Optimisation and Assessment of Airway Clearance in Children with Cystic Fibrosis

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Optimisation and Assessment of Airway Clearance in Children with Cystic Fibrosis

Optimalisatie en onderzoek van airway clearance bij kinderen met cystic fibrosis

Proefschrift

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Nihil Volentibus Arduum

Voor Lisette

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Chapter 1

Optimisation and assessment of airway clearance in children with cystic fibrosis

1.1. CYSTIC FIBROSIS

Cystic Fibrosis (CF) is the most common life-shortening genetic disorder in the white population.[1] It affects approximately 1300 individuals in the Netherlands[2] and 60.000 individuals worldwide. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which is expressed in many organ systems, including the respiratory and gastro-intestinal tracts. Over 1600 mutations of the CFTR gene have been described. The prevalent mutation leading to CF is the deletion of phenylalanine at codon 508 (phe508del, until recently known as ΔF508). This is found in about 70% of the patients. Different mutations in the CFTR gene have varying effects on CFTR function and can result in different phenotypes of the disease. The CFTR protein primarily functions as an ion channel that regulates liquid volume on epithelial surfaces through chloride secretion and inhibition of sodium absorption. Impaired or absent CFTR function leads to a reduced volume of airway surface liquid in the lungs, which negatively affects the mucociliary clearance (MCC). This in turn leads to chronic and severe lung disease that starts in infancy. Treatment of CF is lifelong, complex and intensive. Fortunately, the predicted survival of CF patients has steadily improved over the last decades. The median age of survival in the United States reached 36.9 years in 2006.[3] This progress is due to much better nutritional management, the provision of care through multidisciplinary specialized CF centres, and more effective antibiotics and mucolytic agents.[4]

1.1.2. CF lung disease

CF lung disease is known to develop in the first several months of life in most children. The airways of infants who died with CF in the first days of life appeared to be normal with no evidence of infection, inflammation, or significant mucus plugging.[4,5] The typical features of CF lung disease include mucus plugging of the airways, hypertrophy and hyperplasia of the secretory elements, and chronic infection, primarily with *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[5] Retention of mucus is thought to favour bacterial overgrowth, which then triggers a cycle of repeated or chronic infections associated with intense neutrophilic airway inflammation. Chronic infection and inflammation result in airway wall thickening and plugging of bronchioles with purulent secretions, a process that is thought to begin in the peripheral airways.[4]

At some point in the disease process, cough becomes a prominent symptom in most patients. Patients with early disease may cough during exacerbations only, but with progression of CF lung disease cough becomes chronic. Cough in CF is usually associated with expectoration of sputum. For most patients daily sputum volume increases with age. Blood-streaked sputum and hemoptysis occur more frequently in more advanced disease. Similar to chronic obstructive pulmonary disease, CF patients experience dys-

pnea on exertion and shortness of breath as the lung disease progresses. In end stage lung disease patients often become oxygen dependent (at least at night) with retention of carbon dioxide. In addition they experience progressive worsening of their quality of life (QoL) and exercise tolerance as they have more exacerbations and respiratory therapy is intensifying.[1]

In end stage lung disease most patients have little normal functional lung parenchyma left.[6] Most of the lung volume is occupied by bronchiectasis and/or air trapping. It is worth noting that the onset of these structural abnormalities is in infancy. Bronchiectasis (Figure 1a) is known to be an irreversible pulmonary condition. There is increasing awareness that air trapping (Figure 1b) starts early as well in the disease process and that it is an important component of respiratory failure in end stage lung disease in most patients.[7,6] Respiratory failure still is the major cause of death among patients with CF.

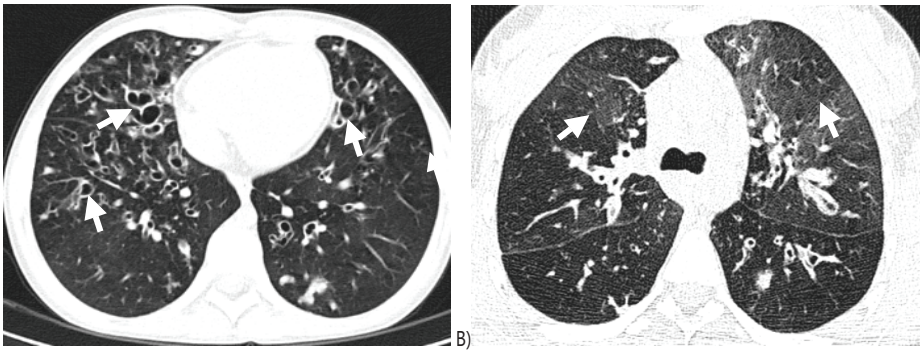

Figure 1.

a) Inspiratory computed tomography scan of CF patient with severe bronchiectasis (see arrows).

b) Expiratory computed tomography scan showing hypodense areas indicating trapped air adjacent to areas of more normal density. Note the contrast between hyper and hypodense areas (see arrows).

1.1.3. CF Gastrointestinal disease

In addition to the CF lung disease most patients usually present with signs and symptoms of gastrointestinal disease as well. Around 10% of the CF patients present in the neonatal period with meconium ileus. Furthermore, cholestatic jaundice can be observed. Pancreatic insufficiency is present in 85% of the CF patients at the time of diagnosis. Infants with CF and pancreatic insufficiency may show a low body mass index, deficiencies of fat-soluble vitamins and failure to thrive. Hepatosplenomegaly and liver cirrhosis can develop later in life and were found to occur in 2% to 37% of all patients. [8,9] Improved survival has led to a growing number of patients with CF-related diabetes, i.e.

around 16% of adolescents and adults. CF-related diabetes could result in more severe disease.[10]

1.1.4. Management of CF patients

The management of CF patients takes place primarily in specialized CF centres. Most centres have different teams for children and for adult patients. A CF team typically includes (pediatric) pulmonologists, (pediatric) gastroenterologists, dieticians, physiotherapists, CF nurses, psychologists, microbiologists, pharmacists, and geneticists. The aim of the CF teams is to provide state of the art management of CF patients, as documented in guidelines and protocols. A key element of the treatment of CF is that the patients are seen frequently, and have easy access to the team in case of problems. In addition to patient care, CF centres participate in clinical and/or basic research on different aspects of CF. Some CF centres will manage some of their patients in assisted care with a general hospital, which can deliver part of the care close to home.

1.1.5. Treatment of CF lung disease

The primary aim of therapy in CF lung disease is to preserve the normal architecture of the lung and to prevent any damage from occurring. Therapy includes frequent treatment with antibiotics, mucoactive drugs and airway clearance techniques (ACT). Aggressive preventive treatment is important because most structural lung damage is irreversible. Treatment is demanding for the patient and parents and is lifelong. Adherence to therapy from infancy into adulthood is the major challenge of the CF team and the patient alike.[4,11]

1.2. MUCOCILIAIRY CLEARANCE

Normal lungs are kept clean and free from infection in part through the process of mucociliary clearance (MCC). Thus, debris and bacteria present in inhaled air become embedded in the mucus on the surface of the airways. This mucus is continuously transported by the cilia on the airway surface out of the lung toward the pharyngeal cavity from where it is swallowed. While the tips of the cilia project into the mucus during their forward propulsive stroke, the greater part of each cilium is bathed by a less viscous fluid, the periciliary liquid (PCL) that lines the airway surface.[12]
For normal MCC it is necessary that the airway epithelial cells are intact, cilia structure and activity are normal, depth and chemical composition of the PCL are optimal, and the rheology of mucus is within the physiological range.[13]

In CF the CFTR dysfunction leads to depletion of the PCL resulting in compressed cilia, which can decrease both mucociliary and cough clearance. [5,14] The deficient MCC contributes to the infection by preventing effective clearance of bacteria out of the respiratory tract. [5] In addition, there is an excessive inflammatory response to pathogens, which mechanism is poorly understood.[7]

In patients with CF more DNA is present in the sputum.[15] This DNA is released by disintegrated inflammatory cells, particularly neutrophils.[16,17] Higher DNA content in CF mucus is associated with higher mucus viscosity.[18]

As part of the inflammatory response neutrophils are releasing large quantities of proteases, such as elastase, which contribute to structural airway damage.[19] This damage further impairs MCC and facilitates the attachment and growth of bacteria, thus reinforcing the vicious circle.[4]

The impaired MCC is an important characteristic of CF that is present early in life and worsens with increasing disease severity.[20,21,13,22] (Figure 2)

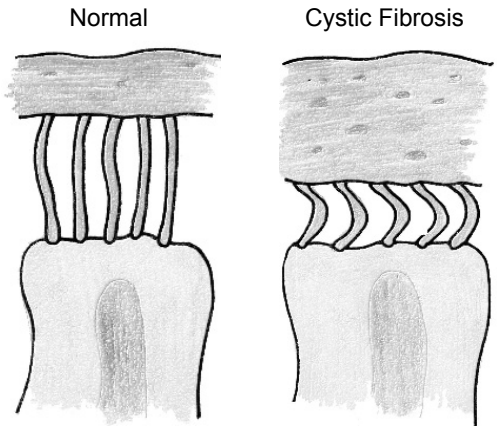

Figure 2. Schematic of ciliated airway epithelial cells expressing normal (left) and mutant (right) CFTR. The normal cel has fully extended and functional cilia and the epithelial cell with mutant CFTR has compressed cilia with increased mucus, decreased epithelial lining fluid and essentially no mucociliary clearance.

Impaired MCC causes mucus to accumulate in the airways. Accumulation of mucus contributes to airflow obstruction. This obstruction increases the work of breathing, produces ventilation-perfusion mismatch, and can result in impaired gas exchange. Retained mucus can further facilitate bacterial growth and thus can worsen inflammation.[23]

For CF patients with compromised MCC cough is an important mechanism to clear the entrapped mucus from the airways.[13] Cough clearance becomes more important when mucus-cilia interaction is compromised.[24] However, cough by itself is relatively inef-

ficient in CF patients to clear the thick, impacted mucus. For this reason a variety of ACT such as forced expiration techniques, PEP mask, autogenice drainage, flutter, and active cycle of breathing techniques have been developed to facilitate MCC. Besides ACT, CF patients use mucoactive drugs, which aim to change the properties of airway secretions.[25]

1.2.1. Airway clearance techniques

ACT are considered the cornerstone in the treatment of CF. Approximately 90% of the CF patients use some form of ACT.[26,27]

The goal of ACT in CF is to improve the removal of mucus from the airways. It has been shown that cough and forced expiration techniques, which are elements of ACT, are effective in clearing secretions from the inner and intermediate lung regions.[28] Effective ACT can improve ventilation-perfusion mismatch, reduce airway resistance, and decrease proteolytic activity in the airway.[29] In general ACT aims firstly to increase expiratory flows in partially obstructed airways and thus to mobilize the sputum. Secondly to increase the lung volume above tidal volume level, which reduces collateral ventilatory resistance and allows air to flow behind secretions aiding their mobilization.

There has been a substantial change in ACT over the last decade.[30] From more passive techniques – for example clapping – physiotherapists are now instructing more active techniques to their patients – like active cycle breathing techniques, autogenic drainage, PEP mask (Figure 3), flutter, and huffing.

Exercise is increasingly regarded as an essential part of the overall physiotherapy management of CF. Exercise increases the respiratory minute volume and recruits lung zones. As a result high flows occur in the central airways, boosting the MCC.[29,31] Exercise in addition improves general fitness, patient's self-esteem, and measures of QoL. Exercise is socially acceptable and helps to normalize the patient's life rather than adding a therapy that accentuates differences from peers.[32]

A meta-analysis has shown that the combination of ACT with exercise is associated with a statistically significant increase in lung function over ACT alone.[33] However, in adults with CF, ACT resulted in a higher production of sputum than exercise alone.[34] Hence, exercise is considered an important add-on strategy to improve MCC, but it cannot replace ACT.

In general ACT are not well standardized. Major differences between centres can be observed in frequency, timing, duration, and intensity. Selection of the best ACT for a patient is determined by many factors such as therapy adherence, use of concomitant medications, and character of the patient. All these factors make it difficult to design and conduct rigorous clinical trials to improve our understanding of the various ACT.

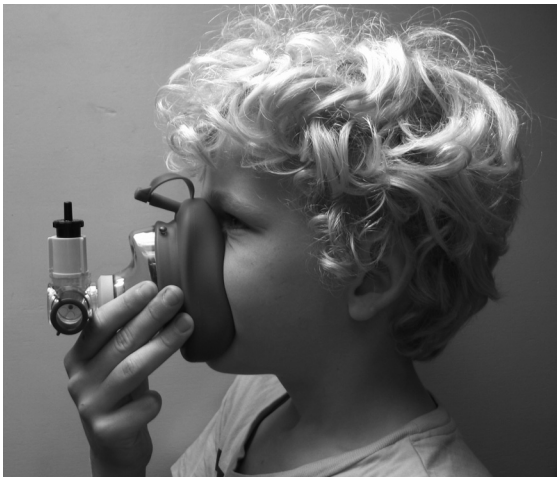

Figure 3. Child with a PEP mask.

Importantly, for ethical reasons it is not possible to conduct double-blind studies in CF patients comparing a specific ACT to no ACT.[29]
Several short- to medium-term studies support the use of ACT in CF. In the Cochrane review 'Chest physiotherapy compared to no chest physiotherapy for CF' it is concluded that ACT have short-term effects in terms of increasing mucus transport. However, no conclusive evidence is available on the long-term effects of ACT.[35]

1.2.2. Mucoactive drugs

Recombinant human deoxyribonuclease (rhDNase) was one of the first drugs specially developed for CF that was shown to be effective.[36] RhDNase is delivered once daily by nebuliser as an aerosol.[37] RhDNase, which is an enzyme, cleaves extra cellular DNA, which is present in high concentrations in purulent CF sputum.
Daily treatment with rhDNase reduces the number of pulmonary exacerbations, improves pulmonary function and QoL, and is well tolerated and safe in patients with mild, moderate and severe CF.[38-44]

1.1.3. RhDNase and ACT

The effectiveness of ACT can be impaired when the mucus is thick and dehydrated.[45] For this reason most patients combine ACT with the use of mucoactive drugs. Though the effectiveness of rhDNase, a mucoactive drug, is well established, very little research has been carried out to determine the time relation between rhDNase nebulisation and ACT. The sequence in which rhDNase and ACT should be used is not known. About 50% of the patients use rhDNase before ACT – and 50% use it after. [26,46] Furthermore, there is

no agreement on the optimal timing of rhDNase nebulisation during the day. The Epidemiologic Registry of Cystic Fibrosis showed that 51.3% of the patients used rhDNase in the morning, 37.4 % in the evening, 8.1% varied between morning and evening, and usage was not specified in 3.2%.[26]

RhDNase is an enzyme with a half-life in rodents of at least 11 hours after inhalation. [47] It requires time to act upon the mucus. In test tube conditions all the sputum moved freely down the tube after 30 minutes.[37] The duration of the half-life in humans is not known. Based on these in-vitro data patients are often advised to use the rhDNase minimally 30 minutes before ACT. Still there are reasons to believe that it may be advantageous to nebulise rhDNase after ACT. As a result of ACT the mucus will be mobilised and airway patency will improve. This is likely to result in a more peripheral and homogenous deposition pattern of nebulised rhDNase.[22,48]

Another treatment option that has been suggested, is to nebulise rhDNase before going to sleep and to do ACT in the morning. Firstly, it would allow sufficient time for rhDNase to act upon the free DNA present in the airway lumen. Secondly, changes in posture during sleep might act like postural drainage therapy; allowing gravity dependent mobilization of the sputum. During sleep adults have an average of 16 position shifts per night.[49] These more or less random changes might be helpful to clear the liquefied mucus from the airway. Postural drainage during sleep is therefore likely to improve MCC. However, a number of theoretical arguments against the nebulisation of rhDNase before sleep have been suggested. Firstly, MCC was depressed during sleep in normal subjects [50] and in subjects with asthma [51]. In addition, it was shown that dehydrated secretions are capable of triggering coughing spells during sleep in CF.[52] Secondly, nightly spontaneous cough might be less effective to expectorate sputum relative to daytime cough. As a result the liquefied sputum instead of being expectorated could even worsen peripheral airway obstruction. Thirdly, when nebulisation before the night should result in increased cough during the night, sleep quality could be reduced.[52] However, the assumptions as discussed above were never systematically studied.

1.3. OUTCOME MEASURES FOR CF LUNG DISEASE

To measure the effect of therapy – in clinical management and clinical studies – sensitive and clinically relevant endpoints are needed. True endpoints are mortality and slope of decline in lung function. With median survival of almost 40 years, mortality is no longer a feasible outcome parameter in CF. Similarly, slope of decline in lung function is not a feasible outcome parameter due to the variability of the measurement and due to the slow annual rate of decline.

Hence, surrogate endpoints must be used to monitor the effect of therapy in clinical management and clinical studies. Surrogate endpoints are outcome measures that first must have reasonable high reproducibility (precision) and must be accurate predictors of primary endpoints. Finally a change in score within an individual must correspond to a change in risk in the right direction for the primary endpoint of interest within that individual; the validity requirement. [53,54,55]

Standardized equipment and techniques should be available with which to feasibly perform the measurement. In addition it is important that the result of the measurement can be expressed numerically. Ideally, the endpoint would also be measured with minimal risk and be inexpensive and easy to perform.[54]

Surrogate endpoints that are used in CF include: function parameters; pulmonary exacerbation rates; QoL measures; growth; sputum cultures; inflammatory markers; nasal potential difference; chest radiograph scores and chest computed tomography (CT) scores (Table 1).[54]

Lung function parameters such as the Forced Expiratory Volume in one second (FEV_1) traditionally have been used as the primary endpoint in many therapeutic studies in CF. Ironically, the improvements in lung function among patients with CF over the past decade have rendered these measurements less useful as clinical trial endpoints, since the annual rate of decline has become very small and its variability remained high.[54] Furthermore, there is interest in endpoints which can reduce the sample size requirements for CF studies.[55] Hence, there is great need for new sensitive and precise surrogate endpoints.[56]

1.3.1. Outcome measures in ACT studies

Standardized outcome measures for ACT are not well developed. There are several problems with ACT studies in CF. First, there is no gold standard by which to compare new outcome measures. Secondly, due to the use of a wide variety of outcome measures studies are difficult to compare and results are often conflicting. And thirdly, most ACT studies are too small and statistically underpowered to provide conclusive evidence.[57]

In three Cochrane reviews about ACT techniques a total of 34 studies were reviewed. In total 809 patients, aged 0-41 years, participated in these studies. In these studies 37 different outcome measures were used.[35,58,59] The most commonly used (>10%) outcome measures were pulmonary function parameters, sputum weight, oxygen saturation, and radiolabelled imaging.

Exercise and admissions were used in three out of the 34 studies (9%). (Figure 4) Vital capacity, peak flow, functional residual volume, RV/TLC, growth, patient preference, intravenous antibiotics administration were used in two (6%) studies. Quality of life, use of antibiotics, gastro-oesophageal reflux, body mass index, days in hospital, symptom

Table 1. Examples of surrogate endpoints that can be used in clinical trials and patient management in CF.

Endpoint	Age (yr)	Risk	Expense	Standardized	Disadvantages	Advantages
Pulmonary exacerbation	All ages	Minimal	Low	Wide variety of definitions	No standardized definition	Clinically relevant endpoint
Quality of Life	> 6	Minimal	Low	Yes (CFQ)	Responsiveness to interventions not well established	- Clinically relevant endpoint - Validated instrument
Infant lung function	< 3	Greater than minimal	High	Yes	- Insensitive to early or regional disease - Requires extensive training - Expensive equipment - Requires sedation	Allows assessment of early obstructive lung disease
Spirometry	> 6	Minimal	Low	Yes	- Poor precision - Poor accuracy - Insensitive to early or regional disease - Unclear association with clinical well-being - High variability	- Most widely used endpoint - Extensive epidemiologic data linking to survival
Respiratory cultures	All ages	Minimal	Low–moderate	Yes	Many patients not able to expectorate sputum, poor sensitivity of oropharyngeal cultures for lower airway micro organisms	Assessment of respiratory pathogens
CT	All ages	Greater than minimal	Moderate–high	Well established scoring system	- Responsiveness not well established - Radiation exposure - Lack of standardization of scanning protocols	- Accurate to detect bronchiectasis - Can be measured across all ages - Sensitive to early disease - Assesses regional disease
Chest radiograph	All ages	Minimal	Low	Several established scoring systems	- Insensitive to early disease - Responsiveness to interventions not well established	Simple, inexpensive

CFQ = Cystic Fibrosis Questionnaire; CT = computed tomography [54]

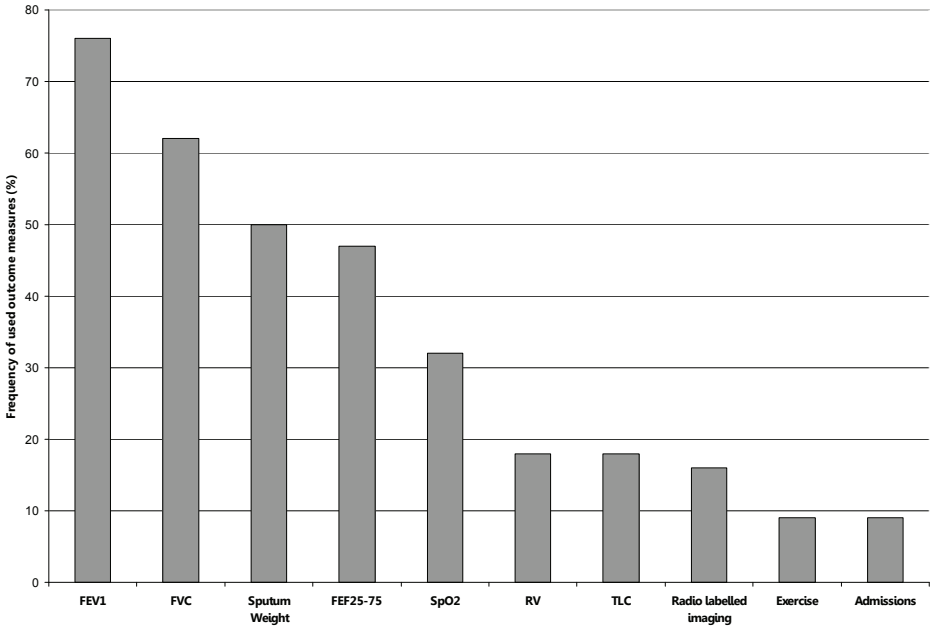

Figure 4. Frequency of most used outcome measures used in 34 studies of Airway Clearance Techniques in Cystic Fibrosis. (Based on [35,58,59])

score, cough score and counting, gas mixing, and other pulmonary lung function tests – for example expiratory rest volume and airway resistance – were used in one study (3%) only. Note that quality of life is used in one study only. A missing but interesting outcome is costs.

In the next section we will discuss the advantages and disadvantages of a number of relevant outcome parameters that can be used in ACT studies. In Table 2 these advantages and disadvantages, as well as the questions to be answered in the future, are summarized.

1.3.1.1. Pulmonary function test

The most widely used pulmonary function test is spirometry. From spirometry various parameters can be obtained. FEV_1 is widely used to evaluate ACT effectiveness.[60] It is questionable whether FEV_1 is a valid endpoint in ACT studies in CF. For one, the measurement of FEV_1 reflects airway patency of especially the central airways. FEV_1 is considered insensitive to detect localized structural damage. In addition it is insensitive to events in the peripheral airways.[61] Furthermore it is unclear whether FEV_1 is able to detect changes in mucus transport.[60,61] During ACT patients can mobilize mucus from the peripheral airways into the more central airways, which action may reduce FEV_1. Hence, FEV_1 seems to be of little value to evaluate the effectiveness of ACT. An interest-

ing alternative parameter that can be obtained from spirometry is the MEF_{25} or FEF_{75}[1]. This flow parameter is considered a sensitive indicator of the condition of the peripheral airways. In addition it is abnormal in an early stage of the disease and has been shown to be responsive to therapy.[41,61]

Table 2. Examples of surrogate endpoints that can be used in ACT studies in CF.

Endpoint	Age (yr)	Standardized	Disadvantages	Advantages	Questions
Spirometry	>6	yes	- No standardized definition - FEV_1 not sensitive in early lung disease	- Not invasive - Clinically relevant	- Responsiveness to ACT interventions
Airway resistance	All ages	Yes		- All ages - Not invasive - Clinically relevant	- Responsiveness to ACT interventions - Correlation with other outcome measures
Oxygen saturation	All ages	No		- All ages - Not invasive - Clinically relevant	- Responsiveness to ACT interventions - Reproducible - Correlation with other outcome measures
Radioactive tracers	?	Yes	- Unethical in children because of radiation exposure - Expensive	- All ages - Clinically relevant	
Sputum collection	>6	No	- Many patients not able to expectorate sputum - Not precise	- Not invasive - Clinically relevant	- Correlation with other outcome measures
Exercise testing	>4	Several used tests	- Insensitive to early disease - No immediate effect	- Not invasive - Clinically relevant	- Responsiveness to ACT interventions
Quality of Life	> 6	Yes (CFQ)		- Not invasive - Clinically relevant - Validated instrument	- Responsiveness to ACT interventions
Cough	All ages	More scoring systems available	- Measuring cough during the day is difficult - Scoring is time consuming	- All ages - Not invasive - Clinically relevant	- Responsiveness to ACT interventions - Reproducible - Correlation with other outcome measures

ACT= airway clearance techniques, CFQ = Cystic Fibrosis Questionnaire

1. MEF_{25} or FEF_{75}: both terms are used in this thesis

1.3.1.2. Airway resistance

Several techniques are available to measure airway resistance. It has been shown that ACT has a positive effect on specific airway resistance as measured by plethysmography [62], suggesting a positive effect of ACT on central airway clearance.[63,64] An alternative and easier tool to measure respiratory system resistance may be the interrupter technique or Rint (Figure 5).[65]

Rint is sensitive to changes in central airway calibre in children with mild respiratory tract infections, it can be performed quickly in the ambulatory setting, and it has been shown to be reproducible.[66,67] Rint measurement is therefore a promising outcome measure to detect changes in the central airways.

