreatic DNase I was approved for human use in the US in 1958. Various uncontrolled clinical studies in patients with respiratory tract infections and one study in CF patients suggested that this agent (administered intravenously and by inhalation) was well tolerated and effective in reducing the viscosity of lung secretions.\[28\] In 1968, aerosolized pancreatic DNase I was reported to be associated with a case of acute and life-threatening bronchospasm.\[29\] Following this and other adverse reports, bovine pancreatic DNase I fell into disuse. The adverse effects observed may have been related to product contamination with digestive pancreatic enzymes (concentrations up to 2% trypsin and chymotrypsin were reported in the final product).\[29\] The entire amino acid sequence of bovine pancreatic DNase I was determined in 1973.\[30\] The crystal structure of the enzyme was established a few years later.\[30\]

The gene for human DNase was cloned from a pancreatic cDNA library in 1988.\[30\] RhDNase was expressed in a Chinese Hamster Ovary (CHO) cell line (designated CHO-DP7). This rhDNase producing cell line was established by co-transfection with two plasmids coding for rhDNase and dihydrofolate reductase. RhDNase is currently synthesized by CHO cells transfected with the protein-encoding sequence of the human DNase gene. Since rhDNase is identical to human DNase it can be used safely without risking the hazards associated with the use of animal proteins. The production of a recombinant drug is still a complex process, however, highly sensitive to disturbances such as
contamination of the cell line.

**Chemical and physical characteristics**

RhDNase is a glycoprotein containing 260 amino acids with a molecular weight of approximately 33,000–38,000 Daltons. The structural features of rhDNase are shown in **figure 1**. Two six-stranded β-pleated sheets packed against each other form the core of the protein. This is surrounded by eight α-helices and six turns. Since rhDNase is a protein it is sensitive to high temperatures. Activity slowly decreases on room temperature and rhDNase is fully inactivated by heat (100°C for 10 minutes). This is why it should be stored in a refrigerator (2°C - 8°C). A single brief exposure (up to 24 hours) to moderately elevated temperatures (up to 30°C) does not affect product stability.

![Figure 1](image)

**Figure 1.** Schematic representation of structural features of human DNase I. Arrows indicate β-sheets, twists indicate α-helices.

**Pharmacodynamics**

In vitro studies showed that rhDNase rapidly and significantly reduces the viscosity of purulent CF sputum. The mechanism by which rhDNase exerts its pharmacological effects is related to its ability to cleave high-molecular-weight DNA in purulent airway secretions. RhDNase administered as an aerosol reduces the viscosity and surface adhesivity (and thus the tenacity) of CF sputum in vitro. A study of aerosolized rhDNase in CF patients confirmed the in vitro observation that the enzyme cleaves high-molecular-weight DNA in infected lung secretions.
Pharmacokinetics and metabolism

From inhalation studies in rodents, the disappearance half-life of rhDNase from the lung is estimated to be around 11 hours. In a study involving cynomolgus monkeys exposed to inhaled rhDNase in three different doses, rhDNase could not be detected (< 2 ng/mL) in the serum of animals exposed to a dose of 0.2 mg/kg, during a post-dose period of 312 hours. Low concentrations (maximum concentration < 40 ng/mL) of rhDNase could be measured in serum in animals exposed to doses of 0.7 and 3.1 mg/kg. These results are consistent with a low level of systemic exposure following inhalation of rhDNase.

Two phase I studies have measured serum DNase concentrations using an assay sensitive to both circulating endogenous DNase and rhDNase. Measurements were performed at several time points following single and multiple inhalation of escalating doses of rhDNase in healthy subjects and in patients with CF. Only very low serum concentrations of DNase were observed. No anti-rhDNase antibodies were detected in each of the studies. Therefore, it was concluded that systemic exposure to inhaled rhDNase was negligible.

Clinical efficacy

Phase I studies – safety and tolerability

Tolerability data from two phase I studies show that aerosolized rhDNase in dosages up to 30 mg/day is well tolerated by healthy volunteers as well as patients with CF. Side effects, if any, were mild and self-limiting.

Aitken and colleagues initially treated twelve healthy subjects and fourteen CF patients with escalating doses during five days. After a two-day interval this was followed by repeated doses of up to 10 mg t.i.d. for another five days. Serum DNase concentrations rose only marginally: from $3 \pm 2$ ng/mL prior to therapy to $5 \pm 2$ ng/mL (mean $\pm$ SD) 6 hours after the last dose on day 12 for healthy subjects, and from $1 \pm 2$ ng/mL to $3 \pm 3$ ng/mL for CF patients. In neither group, anti-rhDNase antibodies were detected.

Hubbard and colleagues gave 13 CF patients a fixed dose of aerosolized rhDNase for six days (4 received 20 mg a day, 5 received 10 mg three times a day, and 4 received 20 mg twice a day). Serum DNase concentrations slightly increased in several patients, yet no more than 10 ng/mL above pre-treatment conditions, regardless of dose. No anti-DNase antibodies were detected. Therefore, systemic exposure to rhDNase was considered negligible. No significant adverse events related to the drug were reported in these studies. Adverse events reported in later phase II studies included pharyngitis, voice alteration, rash and increase in cough. All symptoms were mild and resolved spontaneously. These results have since been confirmed in long-term Phase III studies. Table 2 shows a summary of adverse events that were more frequent in patients treated with rhDNase than in patients with placebo.

<table>
<thead>
<tr>
<th>Study</th>
<th>Adverse Event (% of patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramsey et al.</td>
<td>Pharyngitis (23-24%), voice change (2-16%).</td>
</tr>
<tr>
<td>Fuchs et al.</td>
<td>Voice change (12-16%), rash (10-12%).</td>
</tr>
<tr>
<td>Quan et al.</td>
<td>Rash (6%).</td>
</tr>
<tr>
<td>Shah et al.</td>
<td>Pharyngitis (14%)</td>
</tr>
<tr>
<td>McCoy et al.</td>
<td>Dyspnea (17%), pharyngitis (32%), rhinitis (30%), voice alteration (18%).</td>
</tr>
<tr>
<td>Ranasinha et al.</td>
<td>No difference in adverse events between rhDNase and placebo.</td>
</tr>
<tr>
<td>Shah et al.</td>
<td>No difference in adverse events between rhDNase and placebo.</td>
</tr>
</tbody>
</table>

Table 2. Adverse events. Only adverse events that were significantly more frequent in patients treated with rhDNase than in patients with placebo are noted.

Based on these data, daily dosing of up to 10 mg of rhDNase is generally considered safe. Higher doses might be safe as well. However, this was not systematically studied.
Phase II studies

Two phase II clinical studies have evaluated the efficacy and safety of short-term (10 day) administration of rhDNase. Table 3 shows a summary of phase I and phase II studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Study design</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hubbard et al.</td>
<td>rhDNase 20 mg QD, 10 mg TID or 20 mg BID for 6 days</td>
<td>rhDNase is safe to use, â†’ FEV₁, â†’ FVC</td>
</tr>
<tr>
<td>Aitken et al.</td>
<td>Escalating doses of rhDNase in 1st week, 2 mg TID, 6 mg TID or 10 mg TID for 2nd week.</td>
<td>rhDNase is safe to use, â†’ FEV₁, â†’ FVC</td>
</tr>
<tr>
<td>Ranasinha et al.</td>
<td>rhDNase 2.5 mg BID for 10 days vs. placebo.</td>
<td>RhDNase is safe, adverse events were mild and self-limiting, â†’ FEV₁, â†” FVC</td>
</tr>
<tr>
<td>Ramsey et al.</td>
<td>rhDNase 0.6 mg, 2.5 mg or 10.0 mg QD for 10 days vs. placebo.</td>
<td>RhDNase is safe, adverse events were mild and self-limiting, â†’ FEV₁, â†” FVC only for the 2.5 mg dose</td>
</tr>
</tbody>
</table>

Table 3. Summary of phase I and phase II studies.

Both studies had a randomized, double-blind, placebo-controlled, parallel-group design and included CF patients with mild to moderate respiratory disease, as defined by a baseline FVC ≥ 40% of predicted value. All patients were required to be clinically stable (i.e. no hospitalization or change in antibiotic regimen within 14 days prior to enrolment). Ramsey et al. found that a 10-day administration of twice daily 0.6 mg, 2.5 mg or 10.0 mg rhDNase significantly increased FEV₁ by 9.9 to 14.5% from baseline vs. â†’1.6% for placebo for (p < 0.001 for all 3 doses). FVC increased 9.6 to 11.8% from baseline with rhDNase vs. 0.5% with placebo. A significant effect on FVC was only observed for the 2.5 mg dose.

Similarly, Ranasinha et al. found that a 10-day administration of twice daily 2.5 mg rhDNase improved FEV₁ by 13.5% compared with placebo (p<0.001). Improvement in FVC was not statistically significant.

Treatment with rhDNase was well tolerated by all study participants.

The most common adverse events reported were similar in placebo and rhDNase recipients, and were difficult to distinguish from sequelae associated with the underlying disease. Examples are: pharyngitis; increase in cough; dyspnea; hemoptysis; rhinitis; sputum increase; and wheeze. Only rash and hoarseness or sore throat were found more frequently in the groups receiving rhDNase. These symptoms were mild and self-limiting.

Phase III and IV studies

The efficacy of aerosolized rhDNase in patients with CF has been investigated in a number of well designed dose-ranging and placebo-controlled studies in patients with mild to moderate lung disease (FVC ≥ 40% of the predicted value). Table 4 shows a summary of these phase III and IV studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Study</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuchs et al.</td>
<td>rhDNase 2.5 mg QD, 2.5 mg BID for 24 weeks vs. placebo.</td>
<td>â†’ FEV₁, small â†’ FVC, â†” exacerbation rate</td>
</tr>
<tr>
<td>Ranasinha et al.</td>
<td>rhDNase 2.5 mg BID for 10 days vs. placebo.</td>
<td>â†’ FEV₁, â†” FVC</td>
</tr>
<tr>
<td>Ramsey et al.</td>
<td>rhDNase 0.6 mg, 2.5 mg or 10.0 mg QD for 10 days vs. placebo.</td>
<td>â†’ FEV₁, for all doses, â†’ FVC only for the 2.5 mg dose</td>
</tr>
<tr>
<td>Laube et al.</td>
<td>RhDNase 2.5 mg BID for 6 days vs. placebo.</td>
<td>â†’ FEV₁, â†” FVC</td>
</tr>
<tr>
<td>Quan et al.</td>
<td>rhDNase 2.5 mg QD for 96</td>
<td>â†’ FEV₁, â†” FVC</td>
</tr>
</tbody>
</table>
weeks vs. placebo (in children aged 6 to 10 years).

Shah et al.\textsuperscript{35} rhDNase 2.5 mg BID for 14 days vs. placebo (in advanced lung disease).

McCoy et al.\textsuperscript{36} rhDNase QD for 12 weeks vs. placebo (in advanced lung disease).

Paul et al.\textsuperscript{37} rhDNase 2.5 mg QD for 3 years or no rhDNase

Suri et al.\textsuperscript{38} rhDNase 2.5 mg QD, alternate-day rhDNase 2.5 mg, hypertonic saline BID

Henry et al.\textsuperscript{39} rhDNase 2.5 mg QD for 1 month

Hodson et al.\textsuperscript{40} rhDNase 2.5 mg QD, alternate-day rhDNase 2.5 mg, hypertonic saline BID

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Duration</th>
<th>Lung Function</th>
<th>Inflammation</th>
<th>Exacerbation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shah et al.\textsuperscript{35}</td>
<td>rhDNase 2.5 mg BID</td>
<td>14 days</td>
<td>$\hat{\Delta}^+ FEV_1$, $\hat{\Delta}^+ FVC$</td>
<td>$\hat{\Delta}^+ FEV_1$, $\hat{\Delta}^+ FVC$, $\hat{\Delta}^+$ exacerbation rate</td>
<td>$\hat{\Delta}^+$ exacerbation rate</td>
</tr>
<tr>
<td>McCoy et al.\textsuperscript{36}</td>
<td>rhDNase QD</td>
<td>12 weeks</td>
<td>$\hat{\Delta}^+ FEV_1$, $\hat{\Delta}^+ FVC$, $\hat{\Delta}^+$ exacerbation rate</td>
<td>$\hat{\Delta}^+$ airway inflammation in non-treated group and unchanged in rhDNase group</td>
<td>$\hat{\Delta}^+$ inflammatory mediators in sputum</td>
</tr>
<tr>
<td>Paul et al.\textsuperscript{37}</td>
<td>rhDNase 2.5 mg QD or no rhDNase</td>
<td>3 years</td>
<td>$\hat{\Delta}^+$ airway inflammation in non-treated group and unchanged in rhDNase group</td>
<td>$\hat{\Delta}^+$ inflammatory mediators in sputum</td>
<td>$\hat{\Delta}^+$ inflammatory mediators in sputum</td>
</tr>
<tr>
<td>Suri et al.\textsuperscript{38}</td>
<td>rhDNase 2.5 mg QD, alternate-day rhDNase 2.5 mg, hypertonic saline BID</td>
<td>1 month</td>
<td>$\hat{\Delta}^+ FEV_1$, $\hat{\Delta}^+ FVC$, $\hat{\Delta}^+$ sputum cytology (inflammation)</td>
<td>$\hat{\Delta}^+$ airway inflammation in non-treated group and unchanged in rhDNase group</td>
<td>$\hat{\Delta}^+$ airway inflammation in non-treated group and unchanged in rhDNase group</td>
</tr>
</tbody>
</table>

Table 4. Summary of phase III and IV studies.

$\hat{\Delta}^+$ = significant increase, $\hat{\Delta}^{++}$ = no difference, $\hat{\Delta}^{+++}$ = significant decrease

QD = once daily, BID = twice daily, TID = three times daily

Short term studies

Laube and colleagues investigated the effect of rhDNase on airflow obstruction and mucociliary clearance in CF. Patients were treated with 2.5 mg rhDNase or placebo twice a day for six days. By day 6, FEV\textsubscript{1} and FVC were significantly higher in the rhDNase group, i.e. a mean increase of 9.4 ± 3.5\% and 12.7 ± 2.6\%, respectively, as compared with a decrease of 1.8 ± 1.7\% and an increase of 0.4 ± 1.1\%, respectively, in the placebo group (p<0.05). There were no differences in indices of airflow obstruction and mucociliary clearance, obtained from gamma-camera images.\textsuperscript{47}

Long-term efficacy studies

The first long-term phase III study with rhDNase was a 24-week, multicenter, randomized, double-blind, placebo-controlled trial with a parallel-group design and open-label extension of 24 weeks.\textsuperscript{7} In addition to lung function the study assessed the effect of rhDNase on the number of respiratory tract exacerbations (RTEs) and evaluated the effects of rhDNase on quality-of-life measures.

Fifty-one CF care centers in the U.S. participated and enrolled a total of 968 CF patients aged 5 years and older, of whom 943 had complete data. Randomization was between three treatment arms: rhDNase 2.5 mg once daily, rhDNase 2.5 mg twice daily, or placebo.

Proportion of patients still free of any protocol-defined RTEs at 24 weeks after randomization in the rhDNase groups (78 and 81\% for the once daily and twice daily regimens) exceeded that in the placebo group (72\%); (Figure 2). The relative risk of developing one or more RTEs over the 24-week study period was determined using the Cox proportional hazards model. Compared with that for placebo-treated patients, the age-adjusted relative risks (RR) of an RTE for the once and twice daily rhDNase group were 0.72 (p < 0.05) and 0.63 (p = 0.01), respectively.\textsuperscript{7}
Figure 2. Cumulative proportion of patients who remained free of respiratory tract exacerbations requiring parenteral antibiotic therapy, by treatment group during double-blind study period. Modified from Fuchs et al.

This trial also demonstrated that rhDNase significantly improved FEV\textsubscript{1} from baseline as compared with placebo. The mean percent improvement over 24 weeks for daily and twice daily administration of rhDNase was 5.8 and 5.6%, respectively (p<0.01 for both). After the initial improvement (8 - 9% improvement at day 7), FEV\textsubscript{1} reached a plateau at approximately 6% improvement, which was maintained throughout the remainder of the study; see figure 3. All patients who completed the phase III double-blind study were eligible to participate in a 24-week open-label extension of the trial. The positive effects of rhDNase therapy were maintained throughout the extension.

At the conclusion of the study, antibodies to rhDNase were measurable in 3% and 4% of the patients treated with rhDNase once daily or twice daily, respectively. The presence of antibodies was not associated with an increase in symptoms or adverse events. None of the samples contained DNase-specific IgE antibody.

Figure 3. Mean percent change in FEV\textsubscript{1} from baseline throughout the 24-week study period. From Fuchs et al.
Early lung disease

Quan and colleagues investigated rhDNase in a group of children with CF aged 6 to 10 years, all with well preserved lung function defined as FVC > 80% predicted. The pulmozyme early intervention trial (PEIT) was a 96-week, randomized, double blind, placebo-controlled trial that involved 49 CF centers. Patients received either rhDNase 2.5 mg or placebo once daily. The trial aimed at evaluating whether rhDNase would maintain lung function and reduce RTE in patients with well-preserved lung function. In the rhDNase group FEV1 was maintained at 0.04% Å± 0.8% above baseline at 96 weeks, whereas in the placebo group it decreased 3.2% Å± 0.8% from baseline. This equals a treatment benefit over placebo of 3.2% Å± 1.2% (p = 0.006). At 96 weeks patients receiving rhDNase showed an increase in FEF25-75 of 3.8% Å± 1.6% predicted from baseline, whereas patients receiving placebo showed a decrease of 4.1% Å± 1.7% predicted from baseline. This equals a treatment benefit of 7.9% Å± 2.3% predicted (p = 0.0008) for rhDNase over placebo. For FVC there was no significant difference between the two groups. Regarding the second aim, administration of rhDNase reduced the risk of RTE by 34% (relative risk 0.66 (95% CI 0.44-1.00, p = 0.048).

The PEIT study was the first to show the importance of treating patients with early disease and that the peripheral airways are an key target for treatment.

Advanced lung disease

Most studies on rhDNase involved patients with moderate to mild pulmonary disease (FVC ≥ 40% of predicted value), which represents the majority of the CF population. A small number of studies has been performed in patients with severe lung disease.

In the first study, 70 patients with an FVC ≥ 40% predicted were treated with 2.5 mg rhDNase twice daily or placebo for 14 days in a double-blind, randomized placebo-controlled trial, followed by a six-month open extension period. During the double-blind study period, FEV1 increased by 7% in the rhDNase group and 6% in the placebo group (difference not significant). For FVC these values were 13% in the rhDNase group and 14% in the placebo group (difference not significant). These comparable increases between the rhDNase and the placebo groups were explained as probably due to several factors: admission to hospital and very active chest physiotherapy; participation in the clinical trial; or close follow-up of the patients.

The use of rhDNase in this group of patients with severe lung disease was safe. The authors pointed out, however, that the severely ill patients might be too weak to cough up the liquefied sputum and that rhDNase should only be used in conjunction with a regimen of chest physiotherapy.

The second study assessed the effect of 12-week administration of rhDNase in patients with advanced CF lung disease. This multi-center, double-blind, placebo-controlled study included a total of 320 CF patients in clinically stable condition with an FVC Å≥ 40% predicted. Patients were randomly assigned to receive either 2.5 mg rhDNase once daily or placebo once daily.

Administration of rhDNase significantly improved FEV1. The mean percent change in FEV1, in the rhDNase group was 9.4% compared with 2.1% improvement in the placebo group (p<0.001). For FVC the mean percent improvement in the rhDNase group was 12.4 Å± 18.6% compared with 7.3 Å± 16.5% in the placebo group (p<0.01). There were no differences in the risks of developing RTEs between the placebo and the rhDNase group.