Figure 5. Rint measurement.

1.3.1.3. Oxygen saturation

The primary function of the lung is gas exchange, i.e. the uptake of oxygen from the air and elimination of carbon dioxide. In CF patients gas exchange can be impaired due to airway obstruction. This can result in hypoxemia and hypercapnia.[68-72] Nocturnal oxygen desaturation and reduced resting daytime oxygen saturation have been described in CF patients.[52] Oxygen saturation can be measured by pulse oximetry, which is a non-invasive, reliable and simple method for children of all ages.[73] It can be measured relatively easy with a finger probe. (Figure 6)

Reference values of overnight oxygen saturation are well established for healthy children.[74-80] Surprisingly few studies in children with stable CF have been performed using oxygen saturation.[78,81,82] In one it was shown that structural lung abnormalities on CT correlated with mean nocturnal oxygen saturation.[82] It is not known if oxygen saturation is sensitive to ACT treatment changes in children with CF.

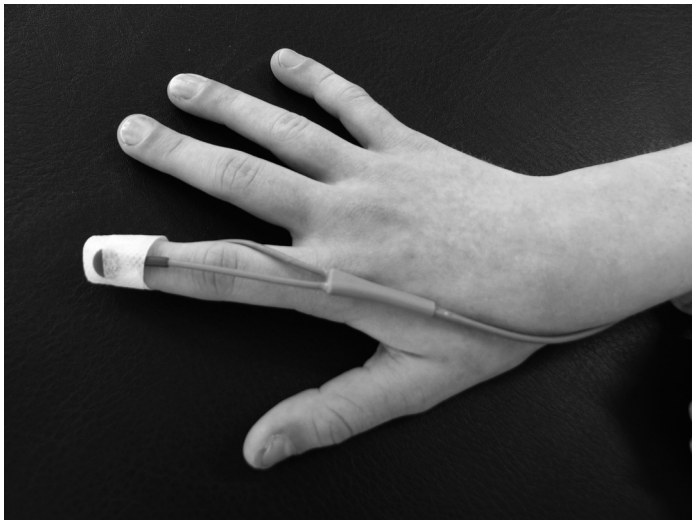

Figure 6. Probe attached to a patient's finger to measure oxygen saturation.

Clearly, oxygen saturation needs further exploration before it can be used as an outcome measure in ACT studies.

1.3.1.4. Radioactive tracers

Measurement of mucus transport by means of radioactive tracers is an attractive method for short term studies in CF.[13,22,60] The use of radioactive tracers in clinical practice is technically demanding, however, and raises some ethical concerns as far as children are concerned. Therefore, this method is not a feasible outcome parameter in larger scale clinical studies.

1.3.1.5. Sputum collection

In many studies sputum weight and/or volume have served as outcome parameters because these are relatively easy measured. Yet this method has some major drawbacks. Patients often are reluctant or not able to expectorate sputum effectively. Then there is the risk of inadvertent swallowing of secretions and of contamination of the secretions by saliva.[60] Furthermore, in younger children it is mostly not possible to obtain reliable sputum samples. As a result sputum weight and/or volume is not a feasible endpoint.

1.3.1.6. Exercise testing

Exercise capacity is an important parameter in children with CF and it correlates with a child's functional capacities.[83] A high level of aerobic fitness in patients with CF is associated with a lesser risk of dying.[84]

Exercise testing is underutilized in studies, which is unfortunate, since it is probably a better predictor of long-term survival than are lung function parameters. One of the problems in performing exercise testing is that many CF centres do not have the specialized laboratory testing equipment or manpower necessary to assess aerobic/anaerobic fitness. For these reasons, field exercise tests such as the shuttle test, or 6-minute walk test, have been introduced. Although they have been validated, there are still little data on reference values and on criteria by which value changes are to be considered clinically significant.[29]
Exercise testing is probably only useful as an outcome measure in long term ACT studies exploring whether functional capacities will improve by an ACT treatment change.

1.3.1.7. Quality of life

Health related QoL as an outcome measure in clinical trials is becoming increasingly important. Registration of new drugs not only requires to demonstrate the pharmacologic activity, but also to demonstrate its effect on QoL. QoL measurement provides a way of incorporating the child's and parent's perspective on how CF and its therapies impact on their physical, social and psychological functioning.[85,86] A CF-specific health-related measure, the Cystic Fibrosis Questionnaire (CFQ), has been developed and validated in many countries, such as the Netherlands.[85,87-89] The CFQ takes developmental stages into account and makes it possible to monitor the health status from the age of 6 years throughout adulthood.
The CFQ has been used in only few ACT studies.[60] The CFQ is still a relatively new instrument and many questions still have to be answered before it can be used on a wider scale. Most importantly, the minimal relevant changes in the various domains have not been well defined. Furthermore, it has not been well validated against other endpoints such as bronchiectasis. Recently, a new inhaled antibiotic was approved for CF based on a study in which the respiratory domain of the CFQ was one of the secondary endpoints.[90] This illustrates that the CFQ is becoming an important instrument in CF. Clearly it is highly relevant to include the CFQ in future ACT studies. It seems that the 'treatment constraint' item from the quality of life domain and the respiratory part from the symptom scale are the most relevant elements for use in ACT studies.

1.3.1.8. Cough

Cough is one of the defensive reflexes of the respiratory tract [91] and it is part of the mucociliary escalator.[13] Cough accompanied by expectoration of sputum is a prominent daily symptom in CF patients.[4] Increased cough is an important symptom of a pulmonary exacerbation.[92,93] This is why cough is included in most definitions of a pulmonary exacerbation and can potentially serve as an objective surrogate parameter for an exacerbation.[93] Surprisingly, cough is rarely used in ACT studies.

Severity of cough in clinical studies has been assessed using a visual analogue scale or the cough symptom score.[94,95] These subjective cough scores correlate only moderately well to more objective measurements such as time spent coughing.[96] Clearly, a more objective method to record cough is important. Currently available cough meters, which consist of electromyogram electrodes and a microphone, are expensive, and data analysis is time consuming and requires a trained investigator.[94,97,98] The manual counting of cough sounds from digital audio recordings is a more patient friendly alternative method that has shown excellent agreement with cough as recorded on video.[99]
Cough in daily life is best measured during sleep. Cough registrations during sleep have been examined in adult patients with CF.[52,72] To our knowledge this method has not yet been applied in children with stable CF. Since cough is an important component of an exacerbation, objective cough measurement in children with CF needs to be further studied.

1.4. ADHERENCE TO ACT

CF treatment is demanding, time consuming and lifelong. Most CF patients require daily intensive treatment at home that can consist of nebulisation of mucolytics and antibiotics, as well as oral antibiotics, pancreatic enzymes, vitamin or nutritional supplements. Furthermore, daily ACT is essential, and so is a healthy lifestyle with adequate nutrition and exercise. Treatment becomes more intensive during disease exacerbations and with progression of the disease. New drugs are currently being developed and some of those will be added to the already impressive daily treatment package. It is not to be wondered at, therefore, that lifelong adherence to this burdensome therapy is a major challenge for the patients, parents and the CF team.[100] Poor adherence to therapy often brings worsening of the disease [100] and is considered an important prognostic factor for progression of the disease.[101] Up to 50% of pediatric populations have been found non-adherent to part of their CF treatment regimens.[102,103] Adherence rates for ACT in children range from 40 to 75% [103-105] and for nebulisation with rhDNase from 57 to 90%.[104,106]
Adherence is a complex issue affected by variables such as: age, knowledge, economical status, psychosocial factors and cognitive functioning.[100,107,108]
Patient knowledge of the disease and treatment regimen as well as understanding of the background of the physicians' recommendations are thought to be critical to adherence. Substantial gaps in knowledge about CF lung disease have been identified in children and their parents.[109] In two studies the level of adherence among children with CF was positively associated with the level of their knowledge of the disease.[110,111]

It would seem important, therefore, to improve in children with CF the knowledge about CF lung disease – with the aim to improve adherence. Unfortunately only a few tools are available to help educate the children in a systematic way.

1.5. SUMMARY OF THE INTRODUCTION

Cystic Fibrosis (CF) lung disease is characterized by the depletion of airway surface liquid and abnormal mucociliary transport. Retention of mucus is thought to favour bacterial overgrowth, which then triggers a cycle of repeated or chronic infections associated with intense neutrophilic airway inflammation. This process causes progressive structural lung damage. Eventually, this leads in most patients to a reduced life expectancy
The primary aim of therapy in CF lung disease is to preserve the normal architecture of the lung and to prevent any damage from occurring. The cornerstone of therapy is treatment with effective antibiotics, mucoactive drugs, and ACT. Little is known on the optimal time relation between rhDNase, a mucoactive drug, and ACT.
Assessment of (new) therapies requires reliable and validated endpoints. More sensitive and accurate endpoints are urgently needed to monitor CF lung disease. There is no gold standard to measure efficacy in ACT studies and currently used outcome measures have several drawbacks. Therefore new outcome measures for ACT studies have to be developed and validated.
CF treatment is demanding, time consuming and life long. The high burden of CF therapy makes that adherence to this therapy is a major challenge for the patients, parents and the CF team. Adherence is affected by variables such as: age, knowledge and socioeconomic factors. To increase patients' knowledge about their disease, education should be an important component in CF therapy.

REFERENCES

1. Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. Am J Respir Crit Care Med 2003;168(8):918-951.
2. Nederlandse Cystic fibrosis Stichting. Baarn; 2008.
3. Cystic Fibrosis Foundation. Patient Registry Annual Data Report. 2006.
4. Tiddens HA, Rosenfeld M. Cystic Fibrosis - Respiratoy Manifestations. In: Taussig LM, Landau LI, editors. Pediatric Respiratory Medicine. Philapdelphia: Mosby Elsevier; 2008.
5. Esther CR, Leigh MW. Cystic Fibrosis - Genetics and Disease Mechanisms. In: Taussig LM, Landau LI, editors. Pediatric Respiratory Medicine. Philapdelphia: Mosby Elsevier; 2008.
6. Loeve M, Lequin MH, Bruijne Md, et al. Measuring trapped air by computed tomography (CT) in CF: In search of the optimal protocol.; 2008; Berlin Germany. p abstract 3827.
7. Davies JC, Alton EW, Bush A. Cystic fibrosis. BMJ 2007;335(7632):1255-1259.
8. Anselmo MA, Lands LC. Cystic Fibrosis - Overview. In: Taussig LM, Landau LI, editors. Pediatric Respiratory Medicine. Philadephia: Mosby Elsevier; 2008.
9. Nash KL, Allison ME, McKeon D, et al. A single centre experience of liver disease in adults with cystic fibrosis 1995-2006. J Cyst Fibros 2008;7(3):252-257.
10. Marshall BC, Butler SM, Stoddard M, et al. Epidemiology of cystic fibrosis-related diabetes. J Pediatr 2005;146(5):681-687.
11. Morgan WJ, Butler SM, Johnson CA, et al. Epidemiologic study of cystic fibrosis: design and implementation of a prospective, multicenter, observational study of patients with cystic fibrosis in the U.S. and Canada. Pediatr Pulmonol 1999;28(4):231-241.
12. Inglis SK, Wilson SM. Cystic fibrosis and airway submucosal glands. Pediatr Pulmonol 2005;40(4):279-284.
13. Robinson M, Bye PT. Mucociliary clearance in cystic fibrosis. Pediatr Pulmonol 2002;33(4):293-306.
14. Rubin BK. Mucus structure and properties in cystic fibrosis. Paediatr Respir Rev 2007;8(1):4-7.
15. Bush A, Payne D, Pike S, et al. Mucus properties in children with primary ciliary dyskinesia: comparison with cystic fibrosis. Chest 2006;129(1):118-123.
16. Chernick W, Barbero G. Composition of tracheobronchial secretions in cystic fibrosis of the pancreas and bronchiectasis. Pediatrics 1959 november:739-745.
17. Costello CM, O'Connor CM, Finlay GA, et al. Effect of nebulised recombinant DNase on neutrophil elastase load in cystic fibrosis. Thorax 1996;51(6):619-623.
18. Zahm JM, Girod de Bentzmann S, Deneuville E, et al. Dose-dependent in vitro effect of recombinant human DNase on rheological and transport properties of cystic fibrosis respiratory mucus. Eur Respir J 1995;8(3):381-386.
19. Meyer KC, Sharma A. Regional variability of lung inflammation in cystic fibrosis. Am J Respir Crit Care Med 1997;156(5):1536-1540.
20. Boucher RC. Evidence for airway surface dehydration as the initiating event in CF airway disease. J Intern Med 2007;261(1):5-16.
21. Boogaard R, de Jongste JC, Merkus PJ. Pharmacotherapy of impaired mucociliary clearance in non-CF pediatric lung disease. A review of the literature. Pediatr Pulmonol 2007;42(11):989-1001.
22. Robinson M, Eberl S, Tomlinson C, et al. Regional mucociliary clearance in patients with cystic fibrosis. J Aerosol Med 2000;13(2):73-86.
23. Hess DR. Airway clearance: physiology, pharmacology, techniques, and practice. Respir Care 2007;52(10):1392-1396.

24. Stannard W, O'Callaghan C. Ciliary function and the role of cilia in clearance. J Aerosol Med 2006;19(1):110-115.
25. King M, Rubin BK. Pharmacological approaches to discovery and development of new mucolytic agents. Adv Drug Deliv Rev 2002;54(11):1475-1490.
26. ERCF. ERCF Annual Report 1998: F. Hoffmann-La Roche Ltd; 2000. 19 p.
27. Konstan MW, Butler SM, Schidlow DV, et al. Patterns of medical practice in cystic fibrosis: part II. Use of therapies. Investigators and Coordinators of the Epidemiologic Study of Cystic Fibrosis. Pediatr Pulmonol 1999;28(4):248-254.
28. Hasani A, Pavia D, Agnew JE, Clarke SW. Regional lung clearance during cough and forced expiration technique (FET): effects of flow and viscoelasticity. Thorax 1994;49(6):557-561.
29. McIlwaine M. Chest physical therapy, breathing techniques and exercise in children with CF. Paediatr Respir Rev 2007;8(1):8-16.
30. Davidson KL. Airway clearance strategies for the pediatric patient. Respir Care 2002;47(7):823-828.
31. Houtmeyers E, Gosselink R, Gayan-Ramirez G, Decramer M. Regulation of mucociliary clearance in health and disease. Eur Respir J 1999;13(5):1177-1188.
32. Orenstein DM, Higgins LW. Update on the role of exercise in cystic fibrosis. Curr Opin Pulm Med 2005;11(6):519-523.
33. Thomas J, Cook DJ, Brooks D. Chest physical therapy management of patients with cystic fibrosis. A meta-analysis. Am J Respir Crit Care Med 1995;151(3 Pt 1):846-850.
34. Salh W, Bilton D, Dodd M, Webb AK. Effect of exercise and physiotherapy in aiding sputum expectoration in adults with cystic fibrosis. Thorax 1989;44(12):1006-1008.
35. van der Schans C, Prasad A, Main E. Chest physiotherapy compared to no chest physiotherapy for cystic fibrosis. Cochrane Database Syst Rev 2000(2).
36. Bakker EM, Tiddens HA. Pharmacoloy, clinical efficacy and safety of recombinant human DNase in cystic fibrosis. Expert Rev Resp Med 2007;1(3):317-329.
37. Shak S, Capon DJ, Hellmiss R, Marsters SA, Baker CL. Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum. Proc Natl Acad Sci U S A 1990;87(23):9188-9192.
38. Jones AP, Wallis CE. Recombinant human deoxyribonuclease for cystic fibrosis. Cochrane Database Syst Rev 2003(3):CD001127.
39. Kearney CE, Wallis CE. Deoxyribonuclease for cystic fibrosis. Cochrane Database Syst Rev 2000;2.
40. Fuchs HJ, Borowitz DS, Christiansen DH, et al. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. N Engl J Med 1994;331(10):637-642.
41. Quan JM, Tiddens HA, Sy JP, et al. A two-year randomized, placebo-controlled trial of dornase alfa in young patients with cystic fibrosis with mild lung function abnormalities. J Pediatr 2001;139(6):813-820.
42. Ramsey BW, Astley SJ, Aitken ML, et al. Efficacy and safety of short-term administration of aerosolized recombinant human deoxyribonuclease in patients with cystic fibrosis. Am Rev Respir Dis 1993;148(1):145-151.
43. Hodson ME, McKenzie S, Harms HK, et al. Dornase alfa in the treatment of cystic fibrosis in Europe: a report from the Epidemiologic Registry of Cystic Fibrosis. Pediatr Pulmonol 2003;36(5):427-432.
44. Hodson ME, Shah PL. DNase trials in cystic fibrosis. Eur Respir J 1995;8(10):1786-1791.
45. Ramsey BW, Smith AL. Reduced incidence of respiratory tract infections in cystic fibrosis patients using aerosolized dornase alfa. Dornase Alfa Clinical Series 1994:1-5.
46. Borsje P, de Jongste JC, Mouton JW, Tiddens HA. Aerosol therapy in cystic fibrosis: a survey of 54 CF centers. Pediatr Pulmonol 2000;30(5):368-376.

47. Green JD. Pharmaco-toxicological expert report Pulmozyme rhDNase Genentech, Inc. Hum Exp Toxicol 1994;13 Suppl 1:S1-42.
48. Regnis JA, Robinson M, Bailey DL, et al. Mucociliary clearance in patients with cystic fibrosis and in normal subjects. Am J Respir Crit Care Med 1994;150(1):66-71.
49. Lorrain D, De Koninck J, Dionne H, Goupil G. Sleep positions and postural shifts in elderly persons. Percept Mot Skills 1986;63(2 Pt 1):352-354.
50. Bateman JRM, Pavia D, Clarke SW. The retention of lung secretions during the night in normal subjects. Clin Sci Mol Med 1978;55:523-527.
51. Hasani A, Agnew JE, Pavia D, Vora H, Clarke SW. Effect of oral bronchodilators on lung mucociliary clearance during sleep in patients with asthma. Thorax 1993;48(3):287-289.
52. Stokes DC, McBride JT, Wall MA, Erba G, Strieder DJ. Sleep hypoxemia in young adults with cystic fibrosis. Am J Dis Child 1980;134(8):741-743.
53. Petrie A, Sabin C. Medical statistics at a glance. Massachusetts: Blackwell Publisher; 2005.
54. Rosenfeld M. An overview of endpoints for cystic fibrosis clinical trials: one size does not fit all. Proc Am Thorac Soc 2007;4(4):299-301.
55. Emond MJ. Statistical Power, Reproducibility, and Accuracy Considerations for Computed Tomography Scores as an Endpoint in Cystic Fibrosis Clinical Trials. Proc Am Thorac Soc 2007;4(4):E1-E6.
56. Ramsey BW. Outcome measures for development of new therapies in cystic fibrosis: are we making progress and what are the next steps? Proc Am Thorac Soc 2007;4(4):367-369.
57. Homnick DN. Making airway clearance successful. Paediatr Respir Rev 2007;8(1):40-45.
58. Elkins MR, Jones A, van der Schans C. Positive expiratory pressure physiotherapy for airway clearance in people with cystic fibrosis. Cochrane Database Syst Rev 2006(2):CD003147.
59. Main E, Prasad A, Schans C. Conventional chest physiotherapy compared to other airway clearance techniques for cystic fibrosis. Cochrane Database Syst Rev 2005(1):CD002011.
60. van der Schans CP, Postma DS, Koeter GH, Rubin BK. Physiotherapy and bronchial mucus transport. Eur Respir J 1999;13(6):1477-1486.
61. Tiddens HA. Detecting early structural lung damage in cystic fibrosis. Pediatr Pulmonol 2002;34(3):228-231.
62. Cochrane GM, Webber BA, Clarke SW. Effects of sputum on pulmonary function. Br Med J 1977;2(6096):1181-1183.
63. Weller PH, Bush E, Preece MA, Matthew DJ. Short-term effects of chest physiotherapy on pulmonary function in children with cystic fibrosis. Respiration 1980;40(1):53-56.
64. Kerrebijn KF, Veentjer R, Bonzet-vd Water E. The immediate effect of physiotherapy and aerosol treatment on pulmonary function in children with cystic fibrosis. Eur J Respir Dis 1982;63(1):35-42.
65. Carter ER, Stecenko AA, Pollock BH, Jaeger MJ. Evaluation of the interrupter technique for the use of assessing airway obstruction in children. Pediatr Pulmonol 1994;17(4):211-217.
66. Merkus P, Arets H, Joosten T, et al. Measurements of interrupter resistance: reference values for children 3-13 years of age. Eur Respir J 2002:907-911.
67. Merkus PJ, Mijnsbergen JY, Hop WC, de Jongste JC. Interrupter resistance in preschool children: measurement characteristics and reference values. Am J Respir Crit Care Med 2001;163(6):1350-1355.
68. Bradley S, Solin P, Wilson J, et al. Hypoxemia and hypercapnia during exercise and sleep in patients with cystic fibrosis. Chest 1999;116(3):647-654.
69. Tepper RS, Skatrud JB, Dempsey JA. Ventilation and oxygenation changes during sleep in cystic fibrosis. Chest 1983;84(4):388-393.
70. Milross MA, Piper AJ, Norman M, et al. Low-flow oxygen and bilevel ventilatory support: effects on ventilation during sleep in cystic fibrosis. Am J Respir Crit Care Med 2001;163(1):129-134.

71. Frangolias DD, Wilcox PG. Predictability of oxygen desaturation during sleep in patients with cystic fibrosis : clinical, spirometric, and exercise parameters. Chest 2001;119(2):434-441.
72. Milross MA, Piper AJ, Dobbin CJ, Bye PT, Grunstein RR. Sleep disordered breathing in cystic fibrosis. Sleep Med Rev 2004;8(4):295-308.
73. Betancourt M, Slade G, Dinwiddie R. Oxygen saturation in cystic fibrosis. Arch Dis Child 1991;66(9):1075-1076.
74. Poets CF, Stebbens VA, Samuels MP, Southall DP. Oxygen saturation and breathing patterns in children. Pediatrics 1993;92(5):686-690.
75. Urschitz MS, Wolff J, Von Einem V, et al. Reference values for nocturnal home pulse oximetry during sleep in primary school children. Chest 2003;123(1):96-101.
76. Moss D, Urschitz MS, von Bodman A, et al. Reference values for nocturnal home polysomnography in primary schoolchildren. Pediatr Res 2005;58(5):958-965.
77. Verhulst SL, Schrauwen N, Haentjens D, et al. Reference values for sleep-related respiratory variables in asymptomatic European children and adolescents. Pediatr Pulmonol 2007;42(2):159-167.
78. Darracott C, McNamara PS, Pipon M, et al. Towards the development of cumulative overnight oximetry curves for children with cystic fibrosis. Journal of Cystic Fibrosis 2004;3(Suppl 1):S53.
79. Gries RE, Brooks LJ. Normal oxyhemoglobin saturation during sleep. How low does it go? Chest 1996;110(6):1489-1492.
80. Uliel S, Tauman R, Greenfeld M, Sivan Y. Normal polysomnographic respiratory values in children and adolescents. Chest 2004;125(3):872-878.
81. Versteegh FG, Bogaard JM, Raatgever JW, et al. Relationship between airway obstruction, desaturation during exercise and nocturnal hypoxaemia in cystic fibrosis patients. Eur Respir J 1990;3(1):68-73.
82. Uyan ZS, Ozdemir N, Ersu R, et al. Factors that correlate with sleep oxygenation in children with cystic fibrosis. Pediatr Pulmonol 2007;42(8):716-722.
83. Gulmans VAM. Exercise in Children with cystic fibrosis; evaluation parameters and training effects. Utrecht: University of Utrecht; 1996.
84. Nixon PA. Role of exercise in the evaluation and management of pulmonary disease in children and youth. Med Sci Sports Exerc 1996;28(4):414-420.
85. Klijn PH, van Stel HF, Quittner AL, et al. Validation of the Dutch cystic fibrosis questionnaire (CFQ) in adolescents and adults. J Cyst Fibros 2004;3(1):29-36.
86. Abbott J, Gee L. Quality of life in children and adolescents with cystic fibrosis: implications for optimizing treatments and clinical trial design. Paediatr Drugs 2003;5(1):41-56.
87. Henry B, Aussage P, Grosskopf C, Goehrs JM. Development of the Cystic Fibrosis Questionnaire (CFQ) for assessing quality of life in pediatric and adult patients. Qual Life Res 2003;12(1):63-76.
88. Bregnballe V, Thastum M, Lund LD, et al. Validation of the Danish version of the revised cystic fibrosis quality of life questionnaire in adolescents and adults (CFQ-R14+). J Cyst Fibros 2008;7(6):531-536.
89. Quittner AL, Buu A, Messer MA, Modi AC, Watrous M. Development and validation of The Cystic Fibrosis Questionnaire in the United States: a health-related quality-of-life measure for cystic fibrosis. Chest 2005;128(4):2347-2354.
90. McCoy KS, Quittner AL, Oermann CM, et al. Inhaled aztreonam lysine for chronic airway Pseudomonas aeruginosa in cystic fibrosis. Am J Respir Crit Care Med 2008;178(9):921-928.
91. Widdicombe J, Fontana G. Cough: what's in a name? Eur Respir J 2006;28(1):10-15.
92. Ferkol T, Rosenfeld M, Milla CE. Cystic fibrosis pulmonary exacerbations. J Pediatr 2006;148(2):259-264.
93. Rosenfeld M, Emerson J, Williams-Warren J, et al. Defining a pulmonary exacerbation in cystic fibrosis. J Pediatr 2001;139(3):359-365.