Post-marketing surveillance

A Cochrane review describes the many studies on rhDNase performed since its introduction on the market. RhDNase is now widely used for treatment of CF lung disease. Long-term clinical data from patient registries have become available for post-marketing surveillance. In 2003, Hodson et al. studied the Epidemiologic Registry of Cystic Fibrosis (ERCF) of patients who had not received rhDNase. Effects on FEV1 and on number of RTEs per year were studied. Patients whose start dates of rhDNase therapy were prior to enrollment in the ERCF or not specified were excluded. In total, 2,023 patients were eligible for analysis of FEV1

Clinical trials have shown that rhDNase significantly improves lung function in patients with CF in general, but also that response can vary between patients. Sanders and colleagues recently investigated possible mechanisms
underlying the variability of response to rhDNase treatment. They compared biochemical and physical properties of sputum obtained from clinical responders and non-responders to rhDNase, and analyzed degradation of sputum by rhDNase. Upon incubation with rhDNase, sputum from clinical responders was extensively degraded in vitro, whereas sputum from clinical non-responders was not degraded: the median decrease in sputum elasticity was 32% and 5%, respectively. Sputum from clinical responders had a significantly higher magnesium concentration than sputum from non-responders (2.0 (1.5-2.6) mM versus 1.3 (1.1-1.5) mM; p = 0.020). Magnesium serves as a cofactor in the enzymatic degradation of DNA by rhDNase. So, to obtain optimal rhDNase activity, a minimum concentration of magnesium is required. When magnesium chloride was added to the sputum samples, the capacity of rhDNase to degrade the sputum dramatically improved. Oral magnesium supplements enhanced the magnesium concentration in sputum from a group of clinical non-responders. Clearly, these interesting observations should be further investigated in clinical studies.

**RhDNase and airway inflammation**

Three studies assessed the effect of rhDNase on airway inflammation. One of these set out to measure neutrophils count, elastase activities and interleukin-8 concentration in the bronchoalveolar lavage (BAL) fluid. Flexible fiberoptic bronchoscopy and BAL were performed at baseline, 18 and 36 months. A total of 105 CF patients (at least 5 years of age) having normal lung function were randomized to receive rhDNase (2.5 mg/day) or no rhDNase. Patients with a normal neutrophil count in BAL fluid at baseline were not randomized and served as a control group. A significant increase in neutrophils was observed over the 3-year study period in both untreated patients and control subjects, whereas the neutrophil count remained unchanged in patients treated with rhDNase. Also, elastase activities and interleukin-8 concentrations increased in untreated patients and remained stable in patients on rhDNase. This study provided evidence that most CF patients with normal lung function develop neutrophilic airway inflammation that increases over time. The authors concluded that treatment with rhDNase in patients with elevated neutrophils counts did not reverse the neutrophilia but rather prevented progression of the neutrophils count in BAL fluid. It may, therefore, be efficacious in modulating the inflammatory process.

A second study investigated the effects of rhDNase and hypertonic saline (HS) on airway inflammation in children with CF. The authors hypothesized that both agents might promote airway inflammation. In a randomized, cross-over trial, 48 children with CF were allocated consecutively to 12 weeks of once-daily 2.5 mg rhDNase, alternate-day 2.5 mg rhDNase and twice-daily HS. Sputum levels of inflammatory mediators such as total interleukin-8 (IL-8), free IL-8, and neutrophils elastase activity were measured before and after each treatment. Neither daily rhDNase nor HS resulted in a change in inflammatory mediator levels over baseline. In summary, this study did not show that rhDNase or HS could worsen airway inflammation in CF.

A third study aimed to determine whether short-term administration of rhDNase would lessen airway inflammation. Twenty patients with CF and chronic lung disease inhaled 2.5 mg of rhDNase daily for 1 month. Before and after the 1-month trial, lung function was measured and sputum was obtained. Sputum total cell and differential counts were measured. After 1 month of rhDNase, mean FEV1 had increased from 62.3% predicted at baseline to 70.8% predicted (p = 0.02); and FVC from 74.4% to 83.9% predicted (p = 0.007). No significant differences were found in sputum cytology before and after rhDNase. The authors concluded that although the administration of rhDNase resulted in significant improvements in FEV1 and FVC, there was no evidence of accompanying changes in airway inflammation.

**CT scan studies in CF**

Lung function parameters have been used as primary end point in most therapeutic studies to date. Nevertheless, thanks to improvements in CF therapy, lung function parameters have become less sensitive endpoints in clinical studies. Cohort studies have shown that computed tomography (CT) in CF is far more sensitive than lung function parameters in detecting disease progression. In addition, CT in CF can identify highly relevant structural lung changes, such as bronchiectasis and air trapping. CT scoring systems have been developed to systematically quantify such structural changes on CT scans. In two clinical studies the effect of rhDNase treatment was evaluated using CT scanning. In a small placebo controlled study by Robinson et al., patients treated with rhDNase showed improvement in quantitative high-resolution CT (HRCT) air trapping measures, whereas the placebo group demonstrated worsening results. Lung function did not show any difference between both groups. In a small study by Nasr et al. some improvement of the CT score in the rhDNase group over placebo was observed. A larger multicenter substudy of the PEIT study did not show any differences in the CT score between the rhDNase group and placebo group.

These limited size studies are interesting but show conflicting results. This is likely to be related to differences in scanning protocols and in the way the images were analysed. Clearly, imaging techniques like CT offer a new and promising platform to evaluate the therapeutic effects of mucoactive drugs like rhDNase.
Aerosol delivery systems

It is a general principle that a registered nebulized drug like rhDNase must be aerosolized using only a nebulizer system approved by regulatory agencies and the responsible manufacturer. Ultrasonic nebulizers have been found unsuitable for delivery of rhDNase. They may inactivate rhDNase or aerosol delivery characteristics may be unacceptable. In general, jet nebulizers do not have these drawbacks.

Two jet nebulizers were extensively evaluated in the early clinical trials with rhDNase, the Marquest Acorn II and Hudson T Updraft II, both used with the PulmoAide compressor. By current standards both systems generate relatively large aerosol particles, partly within the respirable range. There is no established protocol to test equivalence for alternative nebulizers. In principle registration studies are required for any alternative rhDNase - new nebulizer combination. Clearly it is not feasible to run such studies in an orphan disease like CF.

Fiel and colleagues tested the above two systems, as well as the Pari LC/Jet+ used with the Proneb/PariBoy/Inhalierboy compressor as an alternative nebulizer system for the delivery of rhDNase, in a randomized, open label, multi center trial. This study compared improvement in FEV\textsubscript{1} induced by rhDNase inhalation (2.5 mg twice daily) over a treatment period of 15 days. A total of 397 patients > 5 years of age were randomized in the protocol. RhDNase administration, by each of the three aerosol delivery systems, resulted in improvements in pulmonary function tests. FEV\textsubscript{1} improvements ranged from 12.4 to 14.3%, with no significant differences observed between the three aerosol delivery systems. Similar results were obtained for FVC. (see Figure 4)

![Figure 4. Percent change from mean baseline FEV\textsubscript{1} for three different nebulizer systems.](https://www.processtext.com/abcepub.html)

Marquest = Marquest Acorn II jet nebulizer with Pulmo-Aide compressor;
Hudson = Hudson T Updraft II jet nebulizer with Pulmo-Aide compressor;
Pari = Pari LC Jet Plus nebulizer with Pari Inhalier Boy compressor.

Subsequently, Shah and colleagues compared the PulmoAide compressor/Hudson T Updraft nebulizer system with the CR50 compressor/Sidestream nebulizer â€“ a system used by many patients in the UK for the delivery of antibiotics. In vitro and in vivo studies with these two systems were carried out in parallel to evaluate their relative efficiencies.

The PulmoAide/Hudson generated particles with a mass median diameter (MMD) of 6.87 μm as measured by laser diffraction. Thirty-five per cent of the generated aerosols were in the respirable range, nebulizer efficiency was 44% and aerosol delivery was 16% of filling dose. The CR50/Sidestream generated particles with a MMD of 3.42 μm. Seventy-one per cent of the generated aerosols were in the respirable range, nebulizer efficiency was 46% and aerosol...
delivery was 33% of filling dose. These results suggest that the CR50/SideStream combination is the more efficient of the two systems, primarily because it generates smaller particles, a higher proportion of which are delivered in the respirable range.

The clinical study had a multi center, parallel-arm design. CF patients were randomized to either the PulmoAide compressor/Hudson T Updraft nebulizer (n = 87) or the CR50 compressor/SideStream nebulizer (n = 86) for the nebulization of rhDNase (2.5 mg once daily). For both groups, % change from baseline in lung function was measured (absolute % predicted). For the PulmoAide/Hudson group, FEV₁ changed 11% from baseline, FEF₂₅₋₇₅ changed 7% and FVC changed 10%. In the CR50/SideStream group, FEV₁ changed 16% from baseline, FEF₂₅₋₇₅ changed 14% and FVC changed 12%. Differences between the two groups were not significant.

In conclusion, the CR50/SideStream combination was found to be at least as effective as the PulmoAide/Hudson combination in terms of improvement in lung function. The authors suggested a trend for smaller aerosol particles to penetrate more deeply into the smaller airways and thus to induce greater improvement in FEF₂₅₋₇₅ and FEV₁.

A recent innovation is the mesh nebulizer. Its use for the delivery of rhDNase is advised against until regulatory bodies and the responsible manufacturer have authorized its use for this purpose.

**Regulatory affairs**

The FDA approved rhDNase on 30 December 1993. In 1993 rhDNase had also received approval for Europe and Australia.

RhDNase is approved for the management of CF patients with a forced vital capacity (FVC) of greater than 40% of predicted. Studies have shown that for these patients rhDNase improves lung function and decreases exacerbation rate. For patients with severe lung disease (FVC ≤40% predicted) efficacy has not yet been proven.

In Europe, rhDNase has been approved only for CF patients aged 5 years and older, since registration studies were performed in this age category only. In the US, rhDNase has been approved for all ages. Surprisingly, no registration studies have been performed in children below the age of 5 to support this registration.

In a study by Nasr et al., HRCT scores were used to determine efficacy of inhaled rhDNase in CF patients younger than 5 years of age. They were randomly assigned to receive either 2.5 mg of rhDNase or placebo once daily for 100 days. Administration of rhDNase was associated with significant improvement in the HRCT scores compared with placebo.

We may conclude that young CF patients are also likely to benefit from treatment with rhDNase, but studies are needed to establish safety and efficacy especially in those younger than 5 years of age. Clearly, these studies are a challenge since established end points in this age category are lacking.
Conclusion

The major problem in CF lung disease is chronic airway inflammation and infection, starting early in life. Daily sputum mobilization is essential to prevent severe lung damage, but is hindered by the high viscosity of CF sputum. Sputum that is less viscous is easier to clear out from the lungs by mucociliary and cough clearance. RhDNase, an enzyme that breaks down DNA strands in airway secretions, reduces the viscosity and surface adherence of CF sputum when administered as an aerosol.

Several clinical trials have shown that rhDNase is effective as therapy for CF, in patients with mild to moderate lung disease (FVC ≥ 40% predicted). It improves lung function and decreases the exacerbation rate in adults and children with CF. On average patients benefit from regular daily use of rhDNase, but to date it is not possible to predict the response in an individual patient.

Importantly, rhDNase is safe to use. Adverse reactions attributed to rhDNase are rare. If they should occur, they are mostly mild and transient and do not require alterations in rhDNase dosing.

A key issue in aerosol therapy is selecting a nebulizer that is suitable to use with the drug. Physicians should prescribe only devices that are approved for the nebulization of rhDNase and that are maintained well.

Expert commentary

RhDNase is probably the best investigated drug in CF. It has an unbeaten efficacy and safety record. Nevertheless, there is still something to be desired. For one thing, the lack of data in young children is problematic. It cannot be assumed that the drug is safe in a passive infant when studies have been mainly done in active children above 6 years of age. Another problematic issue is that no other than more or less antique delivery systems are available for delivery of this high tech drug. Regulatory agencies and pharmaceutical companies as well as CF caregivers are faced with the issue of how to establish equivalence of newer, faster, and more efficient delivery systems. Finally, new nebulized drugs for CF are coming to the market. It is not feasible to go on adding therapies endlessly. Therapies must be compared so as to establish the most effective treatment package for an individual. Unfortunately, comparative studies are very difficult to run in an orphan disease like CF. Here again, regulatory agencies, pharmaceutical industry and CF caregivers have to work closely together to address this issue in study designs.

Another problematic area is health economics. RhDNase is an expensive drug. On the one hand, its development was very costly, and so is its safe production; on the other hand, the number of CF patients worldwide is relatively small. There is great inequality in to whom the drug can be prescribed. Ideally it is prescribed to all eligible patients. In some countries, however, rhDNase is only reimbursed when a so-called N=1 trial shows a positive effect on lung function parameters in a particular patient. Clearly, such entry criteria aim to reduce the number of patients that will get the drug prescribed. This approach would seem to suggest that it is possible to predict long-term efficacy in an individual when the drug is given during a random period. In addition it suggests the patient would not benefit from if lung function does not improve over the observational period. Unfortunately, for a highly variable disease like CF it is probably impossible to ascertain from an N=1 study whether a drug is effective, since the natural course of disease during the observational period is not known. Even without showing a positive response to therapy, the patientâ€™s decline in lung function may have lessened, or the drug may have prevented an exacerbation. Hence, N=1 studies are not suitable to accurately predict responses for individuals.

Similarly, it has been suggested that the amount of DNA-amount in sputum could predict the efficacy of maintenance treatment rhDNase on FEV1. These tests are not used in current clinical practice yet.

Clearly, it would be helpful to develop predictive tests but, unfortunately, their value can only be tested in large-scale efficacy studies using appropriate end points. Hence, current study designs are not suitable to predict with accuracy the response for an individual.

Five-year view

- New inhaled mucolytic drugs for CF are coming to the market. It is likely that some of these alternative mucolytics will act synergistically with rhDNase. These options have to be included in study designs.

- More efficient, faster, patient-friendly nebulizers have been developed. Equivalence testing of these devices will make it possible to identify the proper devices for the delivery of rhDNase. If this should fail, rhDNase is likely to disappear from the market when other drugs will become available with more patient-friendly and more efficient delivery methods.
More information will need to follow on how to use rhDNase more effectively.

Targeting drugs to specific diseased areas of the lung is already technically possible. In CF, the peripheral airways are the most affected by the disease. Further improvement of treatment might be possible when inhaled medication is deposited specifically in these airways.

Resources for the treatment of CF are restricted. Other expensive drugs are coming to the market. Choices will more and more be based on the best allocation of these resources. Data on health economics for rhDNase will help us make such decisions.

Key issues

- In CF it is important to improve mucociliary clearance.
- RhDNase is effective as therapy for CF, in patients with mild to moderate lung disease (FVC \( \geq 40\% \) predicted).
- RhDNase improves lung function and decreases the exacerbation rate in adults and children with CF.
- Long-term treatment with rhDNase is associated with decreased airway inflammation.
- The use of rhDNase is safe. Side effects that were reported in clinical trials were few, mild and self-limiting.
- RhDNase is registered for use in CF patients aged 5 years and older in Europe and for patients of all ages in the US. Nevertheless, data in young children are lacking.
- For patients with severe lung disease (FVC \( \leq 40\% \) predicted), study results vary.

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Improved treatment response to dornase alfa in cystic fibrosis patients using controlled inhalation. A randomized controlled clinical trial


Abstract

Rationale and objectives

Better treatment of obstructed small airways is needed in CF. This study investigated whether efficient deposition of dornase alfa in the small airways improves small airway obstruction.

Methods

In a multi-centre, double-blind, randomized controlled clinical trial, CF patients on maintenance treatment with 2.5 ml dornase alfa once daily were switched to a smart nebulizer and randomized to small airways deposition (n=24) or large airways deposition (n=25) for 4 weeks. The primary outcome parameter was Forced Expiratory Flow at 75% of Forced Vital Capacity (FEF75).

Results

FEF75 increased significantly by 0.7 SD (5.2% predicted) in the large airways group and 1.2 SD (8.8% predicted) in the small airways group. Intention to treat analysis did not show a significant difference in treatment effect between groups. Per protocol analysis, excluding patients not completing the trial or with adherence <70%, showed a trend (p = 0.06) in FEF75 Z-score and a significant difference (p = 0.04) between groups in absolute FEF75 (L/s) favouring small airways deposition.

Conclusions

Improved delivery of dornase alfa using a smart nebulizer that aids patients in correct inhalation technique resulted in significant improvement of FEF75 in children with stable CF. Adherent children showed a larger treatment response for small airways deposition.

The European Respiratory Journal did not give permission to include the paper Improved treatment response to dornase alfa in cystic fibrosis patients using controlled inhalation. A randomized controlled clinical trial in the e-pub of this thesis.

The paper can be downloaded from http://erj.ersjournals.com/
Small airway deposition of dornase alfa during exacerbations in cystic fibrosis.
A randomized controlled clinical trial


Abstract

Introduction

Small airway obstruction is important in the pathophysiology of cystic fibrosis (CF) lung disease. Additionally, many CF patients lose lung function in the long term as a result of respiratory tract exacerbations (RTE). No trials have been performed to optimize mucolytic therapy during a RTE. We investigated whether specifically targeting dornase alfa to the small airways improves small airway obstruction during RTEs.

Methods

In a multi-centre, double-blind, randomized controlled trial CF patients hospitalized for a RTE and on maintenance treatment with dornase alfa were switched to a smart nebulizer. Patients were randomized to small airway deposition (n=19) or large airway deposition (n=19) of dornase alfa for at least 7 days. Primary endpoint was Forced Expiratory Flow at 75% of Forced Vital Capacity (FEF\textsubscript{75}).

Main Results

Spirometry parameters improved significantly during admission, but the difference in mean FEF\textsubscript{75} between treatment groups was not significant: 0.7 SD, p=0.30. FEV\textsubscript{1}, nocturnal oxygen saturation and diary symptom scores also did not differ between groups.

Conclusions

This study did not detect a difference if inhaled dornase alfa was targeted to small versus large airways during a RTE. Further studies are needed to improve the effectiveness of RTE treatment in CF.

Introduction

Cystic Fibrosis (CF) lung disease is characterized by chronic airway infection and inflammation that start early in life and cause progressive structural lung damage which eventually leads to respiratory failure and early death. Small airways are involved early in the disease process, as indicated by trapped air on CT scans and by lung function tests in young children. In addition small airways disease is an important component of end stage lung disease.

Sputum of CF patients is rich in leukocyte-derived DNA, which contributes to its increased viscoelasticity and leads in turn to small airways obstruction.

Respiratory tract exacerbations (RTEs) are associated with an increase in sputum production. Therefore, effective sputum mobilization is an important component in the treatment of RTEs. Despite this treatment, it is well recognized that many patients do not regain pre-RTE spirometry values at the end of the RTE treatment period. Hence, the effectiveness of treatment during RTEs needs to be further improved. In addition to acute morbidity, repeated exacerbations may have long-term negative impact on lung function and lifespan. Thus, optimal treatment of RTEs is an essential component of CF care. Nevertheless, it is striking that many aspects of RTE treatment are not evidence based. Indeed, reviews indicate that treatment choices are based on empirical evidence, centre preference or previously prescribed therapies. To our knowledge no RCTs have investigated whether mucolytic treatment during RTEs can be optimised.

Dornase alfa is a mucolytic drug that cleaves extracellular DNA through hydrolysis and reduces the viscoelasticity of
CF sputum in vitro. It has been extensively investigated for maintenance treatment in CF and is part of current standard care. Dornase alfa has been shown to improve lung function and reduce the number of pulmonary exacerbations in CF patients but its role in the treatment of RTEs is not well established. Currently, dornase alfa is administered with conventional nebulizers, which are inefficient and deliver a low dose to the lungs; most of the inhaled drug deposits in the large airways and relatively little in the small airways. Highly efficient smart nebulizers have become available that coach patients in proper inhalation technique, and that can be set to target more of the inhaled drug to the small airways.

In a recent study in stable CF patients with normal forced vital capacity (FVC) but with small airways disease as indicated by reduced Forced Expiratory Flow at 75% of Forced Vital Capacity (FEF₇₅), we showed that more efficient delivery of dornase alfa to the small airways leads to a significant improvement in small airway patency. This study used a smart nebulizer that was programmed to preferentially target specific regions of the lung; patients were randomized to receive either small airway deposition or large airway deposition of dornase alfa.

We hypothesized that more efficient delivery of dornase alfa to the small airways during a RTE would also result in more effective treatment of small airways obstruction, resulting in greater improvement in FEF₇₅ and in nocturnal oxygen saturation. To test this hypothesis we conducted a randomized controlled clinical trial in patients with CF admitted for a RTE, comparing large airway deposition with small airway deposition of dornase alfa.

Patients and methods

Study population

CF patients were recruited for the study in two centres: the adult and paediatric departments of the CF centre in Erasmus MC Rotterdam, The Netherlands, and in the Cystic Fibrosis Centre, Verona, Italy. Patients were eligible if they met the following inclusion criteria: age 6 years and older; hospitalised for a RTE; receiving maintenance dornase alfa treatment; able to perform spirometry; and FVC ≥ 40% predicted. Exclusion criteria were: inability to follow instructions of the investigator; concomitant medical conditions that affect inhaled treatment (e.g. cleft palate, severe tracheomalacia); pulmonary complications that might put the patient at risk during the study; and deterioration related to allergic bronchopulmonary aspergillosis (ABPA). A full checklist of inclusion and exclusion criteria is available in the supplement at the end of this chapter.

Study design

This was a randomized, double-blind, multi-centre, parallel-group trial. Primary endpoint was FEF₇₅ since this is a parameter sensitive to changes in the small airways. We assumed the SD of FEF₇₅ values to be 7%, based on a retrospective analysis of improvement in FEF₇₅ in all CF patients that were hospitalized for a RTE in our hospital in the previous two years. A sample size calculation indicated that 80% power to detect a difference of 7% or more in FEF₇₅ between the two treatment groups, with alpha = 0.05, required 16 patients per group (32 total). Since we anticipated that not all patients might complete the study or have analyzable data, we planned to include 38 patients.

Minimal study duration for each patient was 12 days, consisting of a run-in period of five days and a minimal treatment period of seven days. Study treatment continued until the patient was discharged from hospital. During the run-in period patients inhaled dornase alfa using a conventional nebulizer, consistent with current standard care. On day 6 patients were randomly assigned to the small airway deposition group or the large airway deposition group, according to a randomization schedule generated by the study statistician using a random number list (Excel version 1997-2003; Microsoft, Redmond, USA). Randomization was stratified for age and FVC.

This trial was registered in the Dutch trial register (http://www.trialregister.nl) and in the International Standard Randomised Controlled Trial Number Register (number: 50584238). The local ethics committee approved the study. Written informed consent of parents and/or patients was obtained for all participants.

Study treatment

Study treatment consisted of dornase alfa preferentially delivered to the small or the large airways. We used a smart nebulizer device (Akita²® APIXNEB, Activero technologies, Gemuenden, Germany) as previously described. It directs the flow and depth of each inhalation, coaches the patient on correct inhalation technique and controls the fraction of the inspiration time during which aerosol is generated. In order to obtain the two different lung deposition patterns we adjusted three characteristics of the nebulizer treatment: particle size, timing of aerosol bolus and breathing pattern (Figure 1).
For the large airways group, devices were set to simulate conventional nebulizers depositing most of the drug in the large airways: aerosol was generated with a volume median diameter (VMD) of 6.0 μm as measured by laser diffraction using Sympatec HELOS (data on file Activaero GmbH). The devices delivered aerosol-free air before and after the medication bolus, and coached patients to perform controlled normal-depth inhalations.

For the small airways group, the VMD of the aerosol was 3.0 μm. Devices delivered the aerosol bolus directly from the start of the inhalation followed by a bolus of aerosol-free air in the second phase of the inhalation, and coached patients to perform controlled deep inhalations (Figure 1).

The breathing pattern for each patient was based on individual inspiratory capacity and was generated using a microchip-containing smartcard inserted in the device. The investigator carefully instructed each patient on the use of the study device. Patients in both treatment arms received instructions and feedback from the display of the device during each nebulization to ensure that the correct breathing pattern was performed. During the first study
nebulization the investigator was present to observe the handling of the device and inhalation technique, and repeated instructions if necessary. Additional details on device settings and patient feedback, as well as technical details, can be found in the supplement at the end of this chapter.

The daily dose of dornase alfa loaded into the nebulizer was 1.25 mg which was estimated to result in a lung dose that would be 1.5 to 2 times higher than conventional aerosol treatment. The Akita deposits approximately 70% of the loaded dose in the lung, compared to 10-20% for conventional jet nebulizer systems. If the standard dose of 2.5 mg (2.5 ml) had been loaded into the nebulizer, total lung dose of dornase alfa would have been approximately 3-5 times higher than with conventional jet nebulizers. For safety reasons we aimed to deliver 1.5 to 2 times the conventional dose in these unstable patients admitted for a RTE.

Measurements

Spirometry was performed at day 1, 5, 6, 12, and at discharge. Primary endpoint was FEF\(_{75}\), since this parameter is sensitive to changes in the small airways. Spirometry was performed on a Masterscreen electronic spirometer (Viasys, Würzburg, Germany) at least one hour after dornase alfa administration and at a standardized time of day for each patient. Daily chest physiotherapy was performed at the same time of day throughout the study. We did not change patients’ daily routine at study inclusion; the order in which each patient performed daily chest physiotherapy, nebulization of dornase alfa and any other inhaled treatments continued as previously at home. Nocturnal oxygen saturation (SpO\(_2\)) was measured on day 2, 5, 6, and 12 using a MARS pulsoximeter (Novametrix, Model 2001). SpO\(_2\) was considered suitable for analysis when at least 6 hours of successful recording was available. From the stored saturation data mean SpO\(_2\) and two desaturation parameters were computed for each child: D4 desaturations, defined as desaturations of 4% below the mean saturation of the child; and D90 desaturations, defined as desaturations below 90% SpO\(_2\). The number and the time spent in D4 and D90 desaturations were calculated.

Respiratory symptoms were scored in a daily diary as used in previous studies. Patients recorded their day and night-time cough frequencies with a validated cough symptom score. They also rated sputum viscosity, sputum production, sleep quality, and cough frequency on separate visual analogue scales (VAS), each comprising a horizontal line 10 cm in length, anchored by word descriptors at each end. The VAS score was the distance in cm between no symptom and the rating mark placed on the scale by the patient. For both the symptom score and the VAS score a higher score indicates more symptoms.

Statistical analysis

Data were analysed on an intention-to-treat basis. Analyses of between-group comparisons regarding change in lung function variables (FVC, FEV\(_1\), and FEF\(_{75}\)) were performed using repeated measurements ANOVA with adjustment for baseline values and centre. Analyses were performed with SPSS software (version 15.0; IBM, Chicago, IL, USA). Spirometry on day 5 (last day before randomization) was set as baseline and spirometry on day 12 was the endpoint of the study. For the results of nightly oxygen saturation measurements a mixed model analysis was performed. Differences in changes in diary scores were analyzed using a Mann-Whitney test. The accepted level of significance was p=0.05 for all analyses.

Results

Thirty-eight patients were included in the study. All patients were enrolled between October 2006 and February 2009. Characteristics of patients at inclusion were similar for the two treatment groups (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>Large airways (n=19)</th>
<th>Small airways (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>8 /11</td>
<td>9 /10</td>
</tr>
<tr>
<td>Age(\text{yrs})</td>
<td>17.0 (6.9 (\text{â€“} 43.3))</td>
<td>19.8 (9.4 (\text{â€“} 46.4))</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>156.6 (18.1)</td>
<td>161.8 (9.90)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>46.8 (16.6)</td>
<td>52.3 (12.7)</td>
</tr>
<tr>
<td>FVC (Z-score)</td>
<td>â”2.9 (1.4)</td>
<td>â”2.8 (1.5)</td>
</tr>
<tr>
<td>FVC (% pred)</td>
<td>76.9 (17.7)</td>
<td>82.3 (18.2)</td>
</tr>
<tr>
<td>FEV(_1) (Z-score)</td>
<td>â”3.9 (1.5)</td>
<td>â”4.1 (1.8)</td>
</tr>
<tr>
<td>FEV(_1) (% pred)</td>
<td>63.8 (22.4)</td>
<td>63.6 (24.1)</td>
</tr>
<tr>
<td>FEF(_{75}) (Z-score)</td>
<td>â”9.0 (3.9)</td>
<td>â”9.0 (3.3)</td>
</tr>
<tr>
<td>FEF(_{75}) (% pred)</td>
<td>28.1 (27.0)</td>
<td>23.0 (19.4)</td>
</tr>
</tbody>
</table>
Table 1. Descriptives of study population at inclusion in the study. Data are shown as mean (SD) unless otherwise indicated. Median (range). All measurements were performed on day one, except saturation which was measured on day two.

D4 desaturations are defined as desaturations of 4% below the mean saturation of the child.

D90 desaturations are defined as desaturations below 90%

For both D4 and D90 desaturations the number of episodes per hour was calculated as well as the seconds of desaturation per hour.

**Spirometry**

*Figure 2* shows changes of FEF$\text{_{75}}$ during the study period. Mean increase in FEF$\text{_{75}}$, from day 5 at start of randomized treatment to day 12 was 0.4 SD (p=0.32) in the large airways group and 1.2 SD (p=0.02) in the small airways group (*Table 2*). The difference in improvement for the small airways minus large airways group was 0.7 SD (p=0.30). FEV$\text{_{1}}$ results were similar: mean increase in FEV$\text{_{1}}$, during the study period was 0.4 SD (p=0.014) in the large airways group and 0.6 SD (p=0.001) in the small airways group. The difference in improvement for the small airways minus large airways group was 0.2 SD (p=0.33). Individual responses for FEF$\text{_{75}}$ and FEV$\text{_{1}}$ are shown in *figure E1* and E2 of the supplement at the end of this chapter.
Table 2. Spirometry and saturation data after 7 days of study treatment. Data are presented as mean change from baseline at day 5 (day before start of randomized treatment) ± SEM (Anova estimates).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEF75 (Z-score)</td>
<td>0.4 (0.4)</td>
</tr>
<tr>
<td>FEV1 (Z-score)</td>
<td>0.4 (0.1)*</td>
</tr>
<tr>
<td>FVC (Z-score)</td>
<td>0.4 (0.1)*</td>
</tr>
<tr>
<td>mean SpO2 (%)</td>
<td>0.2 (0.3)</td>
</tr>
<tr>
<td>D90 desaturations</td>
<td>â”“80.3 (65.4)</td>
</tr>
</tbody>
</table>

SpO2 is oxygen saturation (measured by pulse oximetry). D90 desaturations are defined as desaturations below 90% SpO2.

Difference is shown for small airways minus large airways group and is presented as mean difference and 95% CI. None of the observed mean differences between study groups were significant.

* P-value ≤ 0.05 for change from baseline within treatment group.

# differences are adjusted for baseline value at day 5 and center.

Nocturnal oxygen saturation

At the first measurement nocturnal oxygen saturation was at the lower limit of normal in both groups: mean SpO2 was 95%. Patients experienced desaturations at a similar frequency in both groups. See table 1 for descriptive statistics on the saturation variables.

Nocturnal oxygen saturation, as assessed by either mean SpO2 or the number or duration of desaturations, did not improve significantly during the study. There was no significant difference in treatment effect on mean SpO2 between the two groups. See also figure E3 of the supplement at the end of this chapter.

Symptom scores

While symptom scores improved significantly during the study period, none of the diary items showed a significant difference between the two groups after 7 days of study treatment (data shown in on line supplement). A composite score of all diary items was also not significantly different.

Discussion

In this randomized, double-blind, multi-centre controlled trial in CF patients hospitalised for a RTE, we did not observe significant differences in spirometry parameters, overnight oxygen saturation or symptom scores between large airway and small airway deposition of dornase alfa.

In both the small and large airway groups spirometry improved significantly during the study period, as expected during a hospital admission for RTE. The magnitude of this improvement was comparable to what has been described in the literature. However, the difference between the two deposition groups was not significant. These findings are in contrast to a recent study by our group that examined the effect of small airway deposition of dornase alfa in 49 stable CF patients who inhaled their once daily dornase alfa for 4 weeks using the same smart nebulizer device. In the per protocol analysis of that study (including only adherent patients) a significant treatment benefit was detected in the primary endpoint (FEF75) in the small airways group compared with the large airways group.

There are a number of possible explanations for these contrasting findings. Firstly it is well recognized that there is increased sputum production during a RTE. Therefore, a higher than usual dose of dornase alfa may be optimal. In addition, central deposition of drugs is increased during a RTE and this may have resulted in under-dosing the small airways even in the small airways group. In our study in stable patients we estimated that the lung dose administered was 3 to 5 times higher than with conventional therapy but, at the time the current study was started the results of that study were unknown. For safety reasons, we therefore chose a lung dose calculated to be no more than twice the dose delivered by a standard nebulizer. In hindsight, because dornase alfa therapy was well tolerated in both studies, a higher dose may have been safe and more effective during an RTE.
Secondly, the study period of 7 days may have been too short. In previous dornase alfa trials a clear treatment effect has been observed after 7-14 days. In our study in stable patients, treatment duration was 4 weeks and a clear difference between the two study groups was observed after two weeks. We decided to include a run-in phase of 5 days in the present study to avoid the great variability in the first days of treatment, when antibiotics are started, chest physiotherapy is intensified and patients may receive supplemental oxygen. We suggest future RTE studies may achieve longer study duration either by randomizing patients directly after admission or by continuing study treatment for at least 14 days.

A third possibility is that the considerable variability in early RTE treatment may have obscured a later effect of dornase alfa on small airways. The intravenous antibiotic and the chest physiotherapy regimens used were personalized to the needs of each patient. Therefore, any additional contribution to improvement during hospital admission by dornase alfa targeted specifically to the small airways may have been too small to detect a difference. Indeed, a previous placebo-controlled study that evaluated the safety and efficacy of dornase alfa administered using a conventional nebulizer in hospitalized CF patients during an RTE did not demonstrate a statistically significant therapeutic effect of rhDNase when added to a regimen of antibiotics and chest physical therapy. The authors speculated that antibiotics and chest physiotherapy may contribute more than concurrent mucolytic therapy to effective treatment of a RTE.

Fourthly, our study may have been underpowered to detect a difference between the two study groups. In our previous study in stable CF patients 49 patients were randomized and the difference between groups just reached statistical significance. In our current study 38 patients were randomized and the small airways group showed a net benefit of 1.2 SD in FEF, at 7 days.

Indeed, there was substantially more variability in spirometry between patients admitted for a RTE than in our study in stable patients, possibly because of the variable pathophysiology of a RTE. Furthermore, spirometry performed during an RTE may be more variable within patients and consequently cause more noise in the spirometry data. Both potential sources of increased variability would make it more difficult to demonstrate a significant treatment effect.

Based on these arguments we feel it is important to further investigate how mucociliary clearance can be improved during a RTE. It is well recognized that many CF patients do not regain lung function to pre-RTE values, and mucus obstruction is likely to play a role in this. Despite worrisome observations of RTEs™ negative long-term impact on the lungs, to our knowledge there are no published randomized trials investigating alternative mucolytic regimens in their treatment. Our study suggests that further investigation should focus on longer treatment with a higher dose of dornase alfa, or dornase alfa in combination with another mucolytic with a different mode of action such as hypertonic saline.

Although RTEs have important clinical and financial implications there are few RCTs™ on antibiotic regimens and none on optimization of mucolytic therapy. Because many aspects are not well investigated, therapeutic decisions are based largely on empirical evidence or centre preference. We find it remarkable that RTE treatment is not evidence-based.

In conclusion, this study did not show a significant difference in FEF, or FEV, when dornase alfa was targeted to the small versus the large airways using a smart nebulizer for 7 days, starting 6 days after admission for a CF respiratory tract exacerbation. Whether longer therapy, a higher dose of dornase alfa or a combination of mucolytic drugs would be more effective in the treatment of RTE requires further investigation.

Acknowledgements

We thank all patients and parents, all medical personnel at the study sites and Ilaria Meneghelli and Marianna Passiu at the CF centre in Verona for their contributions to this study, and we thank Sheila McKenzie (Victoria, Canada) for her critical reading of the manuscript. We thank Activaero GmbH (Gemuenden, Germany) and Roche Netherlands B.V. (Woerden, The Netherlands) for their support and contributions to this study.

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Methods

Checklist of inclusion and exclusion criteria

Inclusion criteria:

- Age 6 years and older;
- Diagnosis of CF confirmed by sweat-test and/or DNA analysis and/or electro physiology testing (nasal potential difference measurement);
- Admission to hospital because of a pulmonary exacerbation requiring treatment with iv antibiotics.

Criteria for a pulmonary exacerbation were based on the definition of exacerbation by Rosenfeld et al.1 and included at least three of the following:

- Decreased exercise tolerance
- Increased cough
- Increased sputum / chest congestion
- School or work absenteeism
- Decreased appetite
- Increased adventitial sounds on lung examination
- Decrease in FEV₁ (% predicted)
- Enrolment in the study between 1 to 5 days after admission for an exacerbation;
- Routine treatment with rhDNase once daily, started at least two weeks before enrolment in the study;
- Ability to perform lung function tests (assessed by trained lung function technician);
- Lung function: FVC ≥ 40% predicted;
- Signed written informed consent.

Exclusion criteria:

- Inability to follow instructions of the investigator;
- Inability to inhale dornase alfa;
- Concomitant medical conditions that affect inhaled treatment (e.g. cleft palate, severe malacia);
- Pulmonary complications that might put the patient at risk to participate in the study;
- Deterioration primarily related to ABPA (allergic bronchopulmonary aspergillosis).

Study Withdrawal Criteria:

Criteria to exclude a patient from further participation were:

- Major adverse event that will affect the study or place the patient at risk;
- Non-compliance with the study protocol, to be decided at the discretion of the investigator;
Failure to produce reproducible lung function tests.

**Power calculation**

We aimed to detect a difference of 7% or more in FEF\(_{75}\) values between the two treatment groups. For use in the sample size calculation we did a retrospective analysis of improvement in FEF\(_{75}\) in the total group of patients that was hospitalized for a CF exacerbation in our hospital in the previous two years. The standard deviation (SD) of the mean improvement in FEF\(_{75}\) was 7%. Considering both factors mentioned above, for an alpha level of 0.05 and a power of 80%, 16 patients per group should be included (32 in total).

**Statistical analysis**

Data were analyzed on an intention-to-treat basis. All patients were included in this analysis, including patients who dropped out of the study or met study stop criteria. For these patients, only available data were used in the model and visits where data were lacking were defined as missing.

**Study treatment**

For large airway deposition we used a particle size of 6.0 μm (volume median diameter (VMD) measured by laser diffraction using Sympatec HELOS, data on file Activaero GmbH). An aerosol bolus technique was used during each inhalation. The Akita2 delivered aerosol-free air before and after the medication bolus using a flow rate of 200 ml/sec. The flow rate during actual nebulization of rhDNase was 60 ml/sec. These settings will lead to an optimal large airway deposition pattern. See also figure 1 of the paper.