94. Hsu JY, Stone RA, Logan-Sinclair RB, Worsdell M, Busst CM, Chung KF. Coughing frequency in patients with persistent cough: assessment using a 24 hour ambulatory recorder. Eur Respir J 1994;7(7):1246-1253.
95. Chang AB, Newman RG, Carlin JB, Phelan PD, Robertson CF. Subjective scoring of cough in children: parent-completed vs child- completed diary cards vs an objective method. Eur Respir J 1998;11(2):462-466.
96. Decalmer SC, Webster D, Kelsall AA, et al. Chronic Cough: How Do Cough Reflex Sensitivity And Subjective Assessments Correlate With Objective Cough Counts During Ambulatory Monitoring? Thorax 2006.
97. Chang AB, Newman RG, Phelan PD, Robertson CF. A new use for an old Holter monitor: an ambulatory cough meter. Eur Respir J 1997;10(7):1637-1639.
98. Munyard P, Busst C, Logan-Sinclair R, Bush A. A new device for ambulatory cough recording. Pediatr Pulmonol 1994;18(3):178-186.
99. Smith JA, Earis JE, Woodcock A. Establishing a gold standard for manual cough counting: video versus digital audio recordings. Cough 2006;2(1):6.
100. Arias Llorente RP, Bousono Garcia C, Diaz Martin JJ. Treatment compliance in children and adults with Cystic Fibrosis. J Cyst Fibros 2008;7(5):359-367.
101. Smith BA, Wood BL. Psychological factors affecting disease activity in children and adolescents with cystic fibrosis: medical adherence as a mediator. Curr Opin Pediatr 2007;19(5):553-558.
102. La Greca A, Schuman WB. Adherence to prescribed medical regimens. In: Roberts MC, editor. Handbook of pediatric psychology. Volume 2nd. New York: Guilford Press; 1995. p 55-83.
103. Passero MA, Remor B, Salomon J. Patient-reported compliance with cystic fibrosis therapy. Clin Pediatr (Phila) 1981;20(4):264-268.
104. Modi AC, Lim CS, Yu N, et al. A multi-method assessment of treatment adherence for children with cystic fibrosis. J Cyst Fibros 2006;5(3):177-185.
105. Myers LB, Horn SA. Adherence to chest physiotherapy in adults with cystic fibrosis. J Health Psychol 2006;11(6):915-926.
106. Zindani GN, Streetman DD, Streetman DS, Nasr SZ. Adherence to treatment in children and adolescent patients with cystic fibrosis. J Adolesc Health 2006;38(1):13-17.
107. Koocher GP, McGrath ML, Gudas LJ. Typologies of nonadherence in cystic fibrosis. J Dev Behav Pediatr 1990;11(6):353-358.
108. Bernard RS, Cohen LL. Increasing adherence to cystic fibrosis treatment: a systematic review of behavioral techniques. Pediatr Pulmonol 2004;37(1):8-16.
109. Modi AC, Quittner AL. Barriers to treatment adherence for children with cystic fibrosis and asthma: what gets in the way? J Pediatr Psychol 2006;31(8):846-858.
110. Gudas LJ, Koocher GP, Wypij D. Perceptions of medical compliance in children and adolescents with cystic fibrosis. J Dev Behav Pediatr 1991;12(4):236-242.
111. Ievers CE, Brown RT, Drotar D, et al. Knowledge of physician prescriptions and adherence to treatment among children with cystic fibrosis and their mothers. J Dev Behav Pediatr 1999;20(5):335-343.

Chapter 2

Outline of the thesis

2.1. TIMING RHDNASE AND AIRWAY CLEARANCE THERAPY

The chapters 3 and 4 describe the studies that are focussed on the timing of rhDNase nebulisation in relation to ACT.
In chapter 3 we describe a study in 25 children with stable CF lung disease that investigates whether inhalation of rhDNase after ACT increases peripheral airway patency more than in the case of inhalation of rhDNase before ACT.
In chapter 4 we describe a study in another group of 25 children with stable CF lung disease. In this study we compare the efficacy and possible side effects of nebulisation of rhDNase before sleep to those of nebulisation after waking up. Efficacy was evaluated with pulmonary function tests. Side effects were monitored using nocturnal oxygen saturation and cough registration.

2.2. OUTCOME MEASURES IN CF LUNG DISEASE

Chapters 5-7 describe studies aimed at developing outcome measures that can be used in ACT studies.
In chapter 5 we investigate cough frequency during two nights in children with clinically stable CF, and examine cough correlation between the two nights with an one week interval and between the cough frequency and oxygen saturation, pulmonary function and CT scores.
In chapter 6 we assess, during two nights, nocturnal saturation profiles in children with clinically stable CF and relate the saturation variables to lung function, cough frequency and structural lung abnormalities as established from CT scan.
In chapter 7 we perform a study to assess whether Rint is a feasible outcome measure for ACT studies.

2.3. PATIENT EDUCATION ON CF LUNG DISEASE

In chapter 8 we describe a study to test a newly developed educational board game – *'Airway'* – aimed at increasing children's knowledge about their CF lung disease. The level of knowledge was tested with a knowledge questionnaire.

Chapter 9 contains a summary of the research presented in this thesis. Chapter 10 contains the general discussion with recommendations for future research.

Chapter 3

RhDNase before airway clearance therapy improves airway patency in children with cystic fibrosis

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ABSTRACT

Introduction

Little is known about the optimal timing of rhDNase nebulization in relation to airway clearance therapy (ACT). Objective: To compare the effects of rhDNase before ACT versus rhDNase after ACT in children with CF.

Methods

Design: randomized, double blind, double dummy, cross over study. Inclusion criteria: CF, stable clinical condition, rhDNase maintenance therapy. Children in Group I inhaled rhDNase 30 minutes before ACT, and placebo directly after ACT in week 1–3. The protocol was reversed during week 4–6. Group II performed the reversed sequence. Patients continued their daily routine ACT. Primary endpoint: MEF_{25} %pred. Pulmonary function tests were performed on days 0, 14, 21, 35 and 42. In weeks 3 and 6 children scored cough and sputum production on daily diary cards.

Results

24 patients completed the study. Mean age 12 years (range 7–19). Mean MEF_{25} %pred was 5.8% higher after 3 weeks of rhDNase before ACT, compared to rhDNase after ACT (58.3% vs 52.5%, p=0.01). There were no significant differences for any of the other variables.

Conclusion

Inhalation of rhDNase before ACT improves peripheral airway patency in children with cystic fibrosis. Since all children were already on maintenance rhDNase therapy before the study, this effect is additional to any existing effect of regular rhDNase.

INTRODUCTION

Cystic fibrosis (CF) lung disease is characterized by the depletion of airway surface liquid and abnormal mucociliary transport. Retention of mucus is thought to favor bacterial overgrowth, which then triggers a cycle of repeated or chronic infections associated with intense neutrophilic airway inflammation.[1] This process causes progressive structural lung damage.[2] Eventually, most patients will then suffer premature death caused by respiratory failure.

Airway clearance therapy (ACT) and nebulisation of recombinant human deoxyribonuclease (rhDNase) are known to aid sputum evacuation. Approximately 90% of CF patients use some kind of ACT.[3] ACT effectively increases sputum mobilization, but the long-term efficacy in terms of outcome compared with unassisted cough alone is unknown.[4] CF sputum is abnormally hydrated, has increased tenacity[5] and contains large amounts of DNA.[6] The DNA is released by disintegrated microorganisms and disintegrated inflammatory cells, particularlyneutrophils.[7] High content of DNA correlates with an increased mucus viscosity.[8] The effectiveness of ACT in CF is counteracted by abnormally thick dehydrated sputum.[9] RhDNase cleaves extra cellular DNA and reduces sputum viscosity.[10] Daily treatment with rhDNase reduces the number of pulmonary exacerbations, improves pulmonary function, and is well-tolerated and safe in patients with mild, moderate, and severe CF.[11–15]

Little is known, however, on optimal timing of rhDNase nebulisation in relation to ACT. A survey of 54 CF centers revealed that half of the patients use rhDNase before, and half after ACT.[16] The Epidemiologic Registry of CF[17] showed that 51% of the patients used rhDNase in the morning, 37% in the evening; and 8% at variable moments; not specified in 3%.

Patients are generally advised to nebulise rhDNase minimally 30 min before ACT, because rhDNase increases the sputum pourability in a time dependent fashion; at 30 min the sputum is liquefied.[10] It may be advantageous, however, to nebulise rhDNase after ACT. ACT induced sputum mobilization is likely to improve large airway patency and this would improve rhDNase deposition in the small airways. Recently a double blind randomized placebo controlled cross over study was conducted in CF patients who were not on maintenance therapy with rhDNase. FEV_1 %pred after 2 weeks of rhDNase therapy did not significantly differ in between the use of rhDNase before or after ACT. Only for patients colonized persistently with pseudomonas rhDNase FEV_1 %pred improved when rhDNase was used after ACT.[18]

The aim of our study was to assess the difference in efficacy between nebulisation of rhDNase before ACT versus nebulisation after ACT in CF patients on maintenance therapy with rhDNase. We hypothesized that inhalation of rhDNase after ACT would increase peripheral airway patency, as reflected by the maximal expiratory flow at 25% of the forced vital capacity (FVC) (MEF_{25} %pred), more than inhalation of rhDNase 30 min before ACT.

MATERIALS AND METHODS

Study Subjects

Patients of the CF center at Erasmus Medical Centre-Sophia Children's Hospital were included in the study when they fulfilled the following criteria: proven CF, at least 5 years of age, ability to perform reproducible spirometry, daily ACT treatment, on maintenance treatment with rhDNase and clinical stability. The latter was defined as no need for intravenous antibiotics and no hospitalizations for at least 1 month prior to the study. CF was defined as clinical symptoms characteristic of CF plus an abnormal sweat test and/or by the presence of two CF mutations. We excluded children who used rhDNase more than once a day, those who were considered to have a poor adherence (<50% of treatments) to nebuliser treatment as judged by the CF team, or those who were mentally retarded. Throughout the study, subjects continued to receive their standard treatment.

Study Design

The study had a randomized, double blind, double dummy, cross over design. All subjects nebulized both rhDNase (2.5 mg of rhDNase in 2.5 ml buffered solution: 8.77 mg/ml sodium chloride and 0.15 mg/ml calcium chloride)[19] and a placebo (2.5 ml of a buffered solution: 8.77 mg/ml sodium chloride and 0.15 mg/ml calcium chloride) once daily for a period of 6 weeks. Placebo was similar to rhDNase in both color and taste. Subjects were randomized in two groups. In the first three weeks group I used rhDNase 30 min before ACT and placebo directly after ACT. In the following 3 weeks the order in which rhDNase and placebo were taken was reversed (Fig. 1). Group II used placebo 30 min before ACT and rhDNase after ACT in the first three weeks, and the reverse sequence thereafter. Weeks 1 and 2 and Weeks 4 and 5 were considered as wash-in and wash-out periods based on several studies.[12,14,20–22] Because patients were on maintenance treatment with rhDNase a true wash-out period without rhDNase was considered unethical and unpractical. Patients were asked not to change their routine ACT technique. Time of the day during which nebulisation and ACT were applied, was kept constant throughout the study.
Placebo and rhDNase were administered using a Sidestream nebuliser (Respironics, Murrysville, PA) and one of the following compressors: Portaneb, Freeway Lite,- Freedom or CR60 (Respironics, Murrysville, PA).
The Erasmus MC Medical Ethical Review Board approved the protocol. The study was performed according to ICH-GCP guidelines.

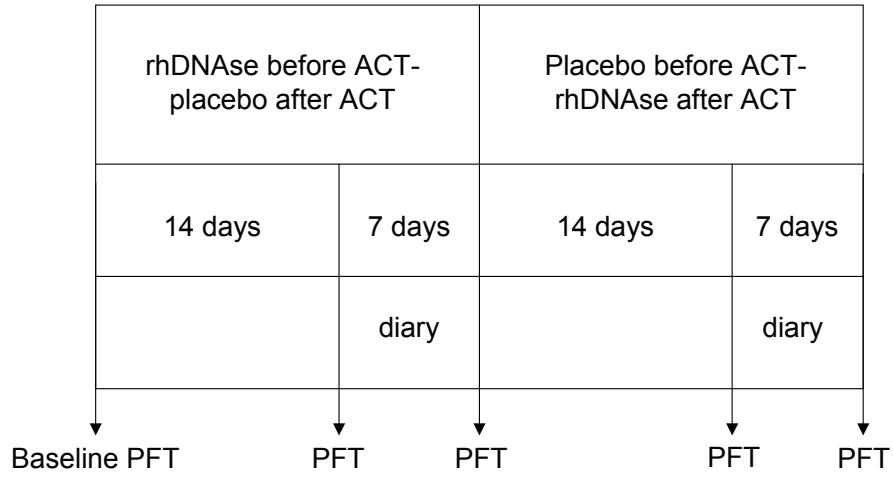

Figure 1. Study design and time points for primary and secondary endpoint measurements for children in group I. In group II the order of the rhDNase and the placebo was reversed.

Equipment Specifications

Pulmonary function tests (PFT) were carried out using a handheld spirometer (MicroLoop, Micro Medical Ltd., Rochester, UK). The following PFT results were obtained: forced expiratory volume in 1 sec (FEV_1 %pred); FVC %pred; mean forced expiratory flow between 25 and 75% of the FVC ($FEF_{25–75}$ %pred) and MEF_{25} %pred. The PFT results were expressed as a percentage of predicted values.[23] Interrupter resistance (Rint) (kPa/L/sec) was measured using the MicroRint (Micro Medical Ltd.). Calibrations of flow were carried out using a 1 L precision pump.

Procedures

PFTs were carried out at the hospital or at the patient's home. Weight and height were measured on Day 0. Rint and spirometry were performed on Days 0, 14, 21, 35, and 42. Rint was always measured first. Throughout the study, measurements were taken approximately at the same time of day and with the same equipment. Before lung function measurements children were asked to clear their throat by coughing. For Rint measurements, children were instructed to sit upright although breathing quietly. The head was positioned in slight extension. The hands of the investigator supported the patient's cheeks and chin to reduce upper airway compliance.[24] One Rint measurement was demonstrated by the investigator before the actual measurements, so as to familiarize children with the sound of the shutter. A minimum of five correct tracings (maximal 10) was obtained, that is, a Rint recording was accepted for further analysis if breathing had

been regular and if the mouth pressure–time curve was consistent of shape.[25] The expiratory Rint ($Rint_e$) was measured, because expiratory interruptions are more sensitive in detecting airway obstruction relative to inspiratory interruptions.[26] Spirometry was performed in triplicate according to ERS guidelines.[27]

Patients continued their medication, such as Tobramycin solution for inhalation (TOBI) and inhaled steroids, throughout the study period. None were using other mucolytics or hypertonic saline during the study.

Patients were asked to keep empty drug vials for vial count. The children were asked to keep a diary during the 3rd and 6th week. Cough frequency during day and nighttime were recorded with the cough symptom score (CSS) (Table 1).[28] Sputum viscosity, sputum production and cough frequency were recorded on a visual analogue scale (VAS).[28] A VAS is a horizontal line, 100 mm in length, anchored by word descriptors at each end. The patients marked on the line the point that represented their perception of symptom severity.

Table 1. Cough Symptom Score

Daytime	
0	no cough
1	cough for one or two short periods
2	cough for more than two short periods
3	frequent cough not interfering with normal activities
4	frequent cough interfering with school or other activities
5	distressing cough most of the day
Night time	
0	no cough
1	cough on waking only/ cough on going to sleep only
2	awoken once or woken early due to coughing
3	frequent waking due to coughing
4	frequent coughs most of the night
5	distressing cough

Data Analysis

Statistical analysis was performed with SPSS version 10.0.

According to the protocol the primary outcome variable was MEF_{25} %pred at the end of each treatment period. Secondary variables were FVC %pred, FEV_1 %pred, $FEF_{25–75}$ %pred, $Rint_e$, and diary scores.

Outcome variables were compared using the paired *t*-test after ensuring that there were no significant carry-over or period effects. Mean diary data for the 3rd and 6th week were analyzed.

In order to obtain a near-normal distribution it was necessary to logarithmically transform the following variables: $Rint_e$, VAS viscosity, VAS sputum amount, and VAS night time coughing. For these latter variables median scores are presented.
With the number of 25 patients the power of the primary outcome variable (MEF_{25} %pred) would be greater than 80% for an effect-size (difference of means/standard deviation) of 0.8 at an alpha (two-sided) of 5%.

RESULTS

Twenty-five patients were enrolled in the study, 12 in group I and 13 in group II. One patient from group I developed a pulmonary exacerbation in the 2nd week and was excluded from the analysis. The mean adherence to study treatment, as calculated from the vial count, was 98 (range 83–100%). Characteristics of the study population, type of ACT and type of nebulisers are summarized in Tables 2 and 3. The groups did not differ for age, sex, and lung function at baseline (Table 2). No carry-over or period effect was observed for any of the endpoints. After 3 weeks 16 patients had a higher MEF_{25} %pred when they nebulized rhDNase before ACT, for one patient it made no difference and in seven patients MEF_{25} %pred had decreased. Overall there was a significant positive effect; MEF_{25} %pred was significantly higher after 3 weeks of nebulising rhDNase

Table 2. Characteristics of the study population (numbers of patients or mean with SD) at baseline for group 1 and 2. In the first three weeks group I used rhDNase 30 minutes before ACT and placebo directly after ACT. In the following three weeks the order in which rhDNase and placebo were taken was reversed. Group II used placebo 30 minutes before ACT and rhDNase after ACT in the first three weeks, and the reverse sequence thereafter.

	Group 1	**Group 2**	
N	11	13	NS
Sex (male/female)	6 / 5	7 / 6	NS
Age (year)	11 (3)	12 (4)	NS
FVC (% predicted)	93 (14)	93 (11)	NS
FEV_1 (% predicted)	88 (16)	88 (11)	NS
MEF_{25} (% predicted)	56 (27)	57 (25)	NS
$FEF_{25-75\%}$ (% predicted)	73 (34)	66 (18)	NS
$Rint_e$ (kPa/L/s	0.6 (0.34)	0.6 (0.22)	NS
Use of TOBI (n)	7	6	NS
Use of steroids (n)	3	6	NS

Table 3. ACT treatment and nebuliser use before and during the study.

ACT (n / %)	
PEP mask	18 / 75%
Flutter	3 / 13%
Active cycle of breathing therapy	1 / 4%
Autogenic Drainage	1 / 4%
Combination	1 / 4%
Nebuliser (n / %)	
Portaneb	17 / 71%
Freeway Lite	3 / 12.5%
Freeway Freedom	3 / 12.5%
CR 60	1 / 4%

before ACT, compared to nebulising after ACT (58.3% vs. 52.5% predicted, P=0.01) (Fig. 2). There were no significant differences in any other endpoints (Tables 4 and 5). Twenty one percent of the patients performed nebulisation and ACT in the morning, 21% in the afternoon, and 58% in the evening. The time of the day nebulisation and ACT were performed did neither correlate with the MEF_{25} %pred nor with the cough scores.

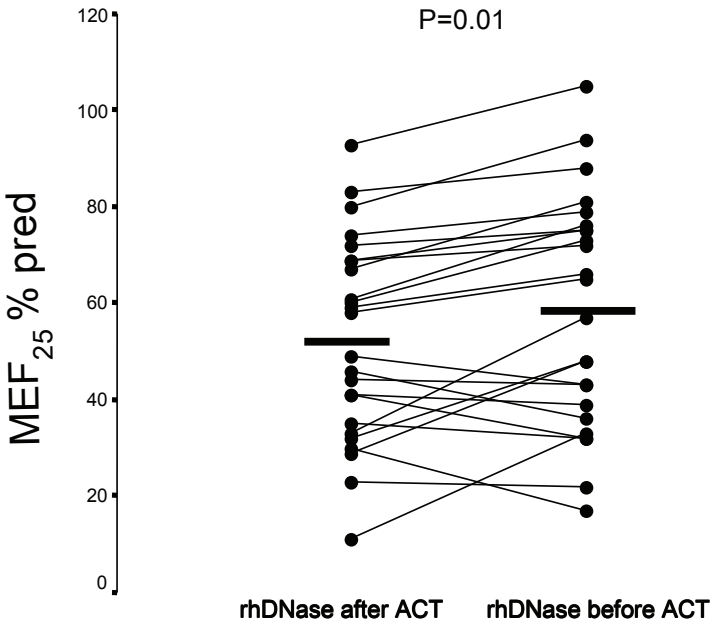

Figure 2. MEF_{25}% pred according to nebulisation of rhDNase after and before ACT after 3 weeks. Lines connect individual data points. Bars represent mean values.

Table 4. Mean or median(*) lung function (%predicted and kPa-L^{-1}.s for $Rint_e$) with range after two and three weeks rhDNase before or after ACT. ‡= p<0.05.

	FVC%	FEV_1%	$MEF_{25\%}$	$FEF_{25-75\%}$	$RINT_e$*
After 2 weeks					
RhDNase after ACT	94.8 (76-131)	89.5 (68-116)	54.2 (18-104)	68.1 (36-118)	0.58 (0.24-1.38)
RhDNase before ACT	92.5 (72-123)	87.1 (57-107)	54.1 (8-103)	67.5 (19-129)	0.55 (0.19-1.84)
p- value	0.12	0.18	0.98	0.84	0.06
After 3 weeks					
RhDNase after ACT	94.1 (75-131)	88.3 (67-105)	52.5 (11-93)	66.6 (36-112)	0.50 (0.21-1.36)
RhDNase before ACT	93.3 (75-127)	89.4 (66-113)	58.3 (17-105)	71.2 (31-129)	0.61 (0.2-2.26)
p-value	0.65	0.58	0.01 ‡	0.07	0.08

Table 5. Mean or median(*) with range diary scores after three weeks rhDNase before or after ACT.

	rhDNase before ACT	rhDNase after ACT	P-value(paired *t*-test)
VAS viscosity*	1.3 (0.2 - 8.4)	1.3 (0.2 - 8.4)	0.73
VAS sputum amount*	0.7 (0 - 5.1)	1.1 (0.1- 6.6)	0.14
VAS day time coughing	1.7 (0 – 5.3)	1.9 (1,0 - 7.4)	0.51
VAS night time coughing*	0.1 (0 - 3.4)	0.2 (0 - 5.7)	0.14
CSS day time	1.4 (0 - 3)	1.6 (0 - 3.1)	0.28
CSS night time	0.4 (0 - 2)	0.7 (0 - 2.6)	0.06

DISCUSSION

This randomized, double blind, double dummy, cross over study aimed at assessing the difference in efficacy between nebulisation of rhDNase before ACT versus nebulisation after ACT. We found that the primary endpoint MEF_{25} %pred after 3 weeks was significantly higher for the before mode. Other lung function parameters did not show significant differences.

So our results suggest improved efficacy of rhDNase when inhaled before ACT, thus refuting our hypothesis that nebulisation after ACT would be more advantageous.

This hypothesis was based on the concept that ACT clean out the large airways in particular. Various studies have shown that the presence of mucus in central airways favors central airway deposition and reduces peripheral deposition of medical aerosols.[29–31] Hence, we expected more efficient peripheral airway deposition when rhDNase would be inhaled after ACT.

Several explanations may account for our result. First, ACT done before nebulisation of rhDNase might mobilize sputum in cartilaginous airways towards the more central airways. Larger amounts of mucus in central airways will lower the amounts of rhDNase

delivered to the peripheral airways and thus reduce its effectiveness to clean out peripheral airways. Second, it has been suggested that ACT may cause partial collapse of the central airways, which would exert immediate negative effects on ventilatory function.[32] Any reduction in the cross sectional area of central airways is likely to increase turbulent airflow and increase impaction of airway particles at these sites.[33] In this case, nebulisation of rhDNase immediately after ACT will result in more rhDNase being delivered to the central and not to the peripheral airways. A third, more likely explanation is that displacement of liquefied sputum in the peripheral compartment does not occur efficiently when rhDNase nebulisation is not followed by ACT. The liquefying effect of rhDNase reaches it maximal impact 30 min after nebulisation.[10] This might therefore be the optimal moment to perform ACT. Therefore, our data suggest that ACT 30 min after rhDNase is needed to remove liquefied sputum from especially the peripheral compartment and to prevent liquefied sputum from obstructing more peripheral airways. Because CF lung disease affects small airways at an early stage, these are an important target for treatment. In the Pulmozyme Early Intervention Study (PEIT), involving rhDNase-naïve patients with normal FVC, but reduced $FEF_{25–75}$ %pred, improved $FEF_{25–75}$ %pred by 8%. This suggests that rhDNase improves clearance of small airways, especially in early lung disease.[13]

The PEIT data favor the idea to deposit as much rhDNase as possible in the peripheral airways. As discussed before, nebulisation of rhDNase should preferably take place when central airways are cleanest. To our surprise we could not find any studies that investigated the diurnal variation of expectorated sputum. We assume that lungs most likely will be cleaner in the afternoon because exercise is thought to facilitate sputum mobilisation. This assumption, however, should be further investigated.