For small airway deposition the particle size was 3.0 μm (VMD measured by laser diffraction, data on file Activaero GmbH). In contrast to the large airway pattern, the small airway breathing pattern was a controlled deep inhalation. The aerosol bolus was delivered directly from the start of the inhalation and was followed by a small bolus of aerosol-free air at the end of inhalation. The Akita2 supplied a constant flow rate of 200 ml/sec. during the complete inhalation maneuver. These settings lead to an optimal small airways deposition.

To ensure that patients performed the correct breathing pattern, the Akita2 showed instructions on a display with each breath. On the display, the number of seconds during which the patient should inhale was counted down. This inhalation time was adjusted to the individual inspiratory capacity (IC) of the patient. In each treatment group a set of five different Smart Cards was used in order to adjust the inhalation volume based on patients' spirometry data. Each patient received the Smart Card that matched with his or her IC. If a patient tried to inhale too fast or too forcefully, a warning message was shown on the display, indicating that the patient should inhale more slowly. Flow was controlled by the Akita2 during each inhalation; therefore a patient could not inhale at a higher flow than programmed for each treatment group.

If pressure at the mouthpiece of the nebulizer was too high (for example if a patient tried to exhale into the mouthpiece instead of inhaling), nebulization was interrupted but continued when pressure was normalized.

All maintenance medications that patients used were continued unchanged.

**Results**

**Spirometry**

Lung function as measured by spirometry improved significantly during the study. However, FEF\(_{75}\) and FEV\(_1\) showed relatively large variability between patients. See figure E1 and E2 of this supplement.
The image shows a graph with multiple lines representing different data sets. The x-axis is labeled "studyday" and ranges from 3 to 12. The y-axis is labeled "FEF75 Z-score" and ranges from -15 to 0.

Each line represents a distinct group or condition, indicated by different colors and markers. The graph illustrates changes in FEF75 Z-score over the study period, with varying trends across different study days.
Figure E1. Individual patient FEF\textsubscript{75} values during hospitalisation. On the X-axis are study days, on the Y-axis FEF\textsubscript{75} (Z-score). Day 1 to 5: run-in, day 6 to 12: study treatment.
Figure E2. Individual patient FEV₁ values during hospitalisation. On the X-axis are study days, on the Y-axis FEV₁ (Z-score). Day 1 to 5: run-in, day 6 to 12: study treatment.

Nocturnal oxygen saturation

Mean SpO₂ showed no significant difference between the two treatment groups, see figure E3 of this supplement.
Figure E3. Mean saturation at baseline, at day 6 and at day 12 of the study. On the X-axis are study days (run-in or treatment), on the Y-axis mean saturation (SpO₂ (%)). Data are presented as mean ± 95% CI. Closed squares represent patients randomised to large airways deposition, open circles represent small airways deposition.

Symptom scores

All symptom scores improved during the study period. However, differences between the treatment groups were not significant. See table E1 of this supplement.

<table>
<thead>
<tr>
<th></th>
<th>Large airways (baseline)</th>
<th>Small airways (baseline)</th>
<th>Large airways (study end)</th>
<th>Small airways (study end)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nocturnal cough</td>
<td>1.1 (0.3 â€“ 1.8)</td>
<td>1.0 (0.0 â€“ 3.0)</td>
<td>0.5 (0.0 â€“ 1.8)</td>
<td>0.6 (0.0 â€“ 1.9)</td>
<td>0.77</td>
</tr>
<tr>
<td>VAS nocturnal</td>
<td>1.1 (0.0 â€“ 4.3)</td>
<td>2.1 (0.1 â€“ 6.9)</td>
<td>0.4 (0.0 â€“ 2.4)</td>
<td>0.7 (0.0 â€“ 4.1)</td>
<td>0.23</td>
</tr>
<tr>
<td>cough</td>
<td>4.3 (6.9)</td>
<td>6.9 (2.4)</td>
<td>2.4 (4.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS sleep</td>
<td>3.0 (0.1 â€“ 5.5)</td>
<td>2.3 (0.0 â€“ 6.6)</td>
<td>1.0 (0.0 â€“ 5.0)</td>
<td>1.6 (0.0 â€“ 5.6)</td>
<td>0.34</td>
</tr>
<tr>
<td>quality</td>
<td>5.5 (1.0 â€“ 3.8)</td>
<td>6.6 (0.0 â€“ 3.0)</td>
<td>5.0 (0.0 â€“ 1.8)</td>
<td>5.6 (0.0 â€“ 1.9)</td>
<td></td>
</tr>
<tr>
<td>Daytime cough</td>
<td>1.8 (0.3 â€“ 3.3)</td>
<td>2.4 (0.8 â€“ 4.0)</td>
<td>1.1 (0.0 â€“ 3.0)</td>
<td>1.4 (0.0 â€“ 3.9)</td>
<td>0.26</td>
</tr>
<tr>
<td>VAS daytime</td>
<td>3.4 (0.2 â€“ 4.9)</td>
<td>4.3 (0.9 â€“ 6.8)</td>
<td>1.6 (0.0 â€“ 3.9)</td>
<td>2.1 (0.0 â€“ 6.0)</td>
<td>0.95</td>
</tr>
<tr>
<td>cough</td>
<td>4.9 (6.8)</td>
<td>6.8 (3.9)</td>
<td>3.9 (6.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS sputum</td>
<td>4.0 (0.3 â€“ 9.0)</td>
<td>4.9 (0.5 â€“ 8.1)</td>
<td>1.1 (0.0 â€“ 9.0)</td>
<td>2.8 (0.0 â€“ 8.1)</td>
<td>0.41</td>
</tr>
<tr>
<td>tenacity</td>
<td>9.0 (8.1)</td>
<td>6.8 (3.9)</td>
<td>6.0 (6.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS sputum</td>
<td>3.6 (0.5 â€“ 1.5)</td>
<td>4.0 (0.5 â€“ 1.9)</td>
<td>1.5 (0.0 â€“ 1.9)</td>
<td>1.9 (0.0 â€“ 1.9)</td>
<td>0.37</td>
</tr>
</tbody>
</table>
amount 6.5) 7.1) 6.4) 7.4)

**Table E1.** Symptom scores at baseline and after 7 days of study treatment.

Data are shown as median (range). VAS scores were expressed on a scale from 0 to 10, with 10 indicating severe symptoms. Both cough symptom scores were expressed on a scale from 0 to 5, with 5 indicating severe symptoms.

P-values for the comparison between large and small airways group in the change from baseline to study end for each diary item

**References**

Small airway deposition of dornase alfa in children with asthma and persistent airway obstruction. A randomized placebo-controlled trial

Bakker E.M., van der Wiel â€“ Kooij E.C., Müllinger B., Kroneberg P., Hop W.C.J, Tiddens H.A.W.M. Submitted to Journal of Allergy and Clinical Immunology

Abstract

Background

The pathophysiology of persistent small airways obstruction in children with asthma is not well understood. Mucus might play a role in this obstruction and therefore mucolytic drugs could be effective.

Objective

To assess the efficacy of dornase alfa in reducing small airway obstruction in children with asthma.

Methods

We conducted a randomized placebo-controlled triple blind clinical trial in 64 children with clinically stable asthma and persistent small airways obstruction. Patients were randomly assigned to dornase alfa or placebo once daily for 3 weeks, administered by a smart nebulizer programmed to maximise deposition in the small airways. Spirometry, Lung Clearance Index (LCI) and fractional concentration of exhaled nitric oxide (FeNO) were measured at inclusion, after 2 and 3 weeks of treatment, and 4 weeks after the study treatment was stopped. Patients scored respiratory symptoms in a diary. Primary endpoint was difference between groups in change in Z-score in forced expiratory flow at 75% of forced vital capacity (FEF\textsubscript{75}).

Results

Mean FEF\textsubscript{75} did not change significantly after 3 weeks of treatment in either group, nor did mean FEV\textsubscript{1}, LCI, FeNO or symptom scores.

Conclusions

Three weeks of treatment with once daily dornase alfa did not reverse persistent small airways obstruction in children with stable asthma. This suggests that either the obstruction is not caused by mucus, or the mucus does not contain increased amounts of extracellular DNA.

Introduction

Approximately 10% of children in the Western world have asthma. The aims of the pharmacological management of asthma are to control asthma symptoms and to normalize pulmonary function. Despite modern anti-asthma treatment using inhaled corticosteroids (ICS) and short- and long-acting ß-2 agonists, 30 to 70% of asthma patients do not gain optimal asthma control. In addition, up to one third of patients continue to show abnormal spirometry values.

Many studies show that small airways pathology persists even in patients who are considered to be well controlled. Major morphological changes have been observed in the airways of patients who died of asthma. More efficient treatment of the small airways could further improve asthma control and improve short and long term lung function outcome.

It has been shown that mucus retention contributes to persistent small airways obstruction in childhood asthma. In children who died from or with asthma, mucus plugs were observed especially in the small airways.
asthma is known to be composed of activated and degenerated inflammatory cells, mucoproteins, and DNA released from disintegrated inflammatory cells.

Recombinant human DNase (dornase alfa) is a mucolytic agent that reduces sputum viscosity by hydrolysing extracellular DNA in sputum. In cystic fibrosis (CF) lung disease maintenance treatment using dornase alfa improves lung function and reduces the number of pulmonary exacerbations.

Smart nebulizers have become available which allow highly efficient treatment of the small airways. In a recent CF study we showed that dornase alfa was effective in reducing small airways obstruction as measured by FEF2. In that study we selected FEF2 as primary outcome parameter because it is sensitive to obstruction in the small airways. It is known that the variability of FEF2 is approximately 1.5-times higher than that of FEV1. What is less well recognized is that FEF2 is decreased more than FEV1 in early lung disease, which outweighs the variability and makes it a sensitive end point as supported by a number of studies.

Several case reports in severe asthma suggest that dornase alfa may contribute to the treatment of asthma. Dornase alfa has been used for therapy-resistant atelectasis in asthmatic children and for the treatment of status asthmaticus. It is unknown whether dornase alfa can reverse persistent small airways obstruction in stable asthmatic patients.

**Methods**

We conducted a randomized placebo-controlled triple blind clinical trial to assess the efficacy of three weeks' treatment with dornase alfa, administered by a smart nebulizer programmed to optimally treat the small airways, on FEF2 in children with stable asthma and persistent small airways obstruction.

**Patient recruitment and randomization**

Study participants were recruited from the outpatient clinic of the department of pediatric pulmonology, Erasmus MC Sophia Children's Hospital Rotterdam and from its specialized asthma clinic Kinderhaven.

Children were eligible to participate in the study if: they were aged 6–18 years, had clinically stable asthma, were on maintenance treatment with at least 400 μg/day inhaled Budesonide or equivalent, and had persistent small airways obstruction on at least two consecutive visits to the outpatient clinic. Small airways obstruction was defined as dissociation between FVC and FEF2 values: FEF2 values were not at least 20% predicted below FVC. Stable asthma was defined as: no change in ICS and no hospital admission for at least 3 months prior to the study. We aimed to include stable asthmatic patients who continued to show airway obstruction despite standard treatment, since in this group of patients there is a need for additional therapy to improve lung function. A full checklist of inclusion, exclusion, and study stop criteria used in the study is available in the supplement at the end of this chapter.

Patients were randomly assigned to three-weeks' treatment with dornase alfa once daily or placebo once daily, based on a randomisation list prepared by the study statistician. Dornase alfa and placebo (NaCl 0.9%) were prepared in identical vials by the hospital pharmacy. Study participants and all study personnel were blinded: patients, the investigator, patient's physicians and lung function technicians were unaware of the treatment assigned. We aimed to assign 2/3 of participants to the dornase alfa group and 1/3 to the placebo group. We chose to create unequal groups (ratio 2:1) to facilitate the planned statistical analysis of correlations between measurements in the intervention group.

This trial was registered in the Dutch trial register (http://www.trialregister.nl) and in the International Standard Randomised Controlled Trial Number Register (number: 71537084). The local ethics committee approved the study. Parental informed written consent and children's assent were obtained prior to the first study measurement.

**Study treatment**

Study treatment consisted of 1.25 ml (1.25 mg) nebulized dornase alfa or placebo once daily. Study drug was administered using the Akita® APIXNEB device (Activaero technologies, Gemüenden, Germany) as previously described. The nebulizer generates particles with a volume median diameter (VMD) of 4.2 μm. The electronic unit controls air flow velocity and aerosol bolus during inhalation. In this publication we will refer to the Akita® APIXNEB device as Akita. The Akita has been used in other studies and was shown to efficiently target specific regions of the lung. It deposits approximately 70% of the loaded dose in the lungs, compared to 10-20% for conventional jet nebulizer systems. Based on these efficiency data we selected a standard daily dose of 1.25 ml of dornase alfa. With this change in filling dose lung deposition was estimated to be 1.5 to 2.5 times higher compared to a conventional nebulizer, but well within a safe range as established in healthy subjects.

For this study the Akita was set to achieve optimal deposition of dornase alfa in the small airways. To accomplish
this, the breathing pattern during nebulisation consisted of controlled deep inhalations. In addition, the aerosol bolus was delivered at the beginning of the inspiration phase and was followed by a small bolus of aerosol-free air at the end of inhalation. A constant low flow rate of 200 ml/s was used. The investigator carefully instructed all patients in both treatment arms on the use of the device at the start of the study. The feedback system from the display of the device gives instructions to guide the patient during each nebulisation and informs the patient on whether the correct breathing pattern is performed. Additional details on feedback to the patient and inhalation settings of the device, as well as technical details can be found in the supplement at the end of this chapter.

Study endpoints

The primary endpoint of this study was difference between the groups in change from baseline FEF75 (Z-score) after 3 weeks of treatment. Secondary endpoints were change in FEV1, FVC, Lung Clearance Index (LCI), fractional concentration of exhaled nitric oxide (FeNO) and change in diary symptom scores.

Study visits were scheduled at inclusion, after 2 weeks, 3 weeks and 7 weeks. The last visit was a follow-up visit 4 weeks after the study medication was stopped to assess the change in lung function after the completion of treatment. At each study visit, spirometry, multiple breath wash out (MBW) and FeNO measurements were performed. Spirometry was measured on a Masterscreen electronic spirometer (Jaeger/Viasys, WÃ¼rzburg, Germany) according to ERS/ATS guidelines. LCI was measured by a MBW test using the Exhalizer®D (Ecomedics, Duernten, Switzerland) according to the methods as described previously. FeNO was measured on a NIOX® (Aerocrine, Solna, Sweden) according to ERS/ATS recommendations.

To monitor possible adverse events during treatment patients were asked to record symptoms in a diary as used in previous studies. This was done during the three weeks of treatment and in the week before the final follow up visit. In the diary three items were scored twice daily: cough, wheezing and shortness of breath. Night-time symptoms were scored in the morning and daytime symptoms were scored in the evening. For each item a score of 0 to 3 points could be given. Consequently, the total score for one day could range between 0 and 18 points. Use of rescue medication was recorded in the diary. In addition, patients were asked to record any unusual symptoms and symptoms for which they visited a physician.

Adherence

The Akita monitors adherence by recording on the smartcard the date, time and number of breaths of each nebulization session. These data are stored on the Smartcard and can be retrieved for analysis afterwards. For each patient we calculated daily dose adherence which expresses the percentage of days a patient adhered correctly to the prescribed once daily nebulization of study drug.

Patients in this study were not aware of the adherence monitoring during their treatment. At the third study visit, when the study treatment was completed, patients visited the outpatient clinic to perform lung function tests and to return the study nebulizer. At the end of this visit we explained to the patients that the Akita had stored data of each treatment on the Smartcard. Next, informed consent was asked for use of the data on the Smartcard.

Estimate of sample size

We aimed to detect a difference of 15% predicted in FEF75 (~ 1.4 Z score) between study arms. Based on a previous study in 25 CF patients by our group it was estimated that for an alpha of 0.05, inclusion of 60 children would result in a power of 84%, which we considered sufficient for this study.

Statistical analysis

Data were analyzed on an intention-to-treat basis. Differences between baseline group characteristics were assessed by an independent samples T-test or Mannâ€“Whitney test, as appropriate. We performed a mixed model analysis to calculate change in lung function in each group and its significance. Analyses of between-group comparisons regarding change in lung function variables (FVC, FEV1, FEF75, LCI, FeNO) were performed using repeated measurements ANOVA (analysis of variance) with adjustment for baseline values and ICS dose. FeNO values were log-transformed prior to analysis. Analyses were performed with SPSS software (version 15.0). For all analyses, two-tailed P values of <0.05 were considered to indicate statistical significance. In addition, a per-protocol analysis was conducted in which patients who violated the study protocol were excluded (Figure 1). Protocol violations were defined as patients who did not complete the trial, had adherence < 70% or for whom adherence data could not be retrieved.
Figure 1. Enrollment, random assignment, follow-up and analysis.

Since both FEF\textsubscript{75} and LCI are indicators of small airways disease, regression analysis was performed to analyze the relation between FEF\textsubscript{75} and LCI.

Spirometry variables were expressed as Z-scores and as % predicted using the reference sets by Stanojevic et al.\textsuperscript{56} for FVC, FEV\textsubscript{1} and FEF\textsubscript{25-75}. These reference sets do not include data for FEF\textsubscript{75}, for which we used the reference values by Zapletal et al.\textsuperscript{57} Changes in absolute values of spirometry variables (L and L/s) were expressed as percentage of baseline values.

For each patient mean daily symptom scores per week were computed. If less than 4 out of 7 days of a week were filled
in, scores for that week were not included in the analysis. Changes in diary scores and number of patients with adverse events were analysed using a Mann-Whitney test and Chi-Square test, respectively.

**Results**

64 patients were included in the study, 59 of whom completed the trial (38 patients in the dornase alfa group and 21 in the placebo group). Patients were enrolled during the period from March 2007 to May 2010. Baseline characteristics of patients in the two treatment groups were similar (Table 1).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Placebo (n = 23)</th>
<th>Dornase alfa (n = 41)</th>
<th>mean difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>14/9</td>
<td>28/13</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>11.7 (2.6)</td>
<td>12.1 (3.4)</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>147.8 (13.7)</td>
<td>147.6 (18.5)</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>43.1 (13.3)</td>
<td>42.6 (15.5)</td>
<td></td>
</tr>
<tr>
<td>FVC (Z-score)</td>
<td>å”0.4 (1.0)</td>
<td>0.1 (0.9)</td>
<td></td>
</tr>
<tr>
<td>FVC (% predicted)</td>
<td>95.8 (11.0)</td>
<td>100.8 (10.1)</td>
<td></td>
</tr>
<tr>
<td>FEV₁, (Z-score)</td>
<td>å”1.0 (1.0)</td>
<td>å”0.8 (0.9)</td>
<td></td>
</tr>
<tr>
<td>FEV₁, (% predicted)</td>
<td>86.9 (11.9)</td>
<td>90.1 (10.6)</td>
<td></td>
</tr>
<tr>
<td>FEF₂₅₋₇₅, (Z-score)</td>
<td>å”3.9 (1.7)</td>
<td>å”3.4 (1.4)</td>
<td></td>
</tr>
<tr>
<td>FEF₂₅₋₇₅, (% predicted)</td>
<td>48.0 (15.4)</td>
<td>52.5 (13.7)</td>
<td></td>
</tr>
<tr>
<td>LCI</td>
<td>7.9 (1.1)</td>
<td>7.9 (1.5)</td>
<td></td>
</tr>
<tr>
<td>FeNO (ppb) Median (range)</td>
<td>23.0 (3.7 å€“ 111.2)</td>
<td>20.5 (3.9 å€“ 97.5)</td>
<td></td>
</tr>
<tr>
<td>ICS dose (Åµg)</td>
<td>843 (365)</td>
<td>844 (280)</td>
<td></td>
</tr>
<tr>
<td>LABA use (%)</td>
<td>78</td>
<td>78</td>
<td></td>
</tr>
</tbody>
</table>

*Table 1. Baseline characteristics of study participants at the start of the study.*

Data are presented as mean (SD) except for gender and FeNO. Dose of inhaled corticosteroids is presented as equivalent dose to budesonide.

**Lung function**

After 3 weeks of treatment, mean FEF₇₅ was not significantly different from baseline within either group (Table 2).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Placebo (n = 23)</th>
<th>Dornase alfa (n = 41)</th>
<th>mean difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEF₇₅ (Z-score)</td>
<td>0.19 (0.34)</td>
<td>å”0.25 (0.25)</td>
<td>å”0.24 (å”1.07 to 0.60)</td>
</tr>
<tr>
<td>FVC (Z-score)</td>
<td>å”0.07 (0.10)</td>
<td>å”0.04 (0.07)</td>
<td>0.06 (å”0.19 to 0.30)</td>
</tr>
<tr>
<td>FEV₁, (Z-score)</td>
<td>å”0.06 (0.13)</td>
<td>å”0.09 (0.09)</td>
<td>0.00 (å”0.32 to 0.32)</td>
</tr>
<tr>
<td>FEF₂₅₋₇₅, (Z-score)</td>
<td>å”0.00 (0.15)</td>
<td>å”0.09 (0.11)</td>
<td>å”0.07 (å”0.44 to 0.30)</td>
</tr>
<tr>
<td>LCI</td>
<td>0.10 (0.34)</td>
<td>å”0.17 (0.24)</td>
<td>0.04 (å”0.59 to 0.66)</td>
</tr>
<tr>
<td>Log₁₀ FeNO (ppb)</td>
<td>0.01 (0.06)</td>
<td>å”0.01 (0.04)</td>
<td>- 0.03# (- 0.16 to 0.10)</td>
</tr>
<tr>
<td>FEF₇₅, PPS analysis (Z-score)</td>
<td>0.28 (0.42)</td>
<td>- 0.29 (0.28)</td>
<td>å”0.52 (- 1.5 to 0.5)</td>
</tr>
</tbody>
</table>

*Table 2. Lung function after 3 weeks of study treatment.*
Data are presented as mean change from baseline ± SEM (Anova estimates). Adjusted differences are shown for dornase alfa minus placebo group (mean Z-score and 95% CI).

# Difference translates into a ratio of geometric means of 0.9 (95%CI: 0.7 to 1.3).

$ Per Protocol

For patients in the dornase alfa group FEF$_75$ decreased 0.25 SD (p=0.32) and for patients in the placebo group FEF$_75$ increased 0.19 SD (p=0.58). No difference in mean FEF$_75$ between groups was found after 3 weeks of treatment (adjusted difference $\approx -0.24$ SD, 95% CI $\approx -1.07$ to $\approx 0.60$, p=0.56) (Figure 2).

Figure 2. Mean change from baseline for FEF$_75$ (Z-score) after 2 and 3 weeks of treatment, and 4 weeks after the study drug was stopped. Data are presented as mean ± SEM (Anova estimates). On the X-axis are weeks of treatment, on the Y-axis change in FEF$_75$ (Z-score). Squares and dotted lines represent dornase alfa, diamonds and solid lines represent placebo.

Individual responses varied widely between patients (Figure 3).
Figure 3. Individual changes from baseline per patient for FEF₇₅ (Z-score) after 2 and 3 weeks of treatment, and 4 weeks after the study drug was stopped. On the X-axis are weeks of treatment, on the Y-axis change in FEF₇₅ (Z-score). Panel A shows FEF₇₅ for patients on dornase alfa, panel B for patients on placebo.

Similar results were found for FVC, FEV₁, and FEF₂₅-₇₅ (Table 2): mean values did not differ significantly from baseline after 3 weeks of treatment, nor was there a significant difference between groups for FVC (p=0.66), FEV₁ (p=0.99) and FEF₂₅-₇₅ (p=0.72).

Due to limited availability of the MBW device, LCI was measured in a subgroup of 44 patients, 16 in the placebo group and 28 in the dornase alfa group. LCI did not change significantly in either group nor was there a significant difference between the changes in the two groups (p=0.91).

LogFeNO did not change significantly from baseline after 3 weeks of treatment in either group (Table 2). For patients in the dornase alfa group logFeNO decreased 0.01 ppb (p=0.77) and for patients in the placebo group logFeNO increased 0.01 ppb (p=0.87). The adjusted difference between the groups was not significant.

**Symptom scores**

Mean symptom scores during the study did not differ significantly between treatment groups (see Figure E1 in the supplement at the end of this chapter) and there were no significant changes in symptom scores within either group. Similarly, there were no differences in percentage of symptom-free days and in use of rescue medication (e.g. F₂₅).
FEF\textsubscript{75} (per protocol analysis)

Fifteen of the 64 patients were excluded from the per protocol analysis: 5 patients did not complete the trial, 9 patients had a daily dose adherence below 70\% and for one patient data on adherence could not be retrieved (Figure 1). Hence, for the per protocol analysis 34 patients in the dornase alfa group and 15 patients in the placebo group were analyzed for the primary endpoint (FEF\textsubscript{75}).

FEF\textsubscript{75} (Z-score) did not differ significantly from baseline after 3 weeks of treatment in either group (Table 2). For patients in the dornase alfa group FEF\textsubscript{75} decreased 0.29 SDS (p=0.31) and for patients in the placebo group FEF\textsubscript{75} increased 0.28 SDS (p=0.50). The difference between the groups was -0.5 SDS (p=0.29) for dornase alfa minus placebo.

At the follow up visit, 4 weeks after the study drugs were stopped, there was a statistically significant difference in mean FEF\textsubscript{75} between the treatment groups in favour of placebo: the adjusted difference was 1.2 SDS (p=0.01), 95\% CI 0.3 \textless 2.2.

Correlations

There was a significant correlation between LCI and FEF\textsubscript{75}. The regression line has a common slope of $\Delta \beta = 0.12$ for all four visits (p=0.02). This indicates that for each increase of 1 SD in FEF\textsubscript{75}, LCI decreases by 0.12 (see Figure E1 in the supplement at the end of this chapter).

Adherence

Mean daily dose adherence was 83\%. Only 9 patients had adherence below 70\% (for detailed information on compliance please see figure E2 and the additional text in the supplement at the end of this chapter).

Discussion

To our knowledge, this is the first randomized double-blind placebo-controlled trial to investigate whether efficient treatment of the small airways with nebulized dornase alfa in asthma patients with stable but persistent small airways obstruction improves short-term lung function outcome or symptom scores. No significant difference between the dornase-treated group and the placebo group was observed. Our results suggest that free DNA in mucus does not play an important role in persistent small airways obstruction in stable asthmatic patients.

This lack of response was unexpected, since there are many arguments to believe that mucus retention contributes to persistent small airways obstruction in childhood asthma and several uncontrolled observations have been published suggesting that dornase alfa may contribute to the treatment of chronic severe or acute asthma. Most studies that showed an effect of dornase alfa were in acute asthma, where cell necrosis and DNA may play a greater role. On the other hand our results are in line with a study in children admitted to the emergency department with acute asthma who did not show improvement after being treated with a single dose of dornase alfa.

The most likely reason why our study did not detect any effect of dornase alfa in children with stable asthma and persistent small airways obstruction is that extracellular DNA does not contribute importantly to persistent small airways obstruction in the patient group studied. However, asthma is a heterogeneous disease, with different phenotypes and complex disease pathophysiology. We cannot exclude the possibility that small airway obstruction caused by DNA retention in mucus plays a role in a subgroup of children with asthma and that this subgroup could benefit from dornase alfa therapy. In accordance with this idea, we observed a wide range in individual responses especially in the dornase alfa group (Figure 3).

Furthermore, as in CF, reducing mucus viscosity by lysing extracellular DNA may be insufficient to improve airway obstruction without a subsequent airway clearance manoeuvre. Since our study did not include airway clearance therapy, liquefied sputum may have remained in the airways instead of being expectorated. Indeed, dornase alfa was shown to be ineffective and potentially harmful in a 24-week study in adult patients with idiopathic bronchiectasis, where FEV\textsubscript{1} decline was greater in patients who received rhDNase compared with placebo.

Alternatively, the persistent small airways obstruction may actually be caused by remodelling of the airway wall rather than mucus present in the lumen. There are limited data on the histology of small airways in children. Autopsy studies in predominantly adult patients have shown hypertrophy and hyperplasia of airway smooth muscle, increase in number of mucous glands and thickening of the reticular basement membrane. Biopsy studies in children with asthma have shown that epithelial damage and angiogenesis, reticular basement membrane thickening and
eosinophilia are present in the larger airways. since specimens were obtained via endobronchial biopsies during bronchoscopy, only larger airways could be examined.

A fourth possible explanation for the lack of response may be that the mass of drug deposited in the small airways was insufficient. However, we used a state of the art nebulizer that is very efficient and administered a lung dose calculated to be up to three times as high as the dose commonly effective in CF. In addition, adherence during the present study was high. Hence, we feel that the lack of a treatment effect was not caused by an insufficient amount of dornase alfa deposited in the small airways.

A puzzling observation is that the per protocol analysis showed a significant difference in FEF\textsubscript{75} in favour of placebo at the follow up visit 4 weeks after the study drug was stopped. Most likely this significant difference at only one point in time during the study is just a chance finding. A less likely explanation is that dehydration of the epithelial lining fluid may play a role in the pathophysiology of small airways obstruction in asthmatics; daily nebulization of placebo may have been more beneficial than dornase alpha to counteract this effect.

In conclusion, this study shows that dornase alfa has no additional value in the treatment of children with stable asthma and persistent small airways obstruction. Since patient responses varied widely, future studies could be aimed at identifying possible subgroups of patients who can benefit from mucolytic medications.

Acknowledgements

We thank all children and parents for participating in this study; we thank our colleagues at the Kinderhaven asthma clinic for their contributions to the study; we thank S. McKenzie (Victoria, Canada), an external consultant to F. Hoffmann-La Roche Ltd, Basel, Switzerland, for critical reading of the manuscript and we thank the lung function technicians at the Erasmus MC aet Sophia ChildrenaETM’s Hospital for their contribution to the spirometry measurements performed in this study.

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Methods

Checklist of inclusion and exclusion criteria

Inclusion criteria:

• Age 6–18 years;
• Asthma diagnosed according to GINA guidelines[3];
• Attending the outpatient clinic for at least one year;
• Treatment with at least 400 µg/day inhaled Budesonide or equivalent (dose constant for at least 3 months) and bronchodilators as needed or daily;
• Clinically stable asthma while using a constant dose of ICS for at least three months;
• Ability to perform lung function tests as assessed by experienced lung function technician;
• Persistent peripheral airways obstruction as assessed by pulmonary function testing, defined as:
  + Dissociation between FVC and FEF\textsubscript{75} values: FEF\textsubscript{75} at least 20% (absolute % predicted) below FVC.
• FVC within normal limits (for this study defined as FVC > 80% pred).

Exclusion criteria:

• Asthma exacerbation with hospital admission in last 3 months;
• Intensive Care Unit (ICU) admission for asthma within the last year;
• Current respiratory tract infection;
• Inability to follow instructions of the investigator;
• Inability to inhale rhDNase;
• Concomitant medical conditions that affect inhaled treatment (e.g. cleft palate, severe malacia);
• Neuromuscular disease;
• Smoking.

Study stop criteria:

• Major adverse event that places the patient at risk or will affect the study;
• Non-compliance with the study protocol, to be decided at the discretion of the investigator;
• Treatment with new pulmonary medication during study period (e.g. antibiotic course, prednisone course).

Randomization

Patients were randomly assigned to three-weeks’ treatment with dornase alfa once daily or placebo once daily. Randomization was performed by the hospital pharmacy according to a randomization schedule prepared by the study statistician. The study was triple blind: patients were blinded, persons who administered the treatment and treated the patient were blinded (investigator and physicians), and the persons who measured the effect size were blinded (lung function technicians). Randomization was stratified for baseline FEF\textsubscript{75} and for ICS dose.
Study treatment

The Akita2 directs the flow and depth of each inhalation, coaches the patient on correct inhalation technique and controls the fraction of the inspiration time during which aerosol is generated. These settings can be optimized for individual patients based on the inspiratory capacity using a microchip-containing smartcard inserted in the device. Thus the Akita2 controls the breathing pattern for each patient and generates an aerosol bolus at a specified time during inhalation.

To optimize small airways deposition, the breathing pattern programmed in the Akita2 was a controlled deep inhalation and the particle size was 4.2 μm (VMD measured by laser diffraction, data on file Activaero GmbH). The aerosol bolus was delivered directly from the start of the inhalation and was followed by a small bolus of aerosol-free air at the end of inhalation. The Akita2 supplied a constant flow rate of 200 ml/sec. during the entire inhalation.

To ensure that patients performed the correct breathing pattern, the Akita2 shows instructions on the display with each breath. On the display, the number of seconds during which the patient should inhale was counted down. This inhalation time was adjusted to the individual inspiratory capacity (IC) of the patient. For each patient a personal Smartcard was produced, containing the optimal inhalation settings for the patients IC to generate small airways deposition.

If a patient tried to inhale too fast, a warning message was shown on the display, indicating that the patient should inhale more slowly. Flow was also controlled by the Akita2 during each inhalation; therefore a patient could not inhale at a higher flow than programmed.

If pressure at the mouthpiece of the nebulizer was too high (for example if a patient tried to exhale into the mouthpiece instead of inhaling), nebulization was interrupted but continued when pressure was normalized.

All maintenance lung medications that patients used were continued unchanged. Patients who needed to be started on new medications (e.g. change in inhaled corticosteroids, antibiotics) during the study were discontinued from the study.

Adherence monitoring

The Akita2 monitors adherence by recording date, time and number of breaths for each nebulized treatment on the smartcard.

The number of breaths required to perform a complete nebulization of 1.25 mg dornase alfa is different for each patient, since this depends on the combination of inspiratory capacity of the patient and the nebulizer output. Therefore the mean number of breaths required to complete one nebulization was calculated for each patient individually. A successfully completed nebulization treatment was defined as one in which the patient performed at least 50% of his required mean number of breaths.

Patients were not informed of the adherence monitoring prior to the study, to ensure that this knowledge did not influence their compliance. At the last study visit, patients were asked for consent to use the data collected on the smart card. Smartcard data were handled anonymously, using only the smartcard number and randomization number as identifiers.

After completion of the study, adherence data were extracted from all smartcards and compliance data and randomization numbers were linked.

Results

Symptom scores

Mean symptom scores during the study did not significantly differ between treatment groups (see table E1).

Adherence

Adherence was high during the study (see figure E2). Since the Akita2 records exact date and time of each nebulization, detailed information on adherence could be gathered. Two indices of adherence were calculated: mean daily dose adherence indicates the percentage of days a patient used medication correctly as prescribed - in this study once daily nebulization of study drug. Total dose adherence indicates the percentage of the 21 doses the patient inhaled during the three weeks of treatment. For a few patients total dose adherence was substantially larger than daily
dose adherence. This is caused by unequal distribution of the doses during the study period. If a patient for example did not inhale any medication the first day and then inhaled twice daily on the next day, the daily dose adherence would be 0% and total dose adherence would be 100%.

<table>
<thead>
<tr>
<th>Week</th>
<th>Group</th>
<th>Mean symptom score</th>
<th>p</th>
<th>Mean % symptom free days</th>
<th>p</th>
<th>Mean rescue beta-2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Placebo</td>
<td>2.0 (0 – 12.9)</td>
<td>0.15</td>
<td>14 (0 – 100)</td>
<td>0.23</td>
<td>0 (0 – 6.4)</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>Dornase</td>
<td>0.4 (0 – 6.7)</td>
<td></td>
<td>57 (0 – 100)</td>
<td></td>
<td>0 (0 – 2.6)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Placebo</td>
<td>1.0 (0 – 4.9)</td>
<td>0.34</td>
<td>71 (0 – 100)</td>
<td>0.35</td>
<td>0 (0 – 3.3)</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Dornase</td>
<td>0.7 (0 – 12.6)</td>
<td></td>
<td>64 (0 – 100)</td>
<td></td>
<td>0 (0 – 3.0)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Placebo</td>
<td>0.4 (0 – 5.3)</td>
<td>0.54</td>
<td>71 (0 – 100)</td>
<td>0.87</td>
<td>0 (0 – 3.3)</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>Dornase</td>
<td>0.4 (0 – 11.0)</td>
<td></td>
<td>71 (0 – 100)</td>
<td></td>
<td>0 (0 – 4.7)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Placebo</td>
<td>0.3 (0 – 6.0)</td>
<td>0.91</td>
<td>77 (0 – 100)</td>
<td>0.88</td>
<td>0 (0 – 2.3)</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Dornase</td>
<td>0.1 (0 – 5.1)</td>
<td></td>
<td>86 (0 – 100)</td>
<td></td>
<td>0 (0 – 2.7)</td>
<td></td>
</tr>
</tbody>
</table>

**Table E1.** Mean daily symptom scores per week, mean percentage of symptom free days per week and mean use of rescue medication per week. Data are presented as median (range).
Figure E1. Correlation between FEF\(_{75}\) and LCI. Circles represent measurements performed at the start of the study, squares after 2 weeks of study treatment, triangles after 3 weeks of treatment and diamonds represent the follow-up visit 4 weeks after the treatment has been stopped.
Figure E2. Adherence during the 3-week treatment period. On the X-axis are individual patients, on the Y-axis adherence (%). Red bars indicate total dose adherence, blue bars indicate daily dose adherence.
Summary and general discussion

Summary

The branching anatomy of the bronchial tree creates a surface area that expands greatly from the trachea to the alveoli. When we breathe the epithelial surface of the lung is exposed to multiple potentially harmful particles such as bacteria and pollutants. Normally, these inhaled particles are efficiently removed from the lung by mucociliary clearance. If mucociliary clearance does not function properly, this can lead to mucus obstruction, infection and inflammation, which can cause chronic lung disease.

Monitoring of lung disease

Since lung disease often starts early in life, sensitive and accurate monitoring techniques to assess pulmonary status are needed to guide and optimize treatment. Spirometry is a monitoring technique widely used in children aged 6 years and older. It is relatively easy to perform, well validated and standardized, but has some limitations. Firstly, with improvements in care for children with chronic lung disease, Forced Expiratory Volume in one second (FEV\textsubscript{1}) now often is normal and therefore not very useful anymore to monitor lung disease. This thesis introduces Forced Expiratory Flow at 75% of Forced Vital Capacity (FEF\textsubscript{75}) as a more sensitive parameter to monitor early lung disease. Secondly, children below the age of 4-6 years are not yet able to perform spirometry. Consequently, there is great need for alternative non-invasive monitoring instruments for young children. This thesis reports a study that evaluated three alternative measurements to determine lung disease in young children with CF: multiple breath wash out (MBW), home nocturnal pulse oximetry and home nocturnal cough recording.

Treatment of lung disease

Cystic fibrosis (CF) is a life-shortening multi system disease in which chronic respiratory disease is the major cause of morbidity and mortality. Impaired mucociliary clearance is an important problem in most patients with CF and therefore enhancing mucus clearance is an important aim of CF therapy. Despite current therapy progressive small airways obstruction is present in most patients with CF. However, the importance of small airway disease is often underestimated. In addition, very few efforts have been focused on how to improve treatment of the small airways using currently available treatment options.

Clinical studies evaluating the efficacy of mucoysis like dornase alfa were conducted in the nineties. The conventional nebulizers used in these studies were very inefficient and deposited most of the inhaled drug in the large airways and thus only small amounts of dornase alfa reached the small airways. Hence, more efficient treatment of the small airways using modern nebulizers set to treat the small airways might increase the efficacy of inhaled medications and thus improve lung function in CF.