As expected, we did not observe a significant change in FEV_1 %pred in accordance with a recent other rhDNase timing study.[18] FEV_1 and FVC are considered insensitive markers in early lung disease compared to MEF_{25} %pred.[34] The latter is more sensitive to changes in the peripheral compartment than are FVC and FEV_1.[34] The $FEF_{25–75}$ %pred showed a trend similar to the MEF_{25} %pred. These two parameters are both thought to reflect the condition of the peripheral compartment. However, $FEF_{25–75}$ %pred is the mean flow value exhaling from 75 to 25% of vital capacity, is thought to be less sensitive for geometrical changes in the peripheral compartment than MEF_{25} %pred. In the PEIT study the treatment effect on FEV_1 %pred in early lung disease was only 3% (P=0.006) and thus substantially smaller than the 8% (P=0.0008) observed for the $FEF_{25–75}$ %pred.[13] The 6% difference in MEF_{25} %pred between the two regimens in the present study was substantial, considering that all patients were on maintenance rhDNase therapy. Timing of rhDNase in the PEIT study was not standardized. We speculate the treatment effect in the PEIT study would have been greater when all patients would have used rhDNase before ACT.

Our results showed a treatment effect after 3 but not after 2 weeks. This is in accordance with the findings of the rhDNase timing study by Fitzgerald et al.,[18] who studied 52 CF patients and did not find a difference in FEF_{25-75} %pred after 2 weeks. The total cross-sectional area of the small airways is several orders of magnitude greater than the total cross-sectional area of the large airways.[35] We speculate that more time is needed to clean a sufficient large number of small airways and obtain the optimum effect. Persistence of MEF_{25} %pred improvement over a longer period of time needs to be addressed in future studies. Though patients were randomly selected from our CF population, they had relatively well preserved lung function. Further studies are needed to determine whether our findings extend to patients with moderate and severe CF lung disease as well. A recent study in patients who were transplanted for end stage lung disease showed that a large percentage of small airways are obstructed by mucus.[36] This suggests that patients with more advanced disease might benefit from effective strategies to treat the peripheral airways. Based on our results we recommend ACT should be done 30 min after inhalation of rhDNase.

In conclusion, the findings from this study favor nebulisation of rhDNase before ACT in children with mild CF lung disease who are on maintenance treatment with rhDNase.

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REFERENCES

1. Ratjen F. Restoring airway surface liquid in cystic fibrosis. N Engl J Med 2006;354:291–293.
2. de Jong PA, Nakano Y, Lequin MH, et al. Progressive damage on high resolution computed tomography despite stable lung function in cystic fibrosis. Eur Respir J 2004;23:93–97.
3. Konstan MW, Butler SM, Schidlow DV, et al. Patterns of medical practice in cystic fibrosis: Part II. Use of therapies. Investigators and coordinators of the Epidemiologic Study of Cystic Fibrosis. Pediatr Pulmonol 1999;28:248–254.
4. McCool FD, Rosen MJ. Nonpharmacologic airway clearance therapies: ACCP evidence-based clinical practice guidelines. Chest 2006;129:250S–259S.
5. ush A, Payne D, Pike S, et al. Mucus properties in children with primary ciliary dyskinesia: Comparison with cystic fibrosis. Chest 2006;129:118–123.
6. Henke MO, Renner A, Huber RM, Seeds MC, Rubin BK. MUC5AC and MUC5B mucins are decreased in cystic fibrosis airway secretions. Am J Respir Cell Mol Biol 2004; 31:86–91.
7. Costello CM, O'Connor CM, Finlay GA, et al. Effect of nebulised recombinant DNase on neutrophil elastase load in cystic fibrosis. Thorax 1996;51:619-623.
8. ahm JM, Girod de Bentzmann S, et al. Dose-dependent in vitro effect of recombinant human DNase on rheological and transport properties of cystic fibrosis respiratory mucus. Eur Respir J 1995;8:381–386.
9. Ramsey BW, Smith AL. Reduced incidence of respiratory tract infections in cystic fibrosis patients using aerosolized dornase alfa. Dornase Alfa Clin Ser 1994:1–5.
10. Shak S, Capon DJ, Hellmiss R, Marsters SA, Baker CL. Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum. Proc Natl Acad Sci USA 1990;87:9188–9192.
11. Kearney CE, Wallis CE. Deoxyribonuclease for cystic fibrosis. Cochrane Database Syst Rev 2000; 2.
12. Fuchs HJ, Borowitz DS, Christiansen DH, et al. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. N Engl J Med 1994;331:637–642.
13. Quan JM, Tiddens HA, Sy JP, et al. A 2-year randomized, placebo-controlled trial of dornase alfa in young patients with cystic fibrosis with mild lung function abnormalities. J Pediatr 2001;139:813–820.
14. Ramsey BW, Astley SJ, Aitken ML, et al. Efficacy and safety of short-term administration of aerosolized recombinant human deoxyribonuclease in patients with cystic fibrosis. Am Rev Respir Dis 1993;148:145–151.
15. Hodson ME, McKenzie S, Harms HK, et al. Dornase alfa in the treatment of cystic fibrosis in Europe: A report from the Epidemiologic Registry of Cystic Fibrosis. Pediatr Pulmonol 2003;36:427–432.
16. Borsje P, de Jongste JC, Mouton JW, Tiddens HA. Aerosol therapy in cystic fibrosis: A survey of 54 CF centers. Pediatr Pulmonol 2000;30:368–376.
17. ERCF. ERCF Annual Report 1998: F. Hoffmann-La Roche Ltd; 2000; 19
18. Fitzgerald DA, Hilton J, Jepson B, Smith L. A crossover, randomized, controlled trial of dornase alfa before versus after physiotherapy in cystic fibrosis. Pediatrics 2005;116:e549–554.
19. Product monograph update 2. 2002; pp 3–65.
20. Shah PI, Bush A, Canny GJ, et al. Recombinant human DNase I in cystic fibrosis patients with severe pulmonary disease: A short-term, doubleblind study followed by 6 months open-label treatment. Eur Respir J 1995;8:954–958.
21. Shah PL, Scott SF, Knight RA, et al. In vivo effects of recombinant human DNase I on sputum in patients with cystic fibrosis. Thorax 1996;51:119–125.

22. McCoy K, Hamilton S, Johnson C. Effects of 12-week administration of dornase alfa in patients with advanced cystic fibrosis lung disease. Pulmozyme Study Group. Chest1996;110:889–895.
23. Zapletal A. Lung function in children and adolescents. Zapletal A, Samanek M, Paul T, editors. Basel: Karger 1987.
24. Hadjikoumi I, Hassan A, Milner AD. Effects of respiratory timing and cheek support on resistance measurements, before and after bronchodilation in asthmatic children using the interrupter technique. Pediatr Pulmonol 2003;36:495–501.
25. Bridge PD, Ranganathan S, McKenzie SA. Measurement of airway resistance using the interrupter technique in preschool children in the ambulatory setting. Eur Respir J 1999;13:792–796.
26. Merkus PJ, Mijnsbergen JY, Hop WC, de Jongste JC. Interrupter resistance in preschool children: Measurement characteristics and reference values. Am J Respir Crit Care Med 2001;163:1350–1355.
27. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J 2005;26:319–338.
28. Chang AB, Newman RG, Carlin JB, Phelan PD, Robertson CF. Subjective scoring of cough in children: Parent-completed versus child-completed diary cards versus an objective method. Eur Respir J 1998;11:462–466.
29. Regnis JA, Robinson M, Bailey DL, et al. Mucociliary clearance in patients with cystic fibrosis and in normal subjects. Am J Respir Crit Care Med 1994;150:66–71.
30. Robinson M, Eberl S, Tomlinson C, et al. Regional mucociliary clearance in patients with cystic fibrosis. J Aerosol Med 2000;13:73–86.
31. Labiris NR, Dolovich MB. Pulmonary drug delivery. Part I: Physiological factors affecting therapeutic effectiveness of aerosolized medications. Br J Clin Pharmacol 2003;56:588–599.
32. Zapletal A, Stefanova J, Horak J, Vavrova V, Samanek M. Chest physiotherapy and airway obstruction in patients with cystic fibrosis—a negative report. Eur J Respir Dis 1983;64:426–433.
33. Stites SW, Perry GV, Peddicord T, et al. Effect of high-frequency chest wall oscillation on the central and peripheral distribution of aerosolized diethylene triamine pentaacetic acid as compared to standard chest physiotherapy in cystic fibrosis. Chest 2006;129:712–717.
34. Tiddens HA. Detecting early structural lung damage in cystic fibrosis. Pediatr Pulmonol 2002;34:228–231.
35. Macklem PT. The physiology of small airways. Am J Respir Crit Care Med 1998;157:S181–183.
36. Burgel PR, Montani D, Danel C, Dusser DJ, Nadel JA. A morphometric study of mucins and small airway plugging in cystic fibrosis. Thorax 2007;62:153–161.

Chapter 4

Recombinant human DNase nebulisation in children with cystic fibrosis: before bedtime or after waking up?

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ABSTRACT

The present study focused on patients with cystic fibrosis (CF), who were on maintenance therapy with recombinant human deoxyribonuclease (rhDNase), with the aim of comparing efficacy and possible side effects of nebulisation of rhDNase when taken before bedtime with efficacy and side effects when taken after waking up.

A randomised, double-blind, double-dummy, crossover study group was used. The inclusion criteria were as follows: 1) CF, 2) stable clinical condition and 3) rhDNase maintenance therapy. Patients in group I inhaled rhDNase before bedtime and a placebo after waking up in weeks 1–2. The protocol was reversed during weeks 3–4. Group II patients performed the reverse of this sequence. Patients continued with their daily routine sputum expectoration. The primary endpoint was classified as the maximal instantaneous forced flow when 25% of the forced vital capacity remained to be exhaled ($MEF_{25\%}$). Pulmonary functions tests were performed on days 0, 7, 14, 21 and 28. At 1, 2, 3 and 4 weeks arterial oxygen saturation and cough frequency were measured during the night.

A total of 24 patients completed the study. The mean (range) age of the patients was 13 (6–19) yrs. $MEF_{25\%}$, taken to be the primary endpoint, did not show a significant difference between nebulisation of rhDNase before bedtime compared with when taken after waking up. Nocturnal cough, oxygen saturation, and other secondary endpoints were not significantly different between the two study periods.

In conclusion, the present study found that it is equally effective and safe to nebulise recombinant human deoxyribonuclease before bedtime compared with when performed after waking up in children with cystic fibrosis, who are on maintenance treatment with recombinant human deoxyribonuclease.

INTRODUCTION

Cystic fibrosis (CF) lung disease is characterised by excess mucus production and impaired mucociliary clearance.[1] This causes the airways to become chronically infected with micro-organisms, leading to chronic airway inflammation and progressive structural lung damage.[2] CF sputum contains large amounts of extracellular DNA released by disintegrated inflammatory cells, particularly neutrophils.[3,4] Patients need daily treatment to reduce the amount of mucus in the airways, though they tend to feel this is a great burden.[5] Airway clearance therapy (ACT) and nebulisation of recombinant human deoxyribonuclease (rhDNase) are the most frequently used methods to mobilise sputum. The rhDNase cleaves extracellular DNA and reduces sputum viscosity, transforming it from a nonflowing viscous gel into a flowing liquid.[6] Daily treatment with rhDNase reduces the number of pulmonary exacerbations, improves pulmonary function, is tolerated well and is safe in mild, moderate and severe CF cases.[7-11] Although the effectiveness of rhDNase nebulisation is well established, little is known regarding optimal timing. The Epidemiologic Registry of Cystic Fibrosis reported that 51% of patients use rhDNase in the morning, 37% use it in the evening and 8% use it at variable times; timing was not specified for 3%.[12]

Theoretically, it could be effective and time efficient for patients to nebulise rhDNase before bedtime. It would allow sufficient time during sleep for rhDNase to act upon the free DNA present in the airway lumen. Its half-life in rodents was found to be 11 h after inhalation.[13] Secondly, postural shifts during sleep might act like postural drainage therapy; allowing gravity-dependent mobilisation of the sputum. Adults were found to have an average of 16 position shifts per night .[14] Postural drainage is reported to be effective when relatively large quantities of mucus of low adhesion are present in the airways.[15] Following nebulisation of rhDNase the mucus will become less viscous. Postural drainage during sleep is therefore likely to improve mucociliary clearance.

Alternatively, there are several theoretical arguments against the nebulisation of rhDNase before bedtime. There are indications that mucociliairy clearance is depressed during sleep in normal subjects[16] and in subjects with asthma[17]. However, in CF patients, the dehydrated secretions are capable of triggering coughing spells during sleep.[18] Secondly, nightly spontaneous cough might be less effective to expectorate the sputum relative to daytime cough or active ACT. Hence, bedtime nebulisation of rhDNase might be detrimental to lung function. Thirdly, nebulisation-induced cough increased during sleep might affect sleep quality.[18] Finally, nebulisation of rhDNase before bedtime might induce ventilation/perfusion mismatch due to additional occlusion of peripheral airways by the more liquid mucus. However, this has never been systematically studied.

The current authors conducted a study in children with CF on maintenance therapy with rhDNase, comparing the efficacy and possible side effects of nebulisation before bedtime with those of nebulisation after waking up. Efficacy was evaluated using pul-

monary function tests. Side effects were monitored using nocturnal oxygen saturation and cough frequency. The present authors hypothesised that nebulisation of rhDNase before bedtime would be safe and more effective in improving peripheral airway flow when compared with nebulisation after waking up.

MATERIAL AND METHODS

Study subjects

Children attending the CF centre in the Erasmus MC, Sophia Children's Hospital (Rotterdam, the Netherlands) were considered eligible for inclusion in the present study when they fulfilled the following criteria: 1) proven CF; 2) minimum age of 5 yrs; 3) the ability to perform reproducible spirometry, forced vital capacity (FVC) >40%; 4) daily ACT, 5) maintenance treatment with rhDNase; and 6) clinical stability. The latter was defined as no need for intravenous antibiotics and no hospitalisations for a minimum of 1 month prior to the study. CF was defined as clinical symptoms characteristic for CF plus an abnormal sweat test and/or the presence of two CF mutations. The current authors excluded children who used rhDNase more than once a day and were considered to have poor therapy compliance (<50% of treatments) as judged by the CF team; or those with learning difficulties.
A total of 152 CF patients were identified. Of these, 49 patients did not meet the study inclusion criteria. Most patients were excluded on the basis that they were too young to perform spirometry. From the remaining 103 patients, 43 were randomly selected and invited to participate. Throughout the study, subjects continued to receive their standard treatment.

Study design

The study had a randomised, double-blind, double-dummy, crossover design. All subjects nebulised both rhDNase (2.5 mg of rhDNase in 2.5 mL buffered solution: 8.77 mg•mL^{-1} NaCl and 0.15 mg•mL^{-1} CaCl)[19] and a placebo (2.5 mL of a buffered solution: 8.77 mg•mL^{-1} NaCl and 0.15 mg•mL^{-1} CaCl) for a period of 4 weeks. Placebo was similar to rhDNase in both colour and taste. Subjects were randomised into one of two groups. Group I used rhDNase before bedtime and placebo in the morning directly after waking up in the first 2 weeks. This order was reversed in the following two weeks (fig. 1). Group II used placebo before bedtime and rhDNase after waking up in the 2 weeks and in the reverse sequence thereafter.
Based on previous studies, weeks 1 and 3 were considered wash-in and wash-out periods.[10,20,21] As patients were on maintenance treatment with rhDNase, a true wash-out

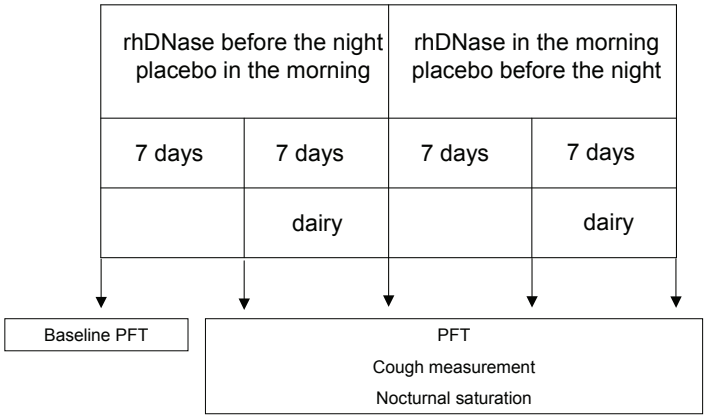

Figure 1. Study design and time points for the primary and secondary endpoint measurements for children in group I. In group II the order of the recombinant human deoxyribonuclease (rhDNase) and the placebo were reversed. PFT: pulmonary function test.

period without rhDNase was considered unethical and unpractical. Patients continued to perform routine ACT during the study period. ACT in the morning was performed 30 min after nebulisation.

Placebo and rhDNase were administered using a Sidestream nebuliser (Respironics, Murrysville, PA, USA) and one of the following compressors: Portaneb, Freeway Lite, Freeway Freedom or CR60 (all Respironics).

The present authors selected the maximal instantaneous forced flow when 25% of the FVC remained to be exhaled ($MEF_{25\%}$) as the primary endpoint, as it is more sensitive to changes in the peripheral compartment in early lung disease compared with FVC and forced expiratory volume in one second (FEV_1).[22]

The study was conducted between September 2005 and June 2006. The Erasmus MC Medical Ethical Review Board approved the protocol. The study was performed according to the International Conference on Harmonisation and World Health Organization Good Clinical Practice standards guidelines.[23]

Equipment specifications

Pulmonary function tests (PFTs) were carried out using a handheld spirometer (MicroLoop; Micro Medical Ltd, Rochester, UK). The following PFT results were obtained: FEV_1, FVC and $MEF_{25\%}$. The PFT results were expressed as a percentage of predicted values.[24] Interrupter resistance (Rint, $kPa \cdot L^{-1} \cdot s^{-1}$) was measured using the MicroRint (Micro Medical Ltd). Calibrations of flow were carried out using a 1–L precision pump.

Procedures

PFTs were carried out at the patient's home. Weight and height were measured on day 0. Rint measurements and spirometry were performed on days 0, 7, 14, 21 and 28, Rint was always measured first. Throughout the study, the lung function measurements were performed using the same spirometer in the afternoon and on the same day of the week for each patient. Before PFTs were performed the children were asked to clear their throat by coughing. For Rint measurements the children were instructed to sit upright while breathing quietly. The head was positioned in slight extension. The hands of the investigator supported the patient's cheeks and chin to reduce upper airway compliance.[25] A test measurement was done before the actual measurements to familiarise children with the sound of the shutter. A minimum of five correct tracings (maximal 10) were obtained. The expiratory Rint($Rint_e$) was measured, because expiratory interruptions are more sensitive in detecting airway obstruction relative to inspiratory interruptions.[26] Spirometry was performed in triplicate according to European Respiratory Society guidelines.[27]

For those patients who used tobramycin solution for inhalation (TSI) the study took place during the 4-week TSI-free interval. To estimate adherence to the study medication, patients were asked to keep empty drug vials for vial count.

Arterial oxygen saturation was determined by pulse oximetry (Mars Pulse oximeter; Respironics) during nights 7, 14, 21, and 28. To measure the nocturnal oxygen saturation profile, a pulse oximeter sensor was attached to either the subject's finger or toe during sleep. Data were stored on the pulse oxymeter. From this recording, the mean oxygen saturation was calculated.

Cough recordings were made on a digital audio player/recorder (Archos™ Gmini 120; Archos, Shenzhen, China) during nights 7, 14, 21 and 28, and then transferred to a computer. The recordings were analysed using free Open Source audio record and edit software, which provided a graphical display for audio analysis applications (Audacity, Boston, USA). A cough episode was identified by having at least one explosive cough present. The duration of each cough episode was counted in seconds (cough seconds (cs)). If several cough sounds occurred successively the duration of the total episode was counted. Cough per hour ($cs \cdot h^{-1}$) was then calculated by summing the total cs and dividing by the total recording time. This measure therefore encompasses an estimate of the length of peals of coughs.[28] Counting of cough sounds from digital audio recordings has excellent agreement with simultaneous video recordings.[29]

During weeks 2 and 4 the children recorded in a diary their day and night-time cough frequencies with a validated cough symptom score (CSS; table 1).[30] They also rated sputum viscosity, sputum production, sleep quality, appetite in the morning and cough frequency on a visual analogue scale (VAS).[30] A VAS is a horizontal line, 10 cm in length, anchored by word descriptors at each end. Rating is done by placing a mark on this line

Table 1. Cough Symptom Score

Daytime	
0	no cough
1	cough for one or two short periods
2	cough for more than two short periods
3	frequent cough not interfering with normal activities
4	frequent cough interfering with school or other activities
5	distressing cough most of the day
Night time	
0	no cough
1	cough on waking only/ cough on going to sleep only
2	awoken once or woken early due to coughing
3	frequent waking due to coughing
4	frequent coughs most of the night
5	distressing cough

in the position that best represents the child's perception. The VAS score is the distance in cm between no symptom (left = 0 cm) and the mark placed by the patient.

DATA ANALYSIS

The pre-planned primary outcome variable was $MEF_{25\%}$. With 24 evaluated patients, the power for this parameter in the comparison of the two treatment schedules would be >80% for an effect-size (difference of mean±SD) of 0.8 at an alpha (two-sided) of 5%. Secondary outcome variables were FVC % pred, FEV_1 % pred, $Rint_e$, oxygen saturation, cough frequency and diary scores.

Outcome variables were compared using the paired t-test after ensuring that there was no significant carry-over or period effects. The mean values of diary scores for weeks 2 and 4 were analysed. Statistical analyses were performed and p = 0.05 (two-sided) was considered the limit of significance in all analyses.

RESULTS

From the 43 randomly selected children, 13 children declined to participate and five did not respond. The study group included 25 children, randomly divided between group I (12) and group II (13). Final analysis was for 24 children, as one child, randomised to group I, withdrew in week 3 due to a common flu. Baseline characteristics are sum-

Table 2. Characteristics of the study population at baseline for group I and II

	Group I	Group II
N	11	13
Sex(male/female)	3 / 8	5 / 8
Age (year)	12.5 (4.5)	13.5 (3.4)
FVC (% predicted)	81 (9)	82 (22)
FEV_1 (% predicted)	74 (12)	76 (27)
MEF_{25} (% predicted)	49 (32)	49 (34)
$Rint_e$ (kPa/L/s)	0.5 (0.21)	0.5 (0.23)
Use of TSI (n)	3	5

Data are presented as n or mean±SD. In the first 2 weeks, group I nebulised recombinant human deoxyribonuclease (rhDNase) before bedtime and placebo in the morning, directly after waking up. In the following 2 weeks the order in which rhDNase and placebo were taken was reversed. Group II nebulised placebo before bedtime and rhDNase after waking up in the first 2 weeks and performed the reverse sequence thereafter. FVC: forced vital capacity; % pred: % predicted; FEV_1: forced vital capacity in one second; $MEF_{25\%}$: maximal instantaneous forced flow when 25% of the FVC remained to be exhaled; $Rint_e$: interrupter resistance exhaled; TSI: tobramycin solution for inhalation.

marised in table 2; type of ACT and nebulisers are listed in table 3. The two randomised groups were comparable at baseline with respect to age, sex and lung function (table 2). No carry-over or period effect was observed for any of the endpoints. Mean adherence to the study treatment, as calculated from the vial count, was 97% (range 82–100%).

The primary endpoint, $MEF_{25\%}$, did not significantly differ (p = 0.25) between after waking up (a) or before bedtime nebulisation (b): the difference (a minus b) after 2 weeks was 3.38 (95% confidence interval (CI): -2.6–9.3%; fig 2).

None of the secondary endpoints or safety parameters significantly differed between the two schedules (tables 4 and 5).

Table 3. Maintenance treatment undertaken by patients during the study

ACT (n / %)	
PEP mask	15 / 62%
Flutter	3 / 13%
Autogenic drainage	4 / 17%
Combination	2 / 8%
Nebuliser (n / %)	
Portaneb	12 / 50%
Freeway Lite	1 / 4 %
Freeway Freedom	9 / 38%
Freeway Elite	1 / 4%
CR 60	1 / 4%

Data are presented as n (%). PEP: positive expiratory pressure. Manufacturer of all nebulisers presented in table: Respironics, Murrysville, PA, USA.

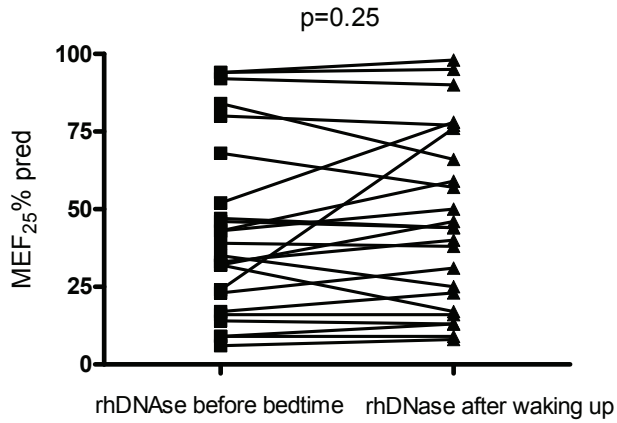

Figure 2. Shows the maximal instantaneous forced flow when 25% of the forced vital capacity remained to be exhaled ($MEF_{25\%}$) as % predicted, according to nebulisation of rhDNase at night-time and in the morning, after 2 weeks. rhDNase: recombinant human deoxyribonuclease.