In this thesis we describe two studies that aimed to improve the treatment of the small airways in CF using dornase alfa. One study aimed to improve small airways treatment in clinically stable CF patients. The other aimed to improve treatment in CF patients who are admitted for a pulmonary exacerbation.

Next to CF, asthma is another important lung disease since approximately 10% of children in the Western world have asthma. In asthma, airway inflammation is treated by inhaled corticosteroids (ICS). The efficacy of ICS in asthma has been well proven and therefore ICS are advised in all medical guidelines. However, despite this treatment 30 â€“ 70% of asthma patients do not gain optimal asthma control and one third of patients continue to have abnormal lung function as measured by spirometry. Mucus retention is thought to contribute to airway obstruction in severe asthma, especially in the small airways. It is not known whether more efficient treatment of mucus obstruction in the small airways in clinically stable patients with asthma and persistent small airways obstruction might further improve lung function. This thesis reports a randomized placebo controlled trial on the efficacy of inhaled dornase alfa in children with clinically stable asthma and persistent small airways obstruction.

Chapter 1 is a general introduction to this thesis and provides the aims of the studies.

Chapter 2 provides a more extensive background on the lung diseases CF and asthma, on monitoring of lung disease and aerosol therapy.

Chapter 3 reports the results of a retrospective study that evaluated longitudinal differences in spirometry parameters between CF patients and healthy controls. In this study, 7367 spirometry measurements from 179 CF patients and 8338
measurements from 1371 healthy children were analysed using quantile regression analysis. We evaluated three spirometry parameters: FVC, FEV₁, and FEF₇₅.

For FVC it is clear that values for CF patients were lower than for healthy children, but a substantial portion of CF patients had FVC values within the normal range. At the age of 8 years approximately 30% of CF patients had FVC values below the P₁₀ of healthy controls. For FEV₁, the difference between CF and healthy children is larger than for FVC. At the age of 8 years almost 50% of CF patients had FEV₁ values below the P₁₀ for healthy controls. For FEF₇₅, the difference between CF and healthy subjects was even larger at all ages than for FVC or FEV₁. At 8 years, the P₁₀ for CF patients was close to the P₁₀ of healthy children. While FEV₁ values increased with age in healthy children, as would be expected with normal growth of the lung, there was almost no increase in FEF₇₅ from 6 to 18 years in CF patients, indicating significant small airways disease. Sample size estimates for a hypothetical study using these data showed that fewer patients are needed in a clinical trial when FEF₇₅ is used as primary endpoint than when FEV₁ or FVC would be used.

We conclude that FEF₇₅ values are substantially more reduced at an early stage of CF lung disease relative to FEV₁ or FVC throughout childhood. Based on these results FEF₇₅ could be a more sensitive marker to detect and monitor early CF lung disease than FEV₁ or FVC both for clinical management as for clinical studies.

In chapter 4 we present the results of a prospective cross-sectional study that aimed to evaluate the feasibility of measuring CF lung disease using multiple breath wash out (MBW), home nocturnal pulse oximetry and home nocturnal cough recording in young children with CF. The second aim of this study was to determine whether these tests could distinguish CF patients from healthy controls. From MBW the lung clearance index (LCI) can be calculated, which is a measure of inhomogeneity of ventilation.

Twenty CF patients and thirty healthy children aged 0-4 years participated in this study. Success rates for saturation and cough measurements were 90% and were similar for CF patients and healthy children. Success rate for MBW was 75% for CF patients and 50% for healthy children. There was a significant difference in mean LCI between healthy children and CF patients (LCI 7.1 vs. 9.3, p < 0.001). Mean nocturnal oxygen saturation was normal in both groups and did not significantly differ between the groups. Similarly, cough showed no differences between both groups.

We therefore conclude that measurements of MBW, nocturnal oxygen saturation and nocturnal cough were feasible in young children; however LCI was the only variable that showed a significant difference between children with CF and healthy children at this young age.

Chapter 5 presents an overview of the literature on dornase alfa for the treatment of CF lung disease. CF sputum shows high concentrations of free DNA released from disintegrating inflammatory cells. The free DNA contributes to the abnormally high viscosity of CF sputum and therefore forms an important target in the treatment of CF lung disease. Dornase alfa is a recombinant human enzyme that is synthesized in a Chinese Hamster Ovary (CHO) cell line. It is a mucolytic drug that enzymatically cleaves extracellular DNA into molecules of shorter length. In vitro studies showed that dornase alfa rapidly and significantly reduces the viscosity of purulent CF sputum. The liquefied sputum is less adhesive to the airway wall and it can be expectorated more easily. Clinical studies have shown that daily nebulization of dornase alfa significantly improves lung function and decreases the frequency of exacerbations in patients with CF.

Chapter 6 describes the results of a multicenter, double-blind, randomized controlled clinical trial on the efficacy of improved small airways deposition of dornase alfa in stable CF patients. All patients included in the study were on maintenance treatment with dornase alfa using a conventional nebulizer. After randomization they were switched to a new type of nebulizer that directed the flow and depth of each inhalation, coached the patient on correct inhalation technique and controlled the fraction of the inspiration time during which aerosol was generated. Patients were randomized to small airway deposition or large airway deposition of dornase alfa. For the large airways group the devices were set to simulate conventional nebulizers and deposit most of the drug in the large airways. For the small airways group the devices were set to deposit dornase alfa preferentially in the small airways. In order to obtain the two different lung deposition patterns we adjusted three characteristics of the nebulizer treatment: particle size, timing of aerosol bolus and breathing pattern. In this study, 24 patients were randomized to 4 weeks of small airways deposition of dornase alfa once daily and 25 patients were randomized to large airways deposition. Spirometry and LCI were measured at inclusion, after 2 and after 4 weeks of study treatment. Respiratory symptoms were scored daily by the patients in a diary. Primary endpoint was FEF₇₅, which is a spirometry parameter sensitive to changes in the small airways.

FEF₇₅ increased significantly by 0.7 SD (5.2% predicted) in the large airways group and 1.2 SD (8.8% predicted) in the small airways group. Intention to treat analysis did not show a significant difference in treatment effect between groups. Per protocol analysis excluding patients not completing the trial or with adherence <70% showed greater improvement in the small airways deposition group. Symptom scores did not show a significant difference between treatment groups. LCI was measured in a subgroup of 29 patients and also did not show a difference between groups.
In conclusion, this study shows that 4 weeks of dornase alfa nebulized using a state of the art nebulizer device resulted in a significant improvement in FEF\textsubscript{75} in children with stable CF lung disease who were on maintenance treatment with dornase alfa. Per protocol analysis demonstrated better treatment response in the small airways group. This study underlines that both adherence and inhalation competence are important determinants to optimize treatment effect of dornase alfa.

In chapter 7 we report the results of a multi-centre, double-blind, randomized controlled clinical trial investigating the efficacy of improved small airways deposition of dornase alfa in CF patients admitted to the hospital for a pulmonary exacerbation. After a run-in of 5 days, patients on dornase alfa maintenance treatment were switched to a smart nebulizer and randomized to small airways deposition (n = 19) or large airways deposition (n = 19) of dornase alfa for a minimum of 7 days. Spirometry and nocturnal oxygen saturation were measured at inclusion, at randomization and after 7 days of study treatment. Spirometry improved significantly in both groups during the study period. We did not observe differences in the primary endpoint FEF\textsubscript{75} and other outcome parameters between the two treatment groups.

We conclude that during a CF exacerbation improved small airways targeting does not result in improved FEF\textsubscript{75}. We speculate that it might be difficult to deposit sufficient drug into the small airways due to increased mucus production and increased airway obstruction in patients with a pulmonary exacerbation. In addition, other treatments such as intravenous antibiotics might be more important for the recovery of an exacerbation than the inhalation of dornase alfa.

Chapter 8 presents the results from a randomized placebo-controlled double blind clinical trial on the efficacy of inhaled dornase alfa in children with clinically stable asthma and persistent small airways obstruction. In this study 41 patients were randomized to dornase alfa and 23 patients to placebo. In both groups study drug was nebulized using a smart nebulizer programmed to optimally treat the small airways.

Spirometry, Lung Clearance Index (LCI) and fractional concentration of exhaled nitric oxide (FeNO) were measured at inclusion, after 2 and 3 weeks of treatment, and at a follow-up visit 4 weeks after study treatment was stopped. Primary endpoint was FEF\textsubscript{75}, since this is a parameter sensitive to changes in the small airways. Patients scored respiratory symptoms in a diary. Mean FEF\textsubscript{75}, FEV\textsubscript{1}, LCI, FeNO or symptom scores did not change significantly after 3 weeks of treatment in both groups.

In conclusion, three weeks of treatment with once daily dornase alfa targeted to the small airways did not reverse persistent small airways obstruction in children with stable asthma. This suggests that the persistent small airways obstruction is not caused by mucus in the small airways. Another explanation can be that the mucus in patients with asthma does not contain increased amounts of extracellular DNA.

General discussion

In this part we discuss the main findings of our studies in the context of current literature and discuss directions for future research.

Endpoints for monitoring and detection of early CF lung disease

The first important finding of our studies is that in children of 6 years and older FEF\textsubscript{75} is a sensitive outcome measure to detect early CF lung disease. End expiratory flow parameters such as the FEF\textsubscript{1} are generated at a low lung volume and are therefore thought to be more sensitive to morphological changes in the small airways and less determined by changes in the large airways. In textbooks it is often stated that FEF\textsubscript{1} is considered to be highly variable and therefore less useful. However, in the study described in chapter 3 we show that in children with CF FEF\textsubscript{1} is substantially more decreased relative to the reference population than FEV\textsubscript{1} or FVC and that abnormalities in FEF\textsubscript{1} occur at a younger age than those in FEV\textsubscript{1} and FVC. We conclude that even though FEF\textsubscript{1} is more variable than FEV\textsubscript{1}, this greater variability is offset by a substantially larger potential effect size on FEF\textsubscript{1}. Thus, there is substantial room for improvement in FEF\textsubscript{1}, making it a more sensitive outcome measure than FEV\textsubscript{1} for use in a clinical trial. Thanks to major improvements in CF care over the last decades, FEV\textsubscript{1} is normal in most schoolchildren and therefore has become relatively insensitive as a primary outcome measure in clinical studies. In contrast, even in most of today’s CF patients aged 6 years or older FEF\textsubscript{3} is decreased reflecting considerable small airways disease. The clinical relevance of FEF\textsubscript{3} as an outcome measure is supported by a number of recent studies. In one such study the sensitivity of FEF\textsubscript{3} to detect structural lung abnormalities on chest computed tomography (CT) scan was compared to that of FEV\textsubscript{1}. The sensitivity of FEV\textsubscript{1} to detect abnormal lung structure on CT as determined by elevated composite CT score, increased air trapping or presence of bronchiectasis, ranged from 19% to 26% while for FEF\textsubscript{3} this ranged from 62% to 75%. The beauty of FEF\textsubscript{3} is that it is generated using routine spirometry which is a well validated, well standardized and relatively cheap test that is widely distributed throughout the world. Based on our findings we strongly recommend including FEF\textsubscript{3} as an important outcome measure to monitor small airways involvement and therapy in CF lung disease.
Unfortunately spirometry is less feasible as a technique to detect CF lung disease in many preschool-aged children. Hence, alternative more feasible lung function tests are needed to detect small airways disease in this age group. Lately multiple breath wash out testing (MBW) has been rediscovered as a means to detect small airways disease. Lung clearance index (LCI) is a key outcome measure derived from the MBW test. An elevated LCI reflects inhomogeneity of ventilation caused by small airways disease. Theoretically, MBW can be performed in infants and preschool children since it requires only tidal breathing. However, the procedure lasts a few minutes and requires a good seal between facemask and face from start to finish. Studies on inhaled drug delivery showed that this tight seal can be a challenge in young children. 

In older children it was suggested that LCI is even more sensitive than FEF, in detecting structural lung abnormalities visible on CT scan: sensitivity for FEF, ranged from 62% to 75% and for LCI from 85 to 94%. Additionally, a prospective study in 57 children with CF aged six to ten years old showed that LCI was more sensitive than spirometry- or plethysmography-related outcome measures in detecting abnormalities visible on chest CT. Two recent intervention studies in CF patients with relatively mild lung disease (FEV, ≥80% predicted) suggested that LCI may be a suitable tool to assess early lung function improvements. The first study showed that four weeks of twice daily hypertonic saline inhalation improved LCI significantly more compared with isotonic saline, whereas spirometry-related outcome measures did not show a difference. The second study showed that LCI improved significantly after 4 weeks of dornase alfa compared with placebo, while FEV did not show an effect. Until now only few clinical intervention studies using LCI as outcome measurement have been done or are currently ongoing (http://www.controlled-trials.com, http://clinicaltrials.gov).

The second important finding of our studies is that the MBW technique is feasible in 75% of children with CF aged 0-4 years without sedation. In addition we showed that LCI was elevated in young CF patients compared with healthy controls and is able to differentiate CF patients from healthy children at an early age. Our findings are in accordance with a number of recent studies in young children. In a prospective study in children with CF below 3 years of age MBW testing and bronchoalveolar lavage (BAL) were done in sedated children within 72 hours. This study showed that at this young age LCI was elevated in 32% of children with CF. Additionally, in CF patients with a positive BAL culture for P. aeruginosa LCI was significantly higher than in patients without this pathogen. Furthermore, a recent prospective study in 48 children with CF demonstrated that LCI measured at preschool age predicts subsequent lung function in children with CF at school age.

Although our study further supports the role of LCI as an outcome measure for early lung disease, many validation steps are still needed before LCI can be considered a valid outcome measure. It is unknown how LCI relates to clinical outcome measures such as frequency of respiratory tract exacerbations (RTEs) and mortality. Furthermore, most LCI studies have been cross-sectional and there are only sparse longitudinal data available to assess the ability of LCI to track disease. Therefore, further validation studies and longer-term follow up are needed.

In summary, FEF, and LCI are promising outcome measures for the detection and monitoring of early CF lung disease. Standardization of MBW testing and further validation of LCI and FEF, against long-term outcomes such as RTE frequency and mortality are still needed. Our study evaluating differential spirometry parameters in children of 6 years and older showed that FEF, is a sensitive outcome measure to detect early CF lung disease. For younger children unable to perform spirometry we showed in a cross-sectional study that it is feasible to perform MBW in unsedated children aged 0 - 4 years and that LCI can detect early CF lung disease at this young age.

The importance of competence in inhalation therapy

The third important new finding of our studies is that by improving inhalation competence the efficacy of dornase alfa in CF increased substantially. Treatment competence is of key importance in nebulizer therapy in addition to the well established importance of treatment adherence. The importance of competence in inhaling drugs has been relatively well studied for anti-asthma drugs delivered by dry powder inhalers (DPIs) and pressurized metered dose inhalers (pMDIs). A less than optimal technique can result in decreased drug delivery and potentially reduced efficacy. Unfortunately, incorrect inhalation technique is common among patients with chronic respiratory disease.

Registration studies for dornase alfa were performed using first generation conventional jet nebulizers. These jet nebulizers are very inefficient for the following two reasons:

Firstly, they do not control the breathing pattern of the patient during inhalation. In general the drug is inhaled while the patient is breathing normally. This breathing pattern results in a centralized deposition pattern of the inhaled aerosol while most of the pathology in CF is localized in the small airways. Secondly, the deposition efficiency of these nebulizers is low and ranges from 10 to 20%. Most of the drug is retained within the nebulizer chamber and a large fraction of the generated aerosol is lost into the environment.

In the study presented in chapter 6, the importance of nebulizer competence was clearly demonstrated. This
randomized controlled clinical trial compared small airways deposition of dornase alfa to large airways deposition in stable CF patients. Both treatment groups showed a considerable improvement in FEF25, four weeks after switching from a conventional nebulizer to a smart nebulizer. The smart nebulizer used in the study directed the flow and depth of each inhalation, coached the patient on correct inhalation technique and controlled the fraction of the inspiration time during which aerosol was generated. This way, the likelihood of making mistakes during nebulization was reduced. Additionally, the device recorded date, time and number of breaths performed for each nebulization. From these data, adherence could be assessed. Adherence during our study was 85%, which is high for an aerosol treatment. FEF25 and FEF75 improved significantly after 4 weeks of study treatment, indicating improved patency of small airways. Importantly, patients in this study were already on dornase alfa maintenance treatment. Hence, the treatment effect was not the result of the introduction of a new drug, but of optimizing inhalation technique and delivery efficiency as well as adherence. Hence, our study suggests that the efficacy of inhaled medication can be improved substantially by optimizing adherence and inhalation technique.

In conclusion, this study underlined the importance of inhaler competence and adherence. We showed that small airway patency of stable CF patients can be improved by switching maintenance dornase alfa treatment from a conventional nebulizer to a smart nebulizer that optimizes breathing pattern and lung deposition.

**Treatment of CF respiratory tract exacerbations**

The fourth important issue that was highlighted by our study in chapter 7 is that optimizing small airway treatment during a respiratory tract exacerbation is difficult. Interestingly, treatment of CF respiratory tract exacerbations (RTEs) is largely not evidence-based and only few randomized clinical trials have been performed on this important aspect of CF care. As 25% of CF patients experiencing a RTE do not regain pre-RTE spirometry values at the end of the RTE treatment period, it is clear that RTE treatment needs to be improved. Effective sputum mobilization is an important component of the treatment of RTEs. We therefore investigated whether efficient small versus large airway treatment with dornase alfa improves small airway obstruction in CF patients during RTEs. In this randomized controlled study we did not observe a difference in treatment effect between small airway and large airway deposition of inhaled dornase alfa during a RTE. There are many reasons that could explain this result. Most importantly it could be that a higher dose of dornase alfa is needed to treat the increased amounts of mucus during a RTE. Additionally, deposition might have been primarily in central airways due to secretions in these airways during an exacerbation. Finally, the treatment period might have been too short. Clearly, alternative personalized strategies are needed to improve current treatment efficacy of RTEs.

**Dornase alfa in asthma**

The fifth important finding of our studies is that maintenance treatment with dornase alfa delivered by a smart nebulizer did not improve small airways patency in children with asthma and persistent small airways obstruction.

Case reports suggest that dornase alfa treatment is effective in acute asthma. However, the only previously reported randomized clinical trial (RCT) on dornase alfa use in children with acute asthma did not show a beneficial effect of adding a single dose of 5 mg dornase alfa to standard treatment in the emergency room. Whether dornase alfa could play a role in maintenance treatment of stable asthma with persistent lung function abnormalities, was never investigated. Therefore we conducted a randomized placebo-controlled double blind clinical trial on the efficacy of a three-week treatment with dornase alfa once daily in children with clinically stable asthma and persistent small airways obstruction. Similar to the study in acute asthma, we did not observe a significant treatment benefit of dornase alfa on small airways obstruction in stable asthmatic patients.

The most likely explanation for this finding is that free extracellular DNA does not contribute to the small airways obstruction in these patients. Since we used a highly efficient smart nebulizer optimizing deposition and inhalation competence we feel that it is unlikely that the dose of dornase alfa delivered to the small airways was too low in our study. Most likely the persistent airway obstruction is caused by mechanisms other than mucus obstruction (e.g. oedema, inflammation, airway wall remodelling, or smooth muscle contraction). Another reason for the contrasting results between the RCTs and the case reports is that the pathophysiology of airway obstruction during severe acute asthma exacerbations is different from that of persistent small airway obstruction in stable patients. The case reports mostly included children with severe airway obstruction who failed to respond to routine therapy.

In conclusion, our study did not show a beneficial effect of dornase alfa in stable asthmatic children who had persistent small airway obstruction.
Directions for future research

Endpoints in paediatric lung disease

In this thesis, two lung function outcome measures have been studied which can be used in the detection and monitoring of CF lung disease: FEF$_{25}$ and LCI.