DISCUSSION

In the current study the authors tested the hypothesis that before-bedtime nebulisation of rhDNase would be safe and prove more effective than nebulisation after waking up. However, the current findings do not support the last part of the hypothesis. Both the primary endpoint $MEF_{25\%}$ and secondary endpoints, such as cough frequency and nocturnal oxygen saturation, did not show significant improvement after 2 weeks of nebulising rhDNase before bedtime, compared with nebulisation after waking up. In addition, cough frequency and nocturnal oxygen saturation were not significantly different between the study periods; this supports the first part of the hypothesis, *i.e.* that nebulisation of rhDNase before bedtime would be safe.

The present authors feel that several possible explanations present themselves as to why no improved efficacy after nebulisation before bedtime was found. First, sputum after nebulisation with rhDNase might still be too viscous to allow any extra positive effect by gravity. Secondly, during sleep mucociliary clearance is depressed[16,17] and breathing patterns change, resulting in reduced minute ventilation and tidal breathing pattern[31,32] as well as an increased airway resistance[33]. If gravity and mucociliary clearance fail to mobilise the viscous sputum spontaneously, it can only be cleared by high expiratory flows.[34] However, sleep is mostly characterised by quiet tidal volume breathing, which is likely to contribute little or nothing to sputum transport. Thirdly, the 2-week treatment period in each arm may have been too short. In a recent study by van der Giessen *et al.*[35], who compared the effect of nebulisation of rhDNase before and after ACT, the primary endpoint $MEF_{25\%}$ showed no significant difference after 2 weeks, but after 3 weeks a significant

Table 4. Results from patients using recombinant human deoxyribonuclease(rhDNase) before bedtime or in the morning after week 1 and 2.

	RhDNase before bedtime	RhDNase morning	p- value
After 1 week			
MEF_{25}%pred	47.2 (30.0)	45.5 (29.9)	0.59
FVC %pred	83.3(18.8)	83.0 (16.8)	0.78
FEV_1%pred	75.5 (21.4)	75.1 (21.6)	0.82
$Rint_e$	0.51 (0.22)	0.55 (0.27)	0.18
Saturation%	95.5 (1.3)	95.8 (1.9)	0.61
Cough (cs/h)	2.7 (4)	3.7 (4)	0.24
After 2 weeks			
MEF_{25}%pred	43.0 (28.4)	46.4 (28.5)	0.25
FVC %pred	83.0 (18.8)	83.1(17.7)	0.97
FEV_1%pred	74.2 (21.8)	75.5 (20.6)	0.38
$Rint_e$	0.54 (0.27)	0.54 (0.23)	0.97
Saturation%	96.1 (1.6)	96.2 (1.4)	0.78
Cough (cs/h)	3.3 (5)	3.7 (8)	0.85

Data are presented as mean±SD unless otherwise stated. $MEF_{25\%}$: maximal instantaneous forced flow when 25% of the forced vital capacity (FVC) remains to be exhaled; % pred: % predicted; FEV_1: forced expiratory volume in one second; $Rint_e$: interrupter resistance exhaled; cs: cough seconds.

increase by 6% was observed when rhDNase was used 30 min before ACT. A longer study period might have resulted in a difference between the two treatment arms. The result of the previous study was not known when the current study started. Extension of the treatment period implied exclusion of those patients treated with TSI, since TSI greatly influences spirometry results.[36] Alternatively, each treatment arm could have been scheduled into the TSI negative or positive treatment period. However, this approach would have made the total study duration for each patient longer with the risk of higher noise and drop-out rates. The absence of differences in nocturnal cough frequency or oxygen saturation between treatment arms suggests no major differences in sputum mobilisation.

A positive finding was that before-bedtime rhDNase administration did not result in increased cough or reduced oxygen saturation during the night in this group of patients with mild-to-moderate lung function abnormalities. As suggested by some physicians, before-bedtime rhDNase could have increased nightly cough periods due to its effect on the sputum. The current authors did not find any increase in $cs \cdot h^{-1}$ when rhDNase was nebulised before bedtime. This might have been the result of the depressed cough reflex during sleep.[37] Such a condition could prevent the liquefied sputum from being evacuated by cough clearance. However, the present authors believe this is unlikely, since oxygen saturation did not differ between the two study periods. Substantial in-

crease of peripheral airway obstruction due to liquefied sputum would have resulted in more severe ventilation/perfusion mismatch and thus, in reduced oxygen saturation. The oxygen saturation finding is therefore in line with the present PFT and cough results. In contrast to the present authors' expectations, the children showed a trend towards better sleep quality and less daytime and night-time cough (table 5) when rhDNase was nebulised before bedtime. Nebulisation of rhDNase before bedtime in patients with mild-to-moderate lung function abnormalities seems therefore to be safe.

Table 5. Diary scores after 2 weeks use of recombinant human deoxyribonulease(rhDNase) taken before bedtime or after waking up

	rhDNase before bedtime	rhDNase morning	p-value
VAS viscosity	2.1 (1.6)	2.5 (1.6)	0.22
VAS sputum amount	1.9 (1.8)	2.1 (1.6)	0.26
VAS daytime coughing	2.2 (1.6)	2.5 (1.7)	0.27
VAS nighttime coughing	1.0 (1.0)	1.3 (1.4)	0.08
CSS daytime	1.3(0.9)	1.6 (0.9)	0.07
CSS nighttime	0.8 (0.8)	1.0 (0.9)	0.27
VAS appetite	3.0 (2.7)	3.3 (2.6)	0.35
VAS sleep quality	1.1 (1.0)	1.5 (1.30)	0.08

Data are presented as mean±SD, unless otherwise stated. VAS: visual analogue scale; CSS: cough symptom score.

How do the current study's results translate to daily treatment for CF patients? Based on the present findings, there are no arguments against the pre-sleep rhDNase treatment. However, long-term studies in larger cohorts and with additional secondary endpoints, such as exacerbation rate, are needed to confirm the current study's results.

The present authors cannot argue that their observations would have been different for children with severe or end-stage lung disease. This must be investigated in a separate controlled trial, using similar monitoring of safety endpoints.

In conclusion, this study showed that for children with cystic fibrosis on maintenance treatment with recombinant human deoxyribonuclease, it is equally effective and safe to nebulise recombinant human deoxyribonuclease before bedtime and to perform airway clearance therapy in the morning as it is to nebulise recombinant human deoxyribonuclease after waking up by airway clearance therapy. It is therefore up to the children themselves to choose the most convenient time to nebulise recombinant human deoxyribonuclease.

REFERENCES

1. FitzSimmons SC. The changing epidemiology of cystic fibrosis. J Pediatr 1993;122:1–9
2. de Jong PA, Nakano Y, Lequin MH, et al. Progressive damage on high resolution computed tomography despite stable lung function in cystic fibrosis. Eur Respir J 2004;23:93–97
3. Costello CM, O'Connor CM, Finlay GA, Shiels P, FitzGerald MX, Hayes JP. Effect of nebulised recombinant DNase on neutrophil elastase load in cystic fibrosis. Thorax 1996;51:619–623.
4. Henke MO, Renner A, Huber RM, Seeds MC, Rubin BK. MUC5AC and MUC5B mucins are decreased in cystic fibrosis airway secretions. Am J Respir Cell Mol Biol 2004;31:86–91.
5. Morgan WJ, Butler SM, Johnson CA, et al. Epidemiologic study of cystic fibrosis: design and implementation of a prospective, multicenter, observational study of patients with cystic fibrosis in the US and Canada. Pediatr Pulmonol 1999;28:231–241.
6. Shak S, Capon DJ, Hellmiss R, Marsters SA, Baker CL. Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum. Proc Natl Acad Sci USA 1990;87:9188–9192.
7. Kearney CE, Wallis CE. Deoxyribonuclease for cystic fibrosis. Cochrane Database Syst Rev 2000;2:CD001127
8. Fuchs HJ, Borowitz DS, Christiansen DH, et al. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. N Engl J Med 1994;331:637–642.
9. Quan JM, Tiddens HA, Sy JP, et al. A two-year randomized, placebo-controlled trial of dornase alfa in young patients with cystic fibrosis with mild lung function abnormalities. J Pediatr 2001;139:813–820.
10. Ramsey BW, Astley SJ, Aitken ML, et al. Efficacy and safety of short-term administration of aerosolized recombinant human deoxyribonuclease in patients with cystic fibrosis. Am Rev Respir Dis 1993;148:145–151.
11. Hodson ME, McKenzie S, Harms HK, et al. Dornase alfa in the treatment of cystic fibrosis in Europe: a report from the Epidemiologic Registry of Cystic Fibrosis. Pediatr Pulmonol 2003;36:427–432.
12. ERCF. ERCF Annual Report 1998: F. Hoffmann-La Roche Ltd; 2000
13. Green JD. Pharmaco-toxicological expert report Pulmozyme rhDNase Genentech, Inc. Hum Exp Toxicol 1994;13: Suppl. 1 S1–42.
14. Lorrain D, De Koninck J, Dionne H, Goupil G. Sleep Positions and postural shifts in elderly persons. Percept Mot Skills 1986;63:352–354.
15. Houtmeyers E, Gosselink R, Gayan-Ramirez G, Decramer M. Regulation of mucociliary clearance in health and disease. Eur Respir J 1999;13:1177–1188.
16. Bateman JRM, Pavia D, Clarke SW. The retention of lung secretions during the night in normal subjects. Clin Sci Mol Med 1978;55:523–527.
17. Hasani A, Agnew JE, Pavia D, Vora H, Clarke SW. Effect of oral bronchodilators on lung mucociliary clearance during sleep in patients with asthma. Thorax 1993;48:287–289.
18. Stokes DC, McBride JT, Wall MA, Erba G, Strieder DJ. Sleep hypoxemia in young adults with cystic fibrosis. Am J Dis Child 1980;134:741–743.
19. Pulmozyme. Product monograph update 2. Gardiner-Caldwell Communications, 2002; pp. 3–65.
20. Shah PL, Scott SF, Geddes DM, Hodson ME. Two years experience with recombinant human DNase I in the treatment of pulmonary disease in cystic fibrosis. Respir Med 1995;89:499–502.
21. Shah PI, Bush A, Canny GJ, et al. Recombinant human DNase I in cystic fibrosis patients with severe pulmonary disease: a short-term, double-blind study followed by six months open-label treatment. Eur Respir J 1995;8:954–958.

22. Tiddens HA. Detecting early structural lung damage in cystic fibrosis. Pediatr Pulmonol 2002;34:228–231.
23. ICH (1996). International Conference on Harmonisation (ICH) Tripartite Guideline. ICH, Gereva, 1996.
24. Zapletal A, Samanek M, Paul T. Lung Function in Children and Adolescents (Progress in Respiratory Research). Basel, Karger; 1987.
25. Hadjikoumi I, Hassan A, Milner AD. Effects of respiratory timing and cheek support on resistance measurements, before and after bronchodilation in asthmatic children using the interrupter technique. Pediatr Pulmonol 2003;36:495–501.
26. Merkus PJ, Mijnsbergen JY, Hop WC, de Jongste JC. Interrupter resistance in preschool children: measurement characteristics and reference values. Am J Respir Crit Care Med 2001;163:1350–1355.
27. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J 2005;26:319–338.
28. Smith J, Owen E, Earis J, Woodcock A. Cough in COPD: correlation of objective monitoring with cough challenge and subjective assessments. Chest 2006;130:379–385.
29. Smith JA, Earis JE, Woodcock A. Establishing a gold standard for manual cough counting: video versus digital audio recordings. Cough 2006;2:6.
30. Chang AB, Newman RG, Carlin JB, Phelan PD, Robertson CF. Subjective scoring of cough in children: parent-completed vs child-completed diary cards vs an objective method. Eur Respir J 1998;11:462–466.
31. Tepper RS, Skatrud JB, Dempsey JA. Ventilation and oxygenation changes during sleep in cystic fibrosis. Chest 1983;84:388–393.
32. George CF, West P, Kryger MH. Oxygenation and breathing pattern during phasic and tonic REM in patients with chronic obstructive pulmonary disease. Sleep 1987;10:234–243.
33. Henke KG, Badr MS, Skatrud JB, Dempsey JA. Load compensation and respiratory muscle function during sleep. J Appl Physiol 1992;72:1221–1234.
34. van der Schans C. Physiotherapy and Bronchial Mucus Transport. Groningen, Rijksuniversiteit, 1991.
35. van der Giessen LJ, de Jongste JC, Gosselink R, Hop WC, Tiddens HA. RhDNase before airway clearance therapy improves airway patency in children with CF. Pediatr Pulmonol 2007; 42: 624–630.
36. Ramsey BW, Pepe MS, Quan JM, et al. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. Cystic Fibrosis Inhaled Tobramycin Study Group. N Engl J Med 1999;340:23–30.
37. Widdicombe J, Fontana G. Cough: what's in a name? Eur Respir J 2006;28:10–15.

Chapter 5

Nocturnal cough in children with stable cystic fibrosis

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ABSTRACT

Introduction

To date no studies have been published on nocturnal cough frequency in children with stable CF. Aim of the study was to assess nocturnal cough frequency in children with CF. In addition nocturnal cough frequency was correlated with parameters of disease severity.

Methods

During two nights cough was recorded with a digital audio recorder in 25 patients (mean age 13 years; range 6-19) with clinically stable CF. In addition oxygen saturation was measured. The day following the recording spirometry was carried out. CT scores were obtained from the most recent routine CT scan. Cough was expressed in cough seconds (cs) and in cough seconds per hour (cs/h).

Results

Data shown are median values and interquartile range (IQR).
First night: 8 cs (IQR 3-52); 0.9 cs/h (IQR 0.3-6.1) Second night: 6 cs (IQR 2-32); 0.6 cs/h (IQR 0.1-3.4). Cs in the 1st night did not correlate significantly with cs in the 2nd night. Only for the 2nd night a strong correlation was found between cs/h and the FEV1 %pred (r_s= -0.75, p<0.001) and FEF_{75} %pred (r_s= -0.71, p<0.001). Bronchiectasis score correlated borderline with the mean cs/h of both nights (r_s= 0.39, p=0.08). During both nights cough was significantly higher in the first hour of sleep (p≤0.04).

Conclusion

Frequency of nocturnal coughing in children with CF was higher than that described for normal children. Nocturnal cough tended to be more severe in children with more advanced CF lung disease. Nocturnal cough was more severe in the first hour of sleep and varied from night-to-night.

INTRODUCTION

Cystic Fibrosis (CF) lung disease is characterized by abnormal mucus and by impaired mucociliary clearance.[1] This impairment can lead to pulmonary exacerbations, characterized by respiratory symptoms such as increased cough, higher sputum production, and fatigue. Pulmonary exacerbations are used as outcome measures in many clinical trials, in spite of the fact that objective criteria for an exacerbation are hard to define.[2] Increased cough is highly associated with a pulmonary exacerbation[2] and could therefore be used as an objective surrogate parameter for an exacerbation.

Severity of cough in clinical studies has been assessed using a visual analogue scale (VAS) or the cough symptom score (CSS).[3,4] These subjective cough scores correlate only moderately well to more objective measurements such as time spent coughing.[5] Clearly, a more objective method to record cough would be preferred. Currently available cough meters are expensive, and data analysis is time consuming and requires a trained investigator.[3,6,7] The manual counting of cough sounds from digital audio recordings is a more patient friendly alternative method that has shown excellent agreement with cough as recorded on video.[8] Various methods have been described to quantify cough. Either the number of explosive phases or the cough seconds are counted. It is thought that these cough parameters are interchangeable.[9] Recently a new method for quantifying cough in cough seconds was developed.[10,11] This method can be further improved with the use of modern digital audio recording and by using dedicated software to analyse the recordings.

Currently, the best feasible and objective way of measuring cough in daily life is during sleep. Nocturnal cough in healthy children is unusual and often related to upper airway infections.[12] Asthma and asthma-like symptoms, protracted bronchitis, and upper airway cough syndrome were detected in order of frequency as the reason for chronic cough in children.[13] Postnasal drip syndrome and gastro-oesophageal reflux are in contrast to adults rare as a cause of isolated chronic cough in children.[14]

Cough during sleep has been examined before in adult patients with CF.[15,16] To our knowledge this has not yet been studied in children with stable CF. We therefore assessed cough frequency during two nights in children with clinically stable CF, and examined cough correlation between the two nights with a one week interval and between the cough frequency and oxygen saturation, pulmonary function and CT scores.

MATERIAL AND METHODS

Study subjects

Patients of the CF centre at Erasmus Medical Centre – Sophia Children's Hospital were eligible when they fulfilled the following criteria: proven CF, defined as clinical symptoms characteristic for CF plus an abnormal sweat test and/or by the presence of two CF mutations; at least five years of age; ability to perform reproducible spirometry; and clinical stability. The latter was defined as: absence of symptoms of a common cold; no need for intravenous antibiotics; and no hospitalizations for at least one month prior to the study. Throughout the study, subjects continued to receive their standard treatment. This study was a sub study of a randomized controlled cross-over trial on the effect of the administration of rhDNase before or after the night.[17] Cough was included as a safety endpoint. One group used rhDNase before bedtime and placebo (2.5 ml of a buffered solution: 8.77 mg/ml sodium chloride and 0.15 mg/ml calcium chloride) in the morning directly after waking up in the first two weeks. This order was reversed in the following two weeks. A second group used placebo before bedtime and rhDNase after waking up in the first two weeks, and in reverse sequence thereafter. All subjects performed airway clearance therapy 30 minutes after the morning nebulisation.

For the present analysis we only used the subjects' cough registrations for the two weeks when placebo was inhaled before the night and rhDNase in the morning. Nebulisation of placebo is thought to have no influence on sputum viscosity and thus is unlikely to have any effect on cough.

The study was conducted between September 2005 and June 2006. The Erasmus MC Medical Ethical Review Board approved the protocol. The study was performed according to ICH-GCP guidelines.

Equipment specifications

Pulmonary function tests (PFT) were carried out using a handheld spirometer (MicroLoop, Micro Medical Ltd, Rochester, UK). The following PFT results were obtained: forced expiratory volume in one second (FEV_1); forced vital capacity (FVC); and FEF_{75}. The PFT results were expressed as percentages of predicted values.[18]

Procedures

Cough recordings

Recordings (32 bits mp3 format) were made at home during nights 7 and 14 in those weeks when placebo was inhaled before sleep. A digital audio player/recorder (Archos ™ Gmini 120, Shenzhen, China) was placed on a stable surface as close as possible to

the patient. The recording was for obvious logistical reasons started when children were put to bed.

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

The investigator (LvdG) identified by ear all sounds detected by the software on the digital recordings. The cough counting was started when the children stopped making noise like talking, singing or making noisy movements and when quiet breathing could be heard. The beginning of a cough episode was defined as the moment when a clear explosive cough was heard. Next, duration of this cough episode following the initial cough was computed in seconds. Finally, from total duration of cough seconds for that night and total time of the recording we computed the cough seconds per hour (cs/h). In case multiple cough sounds occurred in quick succession, we assumed this as one episode, lasting from the onset of the first cough sound up to and including the last of the quickly succeeding cough sounds.[11,19] An example of cough analysis is displayed in Figure 1.

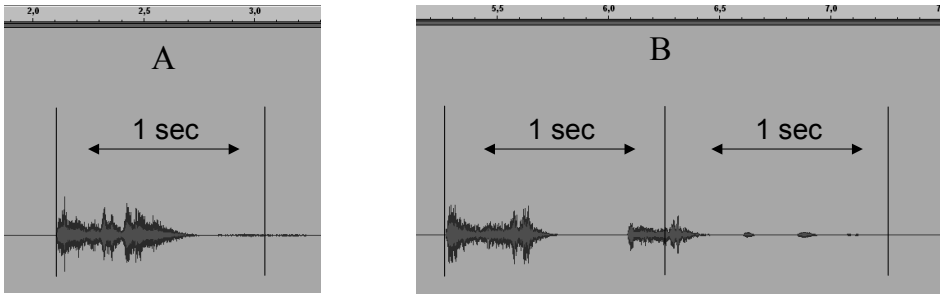

Figure 1. Example of cough analysis. The cough duration in example A is calculated as one cough second. The cough in period B is calculated as two seconds; if there were several coughs occurred successively, the number of seconds in which these sounds fell were counted.

Pulmonary function tests

PFT took place at the patient's home and were performed by LvdG. Weight and height were measured on day 0. On the days following the nightly cough registration, spirometry was performed in triplicate according to ERS guidelines.[20]

CT score

Severity of the structural lung changes was estimated using the Brody-II CT score.[21] This system typically evaluates the following qualities: bronchiectasis; airway wall thickening; mucus plugging; and opacities on inspiratory images, and air trapping on expiratory images. Scores were expressed as percentages of the maximum possible score on a

scale from 0 (no disease) to 100 (maximum lung disease). For scoring we used the CT scan obtained at the patient's most recent annual routine evaluation prior to the study. A single experienced independent observer (ML) who was unaware of the cough registrations scored all CT scans in random order.

Oxygen saturation

Oxygen saturation was measured by pulse oximetry (Mars Pulse oximeter, Respironics, Murrysville, Pennsylvania, USA), simultaneously with the cough measurements. A Y-sensor™ measuring the oxygen saturation was positioned at a finger or a toe. Mean oxygen saturation was computed from artifact free time.

Diary

During the second week the children were asked to keep a diary. Cough frequency during night time was estimated with the CSS the day after the recording (Table 1).[4] The patients themselves scored sputum viscosity, sputum production, sleep quality, appetite in the morning and cough frequency on a visual analogue scale (VAS).[4] A VAS is a horizontal line, 100 mm in length, anchored by word descriptors at each end. Scoring is by placing a mark on the line that best represents one's perception of the issue.

Table 1. Cough Symptom Score – Night time

Night time	
0	no cough
1	cough on waking only/ cough on going to sleep only
2	awoken once or woken early due to coughing
3	frequent waking due to coughing
4	frequent coughs most of the night
5	distressing cough

Data analysis

Statistical analysis was performed with SPSS version 11.0.

Cough frequency and the cs/h were expressed as median values and interquartile range (IQR). Spearman rank correlation coefficient (r_s) was used to examine relations between cs/h of the two different nights and between cs/h and lung function, age and the CT score. The mean cs/h of the two study nights was used to investigate correlations with parameters (age and CT-score) that were similar for the first and second night. For other parameters with more day to day variability we analyzed the correlations between cs/h and those parameters for each of the 2 nights separately. The relations between cs/h on one hand and PFT parameters and oxygen saturation on the other hand for each of the

two study days were evaluated using repeated measures ANOVA. In this analysis cs/h data were logarithmically transformed to obtain approximately normal distributions. Values of cs/h equal 0 were replaced by 0.05 before this transformation, which only has a minor effect on the results.
The Wilcoxon Rank test and the Friedman test were used to compare the consecutive hours of sleep.
Differences were considered significant at a value of $p<0.05$.

RESULTS

The CF-centre at the Erasmus MC-Sophia Children's Hospital follows 152 CF-patients. Forty-nine of them did not meet the study inclusion criteria. Of the remaining 103, 43 children were randomly selected and invited to participate in the study. Of these, 13 declined to participate, and 5 did not respond. The study group therefore consisted of 25 children. Final analysis was done on 24 children, as one child withdrew in the third week of the study due to a common cold. Characteristics of the study population are summarized in Table 2.

Table 2. Characteristics of the study population (numbers of patients or mean with range) at baseline

Gender (male/female)	7 / 15
Age (years)	12.8 (6.3-18.9)
FVC %pred	80 (47-114)
FEV_1 %pred	72 (32-105)
FEF_{75} %pred	45 (6-112)
CT score	15 (0-37)
Bronchiectasis score	14 (0-47)

The mean total recorded time from the first night was 557 minutes (range 419-724) and from the second night 562 minutes (range 403-713).
Cough recordings for 2 children were unsuccessful in the first week, and for 3 in the second week. The 5 unsuccessful recordings were due to parents failing to operate the digital audio recorder.
During the 1st night a median of 8 cough seconds (IQR 3-52) and 0.9 cs/h (IQR 0.3-6.1) were recorded and during the 2nd night a median of 6 cough seconds (IQR 2-32) and 0.96 cs/h (IQR 0.1-3.4). During the 1st night 19 out of 22 children had one or more cough seconds and during the 2nd night 17 out of 21 children had one or more cough seconds.

Cough seconds during the 1st night did not significantly correlate with the cough seconds during the 2nd night (r_s=0.29, p=0.20). The same applied to cs/h (r_s=0.35, p=0.12). See Figure 2.

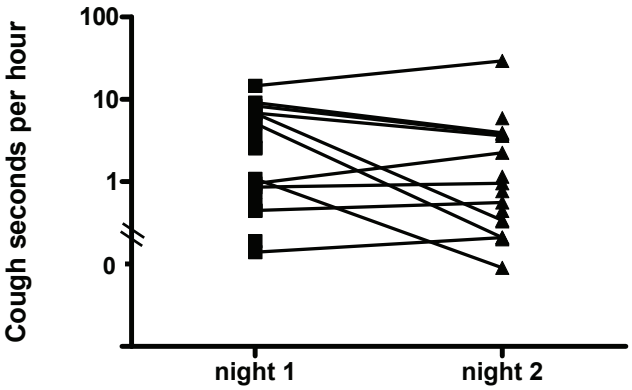

Figure 2. Cough seconds per hour (on a log scale) during night 1 and 2. No significant relation between these nights were observed (r_s 0.35, p=0.12). Individual data points are connected with straight lines.