We showed that FEF$_{25}$ is more sensitive than FEV$_{1}$ to detect small airways disease in CF. However, FEV$_{1}$ is well validated and has been shown to correlate with survival and exacerbation rate, while these data are lacking for FEF$_{25}$. Future studies should therefore investigate whether FEF$_{25}$ is related to true clinical efficacy measures such as exacerbations, hospitalizations or survival. Such studies will be relatively easy to execute since spirometry is widely used and spirometry parameters are available in many datasets.

Validation studies are also needed for LCI. Most interesting will be longitudinal comparisons between changes in FEF$_{25}$ and changes in LCI to see to what extent these two parameters yield identical or complementary information. While spirometry is well standardized, further standardization of MBW measurement techniques is needed. To date, there is no international consensus on equipment or procedures for MBW. For this reason it was suggested that each centre should produce its own reference values. Recent ATS/ERS guidelines on pulmonary function testing in preschool children include a section on LCI. This document only gives general recommendations on how to perform MBW. It is stressed that, because only a few centres have experience with MBW, proposals regarding equipment, procedures, analysis, and interpretation must be considered tentative.

Most MBW studies published over the past decade were performed using mass spectrometry in infants and children. However, mass spectrometry equipment is expensive and custom-built and its use is therefore restricted to few specialised research centres. As an alternative, ultrasonic technology was introduced and a commercial device (Exhalyzer D, Eco Medics AG, Switzerland) with a mainstream ultrasonic transducer was marketed a few years ago. This system was improved (ndd medical technologies, Switzerland) using a sidestream ultrasonic transducer where the measurement of molar mass is not influenced by temperature and humidity; only flow is sampled from a mainstream ultrasonic transducer. Since it is unlikely that centers will use identical equipment, guidelines for both mass spectrometry and ultrasonic equipment should be developed. Furthermore, studies comparing different types of equipment are needed to facilitate multi-center studies. In addition there is a need for standardization of the MBW procedure. Recent guidelines advise that measurements should be done with the child seated in an upright position, while most of the studies to date in infants and young children have been performed in sedated and supine children.

Our study showed that the MBW test is feasible in 50 to 75% of very young children. The most important challenge of the test remains the time during which a perfect seal between face and mask can be maintained. Recent studies suggest that the nitrogen washout test could replace the currently used wash-in and washout MBW procedure using inert gasses. Since no wash-in phase is needed, this will reduce the time needed to perform a MBW test. It is likely that this reduction in time will further improve the acceptability of the MBW procedure.

Future studies on inhaler competence and small airway treatment in CF

Our RCT that compared small airways deposition of dornase alfa to large airways deposition in stable CF patients clearly showed the importance of optimizing inhalation competence and adherence.

Future studies on inhaler competence could focus on optimizing CF treatment with drugs other than dornase alfa. For example, it is very likely that patients may benefit from improved administration of inhaled antibiotics that are used to treat bacterial infections in CF. The total surface area of small airways is large. We speculate that the delivered concentration of antibiotics is insufficient with currently used nebulizers, resulting in a suboptimal treatment effect. The use of smart nebulizers to deposit more of the antibiotic in the small airways may result in more effective eradication and suppression of infectious organisms such as P. Aeruginosa. This could theoretically improve the treatment of CF patients chronically infected with P. Aeruginosa and other microorganisms.

Furthermore, insights on inhaler competence and aerosol deposition gained in our studies are relevant for other paediatric lung diseases such as asthma. It would for example be worthwhile to investigate whether more efficient deposition of inhaled corticosteroids into the small airways can improve lung function and symptoms in children with difficult-to-treat asthma. It is striking that 30 to 70% of asthma patients currently do not gain optimal asthma control despite maintenance treatment.

Future studies on the treatment of RTEs in CF

When reading current literature and guidelines on treatment of CF lung disease, we were surprised to find that...
treatment for RTEs in CF largely is not evidence-based. Very few studies have been performed on this important component of CF care. A recent guideline on the treatment of RTEs in CF underlined this lack of evidence, as for many topics it could not give an evidence-based recommendation. Hence, this offers a great opportunity for further improvement of the therapy of CF lung disease.

Generally, treatment of a RTE consists of intravenous (IV) antibiotics, intensified airway clearance therapies and continuation or intensification of maintenance therapies.

Regarding treatment with antibiotics, studies should investigate the optimal duration of IV antibiotic treatment, the type and number of antibiotics to be used and whether simultaneous use of inhaled and IV antibiotics is safe and efficacious.

Regarding mucus clearance during exacerbations we feel it is important to further investigate treatment with a higher dose of dornase alfa delivered by smart nebulizer in comparison to treatment with a conventional nebulizer, or dornase alfa in combination with other mucolytics such as hypertonic saline that work through a different mode of action. Additionally, the optimal combination of mucolytic drugs and timing of airway clearance therapy could be investigated during exacerbations.

Finally, it is current practice to continue most chronic medications during a RTE. In the recent guideline it is stated that the authors ‘found no compelling reason why any recommended chronic therapy should be discontinued during treatment of a pulmonary exacerbation.’ From the perspective of our studies it would be interesting to study the effect of improved small airway deposition of inhaled antibiotics in addition to i.v. antibiotics, to optimize antibiotic penetration into the lungs.

**Future studies on the use of off-label dornase alfa**

We studied off-label use of dornase alfa in children with asthma and persistent airway obstruction. Case reports suggest that dornase alfa treatment is effective in RSV bronchiolitis, acute asthma with atelectasis, primary ciliary dyskinesia, pertussis, atelectasis and mucus retention in premature neonates, and plastic bronchitis in acute chest syndrome of sickle cell disease.

In contrast to these case reports, the few RCTs on dornase alfa use in non-CF pediatric lung diseases that have been performed until now, mostly have not shown a beneficial effect. Infants hospitalized with RSV bronchiolitis, children with airway malacia and lower respiratory tract infection, and children with a moderate to severe acute asthma exacerbation did not benefit from treatment with dornase alfa. Similar to these studies, our study described in chapter 8 did not show a beneficial effect of dornase alfa on small airway obstruction in children with stable asthma. The only pediatric study on off-label use of dornase alfa that did show a positive effect investigated whether it could prevent atelectasis in children who were on a ventilator post-operatively. Dornase alfa reduced ventilation time, incidence of atelectasis, length of stay on the pediatric intensive care unit, and mean costs.

Other patients who need artificial ventilation may also benefit from dornase alfa treatment. For example in prematurely born neonates, each additional day spent on the ventilator increases the risk of bronchial pulmonary dysplasia (BPD). Shorter ventilation time might therefore lead to relevant reduction in morbidity in this vulnerable group of patients.

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Nederlandse samenvatting

Samenvatting

De belangrijkste functie van de long is het opnemen van zuurstof en het afvoeren van koolstofdioxide. Via de brochiaalboom wordt daarom continu lucht van en naar de longblaasjes vervoerd. De brochiaalboom is een steeds weer vertakkend systeem van luchtwegen. Door de vele vertakkingen neemt de totale oppervlakte van de luchtwegen sterk toe vanaf de luchtpijp naar de longblaasjes. Als we ademen wordt het luchtwegoppervlak blootgesteld aan potentieel gevaarlijke deeltjes in de lucht, zoals stof, luchtverontreiniging of bacteriën. Normaalgesproken worden deze deeltjes uit de luchtwegen verwijderd door een complex proces genaamd mucociliaire klaring. Dit proces verloopt via de volgende stappen:

- Op het oppervlak van de luchtwegen ligt een dun laagje slijm (mucus).
- De ingeademde deeltjes blijven plakken in de mucuslaag.
- De deeltjes worden vervolgens door de trilhaartjes op het oppervlak van de luchtwegen getransporteerd naar de keel of mondholte, en worden hierna doorgeslikt of samen met het slijm uitgehoest.

Als de mucociliaire klaring niet goed werkt, hoopt de mucus zich op en worden ingeademde deeltjes niet goed uit de luchtwegen verwijderd. Dit kan leiden tot luchtwegobstructie, -infectie en -ontsteking, met chronische longziekte tot gevolg.

Het meten van longziekte

Omdat longziekte soms al op jonge leeftijd begint en in de loop van de tijd kan verergeren, is het belangrijk om de ernst van ziekte goed te kunnen meten en te kunnen monitoren. Hiervoor zijn verschillende longfunctietesten beschikbaar. Een longfunctietest die veel gebruikt wordt bij kinderen van 6 jaar en ouder is spirometrie. Bij deze test wordt de patiënt gevraagd om eerst zo diep mogelijk in te ademen, en daarna zo hard en zo snel mogelijk uit te blazen in de spirometer. Uit de spirometrie kunnen een aantal belangrijke parameters worden afgeleid:

- Forced vital capacity (FVC) is het maximale volume aan lucht (in liters) dat uitgeademd kan worden na een maximale inademing. Een te lage FVC kan wijzen op verlies van longvolume.
- Forced expiratory volume in 1 second (FEV₁) is het maximale volume aan lucht (in liters) dat uitgeademd kan worden in de eerste seconde van de geforceerde uitademing na een maximale inademing. De FEV₁ is een parameter die vooral gevoelig is voor obstructie in de grote luchtwegen.
- Forced Expiratory Flows at 25, 50 and 75% of FVC (respectievelijk FEF₂₅, FEF₅₀ en FEF₇₅) zijn stroomsnelheden van de uitgeademde lucht gemeten op verschillende afkappunten van de geforceerde uitademing (in liters per seconde). Deze parameters zijn gevoelig voor obstructie in de kleine luchtwegen.

Spirometrie is relatief makkelijk te verrichten, is goed gevalideerd en gestandaardiseerd, maar heeft ook een aantal beperkingen.

Ten eerste is door de vele verbeteringen in de zorg voor kinderen met chronische longziekten de FEV₁ nu vaak normaal op de kinderleefleeftijd en daardoor niet meer nuttig in de monitoring van de longziekte. In dit proefschrift introduceren we daarom FEF₇₅ als een gevoeliger parameter om beginnende longziekte te detecteren. Ten tweede zijn kinderen jonger dan 4-6 jaar niet in staat om spirometrie uit te voeren. Het is dus noodzakelijk om te zoeken naar meetmethoden die wel bij jonge kinderen verricht kunnen worden. In dit proefschrift evalueren wij drie alternatieve metingen van longziekte bij jonge kinderen: de multiple breath wash out (MBW) techniek, meting van de zuurstofsaturatie van het bloed gedurende de slaap en meting van nachtelijk hoesten.

Behandeling van longziekten

In dit proefschrift beschrijven we studies bij twee verschillende longziekten: taaislijmziekte (cystic fibrosis; CF) en astma. Bij beide ziekten worden medicijnen bij voorkeur toegediend als inhalatiemedicatie. Deze toediening heeft namelijk het voordeel dat het medicijn direct in de longen terecht komt. Hierdoor heeft geïnhaleerde medicatie snel
effect en is meestal een lagere dosis nodig dan bijvoorbeeld bij het slikken van een tablet om toch hetzelfde effect te bereiken. De nevel van medicijn die een patiënt inhaleert, wordt ook wel aerosol genoemd.

**Cystic Fibrosis**

CF is een erfelijke ziekte waarbij chronische longziekte de meeste klachten veroorzaakt en uiteindelijk leidt tot vroegtijdig overlijden. Zoals de Nederlandse benaming aëtauilzijeïnkteïn™ al zegt, is het slippert dat de luchtwegen bedekt bij CF te visueel ofwel taa. Verstoorde mucociliaire klaring is een belangrijk probleem bij de meeste patiënten met CF en daarom is therapie gericht op verbeteren van mucus klaring een belangrijk onderdeel van de behandeling. Patiënten gebruiken hiervoor medicijnen die het taa slippert dunner maken, waardoor het makkelijker uit de luchtwegen opgeruimd kan worden. Daarnaast doen de patiënten luchtwegfysiotherapie om het ophoesten van slijm te bevorderen. Ondanks intensieve therapie komt schade aan de kleine luchtwegen bij veel patiënten met CF voor. Het belang van deze schade aan de kleine luchtwegen wordt soms onderschat. Ook is nog weinig onderzoek verricht naar de verbetering van behandeling van de kleine luchtwegen.

Het meest gebruikte slijnwenduunnde medicijn bij CF patiënten is DNase (ook wel bekend als dornase alfa, merknaam Pulmozyme®). DNase wordt toegediend met een vernevelaar. Dit is een apparaat dat een vloeibaar medicijn onzett in een nevel, die vervolgens kan worden gemaakt inhaleerd. De klinische studies naar de werkzaamheid van DNase werden verricht in de jaren â€™90. De vernevelaars die in deze studies gebruikt werden, waren relatief inefficiënt. Daarnaast kwam een groot deel van de geâ€“ nhaleerde medicatie in de grote luchtwegen terecht en maar weinig medicatie in de kleine luchtwegen. Daarom verwachten wij dat verbeterde behandeling van de kleine luchtwegen met modernere vernevelaars de effectiviteit van geâ€“ nhaleerde medicatie verhogen en daarmee de longfunctie van patiënten met CF kan verbeteren.

In dit proefschrift beschrijven we twee studies die tot doel hadden de behandeling van de kleine luchtwegen met DNase bij patiënten met CF te verbeteren. Eén studie onderzocht dit bij stabiele CF patiënten en de andere studie bij CF patiënten die waren opgenomen in het ziekenhuis voor CF patiënten met CF te vernevelen. Eén studie onderzocht dit bij stabiele CF patiënten en de andere studie bij CF patiënten die waren opgenomen in het ziekenhuis vanwege een toename van de luchtwegklachten oftewel exacerbatie.

**Asthma**

Een andere belangrijke longziekte is astma, omdat ongeveer 10% van de kinderen in de Westerse wereld astma heeft. Astma is een complex multifactoriële ziekte waarbij obstuctie en zwelling van de luchtwegwand, samenbreken van de spiertjes rondom de luchtweg en verminderde mucociliaire klaring een rol spelen. Astma wordt gekarakteriseerd door een wisselende luchtwegobstructie. Klachten die kunnen optreden bij patiënten zijn kortademigheid, een snelle ademhaling, hoest, verminderde mogelijkheid tot het verrichten van inspanning en een verstoorde slaap.

De luchtwegobstructie bij astma wordt behandeld met geâ€“ nhaleerde corticosteroiden. De werkzaamheid van inhalatiecorticosteroiden is duidelijk vastgesteld in klinische studies. Toch bereikt ondanks behandeling met inhalatiecorticosteroiden 30 tot 70% van alle patiënten geen optimale controle van hun astma en houdt een derde van alle patiënten een afwijkende longfunctie. Mucus retentie draagt bij aan de luchtwegobstructie bij astma, met name in de kleine luchtwegen. Het is nog niet bekend of betere behandeling van mucus obstructie in de kleine luchtwegen de longfunctie van kinderen met astma kan verbeteren. Daarom onderzochten wij of inhalatie van DNase leidt tot verbetering van de longfunctie en van de klachten bij kinderen met astma.

**Hoofdstuk 1** is een algemene introductie van dit proefschrift waarin ook de doelen van de studies worden beschreven.

**Hoofdstuk 2** geeft meer achtergrondinformatie over de longziekten CF en astma, over het meten van de ernst van longziekte en over inhalatiemedicatie.

**Hoofdstuk 3** beschrijft de resultaten van een retrospectieve studie naar longitudinale verschillen in de spirometrie parameters FVC, FEV, en FEF, tussen CF patiënten en gezonde kinderen. We wilde deze drie parameters vergelijken om te zien welke parameter het gevoeligst is om beginnende longziekte op te sporen. In deze studie werden 7367 spirometrie metingen van 179 CF patiënten en 8338 metingen van 1371 gezonde kinderen geanalyseerd. Hieruit bleek dat de FVC van CF patiënten gemiddeld lager is dan die van gezonde kinderen, maar een relatief groot deel van de CF patiënten FVC waarden heeft die nog binnen de normale range vallen. Voor FEV, is het verschil tussen CF patiënten en gezonde kinderen groter en zijn er meer kinderen die niet meer binnen de normale range vallen met hun FEV. Voor FEF, is het verschil tussen CF patiënten en gezonde kinderen nog groter dan voor FVC of FEV.

Bijvoorbeeld van de leeftijd van 8 jaar heeft bijna 90% van de CF patiënten een afwijkende FEF,.. En terwijl de FEF, waarden bij de gezonde kinderen toenemen met de leeftijd, zoals verwacht mag worden met normale groei van de long, neemt de FEF, bij de CF patiënten bijna niet toe. Dit wijst op de aanwezigheid van schade aan de kleine luchtwegen.

We concluderen dat op relatief jonge leeftijd FEF, aanzienlijk meer verlaagd is dan FEV, of FVC bij kinderen met CF.
Hoofdstuk 4 presenteren we de resultaten van een prospectieve cross-sectionele studie die evalueerde of breath wash out (MBW), meting van de zuurstofsaturatie van het bloed gedurende de nacht en meting van nachtelijk hoesten uitvoerbaar zijn bij jonge kinderen van nul tot en met 4 jaar. Daarnaast onderzochten we of deze testen onderscheid konden maken tussen CF patiënten en gezonde kinderen. Uit de MBW test kan de lung clearance index (LCI) worden berekend, wat een maat is voor inhomogeniteit van ventilatie.

Twintig kinderen met CF en dertig gezonde kinderen van 0 tot en met 4 jaar oud namen deel aan deze studie. Het succespercentage voor de metingen van saturatie en hoest was 90% voor beide groepen kinderen. Het succespercentage voor MBW was 75% voor CF patiënten en 50% voor gezonde kinderen. Er was een significant verschil in gemiddelde LCI tussen gezonde kinderen en CF patiënten (LCI respectievelijk 7.1 en 9.3, p < 0.001). De gemiddelde nachtelijke zuurstofsaturatie was normaal in beide groepen en toonde geen verschil aan tussen CF patiënten en gezonde kinderen. Ook de meting van het nachtelijk hoesten toonde geen verschil tussen de groepen.

Wij concluderen daarom dat metingen van MBW, van de nachtelijke zuurstofsaturatie en van nachtelijk hoesten uitvoerbaar zijn bij jonge kinderen. LCI was de enige parameter die een duidelijk verschil liet zien tussen kinderen met CF en gezonde kinderen. Daarom kan LCI een goede parameter zijn om in een vroeg stadium CF longziekte op te sporen.

Hoofdstuk 5 geeft een overzicht van de literatuur over het medicijn DNase bij de behandeling van CF longziekte. Het sputum van CF patiënten bevat hoge concentraties vrij DNA, dat afkomstig is van afweercellen die kapot zijn gegaan. Deze lange sliben DNA-eisen dat aan de abnormale taaiheid van het sputum en daarom is dit vrije DNA een belangrijk aangrijpingspunt voor de behandeling. Recombinant humaan DNase (rhDNase; Pulmozyme®) is een recombinaat menselijk enzym dat het vrije DNA opknipt in moleculen van kortere lengte. Het is een slijmverdunnend middel dat ontwikkeld is voor de behandeling van longziekte bij CF. DNase vermindert de viscositeit van het CF sputum, waardoor het minder blijft plakken aan de luchtwegwand en makkelijker geklaard kan worden uit de luchtwegen. Klinische studies hebben aangetoond dat dagelijkse verneveling met DNase de longfunctie verbetert en het aantal exacerbaties verminderd bij patiënten met CF.