PFT parameters correlated very strongly between the two study days; FVC %pred (r_s=0.95, p<0.001), FEV_1 %pred (r_s=0.91, p<0.001), FEF_{75} %pred (r_s=0.94, p<0.001).
Figure 3 shows cough seconds as a function of the first eight hours spent in sleep during the second night. Cough is significantly higher in the first hour of sleep relative to all consecutive hours (all p ≤0.04) with the exception of the 7th hour of sleep during the first night (p=0.27). There were no significant differences in cough seconds between the other hours of sleep.

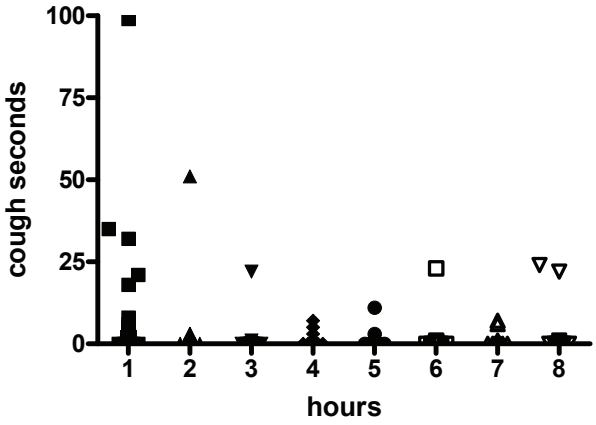

Figure 3. Cough seconds during the first eight consecutive hours of sleep at night 2. Each symbol represents the cough seconds of one patient at that specific hour.

For the 2nd night, but not for the first, we found a strong correlation between cs/h and the FEV_1 %pred (r_s= -0.75, p<0.001) and FEF_{75} %pred (r_s= -0.71, p<0.001). No significant correlation could be found between cs/h and: FVC %pred; or the mean oxygen saturation. Further analysis evaluating both nights simultaneously using repeated measures ANOVA showed that the relation between FEF_{75} %pred versus cs/h did not significantly differ between the two evaluated nights. There was a significant common correlation between FEF_{75} %pred and cough seconds per hour. For a decrease of the FEF_{75} %pred by 10 percentage points the cs/h increases by a factor 1.4 (p=0.002; 95% CI 1.15-1.73). See Figure 4.

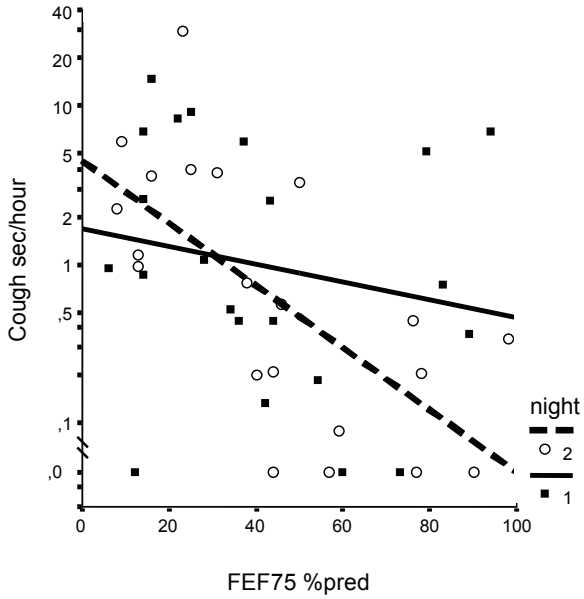

Figure 4. Scatter plot of cough sec/hour versus FEF_{75} %pred for the two study nights. Lines represent regression lines. Note the logarithmically scaled vertical axis.

There was a moderate correlation between the mean cs/h of the two nights and age (r_s=0.46, p=0.04). No correlation was found between total CT score and the mean cs/h of the two nights. The bronchiectasis score showed borderline significant correlation with the mean cs/h of the two nights (r_s=0.39, p=0.08). The correlation for age and CT was r_s=0.67, p=0.001, and correlation between age and bronchiectasis score r_s=0.73, p<0.000.

We did not find correlations between cs/h and the subjective VAS score for nocturnal cough or sleep quality. The CSS, on the contrary, showed a moderate significant correlation with cs/h (r_s=0.55, p= 0.01).

Finally, most of the children slept for at least eight hours. We therefore repeated all analyses just using the data for the first eight hours. This resulted in similar outcomes.

DISCUSSION

To our knowledge this is the first study which quantifies the nocturnal cough frequencies in children with clinically stable CF. We found that more than 80% of the children were coughing during the two nights that cough was recorded. This is substantially higher than the 5% reported in 'normal' children.[12] Of 41 children studied, one had one episode of coughing and one child had three episodes of coughing. Cough was also studied in a cohort of children with primary ciliary dyskinesia.[22] The median cough frequency, defined as total number of cough episodes (<2 sec between individual coughs), was similar to that found in our study. Our study, however, showed a wider range of coughing in the children with CF relative to the range of coughing in primary ciliary dyskinesia (median: 5.5; IQR 3-7.3).

Total time spent coughing in our study was substantially lower than what we had expected with a maximum of 115 sec during the first night and 198 sec during the second night. Thus, from the above we can conclude that children with clinically stable CF are coughing more than normal children during the night. However, the total duration of cough was relatively low.

Surprisingly, cough during the 1st night did not correlate to cough during the 2nd night. This suggests that within subjects cough can vary considerable from night to night. Two possible reasons for this lack of correlation present themselves. First, cough frequency was too low and so insensible to measure correlation. Second, multiple causes, like gastro-oesophageal reflux may underlie cough in CF.[23] We observed a variable correlation between lung function parameters and cough for the two nights. This most likely can be explained by the combination of the variability in the cough measurement and chance. In other studies lung function parameters did not correlate with the cough frequency, probably as a result of variability in cough.[22,24]. In contrast, PFT parameters after the 1st recording night correlated well to those after the 2nd night. FEF_{75} %pred is significantly related to cs/h for both nights when analysed simultaneously. FEF75 %pred is more sensitive than FEV1 %pred to changes in the peripheral airways, which are involved early in the course of disease.[25] The night-to-night cough variability raises a question about the sensitivity of nocturnal cough as an endpoint in clinical studies. In future studies the minimum number of nights needed to compute a reliable mean should be investigated. Furthermore, the low cough frequency in many children also raises the question if cough is a reliable outcome measure for example in CF treatment studies, because most of the children cannot 'improve' their nocturnal cough frequency. In one study in

children with a pulmonary exacerbation no change in diurnal and nocturnal objective cough frequency could be found after treatment with antibiotics.[24]

A striking observation was that nocturnal cough dominated in the first hour of sleep and that frequency then decreased with time. Less coughing later during the night might be the result of reduced cough reflex[26] or depressed mucociliary clearance during sleep[27]. It has been shown in other studies that nocturnal cough is lower than diurnal cough.[3,12,28,29]

A limitation of our study is that we do not know for sure whether this increased cough was present before and / or after the children fell asleep. For obvious logistical reasons the audio recording was started when children were put to bed. The cough registration was started when the children did not produce any noise. We think it is likely that this moment is close to the moment that the children fall asleep. However, this has never been systematically studied.

Therefore, it might be useful in clinical studies to analyse cough in the first hours separately from cough in the consecutive hours.

In our study age and nocturnal cough are correlated; i.e. the older the child the more the child coughed. The most likely explanation for this correlation is that lung disease is more severe in older patients. We showed previously that structural damage CT scores progresses with age.[30] Indeed, for the present cohort we observed a trend for a positive correlation between the bronchiectasis score and the cs/h; suggesting that cough frequency is linked to severity of CF lung disease.

The moderate correlation observed between objective cough measurement and the subjective night time CSS, is in accordance with other studies in children with CF and also in children with asthma.[3,5,31,32] In our study seven children were coughing more than once during one or more hours. Their CSS scores were 1 or 2 (table 1) rather than 3 or higher, which would have reflected the true cough frequency. It would seem they are mostly not aroused from sleep by coughing, and therefore underestimate their nocturnal cough. For this reason the subjective CSS for nocturnal cough cannot replace the objective cough measurement. In another study in children with CF it was concluded as well that these children did not assess their night time cough frequency well.[24]

In this study we cannot exclude that the nebulisation of placebo influenced our observations to some extent. However, we think this is unlikely since the placebo for DNase that we used has been used in many studies and is considered ineffective to affect mucociliary clearance or cough.[33]

In conclusion, the frequency of nocturnal coughing in children with clinically stable CF was substantially higher than that is described for normal children. In addition, nocturnal cough tended to be more severe in patients with more advanced disease as indicated by lung function parameters. Nocturnal cough was more severe in the first hour of sleep and varied considerably from night-to-night. To use nocturnal cough as an endpoint in clinical studies its night to night variability should be taken into account.

More studies are needed to assess if cough frequency is sensitive to treatment changes and to determine the optimal number of nights that have to be assessed before nocturnal cough counting in CF can be used as a reliable surrogate endpoint in clinical studies.

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REFERENCES

1. FitzSimmons SC. The changing epidemiology of cystic fibrosis. J Pediatr 1993;122(1):1-9.
2. Rosenfeld M, Emerson J, Williams-Warren J, et al. Defining a pulmonary exacerbation in cystic fibrosis. J Pediatr 2001;139(3):359-365.
3. Hsu JY, Stone RA, Logan-Sinclair RB, et al. Coughing frequency in patients with persistent cough: assessment using a 24 hour ambulatory recorder. Eur Respir J 1994;7(7):1246-1253.
4. Chang AB, Newman RG, Carlin JB, Phelan PD, Robertson CF. Subjective scoring of cough in children: parent-completed vs child- completed diary cards vs an objective method. Eur Respir J 1998;11(2):462-466.
5. Decalmer SC, Webster D, Kelsall AA, et al. Chronic Cough: How Do Cough Reflex Sensitivity And Subjective Assessments Correlate With Objective Cough Counts During Ambulatory Monitoring? Thorax 2006.
6. Chang AB, Newman RG, Phelan PD, Robertson CF. A new use for an old Holter monitor: an ambulatory cough meter. Eur Respir J 1997;10(7):1637-1639.
7. Munyard P, Busst C, Logan-Sinclair R, Bush A. A new device for ambulatory cough recording. Pediatr Pulmonol 1994;18(3):178-186.
8. Smith JA, Earis JE, Woodcock A. Establishing a gold standard for manual cough counting: video versus digital audio recordings. Cough 2006;2(1):6.
9. Kelsall A, Decalmer S, Webster D, et al. How to quantify coughing? Correlations with quality of life in chronic cough. Eur Respir J 2008.
10. Smith JA. Cough frequency and patterns of cough in cystic fibrosis. J R Soc Med 2006;99 Suppl 46:17-22.
11. Smith J, Owen E, Earis J, Woodcock A. Cough in COPD: correlation of objective monitoring with cough challenge and subjective assessments. Chest 2006;130(2):379-385.
12. Munyard P, Bush A. How much coughing is normal? Arch Dis Child 1996;74(6):531-534.
13. Asilsoy S, Bayram E, et al. Evaluation of chronic cough in children. Chest 2008;134(6):1122-1128.
14. Chang AB, Widdicombe JG. Cough throughout life: children, adults and the senile. Pulm Pharmacol Ther 2007;20(4):371-382.
15. Stokes DC, McBride JT, Wall MA, Erba G, Strieder DJ. Sleep hypoxemia in young adults with cystic fibrosis. Am J Dis Child 1980;134(8):741-743.
16. Milross MA, Piper AJ, Dobbin CJ, Bye PT, Grunstein RR. Sleep disordered breathing in cystic fibrosis. Sleep Med Rev 2004;8(4):295-308.
17. van der Giessen LJ, de Jongste JC, Gosselink R, Hop WC, Tiddens HA. RhDNase before airway clearance therapy improves airway patency in children with CF. Pediatr Pulmonol 2007;42(7):624-630.
18. Zapletal A. Lung Function in Children and Adolescents. Zapletal A, Samanek M, Paul T, editors. Basel: Karger; 1987.
19. Morice AH, Fontana GA, Belvisi MG, et al. ERS guidelines on the assessment of cough. Eur Respir J 2007;29(6):1256-1276.
20. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J 2005;26(2):319-338.
21. Brody AS, Klein JS, Molina PL, et al. High-resolution computed tomography in young patients with cystic fibrosis: distribution of abnormalities and correlation with pulmonary function tests. J Pediatr 2004;145(1):32-38.
22. Zihlif N, Paraskakis E, Lex C, Van de Pohl LA, Bush A. Correlation between cough frequency and airway inflammation in children with primary ciliary dyskinesia. Pediatr Pulmonol 2005;39(6):551-557.

23. Irwin RS, Boulet LP, Cloutier MM, et al. Managing cough as a defense mechanism and as a symptom. A consensus panel report of the American College of Chest Physicians. Chest 1998;114(2 Suppl Managing):133S-181S.
24. Hamutcu R, Francis J, Karakoc F, Bush A. Objective monitoring of cough in children with cystic fibrosis. Pediatr Pulmonol 2002;34(5):331-335.
25. Tiddens HA. Detecting early structural lung damage in cystic fibrosis. Pediatr Pulmonol 2002;34(3):228-231.
26. Widdicombe J, Fontana G. Cough: what's in a name? Eur Respir J 2006;28(1):10-15.
27. Houtmeyers E, Gosselink R, Gayan-Ramirez G, Decramer M. Regulation of mucociliary clearance in health and disease. Eur Respir J 1999;13(5):1177-1188.
28. Power JT, Stewart IC, Connaughton JJ, et al. Nocturnal cough in patients with chronic bronchitis and emphysema. Am Rev Respir Dis 1984;130(6):999-1001.
29. Li AM, Tsang TW, Chan DF, et al. Cough frequency in children with mild asthma correlates with sputum neutrophil count. Thorax 2006;61(9):747-750.
30. de Jong PA, Lindblad A, Rubin L, et al. Progression of lung disease on computed tomography and pulmonary function tests in children and adults with cystic fibrosis. Thorax 2006;61(1):80-85.
31. Kerem E, Wilschanski M, Elfring GL, et al. Quantitive cough assessment in Cystic Fibrosis. Journal of Cystic Fibrosis 2008;7(2):S59.
32. Archer LN, Simpson H. Night cough counts and diary card scores in asthma. Arch Dis Child 1985;60(5):473-474.
33. Quan JM, Tiddens HA, Sy JP, et al. A two-year randomized, placebo-controlled trial of dornase alfa in young patients with cystic fibrosis with mild lung function abnormalities. J Pediatr 2001;139(6):813-820.

Chapter 6

Nocturnal oxygen saturation in children with stable cystic fibrosis

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Submitted

ABSTRACT

Background

Hypoxemia during sleep is a common finding in Cystic Fibrosis (CF) patients with more advanced lung disease. Nightly hypoxemia is associated with frequent awakenings and poor sleep quality. For children with CF, data of nocturnal oxygen saturation are sparse.

Objective

To assess the oxygen saturation profile during sleep in 25 clinically stable children with early CF lung disease and to correlate these data with spirometry, cough frequency, sleep quality and CT-scan scores.

Method

During two nights cough was recorded with a digital audio recorder in 25 clinically stable CF patients (mean age 13 years; range 6-19). In addition oxygen saturation was measured. The day following the recording spirometry was carried out. CT scores were obtained from the most recent routine CT scan.

Results

22 patients were included in the study. Mean age (range) was 13 (6-18) years. Spirometry was FVC% 84 (range 52-114), FEV_1% 77 (range 43-115) and FEF_{75}% 50 (range 12-112). The mean SO_2 was 95.6% for the first and 96.2% for the second night. Mean SO_2 between the two nights correlated strongly (r_s=0.84, $p<0.001$). Positive correlation was observed between mean SO_2 of the two nights (mean*SO_2) and FVC, FEV_1 and FEF_{75}. Correlations were found between mean*SO_2 and the total CT score (r_s=-0.45, p=0.05) and the bronchiectasis subscore (r_s=-0.48, p=0.03).

Conclusion

Nocturnal oxygen saturation in children with stable CF is lower than that in healthy children, and is correlated with lung function parameters and CT scores. Monitoring oxygen saturation during one night is sufficient to get a representative recording.

INTRODUCTION

Cystic fibrosis (CF) is characterized by chronic airway inflammation and infection that start early in life and lead to airway obstruction by mucus impaction and remodeling. This results in gas trapping, ventilation/perfusion mismatch, higher airflow resistance, and increased work of breathing. Patients with more advanced disease may show hypoxemia and hypercapnia during exercise and sleep but not during daytime.[1-5] However, nocturnal oxygen desaturation and reduced resting daytime oxygen saturation has been described in these patients as well.[6] Nightly hypoxemia is associated with frequent awakenings and poor sleep quality. As a result these patients suffer from morning tiredness, chronic headache and impaired neurocognitive function.[6-8] Furthermore, chronic or intermittent hypoxia is associated with developmental impairment, and has a negative effective on behaviour and academic achievement.[9] In addition in one study it was shown structural lung abnormalities on CT correlated with mean nocturnal oxygen saturation.[10] Hence, we think that nightly oxygen saturation profiles are a clinically relevant surrogate endpoint that can be used in clinical studies.

Reference values of overnight oxygen saturation are available for healthy children,[11-17] yet sparse for children older than 6 years with stable CF.[10,15,18]

We therefore set out to assess nocturnal saturation profiles in children with clinically stable CF during two nights and to relate these saturation variables to lung function, cough frequency and structural lung abnormalities as established from CT scan.

MATERIAL AND METHODS

Study subjects

Patients followed by the CF centre at Erasmus Medical Centre – Sophia Children's Hospital were eligible for inclusion when they fulfilled the following criteria: proven CF; at least five years of age; ability to perform reproducible spirometry; and clinical stability. The latter was defined as no need for intravenous antibiotics and no hospitalizations for at least one month prior to the study. CF was defined as clinical symptoms characteristic for CF plus an abnormal sweat test and/or by the presence of two CF mutations. Throughout the study, subjects continued to receive their standard treatment.

This was a sub study of a randomized controlled cross over trial in which we evaluated effects of administration of rhDNase or a placebo (saline 0.9%) before or after the night during two periods of two weeks.[19] For this sub study we included the saturation profiles during the 7th and the 14th night in the two weeks when placebo was inhaled before the

night and rhDNase in the morning. Nebulisation of saline 0.9% was assumed not to have major effects on sputum viscosity and the nightly saturation profile.
The Erasmus MC Medical Ethical Review Board approved the protocol. The study complied with ICH-GCP guidelines.

Procedures

Pulse oximetry

Oxygen saturation (SO_2), determined by pulse oximetry (Mars pulse oximeter, Model 2001, Respironics, Murrysville, PA, USA), was measured at home during nights 7 and 14 in the two-weekly trial schedule. (Figure 1) The Mars oximeter has a motion artifact rejection system. Patients and parents were trained how to position a Y-sensor™ correctly at a finger or a toe and how to operate the oximeter in the home situation. The acoustic signals for pulse volumes and desaturation alarms were silenced during registration. Registration was started when the child went to sleep and was stopped at waking up in the morning.

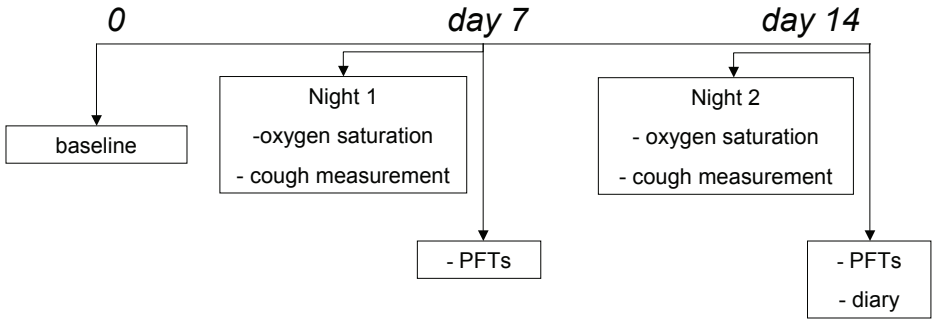

Figure 1. Time schedule of the study.

The SO_2 signal was recorded every eight seconds and analyzed with NovaMetrix software™ and Microsoft Excel. Total recording time (TRT) and artifact free recording time (AFRT) were calculated. If AFRT was less than 5 hours, the recording was excluded from further analysis, as it was thought not to be long enough to include at least three full sleep cycles.[20] The mean SO_2 was used for baseline calculation. Desaturation events were defined as a SO_2 below ≤90% (D_{90}) and/or a fall in SO_2 relative to the mean SO_2 by ≥4% (D_4), which could appear every eight seconds. From the event data we calculated the total times (in seconds) of D_{90} (T_{90}) and D_4 (T_4)as well as the oxygen desaturation index (ODI), defined as the total number of D_{90} or D_4 per hour.

Pulmonary function tests

Pulmonary function tests (PFT) by means of spirometry was performed on the days immediately following the nocturnal oxygen saturation measurements. (Figure 1) We used a handheld spirometer (MicroLoop, Micro Medical Ltd, Rochester, UK). Spirometry was performed in triplicate according to ERS guidelines.[21] The following PFT parameters were used for analysis: forced expiratory volume in one second (FEV_1); forced vital capacity (FVC); and maximal expiratory flow when 75% of FVC was expired (FEF_{75}). The values obtained were expressed as percentages of predicted values. [22]

CT score

Severity of structural lung changes was estimated using the Brody-II CT score. [23] This system evaluates the following qualities: bronchiectasis; airway wall thickening; mucus plugging; and opacities on inspiratory images as well as air trapping on expiratory images. The expiratory slices were taken at the following anatomical levels: between the lung apex and the top of the aortic arch, just below the carina, and at top of the diaphragm. Total CT scores and subscores were expressed as percentages of the maximal possible score on a scale of 0 (no disease) to 100 (maximal lung disease). The CT scan obtained at a patient's most recent annual routine evaluation was scored. All scans were de-identified and randomly scored by one experienced, independent observer who was blinded to clinical background of the scans.

Cough recording

Nocturnal cough was recorded simultaneously with the oxygen saturation measurements. Sound was recorded (32 bits mp3 format) using a digital audio player/recorder (Archos™ Gmini 120, China). The recorder was positioned on a stable surface as close as possible to the patient. Cough recordings were transferred from the digital recorders to a personal computer. Recordings were analyzed using free software (Audacity, Boston, USA). First, cough episodes were identified during which at least one explosive cough was present. Next, duration (in seconds) of each cough episode was computed. Finally, durations of cough episodes for that night were summed and divided by the total time of the recording. Cough frequency was thus expressed in seconds of cough per hour (cs/h). If several cough sounds occurred successively, duration of the timeframe in which these sounds fell was counted. [24]

Diary

The children were asked to keep a diary for every day of the second trial week. They scored sleep quality on a visual analogue scale (VAS).[25] This is a horizontal line, 100 mm in length, anchored by word descriptors at each end. Rating is by placing a mark on this line in the position that best represents the child's perception. The VAS score is the

distance in cm between 'no symptom' (left = 0 cm) and the mark placed by the child. The children marked on the line the point that represents best their perception of sleep. A low VAS score indicated good sleep quality.

Data analysis

Statistical analysis was performed with SPSS version 11.0.
Variables that were not normally distributed are expressed as median values. Relations between mean SO_2 on the one hand, and lung function, cs/h and desaturation parameters (T_{90}, D_{90}, D_4, T_4, ODI_4, ODI_{90}) on the other hand were evaluated for the two study nights using repeated measures ANOVA. Spearman rank correlation coefficients (r_s) were used to examine relations between mean oxygen saturation and age and the CT score. For this analysis we used the average of the two mean oxygen saturation values of the two nights defined as mean*SO_2. The mean outcomes of the VAS score obtained during the second week were used for analysis.
The cough seconds per hour, T_{90}, D_{90} and ODI_{90} were log transformed to get approximate normal distributions. Differences were considered significant at a value of $p<0.05$.

RESULTS

The CF-centre at the Erasmus MC-Sophia Children's Hospital follows 152 CF-patients. Forty-nine of them did not meet the study inclusion criteria. The project statistician sorted the remaining eligible 103 children in a random order. The first 43 were invited to participate in the study. Of these, 13 declined to participate, and 5 did not respond. The study group therefore consisted of 25 children. Analysis was done on the data of 24 children, as one child was withdrawn from the study due to a common cold. The first saturation measurement was successful in 22 of the 24 patients: two patients were excluded from analysis since the AFRT was less than 5 hours. The second measurement was successful in 20 patients: for two patients AFRT was less than 5 hours and for two patients data recording had failed, probably due to faulty fitting of the finger probe. Data of 22 children were included in the final analysis. Table 1 shows characteristics of these children.

Desaturation

Saturation parameters and their correlations are summarized in Table 2. During the first night 18 out of 22 children (82%) had one or more D_4 events (range 1-50) and 12 children (55%) had one or more D_{90} events (range 1-85). During the second night 11 out of

20 children (55%) had one or more D_4 events (range 1-76) and 8 children (40%) had one or more D_{90} events (range 1-107).

Table 1. Baseline characteristics of the study population (n=22). Numbers are expressed as mean (range).

Sex (male/female)	7 / 15
Age (years)	13 (6.3-18.7)
FVC %pred	84 (52-114)
FEV_1 %pred	77 (43-115)
FEF_{75} %pred	50 (12-112)
Brody CT score (%)	13 (0-37)
Bronchiectasis score (%)	13 (0-47)

Table 2. Saturation parameters and recording time (median (range)) for nights 1 and 2 separately. The right-hand column shows correlations between the two nights (r_s,p). ‡= $p<0.05$

	Night 1	**Night 2**	**Correlation (r_s,p)**
Mean saturation (%)	96.2 (90.1-97.6)	96.3 (92.9-98.2)	0.84, <0.001‡
AFRT (min)	530 (300-736)	574 (383-715)	0.50, 0.02 ‡
TRT (min)	579 (300-745)	573 (383-720)	0.60, 0.01 ‡
D_4	2 (0-50)	1(0-76)	0.37, 0.11
ODI_4	0.2 (0-9.9)	0.1 (0-8.0)	0.44, 0.05
T_4 (sec)	12 (0-2928)	60 (0-4288)	0.30, 0.19
D_{90}	1 (0-85)	0 (0-107)	0.35, 0.14
ODI_{90}	0.1 (0-8.9)	0.0 (0-11.2)	0.36, 0.12
T_{90} (sec)	8 (0-8376)	0 (0-4816)	0.39, 0.88

Abbreviations: AFRT: artifact free recording time; TRT: total recording time; D4:Fall in SO2 by ≥4%; D90: Fall in SO2 to ≤90%; ODI4: Oxygenation desaturation index per hour of D4; ODI90: Oxygenation desaturation index per hour of D90; T4: total time in seconds of D4; T90: total time in seconds of D90

Mean SO_2

For each patient the mean value of the recorded SO_2 was determined. The mean of these mean SO_2 across patients was 95.6% for the first night and 96.2% for the second night (Table 2). Mean SO_2 in the first night correlated strongly (r_s=0.84, $p<0.001$) (Figure 2a) with that in the second night, however the agreement between the two was moderate (ICC=0.70). The Bland Altman suggests that the variability is somewhat less for the lower mean SO_2.(Figure 2b)

Repeated measures ANOVA showed that the relations between mean SO_2 versus PFTs (FVC, FEV_1 and FEF_{75} %pred) and the resting SO_2 did not significantly differ between the

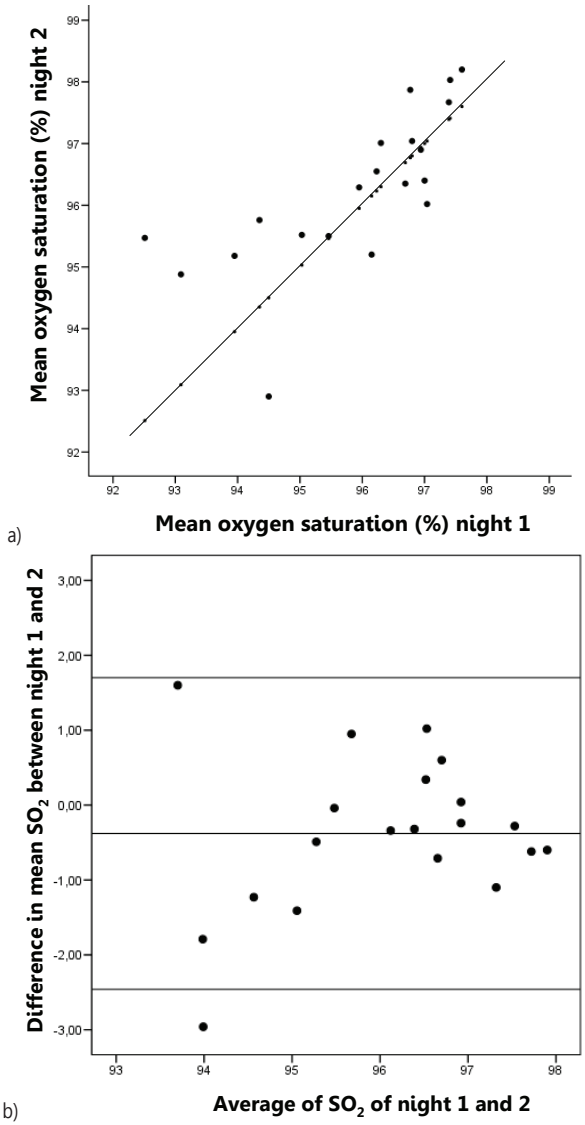

Figure 2.

a) Correlation between mean oxygen saturation of the 1st versus the 2nd night. Line represents line of identity.

b) Bland-Altman Plot. The differences in mean oxygen saturation (SO_2) of night 1 and 2 plotted against the averages of the two measurements. Top and bottom and horizontal lines represent +/-2 standard deviation (-2.46 to 1.70). Line in the middle represents the mean difference (-0.38).

two evaluated nights. For further analysis we therefore used the mean SO_2 of the two nights defined as mean*SO_2.

A positive correlation was observed between mean*SO_2 and FVC. For a decrease of FVC %pred by 10 percentage points, the mean*SO_2 decreased by 0.6 percentage points high-

er (p=0.007; 95% CI 0.2%-1%). For a decrease of FEV_1 and FEF_{75} by 10% the mean*SO_2 decreased by 0.5% (p=0.004; 95% CI 0.2%-0.7%) and 0.3% (p=0.02; 95% CI 0.1%-0.5%) respectively. (Figures 3, 4 and 5)

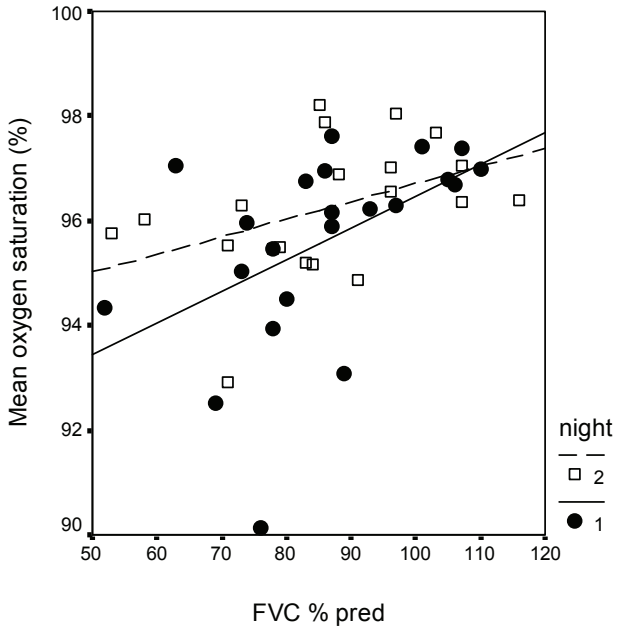

Figure 3. Scatter plot of mean oxygen saturation versus FVC %pred for the two study nights. Lines represent regression lines; no significant difference between the two evaluated nights.

There was no significant correlation between mean*SO_2 and *cs/h (p=0.81) when evaluating both nights.

There was a moderate correlation between the mean*SO_2 and the total CT score (r_s=-0.45, p=0.05) and the bronchiectasis subscore (r_s=-0.48, p=0.03). No significant correlation was found between mean*SO_2 and the air trapping subscore (r_s=-0.18, p=0.94). Also no correlation was found between mean*SO_2 and age (r_s=0.29, p=0.22).

Sleep quality

We could not establish significant correlations between the subjective VAS score sleep quality and saturation parameters. The TRT was moderately correlated with the VAS score sleep quality (r_s=-0.45 p= 0.05).

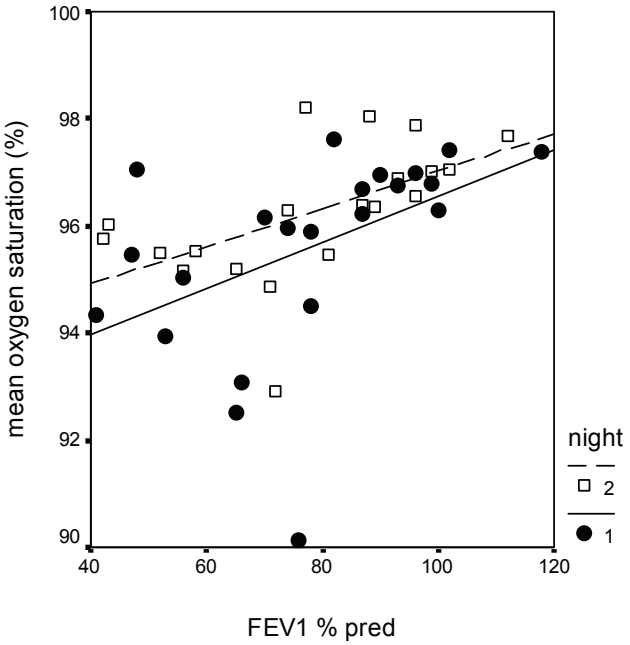

Figure 4. Scatter plot of mean oxygen saturation versus FEV_1 %pred for the two study nights. Lines represent regression lines; no significant difference between the two evaluated nights.

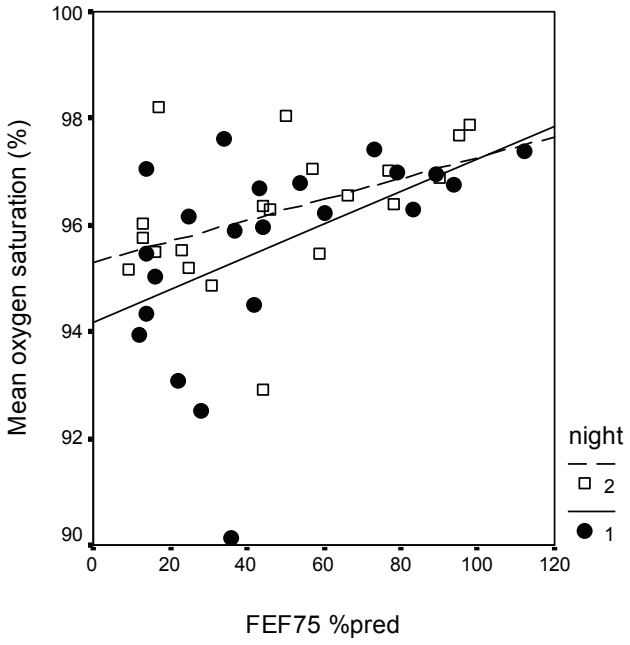

Figure 5. Scatter plot of mean oxygen saturation versus FEF_{75} %pred for the two study nights. Lines represent regression lines; no significant difference between the two evaluated nights.

DISCUSSION

In this study we recorded SO_2 profiles during two nights in children with clinically stable CF. The mean values were 95.6% and 96.2%, respectively, and thus lower than what has been reported 96.5-99.3% for healthy children. [11-13,15-17] In addition, we found correlations between mean SO_2 and other parameters of disease severity, such as lung function and CT scores.

The oxygen saturation we observed is comparable to that reported for a group of 72 CF patients aged 1-18 years (mean SaO_2 96.1%) in 2004 and for a group of 24 patients aged 8-13 years (mean SaO_2 96.1%) in 2007, but slightly higher than that for a group of 24 CF patients aged 10-22 years (mean SaO_2 94%) in 1990.[10,15,18] The latter might be explained by the younger age of our subjects and by a better clinical condition of patients today relative to 17 years ago. The subjects in the study by Versteegh and colleagues in 1990 had a mean FEV_1 of 57% (range 24-111), which is 20% below that of the patients in our study. The strong correlation (r_s=0.84) between the two study nights we found for oxygen saturation is in accordance with the finding of Versteegh and colleagues, who found a correlation of r_s=0.83. This would seem to implicate that for the mean SO_2 the recording of one night is in general sufficient to get a representative recording. But, using the mean SO_2 based on one night, gives a chance for missing low mean SO_2 from other nights. See Figure 2. However, for desaturation parameters such as the D_4 and D_{90} it still might be useful to measure two nights.

In this study we report frequency of desaturations at night in children with CF. This frequency ranged from 40-55%, whereas D_{90} events were reported in no more than 3 to 37% of healthy children.[11-14] Hence, children with CF seem to have more desaturation events than healthy children, which is similar as in the study of Uyan et al.[10]

In this study a correlation was found between lung function parameters and the mean SO_2, which confirms previous observations.[4,10,18,26,27] Versteegh and colleagues found a FEV_1% of ≤ 65% to be associated with nocturnal desaturation, defined as SO_2 ≤ 90%.[18] In a study in adults with CF no thresholds were found that could reliably predict desaturation during the night.[4] The same was true for our study. In the first night 5 out of 6 children with a FEV_1 ≤65 %pred had D_{90} events. Yet, 7 of the 16 children with a FEV_1 >65% also experienced D_{90} events. Although all children with a FEV1 %pred >82% had a mean SO_2 >96%, 67% of these children had D90 events during the first night. Therefore, although lung function parameters are correlated with oxygen saturation, they are of limited use in predicting desaturation periods. See Figure 6.

In this study nocturnal saturation was correlated with CT scores. We showed that low mean saturation is associated with structural lung damage, as reflected by a high total CT score or bronchiectasis subscore. This correlation is comparable with one other study in children with CF aged 8-13 years. They found a correlation of 0.67 between total CT

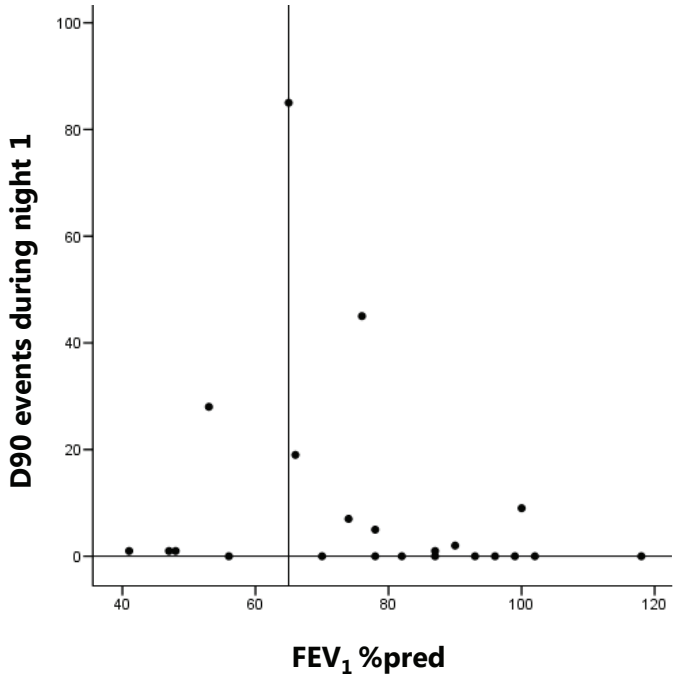

Figure 6. Relationship between FEV_1 %pred and desaturation events during the first night. Note: Area left of the vertical line represents FEV_1 <65 %pred, reported to be associated with nocturnal desaturation.[18]
D90= Fall in oxygen saturation to ≤90%.

score (using the Helbich score) and oxygen saturation.[10] Two other studies showed correlation coefficients from 0.35 to 0.57 between mean SO_2 and chest radiographs.[4,10,15] In one of these, CF patients had a mean FEV_1 of 58% (range 21-113).[4] The other study does not provide details on lung function measurements. Structural abnormalities on CT are considered an important surrogate endpoint for reflecting the severity of CF lung disease.[28] Our findings in this cross sectional study suggests that reduced nocturnal oxygen saturation in stable CF patients can be an indicator of structural abnormalities on CT. However, the air trapping subscore did not correlate with the mean*SO_2. This can be explained by the fact that we used only 3 expiratory slices to estimate the severity of air trapping. Recently, we showed that the use of only 3 slices underestimated the severity of air trapping for the Brody II score and increased the variability of the mean air trapping score. In addition, we did not use a controlled volume procedure, which may have introduced some extra variability in the estimate of the severity of air trapping.[29] Finally, air trapping as observed on CT may not be related to ventilation perfusion mismatch. Further longitudinal studies would be needed, however, to establish whether nocturnal oxygen saturation is able to track CF disease progression as accurately as CT.

We think it is highly relevant to monitor nocturnal oxygen saturation more closely in CF. It has been well recognised that sleep problems may have significant effects on daytime functioning.[5,7] In addition, even milder levels of desaturation have been associated with lower IQ and Attention Deficit Hyperactivity Disorder symptoms in children with other disorders than CF.[9] As shown by our results even stable CF patients have suboptimal nocturnal oxygen saturation and thus might be at risk for such disorders. Currently it is unclear what level of oxygen desaturation becomes important during sleep.[30] Clearly, the relation between subnormal oxygen saturation profiles in CF on sleep quality, IQ and behaviour needs to be further investigated.

The potential value of saturation profiles as an objective endpoint to evaluate the effect of therapeutic interventions has not been studied systematically. We speculate that the observed suboptimal nocturnal saturation profiles reflect especially peripheral airway obstruction as indicated by the reduced FEF_{75}. Intravenous antibiotic treatment and rigorous physiotherapy are likely to improve nocturnal saturation. Nocturnal oxygen saturation could even be a more clinically relevant surrogate endpoint than spirometer derived parameters in relation to the importance of good sleep quality.

In conclusion, oxygen saturation in children with stable CF is lower than that in healthy children, and is correlated with lung function parameters and CT scores. Monitoring oxygen saturation during one night is sufficient to get a representative recording. While desaturation periods were observed in some patients with well-preserved lung function, it did not occur in other patients with more advanced disease.

The value of nocturnal oxygen saturation as a surrogate endpoint should be further investigated in longitudinal interventional studies in larger cohorts.

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REFERENCES

1. Bradley S, Solin P, Wilson J, et al. Hypoxemia and hypercapnia during exercise and sleep in patients with cystic fibrosis. Chest 1999;116(3):647-654.
2. Tepper RS, Skatrud JB, Dempsey JA. Ventilation and oxygenation changes during sleep in cystic fibrosis. Chest 1983;84(4):388-393.
3. Milross MA, Piper AJ, Norman M, et al. Low-flow oxygen and bilevel ventilatory support: effects on ventilation during sleep in cystic fibrosis. Am J Respir Crit Care Med 2001;163(1):129-134.
4. Frangolias DD, Wilcox PG. Predictability of oxygen desaturation during sleep in patients with cystic fibrosis : clinical, spirometric, and exercise parameters. Chest 2001;119(2):434-441.
5. Milross MA, Piper AJ, Dobbin CJ, Bye PT, Grunstein RR. Sleep disordered breathing in cystic fibrosis. Sleep Med Rev 2004;8(4):295-308.
6. Stokes DC, McBride JT, Wall MA, Erba G, Strieder DJ. Sleep hypoxemia in young adults with cystic fibrosis. Am J Dis Child 1980;134(8):741-743.
7. Dancey DR, Tullis ED, Heslegrave R, Thornley K, Hanly PJ. Sleep quality and daytime function in adults with cystic fibrosis and severe lung disease. Eur Respir J 2002;19(3):504-510.
8. Jankelowitz L, Reid KJ, Wolfe L, et al. Cystic fibrosis patients have poor sleep quality despite normal sleep latency and efficiency. Chest 2005;127(5):1593-1599.
9. Bass JL, Corwin M, Gozal D, et al. The effect of chronic or intermittent hypoxia on cognition in childhood: a review of the evidence. Pediatrics 2004;114(3):805-816.
10. Uyan ZS, Ozdemir N, Ersu R, et al. Factors that correlate with sleep oxygenation in children with cystic fibrosis. Pediatr Pulmonol 2007;42(8):716-722.
11. Poets CF, Stebbens VA, Samuels MP, Southall DP. Oxygen saturation and breathing patterns in children. Pediatrics 1993;92(5):686-690.
12. Urschitz MS, Wolff J, Von Einem V, et al. Reference values for nocturnal home pulse oximetry during sleep in primary school children. Chest 2003;123(1):96-101.
13. Moss D, Urschitz MS, von Bodman A, et al. Reference values for nocturnal home polysomnography in primary schoolchildren. Pediatr Res 2005;58(5):958-965.
14. Verhulst SL, Schrauwen N, Haentjens D, et al. Reference values for sleep-related respiratory variables in asymptomatic European children and adolescents. Pediatr Pulmonol 2007;42(2):159-167.
15. Darracott C, McNamara PS, Pipon M, et al. Towards the development of cumulative overnight oximetry curves for children with cystic fibrosis. Journal of Cystic Fibrosis 2004;3(Suppl 1):S53.
16. Gries RE, Brooks LJ. Normal oxyhemoglobin saturation during sleep. How low does it go? Chest 1996;110(6):1489-1492.
17. Uliel S, Tauman R, Greenfeld M, Sivan Y. Normal polysomnographic respiratory values in children and adolescents. Chest 2004;125(3):872-878.
18. Versteegh FG, Bogaard JM, Raatgever JW, et al. Relationship between airway obstruction, desaturation during exercise and nocturnal hypoxaemia in cystic fibrosis patients. Eur Respir J 1990;3(1):68-73.
19. van der Giessen LJ, Gosselink R, Hop WC, Tiddens HA. Recombinant human DNase nebulisation in children with cystic fibrosis: before bedtime or after waking up? Eur Respir J 2007;30(4):763-768.
20. Roffwarg HP, Muzio JN, Dement WC. Ontogenetic Development of the Human Sleep-Dream Cycle. Science 1966;152:604-619.
21. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J 2005;26(2):319-338.

22. Zapletal A. Lung Function in Children and Adolescents. Zapletal A, Samanek M, Paul T, editors. Basel: Karger; 1987.
23. Brody AS, Klein JS, Molina PL, et al. High-resolution computed tomography in young patients with cystic fibrosis: distribution of abnormalities and correlation with pulmonary function tests. J Pediatr 2004;145(1):32-38.
24. Smith J, Owen E, Earis J, Woodcock A. Cough in COPD: correlation of objective monitoring with cough challenge and subjective assessments. Chest 2006;130(2):379-385.
25. Chang AB, Newman RG, Carlin JB, Phelan PD, Robertson CF. Subjective scoring of cough in children: parent-completed vs child- completed diary cards vs an objective method. Eur Respir J 1998;11(2):462-466.
26. Coffey MJ, FitzGerald MX, McNicholas WT. Comparison of oxygen desaturation during sleep and exercise in patients with cystic fibrosis. Chest 1991;100(3):659-662.
27. Milross MA, Piper AJ, Norman M, Willson GN, Grunstein RR, Sullivan CE, Bye PT. Predicting sleep-disordered breathing in patients with cystic fibrosis. Chest 2001;120(4):1239-1245.
28. Tiddens HA, de Jong PA. Imaging and clinical trials in cystic fibrosis. Proc Am Thorac Soc 2007;4(4):343-346.
29. Loeve M, Lequin MH, Bruijne Md, Hartmann I, Hop W, Tiddens HA. Measuring trapped air by computed tomography (CT) in CF: In search of the optimal protocol.; 2008; Berlin Germany. p abstract 3827.
30. Urquhart DS, Montgomery H, Jaffe A. Assessment of hypoxia in children with cystic fibrosis. Arch Dis Child 2005;90(11):1138-1143.

Chapter 7

Interrupter resistance: a tool for evaluating airway clearance in children with cystic fibrosis

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Submitted

ABSTRACT

Background

Airway clearance therapy (ACT) is used to improve expectoration in children with Cystic Fibrosis (CF). So far no gold standard is available to evaluate the efficacy of ACT. Resistance of the respiratory system can be measured with Interrupter technique ($Rint_e$).

Object

To assess the feasibility and sensitivity of $Rint_e$ to detect changes in airway resistance in relation to ACT

Methods

$Rint_e$ was performed directly before and after ACT in children with CF. The difference in $Rint_e$ ($\Delta Rint_e$) was used as a measure of response to ACT.
Sputum production was estimated with a semi quantitative scoring system.

Results

Eighteen children participated. Mean age (range) was 15 (7-19) years. $\Delta Rint_e$ correlated with the sputum production score during ACT (r_s=0.67, p=0,002) and with the daily sputum production score (r_s=0.62, p=0.007)

Conclusion

$Rint_e$ tends to improve after ACT and is negatively correlated to the sputum production.

INTRODUCTION

Airway clearance techniques (ACT) are considered cornerstone therapy in Cystic Fibrosis (CF) to improve mucociliary clearance.[1] Approximately 90% of all CF patients use some form of ACT.[2, 3] A Cochrane review demonstrated ACT to be effective in increasing mucus transport in the short term. No evidence was found with respect to long-term effects. Endpoints in the reviewed studies were sputum weight, total lung capacity, functional residual capacity and transport rate of radioactive tracers.[4]

A clinical applicable gold-standard method for evaluating the short-term efficacy of ACT in children with CF is still lacking. Combined measurement of mucus transport – using radioactive tracers – and expectorated mucus volume is probably the most reliable method to date.[1, 5, 6] The use of radioactive tracers in clinical practice, however, raises ethical concerns, and is technically difficult. Therefore, they are not a suitable outcome parameter. Monitoring of sputum weight or volume has its drawbacks as well. Patients often are reluctant to expectorate and there is the risk of inadvertent swallowing of secretions and of contamination of the secretions by saliva.[5] Furthermore, in younger children expectorated sputum is often not available and cannot be used as a study endpoint.