Hoofdstuk 6 beschrijft de resultaten van een multicenter, gerandomiseerde, dubbelblinde studie naar de effectiviteit van verbeterde behandeling van de kleine luchtwegen met DNase bij stabiele kinderen met CF. Alle CF patiënten die meededen aan de studie, gebruikten vÅ‘A’r meting van de lung clearance index (LCI) in de studie al DNase als onderhoudsmedicatie met een gewone vernevelaar. De patiënten werden in de studie gerandomiseerd naar toediening van DNase voornamelijk gericht op de kleine luchtwegen of op de grote luchtwegen. Beide groepen gebruikten een nieuw soort vernevelaar, die de snelheid en de diepte van de inademing stuurde, de patiënt coachte om op de juiste manier in te ademen en bepaalde de inhoud van de inademingstijd die aeroloser werd geproduceerd. De behandeling gericht op de grotere luchtwegen lijkt op de huidige behandeling met een standaard vernevelaar. De behandeling gericht op de kleine luchtwegen is de nieuwe behandeling. Om de twee verschillende behandelingen te genereren, pasten we drie eigenschappen van de vernevelaar aan: de deeltjesgrootte van de gAz inhaleerde medicatie, de timing van de aerosol bolus en de ademhalingspatroon. In deze studie werden 24 patiënten gerandomiseerd naar 4 weken behandeling met DNase gericht op de kleine luchtwegen en 25 patiënten naar 4 weken behandeling met DNase gericht op de grotere luchtwegen. Er werd longfunctie gemeten met behulp van spirometrie aan het begin van de studie, na 2 weken en na 4 weken behandeling.

FEF75, verbeterde significant in beide groepen: met 0.7 standaard deviatie (SD) in de groep die de grotere luchtweg behandeling kreeg en met 1.2 SD in de groep die de behandeling gericht op de kleine luchtwegen kreeg. Als we voor de twee behandelgroepen alle kinderen die met de onderzoeksbehandeling gestart waren vergeleken, vonden we geen verschil tussen de twee groepen. Maar weken we alleen naar kinderen die de 4 weken behandeling hadden afgemaakt en minstens 70% van de medicatie daadwerkelijk ingenomen hadden, dan zagen we dat de behandeling gericht op de kleine luchtwegen een significant grotere verbetering van de FEF75 gaf. Klachtenscores die de kinderen bijhielden in een dagboekje, lieten geen verschil zien tussen de twee behandelgroepen.

Wij concluderen dat 4 weken behandeling met DNase verneveld met een nieuw soort vernevelaar leidt tot een significante verbetering van de FEF75 bij kinderen met CF. Een vergelijking van de twee behandelingen (DNase gericht op de kleine luchtwegen of gericht op de grote luchtwegen) in de groep kinderen die minstens 70% van de medicatie hadden ingenomen, liet zien dat de kleine luchtweg behandeling significant beter was. Dit onderzoek benadrukt dat het op de juiste manier inhaleren van medicatie van belang is om een optimaal effect van de behandeling met DNase te verkrijgen.

In hoofdstuk 7 vermelden we de resultaten van een multicenter, gerandomiseerde, dubbelblinde studie naar de effectiviteit van verbeterde behandeling van de kleine luchtwegen met DNase bij kinderen met CF die waren opgenomen in het ziekenhuis vanwege een toename van de luchtwegklachten. Zoâ€™t in toename van klachten wordt...
een exacerbatie genoemd. Na een run-in periode van 5 dagen waarin alle patiënten de gebruikelijke behandeling voor een exacerbatie kregen, werden de patiënten gerandomiseerd naar inhalatie van DNase gericht op de kleine luchtwegen (n = 19) of inhalatie van DNase gericht op de grotere luchtwegen (n = 19) gedurende minimaal 7 dagen. Spirometrie en zuurstofsaturatie van het bloed tijdens de slaap werden gemeten bij de start van het onderzoek, op de dag van randomisatie en na 7 dagen behandeling. De longfunctie, gemeten met behulp van spirometrie, verbeterde significant in beide behandelgroepen. We konden geen verschil aantonen in FEF75, of andere uitkomsten tussen de twee groepen. We concluderen daarom dat gedurende een CF exacerbatie de behandeling met DNase gericht op de kleine luchtwegen niet beter werkt dan de standaard behandeling. Misschien komt dit doordat er tijdens een exacerbatie meer slijm in de luchtwegen aanwezig is, waardoor het niet goed lukt om voldoende DNase in de kleine luchtwegen terecht te laten komen. Daarnaast zou het zo kunnen zijn dat tijdens een exacerbatie de andere behandelingen, zoals antibiotica per infuus, belangrijker zijn voor het herstel dan de behandeling met DNase.

Hoofdstuk 8 beschrijft de resultaten van een gerandomiseerde, placebo-gecontroleerde, dubbelblinde studie naar de effectiviteit van DNase bij kinderen met stabiel astma en blijvende obstructie van de kleine luchtwegen. In deze studie werden 41 kinderen gerandomiseerd naar DNase en 23 kinderen naar placebo. In allebei de groepen gebruikten de kinderen een vernevelaar die was ingesteld om de kleine luchtwegen optimaal te behandelen.

Spirometrie, lung clearance index (LCI) en de concentratie van uitgeademd stikstofoxide (fractional concentration of exhaled nitric oxide; FeNO) werden gemeten bij de start van de studie, na 2 en na 3 weken behandeling. Daarnaast hielden de kinderen dagelijks een dagboekje bij over hun luchtwegklachten. FEF75, FEV1, LCI, FeNO en klachtenscores lieten geen verschil zien tussen de twee behandelgroepen na drie weken behandeling.

Concluderend kunnen we zeggen dat drie weken behandeling met DNase gericht op de kleine luchtwegen niet leidt tot verbetering van de luchtwegobstructie bij kinderen met astma. Een verklaring hiervoor kan zijn dat de obstructie niet veroorzaakt wordt door slijm in de luchtwegen, of dat DNase minder goed werkt op het slijm van kinderen met astma dan op het slijm van kinderen met CF.
Dankwoord

Het is zover: mijn proefschrift is af! Dit proefschrift en de hierin beschreven studies zijn tot stand gekomen met de hulp van een heleboel mensen. Een mooi moment om hen allemaal te bedanken.

Allereerst de kinderen en ouders die hebben meegedaan aan de onderzoeken. Ik vond het erg leuk om jullie te leren kennen. We hopen dat we door het doen van wetenschappelijk onderzoek de behandeling van kinderen met een longziekte verder kunnen verbeteren. Jullie hulp is daarbij onmisbaar!

Prof.dr. H.A.W.M. Tiddens, beste Harm. Bedankt voor je enthousiaste begeleiding. Jouw energie en gedrevenheid lijken geen grenzen te kennen! Je gaf me de mogelijkheid om studies met veel vrijheid en zelfstandigheid op te zetten en uit te voeren, waardoor ik veel heb kunnen leren. En ondanks jouw ongelooflijk drukke agenda maakte je altijd tijd om te overleggen of papers te corrigeren. Ook de mogelijkheden die je me bood om mijn blik te verbreden heb ik enorm gewaardeerd, zoals het bezoeken van diverse congressen, deelnemen aan cursussen of workshops en natuurlijk de twee maanden stage bij het aerosol lab van de McMaster University, Hamilton, Canada.

Prof.dr. J.C. de Jongste, beste Johan. Bedankt voor de mogelijkheid om onderzoek te doen op jouw afdeling en voor je altijd constructieve commentaren bij bijvoorbeeld de research besprekingen op vrijdag.

Dr. M.W.H. Pijnenburg, beste Mariëlle. Mijn enthousiasme voor wetenschappelijk onderzoek begon bij jou. Als afstudeerstudent kwam ik onder jouw vleugels mijn scriptie schrijven. Dankzij jouw warme en enthousiaste begeleiding had ik een geweldige tijd op de afdeling kinderlongziekten. Je gaf me veel vertrouwen en zelfstandigheid, waardoor ik zelf heb kunnen ervaren hoe veelzijdig en leuk het doen van onderzoek kan zijn.

Prof.dr. C.K. van der Ent, prof.dr. A.J. van der Heijden en prof.dr. I.K.M. Reiss wil ik graag bedanken voor de bereidheid om plaats te nemen in de kleine commissie.


Dr.ir. W.C.J. Hop, beste Wim. Bedankt voor al je hulp bij de statistiek. Ondanks het feit dat er erg veel onderzoekers bij jou langskomen voor advies, wist je altijd precies wat ik aan het onderzoeken was. Bedankt ook voor je äº no nonsenseÂ™ kijk op de statistiek.

Prof. B.M. Assael, dear Bennie, thank you for the cooperation on the PIPES and DEPTH studies. And thank you for inviting me to the Centro Fibrosi Cistica di Verona, I really enjoyed your pasta at the Non Solo Pasta meeting!

Sonia Volpi, thank you for all your help in performing the studies. Thank you for your enthusiastic company on conferences and for your great hospitality the times I visited Verona. I will never forget your Melanzane!

Dear Elena, Ilaria and Marianna at the Centro Fibrosi Cistica di Verona, thank you for all your help performing the studies. I enjoyed meeting you and working together.

Dr. P.J.F.M. Merkus, beste Peter, bedankt voor je altijd positieve en enthousiaste samenwerking. Eerst in Rotterdam, maar vooral ook in Nijmegen, waar we samen de PIPES studie hebben gedaan.

Coosje Sintnicolaas, dank voor je bijdrage aan de PIPES studie in Nijmegen, je enthousiasme en de altijd prettige samenwerking.

S.G. McKenzie, dear Sheila. Thank you for the critical reading of my manuscripts. Your comments were always friendly and helpful. I enjoyed meeting you on the ECFS conference!

Het onderzoek was niet mogelijk geweest zonder de financiële steun van Roche B.V., Stichting AstmaBestrijding en
de Stichting Vrienden van het Sophia. Ik wil hen graag bedanken voor de mogelijkheid om onze onderzoeksvragen in alle vrijheid uit te werken en onze studies uit te voeren.

I would also like to thank Activaero GmbH for their support and great collaboration on the three clinical studies we performed using the Akita device. Bernhard, Philipp and Jens: thank you for the opportunity to visit you and for the many nice conference dinners!

Dan natuurlijk de afdeling kinderlongziekten, waar ik met zo veel plezier onderzoek heb gedaan:

Kamer Sb-2666: â€œgedae! onderzoekskamer! In wisselende samenstelling heb ik hier mogen samenwerken met geweldige collegaë®TM's:

Ruben Boogaard, collega van het eerste uur en fijne paranimf. We hebben vele hoogte- en dieptepunten van ons onderzoekstraject gedeeld. Bedankt voor je hulpvaardigheid, je luisterend oor en je altijd positieve houding. Ik bewaar goede herinneringen aan onze etentjes verspreid over heel Rotterdam, en hoop dat we die traditie in ere blijven houden!

Els van der Wiel, dank voor al je hulp gedurende mijn onderzoekstraject: de bergen met formulieren en ingewikkelde regelgeving die komen kijken bij het doen van wetenschappelijk onderzoek, het stug doorzetten bij alle hordes die wij tegenkwamen bij onze pogingen tot het gebruiken van data uit het elektronisch patientendossier voor onderzoek, samen patiÃ©nten includeren voor de klinische studies, gezellige congresbezoeken en natuurlijk ons gezamenlijke bezoek aan het CF centrum in Verona.

Eveline Nieuwhof, bedankt voor al je hulp, vooral tijdens de FLY-studie. Mede dankzij jouw inzet hebben we bij 50 kinderen de lung clearance index kunnen meten, en dat vroeg bij eigenwijze peuters geregeld veel van onze creativiteit!

Lianne van der Giessen, bedankt voor al je tips wat betreft het meten van hoest en saturatie. Fijn dat ik op jouw ervaringen verder kon bouwen! Dank ook voor je altijd positieve houding en je streven naar optimale zorg voor CF patiÃ©nten.

Carmelo Gabriele, thank you for the enthusiastic cooperation on your studies performed at the department of pediatric pulmonology. It started with â€œblazen, blazenâ€, and soon you conducted multiple interesting studies on FeNO!

Ook mijn directe collegaë®TM's Daan, Esther, Leonie, Martine en Sandra wil ik bedanken voor de gezelligheid en voor het meedenken met de praktische vragen die we tijdens het doen van onderzoek tegenkwamen.


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Mijn collega-onderzoekers Jeroen, Sandra, Danaïdel, Ruben, Ralph, Emile, Lizet, Iris, Saskia, Hester, Denise, Nienke, Marjolein, Frans, Chris, Carine, Mirjam en alle anderen. Dank voor de gezamenlijke lunches, de drankjes op de Â´VOBSâ€”(vrijdagmiddag onderzoekersborrel Sophia), en het heerlijk even met jullie kunnen mopperen als er weer eens iets tegen zat op onderzoeksgebied.

Collegaë®TM's in het Maasstad Ziekenhuis: wat is de kindergeneeskunde een leuk vak en wat is het fijn ommet jullie te mogen samenwerken! Mede dankzij jullie ga ik elke dag met veel plezier naar mijn werk. Carsten Lincke, dank voor je vertrouwen en voor je rustige en plezierige manier van opleiden. Alle kinderartsen: jullie zorgen samen voor een open werksfeer en een ideale werk- en opleidingsomgeving voor arts-assistenten. Bedankt voor de opbouwende feedback en
de mogelijkheid om me te ontwikkelen als klinische dokter. Collega-a(n)ios: bedankt voor de collegiale sfeer en de fijne dagelijkse samenwerking!


Maaikje, Myrthe, Ruchi en Wendy: bedankt voor onze jarenlange vriendschap en alle steun tijdens mijn werk als onderzoeker en als ANIOS. Studeren, co-schappen, eerste baan, samenwonen, trouwen, kinderen krijgen: we hebben het allemaal met elkaar gedaan. Dat er nog vele etentjes, high tea’s en kinderborrels mogen volgen.

Ook mijn familie en schoonfamilie wil ik op deze plek bedanken. Paps en mam, bedankt voor het veilige nest dat jullie ons altijd gegeven hebben en de mogelijkheden die jullie me hebben geboden om me te ontwikkelen. Paul, Merel en Alex: het is fijn om jullie (schoon-) zus te zijn! Bedankt voor jullie interesse en de gezellige etentjes op het Verdipad.


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**Curriculum vitae**

Edith Marije van den Beukel – Bakker was born in Gouda on January 14th, 1979. She graduated from secondary school at the åfComenius Collegeã in Capelle aan den IJssel in 1997. In the same year she started her medical training at the Medical Faculty of the Erasmus University of Rotterdam. She received a scholarship from the university in 2000 to take part in the board of the students’ association SSR-Rotterdam. In 2002 she performed a research project åfTitrating steroids on exhaled nitric oxide in children with asthmaã, supervised by dr. M.W.H. Pijnenburg at the department of Pediatric Pulmonology, Erasmus MC åfSophia Childrenå€™s Hospital, which greatly increased her enthusiasm for clinical research. She was elected for an international exchange program of the IFMSA (International Federation of Medical Studentså€™ Associations) and spent two months of clinical internships at the Taipei Medical University Hospital, Taiwan.

After returning to Rotterdam, Marije was asked to work six months on another clinical research project investigating the use of exhaled nitric oxide in children with asthma and subsequently started her internships. She chose her final rotation at the department of general pediatrics of the Sophia Childrenå€™s Hospital and obtained her medical degree in 2005 (cum laude). In the same year she started as a PhD student at the department of Pediatric Pulmonology on the project åfDetection and treatment of small airway diseaseã, supervised by prof.dr. H.A.W.M. Tiddens. The results of the studies performed in this project are described in this thesis. In 2011 she worked half a year as a resident on the department of neonatology of the Sophia Childrenå€™s Hospital and in April 2012 she started to work as a resident at the department of pediatrics of the Maasstad Hospital in Rotterdam. She lives together with her husband Dirk and their two children Lennard (2009) and Vera (2011).

**Curriculum vitae**


Nadat zij was teruggekomen uit Taiwan, werkte zij als wetenschappelijk onderzoeker op de afdeling kinderlongziekten...

**List of publications**


9. Bakker EM, Borsboom GJMM, van der Wiel â€“ Kooij EC, Rosenfeld M, Tiddens HAWM. Forced expiratory flow at 75% of vital capacity (FEF75) is a sensitive and early marker of cystic fibrosis lung disease. Submitted.


# Portfolio

**PhD Portfolio: Summary of PhD training and teaching**

**Erasmus MC**  
Department:  
Pediatric Pulmonology and Allergology PhD  
period: 1 March 2005 – 1 December 2010  

Promotor:  
Prof.dr. H.A.W.M. Tiddens  
Co-promotor:  
Prof.dr. J.C. de Jongste  

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| NKFK/Nihes course                    | 2007 | 1.0             |
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| Weekly research meeting, department  | 2005-2010 | 2.0            |
| Pediatric                             |      |                 |
Pulmonology

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conference. Oral presentation and poster presentation
ISAM 2011 1.2
conference. Oral presentation, 2nd prize ‘young investigator best oral presentation’

**Teaching activities**

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**Other**

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**Total** 40.9

**Abbreviation list**

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<th>ACT</th>
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<tr>
<td>allergic bronchopulmonary aspergillosis</td>
<td>airway clearance techniques</td>
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<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>AE</td>
<td>adverse event</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>ASL</td>
<td>airway surface liquid</td>
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<tr>
<td>BAL</td>
<td>bronchoalveolar lavage</td>
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<td>CEV</td>
<td>cumulative expired volume</td>
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<tr>
<td>CF</td>
<td>cystic fibrosis</td>
</tr>
<tr>
<td>CFTR</td>
<td>cystic fibrosis transmembrane conductance regulator</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
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<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonuclease</td>
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<td>DPI</td>
<td>dry powder inhaler</td>
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<td>EPD</td>
<td>electronic patient dossier</td>
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<td>FEF&lt;sub&gt;25-75&lt;/sub&gt;</td>
<td>forced expiratory flow between 25% and 75% of FVC</td>
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<td>FeNO</td>
<td>fractional concentration of exhaled nitric oxide</td>
</tr>
<tr>
<td>FEV₁</td>
<td>forced expiratory volume in 1 second</td>
</tr>
<tr>
<td>FRC</td>
<td>functional residual capacity</td>
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<td>FVC</td>
<td>forced vital capacity</td>
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<tr>
<td>GSD</td>
<td>geometrical standard deviation</td>
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<td>HS</td>
<td>hypertonic saline</td>
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<tr>
<td>ICC</td>
<td>Intra class Correlation Coefficient</td>
</tr>
<tr>
<td>ICS</td>
<td>inhaled corticosteroids</td>
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<td>infant pulmonary function test</td>
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</tr>
<tr>
<td>MBW</td>
<td>multiple breath wash out</td>
</tr>
<tr>
<td>MMAD</td>
<td>mass median aerodynamic diameter</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
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<tr>
<td>NaCl</td>
<td>sodium chloride</td>
</tr>
<tr>
<td>PCL</td>
<td>periciliary liquid layer</td>
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<tr>
<td>pMDI</td>
<td>pressurized metered dose inhaler</td>
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<tr>
<td>RCT</td>
<td>randomized clinical trial</td>
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<tr>
<td>rhDNase</td>
<td>recombinant human DNase</td>
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<tr>
<td>r</td>
<td>Spearman’s correlation coefficient</td>
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<td>RTE</td>
<td>respiratory tract exacerbation</td>
</tr>
<tr>
<td>SAE</td>
<td>serious adverse event</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SpO₂</td>
<td>oxygen saturation</td>
</tr>
<tr>
<td>VAS</td>
<td>visual analogue scale</td>
</tr>
<tr>
<td>VMD</td>
<td>volume median diameter</td>
</tr>
</tbody>
</table>
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Cover: Lung tissue. Coloured scanning electron micrograph (SEM) of the inner surface of a bronchiole, the tiny airways within the lung. The epithelium consists of ciliated cells (pink), and non-ciliated Clara cells (green) that secrete mucus (brown). Particles entering the lung together with the air are immobilized by the mucus and transported by the cilia out of the lung.


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