Standard pulmonary function tests, such as flow volume measurement, were frequently used to evaluate chest physiotherapy effectiveness.[5] However, the measurement of airflow and simple lung volumes do not appear to reflect changes in mucus transport and are relatively insensitive to airway clearance manoeuvres. For this reasons these tests are considered to be inadequate to evaluate ACT in short term studies. [5] A promising observation was that ACT has a positive effect on specific airway conductance (sGaw) as measured by plethysmography [7] suggesting a positive effect of ACT on central airway clearance.[8, 9] An alternative and easier tool to measure respiratory system resistance may be the interrupter technique or Rint.[10] Rint is sensitive to changes in airway calibre in children with mild respiratory tract infections and can be performed quickly in the ambulatory setting.[11] $Rint_e$ measurements have been shown to be reproducible.[12] Validation studies against measurement of airway resistance with body plethysmograph showed a good correlation between the two methods.[12, 13] We performed a study to assess whether Rint is a feasible tool for ACT studies, hypothesizing that ACT clears out mainly central airways and thus lowers airway resistance as measured by Rint.

MATERIAL AND METHODS

Study subjects

Eligible subjects were children with CF aged 6 years or older, hospitalized for pulmonary exacerbation in Erasmus MC Sophia Children's Hospital in Rotterdam, the Netherlands and who were able to perform ACT. Exclusion criteria were: inability to perform reproducible pulmonary lung function tests; being in the process of learning a new form of ACT; inability to expectorate sputum.

Study design

Rint measurements were done before and after routine ACT in the first week of a hospital admission for a pulmonary exacerbation. ACT techniques used are shown in Table 1. Children were asked to score their daily sputum expectoration score (DSE) by a semi-

Table 1. Form of ACT

	N / %
PEP mask	4 / 22%
Autogenic drainage	9 / 50%
Flutter® VRP1	2 / 11%
Combination	3 / 17%

quantitative scoring system, ranging from 0=no sputum to 3=more than a cup of sputum a day (Table 2). The physiotherapist, who performed the ACT, documented the "ACT related sputum expectoration score" (ASE) using a semi quantitative scoring system ranging from 0=no sputum to 4=more than 10 times sputum expectoration during ACT (Table 3). Duration of ACT was standardized 30 minutes. Pulmonary function tests (PFT) were carried out in the first week after admission.

Table 2. Daily sputum expectoration score (DSE)

Score	**Description**	**N / %**
0	nothing – one tablespoon a day	3 / 16
1	one tablespoon – half a cup	10 / 56
2	half a cup – 1 cup	5 / 28
3	more than a cup	0 / 0

Table 3. ACT sputum expectoration (ASE)

Score	Description	N / %
0	0 times expectorate sputum	2 / 11
1	1-3 times expectorate sputum	2 / 11
2	4-6 times expectorate sputum	4 / 22
3	7-9 times expectorate sputum	2 / 11
4	> 10 times expectorate sputum	8 / 44

Equipment specifications

We used the MicroRint (Micro Medical Ltd, Rochester, UK), a portable device connected to a palmtop computer. MicroRint has an online display showing: mouth pressure; time of shutter closure; Rint values; and median value of all Rint data recorded during one session. Measurements of the interrupter resistance (Rint) are based on measurements of tidal airflow before and mouth pressure after closure of a fast shutter. The ratio between the pressure difference and airflow equals the interrupter resistance and reflects respiratory resistance.

Pulmonary functions tests (PFT) were done using a Jäger diagnostic system (Masterlab, Jäger, Germany). Flow was calibrated daily using a 1-L precision pump. The following PFT results were obtained: forced expiratory volume in one second (FEV_1); forced vital capacity (FVC); and expiratory flow at 25% of the actual forced vital capacity (MEF_{25}). Spirometry was performed in triplicate according to ERS guidelines.[14] The PFT results were expressed as a percentage of predicted values.[15]

Procedure

The physiotherapist was trained by a certified lung function technician until she was able to perform the Rint measurements according to their standards. Rint measurements were carried out in the morning in the patient's room by one investigator (LG). Children were asked to sit quietly for 10 minutes prior to the procedure and then clear their throat by coughing. The hands of the investigator supported the patient's cheeks and chin to reduce upper airway compliance.[16] To accustom the children to the sound of the shutter its functioning was demonstrated to the children before the actual measurements. Children were instructed to sit upright and breathe quietly during measurements. Next, a minimum of 5 and a maximum of 10 correct tracings were collected during the expiratory phase ($Rint_e$) of the breathing cycle. A correct tracing consisted of a regular breathing pattern and a consistent shape of the mouth pressure-time curve.[17,18] Expiratory interruptions are considered to be more sensitive than inspiratory interruptions to detect intrathoracic airway obstruction.[12] During $Rint_e$ measurements the head

was positioned in a slight extension and a nose clip was used. The hands of the observer supported the cheeks and chin to reduce the effect of upper airway compliance on airway resistance.[16,19] After this measurement routine ACT was conducted for 30 minutes. Finally, $Rint_e$ measurements were repeated 10 minutes after ACT completion.

Data analysis

Statistical analysis was performed with SPSS version 10.0.
$Rint_e$ before and after ACT were expressed as median values to reduce the effect of possible outliers.[20] The change in $Rint_e$ ($\Delta Rint_e$) was calculated for each individual patient as the difference between $Rint_{e\ (before)}$ and $Rint_{e\ (after)}$. Hypothesizing a decrease of resistance following ACT, the values of $Rint_{e\ (before)}$ and $Rint_{e\ (after)}$ were compared using a paired T-test. Spearman rank correlation coefficient (r_s) was used to examine relations between $\Delta Rint_{e,}$ lung function, age, $Rint_{e\ (before),}$ sputum production during ACT, and daily sputum production. Power calculation showed that 17 patients were needed to detect a correlation of 0.7 at alpha is 0.05 with a power of 90%.

RESULTS

$Rint_e$ measurements could be performed without difficulty in all 18 eligible children (7 male; 11 female). The distribution of DSE and ASE scores are shown in Tables 2 and 3. The patient characteristics are shown in Table 4. There was a trend towards a reduction of the $Rint_{e\ (after)}$ relative to the $Rint_{e\ (before)}$ (95% CI –0.005 to 0.093 kPa/L/s; p=0.07). Sixty-one percent (n=11) of the children improved their $Rint_e$ after ACT, 5% (n=1) had no difference and 33% (n=6) had deterioration in $Rint_e$. The median drop in resistance was 6%. $\Delta Rint_e$ varied between –0.13 and 0.26 kPa/L/s. $\Delta Rint_e$ correlated significantly with the ACT sputum production score (r_s=0.67, p=0.002) (Figure 1) and with the daily sputum production score (r_s=0.62, p=0.007) (Figure 2). $Rint_{e\ (before)}$ correlated significantly with the daily sputum production score (r_s=0.67, p=0.002). No significant correlation was found between $\Delta Rint_e$ and baseline FVC, FEV_1 or MEF_{25}. The daily sputum produc-

Table 4. Patient characteristics

	Mean (range)
Age (yrs)	14.7 (6.8-19.7)
FVC (%pred)	81 (37-123)
FEV_1 (%pred)	68 (31-113)
MEF_{25} (%pred)	26 (5-58)
$Rint_e$ (kPa/L/s)	0.60 (0.26-1.46)

tion score and the ACT sputum production score were significantly correlated (r_s=0.7, p=0.002).

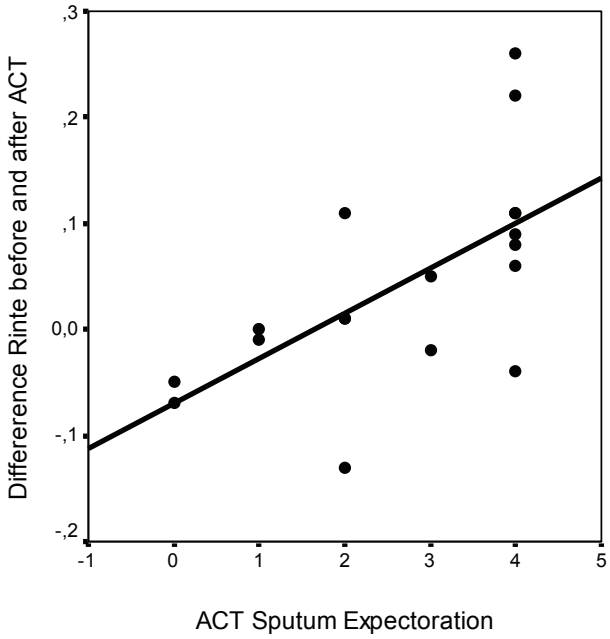

Figure 1. Correlation between $\Delta Rint_e$ and the ACT sputum expectoration score (r_s=0.67, p=0.002).

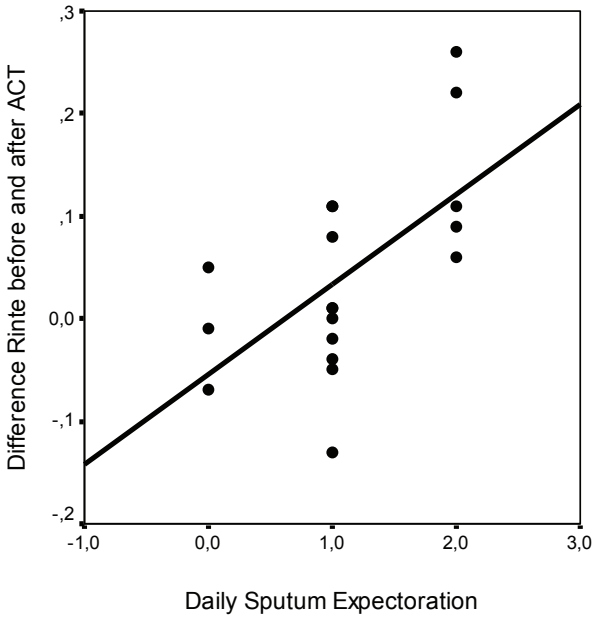

Figure 2. Correlation between $\Delta Rint_e$ and the daily sputum expectoration score (r_s=0.62, p=0.007).

DISCUSSION

The objective of this pilot study was to assess the feasibility of $Rint_e$ to detect changes in respiratory resistance in relation to ACT.

$Rint_e$ measurements were feasible in all eligible patients. ACT had a borderline effect on $Rint_e$ and changes in $Rint_e$ correlated significantly with the reported amounts of expectorated mucus.

The relation between $Rint_{e\ (before)}$ and $Rint_{e\ (after)}$ is probably not straightforward. $Rint_{e\ (after)}$ was even higher than $Rint_{e\ (before)}$ in the five children who were not able to mobilize sputum during ACT. In these patients, sputum might have been mobilized from the peripheral airways into the more central airways, thereby increasing central airway resistance. Patients with higher ASE or DSE scores had the greatest fall in respiratory resistance. Low sputum scores were associated with wider variability in decline of $Rint_e$. This suggests that $Rint_e$ is a more suitable outcome variable in patients with greater sputum production. One other study investigated the $Rint_e$ before and after ACT in children with CF. They found in 77% a fall in $Rint_e$. In this study the amount of sputum was not measured.[21]

We could not demonstrate a correlation between sputum production and expiratory flow rate. Our findings are in agreement with those from other studies that investigated the effect of ACT on lung function.[7-9, 22, 23] ACT previously was shown to improve specific airway conductance (sGaw) and to reduce functional residual capacity.[7, 23] However, no relation was observed between sputum production and sGaw increase.[7] The respiratory resistance can be underestimated by $Rint_e$ in patients with more severe airways obstruction because alveolar pressures will be underestimated when time constants are too long for equilibration between alveolar and mouth pressure to occur.[24] We therefore speculate that the difference in $Rint_e$ following ACT is underestimated in those subjects with more severe obstruction.

Healthy children show 8.5% fall in $Rint_e$ after bronchodilatation.[25] Children with CF varied greatly in their response with $Rint_e$ after bronchodilatation. These authors conclude that $Rint_e$ cannot be used as a substitute for spirometry in school children with CF undergoing bronchodilator challenge.[26] The fall in our study was 6%, but the clinical relevance of this order of magnitude for children with CF cannot be confirmed at this moment.

We have assumed a causal relationship between the measured difference in $Rint_e$ and ACT. However, we assume that the observed differences might not have been related to the mobilization of sputum, but rather to, for example, physiological fluctuations of $Rint_e$.

For future studies we recommended to combine measurement of $Rint_e$ with Sgaw measurement or with measurements of ventilation inhomogeneity, so as to exclude other explanation for the observed fluctuations in resistance. Measurement with forced ex-

piration manoeuvres are not recommended because they can result in sputum expectoration and thus influence the respiratory resistance. Then, our study design did not provide for blinding of the investigator for timing of ACT. In future studies it would be important to blind the investigator for the timing of ACT. Another limitation of our study is that $Rint_e$ was only compared to a non-validated semi–quantitative scoring system for sputum volume; sputum weight or volume did not serve as a golden standard. Because it is easy in use, cheaper than monitoring sputum weight and gives a sufficient insight in the amount of sputum evacuation of the patient, it is worthwhile to validate this scoring system with sputum weight or volume in the future.

In conclusion, $Rint_e$ is a feasible method that may serve as an outcome variable for short-term ACT studies. $Rint_e$ tends to improve after ACT and is negatively correlated to the sputum production.

REFERENCES

1. Robinson M, Bye PT. Mucociliary clearance in cystic fibrosis. Pediatr Pulmonol. 2002;33(4):293-306.
2. ERCF. ERCF Annual Report 1998: F. Hoffmann-La Roche Ltd; 2000.
3. Konstan MW, Butler SM, Schidlow DV, et al. Patterns of medical practice in cystic fibrosis: part II. Use of therapies. Investigators and Coordinators of the Epidemiologic Study of Cystic Fibrosis. Pediatr Pulmonol. 1999;28(4):248-254.
4. van der Schans C, Prasad A, Main E. Chest physiotherapy compared to no chest physiotherapy for cystic fibrosis. Cochrane Database Syst Rev. 2000(2).
5. van der Schans CP, Postma DS, Koeter GH, Rubin BK. Physiotherapy and bronchial mucus transport. Eur Respir J. 1999;13(6):1477-1486.
6. Robinson M, Eberl S, Tomlinson C, et al. Regional mucociliary clearance in patients with cystic fibrosis. J Aerosol Med. 2000;13(2):73-86.
7. Cochrane GM, Webber BA, Clarke SW. Effects of sputum on pulmonary function. Br Med J. 1977;2(6096):1181-1183.
8. Weller PH, Bush E, Preece MA, Matthew DJ. Short-term effects of chest physiotherapy on pulmonary function in children with cystic fibrosis. Respiration. 1980;40(1):53-56.
9. Kerrebijn KF, Veentjer R, Bonzet-vd Water E. The immediate effect of physiotherapy and aerosol treatment on pulmonary function in children with cystic fibrosis. Eur J Respir Dis. 1982;63(1):35-42.
10. Carter ER, Stecenko AA, Pollock BH, Jaeger MJ. Evaluation of the interrupter technique for the use of assessing airway obstruction in children. Pediatr Pulmonol. 1994;17(4):211-217.
11. Merkus P, Arets H, Joosten T, et al. Measurements of interrupter resistance: reference values for children 3-13 years of age. Eur Respir J. Vol 20; 2002:907-911.
12. Merkus PJ, Mijnsbergen JY, Hop WC, de Jongste JC. Interrupter resistance in preschool children: measurement characteristics and reference values. Am J Respir Crit Care Med. 2001;163(6):1350-1355.
13. Phagoo SB, Watson RA, Pride NB, Silverman M. Accuracy and sensitivity of the interrupter technique for measuring the response to bronchial challenge in normal subjects. Eur Respir J. 1993;6(7):996-1003.
14. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-338.
15. Zapletal A. Lung Function in Children and Adolescents. Basel: Karger; 1987.
16. Hadjikoumi I, Hassan A, Milner AD. Effects of respiratory timing and cheek support on resistance measurements, before and after bronchodilation in asthmatic children using the interrupter technique. Pediatr Pulmonol. 2003;36(6):495-501.
17. Beydon N, Davis SD, Lombardi E, et al. An official american thoracic society/european respiratory society statement: pulmonary function testing in preschool children. Am J Respir Crit Care Med. 2007;175(12):1304-1345.
18. Bridge PD, Ranganathan S, McKenzie SA. Measurement of airway resistance using the interrupter technique in preschool children in the ambulatory setting. Eur Respir J. 1999;13(4):792-796.
19. Oswald-Mammosser M, Llerena C, Speich JP, Donata L, Lonsdorfer. Measurements of respiratory system resistance by the interrupter technique in healthy and asthmatic children. Pediatr Pulmonol. 1997;24(2):78-85.
20. Bridge PD, McKenzie SA. Airway resistance measured by the interrupter technique: expiration or inspiration, mean or median? Eur Respir J. 2001;17(3):495-498.

21. Morrison L, Ball R, Conway SP, Brownlee KG. Airway Resistance Measurements in Children with Cystic Fibrosis. Physiotherapy. 2003;89(11):629-636.
22. Desmond KJ, Schwenk WF, Thomas E, Beaudry PH, Coates AL. Immediate and long-term effects of chest physiotherapy in patients with cystic fibrosis. J Pediatr. 1983;103(4):538-542.
23. Newton DA, Stephenson A. Effect of physiotherapy on pulmonary function. A laboratory study. Lancet. 1978;2(8083):228-229.
24. Oswald-Mammosser M, Charloux A, Donato L, et al. Interrupter technique versus plethysmography for measurement of respiratory resistance in children with asthma or cystic fibrosis. Pediatr Pulmonol. 2000;29(3):213-220.
25. Chan EY, Bridge PD, Dundas I, et al. Repeatability of airway resistance measurements made using the interrupter technique. Thorax. 2003;58(4):344-347.
26. Davies PL, Doull IJ, Child F. The interrupter technique to assess airway responsiveness in children with cystic fibrosis. Pediatr Pulmonol. 2007;42(1):23-28.

Chapter 8

Playing the board game ***'Airway'*** increases children's knowledge about cystic fibrosis lung disease

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Submitted

ABSTRACT

Introduction

More than half of the pediatric Cystic Fibrosis (CF) population is non-adherent to CF treatment. Inadequate knowledge of the disease and treatment are factors that might influence compliance negatively. To increase patients' knowledge about CF lung disease we developed a board game named '*Airway'*. The effect of playing *'Airway'* was evaluated in a randomized controlled study.

Objective

To increase pediatric CF patients' knowledge of CF lung disease.

Patients and methods

'Airway' is played by a pediatric physiotherapist and one child. To study the effect on knowledge by playing *'Airway'* we performed a randomized controlled study. Inclusion criteria: proven CF, age 7-13 years. Children were randomized into two groups. Both groups were assessed twice with a written knowledge questionnaire including 16 multiple choice questions about airways, CF and treatment. Only children in the intervention group played 'Airway' in between the two questionnaire rounds.

Results

Thirty-seven children participated, 17 in the intervention group, 20 in the control group. Patient characteristics: boys n = 9 and 10, mean age 10.5 (range 8-13) and 10 (8-13) years, respectively. The mean percentage of correct answers to the questionnaire increased by 13% for the intervention group and by 5% for the control group (p=0.03).

Conclusion

Playing the board game *'Airway'* increases children's knowledge about CF lung disease.

INTRODUCTION

Cystic fibrosis (CF) is the most common autosomal recessive disease in the Caucasian population. It is a complex illness, involving many organ systems such as the respiratory and gastro-intestinal tracts. In the lungs, CF is characterized by abnormal mucus accumulation. As a result the airways become chronically infected with bacteria, so that lung function progressively deteriorates.[1] This eventually leads to premature death due to respiratory failure in the majority of the patients. Treatment of the airways is initiated immediately after diagnosis and has to be continued life long. Treatment intensity is stepped up gradually with increasing age and generally represents a great burden to the patients.[2] Home treatment for CF lung disease consists of oral and nebulised antibiotics, daily airway clearance therapy (ACT) and physical exercise, and nebulisation of mucolytics such as rhDNase and hypertonic saline. In addition most patients have pancreatic insufficiency and thus require daily pancreatic enzymes and vitamins. Compliance to treatment is an important factor that determines morbidity and mortality.[3] Not surprisingly, the highly complex nature of CF treatment is a major challenge to compliance. Up to 50% of pediatric populations have been found non-adherent to their CF treatment regimens.[4, 5] Compliance rates for ACT in children range from 40 to 75% [5-7] and for nebulisation with rhDNase from 57 to 90% [6, 8]. Compliance is a complex issue affected by variables such as: age, inadequate knowledge; psychosocial resistance and cognitive functioning.[9, 10]
Both knowledge of the disease and treatment regimen and understanding of the background of the physicians' treatment recommendations are thought to be critical to compliance. Substantial gaps in knowledge about CF lung disease have been identified in children and their parents.[11] In two studies the level of compliance among children with CF was positively associated with the level of their knowledge of the disease.[12, 13] In another study no such relationship was found.[11]
We aimed to test a purpose-developed instrument which could potentially increase children's knowledge about their CF lung disease: a board game named *'Airway'*. We hypothesized that playing this game would increase children's knowledge about their CF lung disease.

MATERIAL AND METHODS

Board game

'Airway' was developed in close collaboration with primary education teacher-training college "Thomas More" (Rotterdam, the Netherlands) and educational publisher Xplore (Hoevelaken, the Netherlands). The language of *'Airway'* is Dutch, but during develop-

ment we took into account that easy translation in other languages would be feasible. *'Airway'* is played by the child and a trained physiotherapist, and involves tasks and questions focussed on CF, sputum physiology, infection and treatment. Interaction and discussion are facilitated during the game. *'Airway'* takes approximately 45 minutes to play. Figure 1 shows an example of one of the tasks.

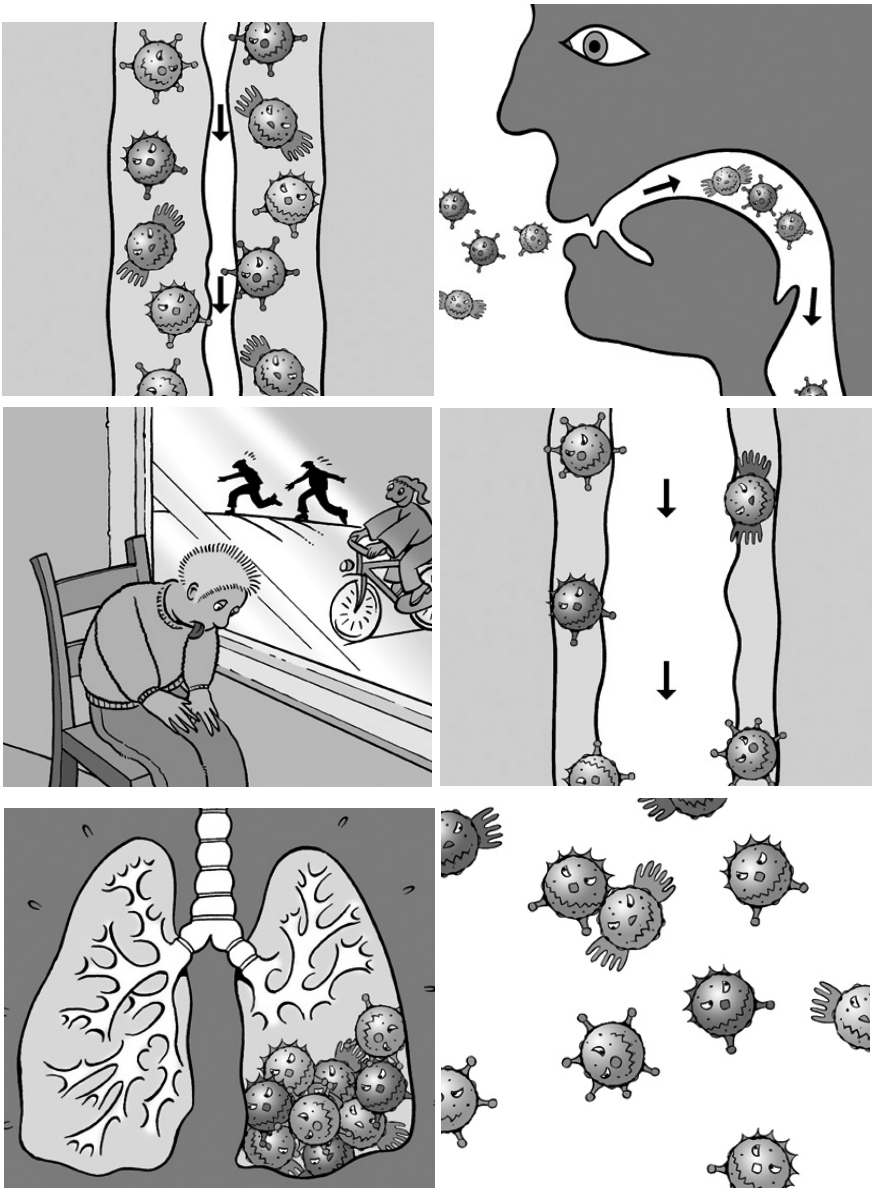

Figure 1. Example of task 'place the pictures in the right order' in the board game *'Airway'*.

Knowledge test

To test children's knowledge level of CF lung disease a dedicated Knowledge Questionnaire (KQ) was developed in close collaboration with primary education teacher-training college "Thomas More" (Rotterdam, the Netherlands). It consists of 16 multiple-choice questions with each three possible answers. The questions are focussed on lung function and anatomy, the effect of CF on the lungs, and treatment of CF lung disease. Questions about emotional aspects of the disease were not included in the KQ. The KQ was tested in six healthy primary school children aged 7 to 13 years. These children were asked to provide feedback on the wording of the questions.

Study subjects

Patients followed by the CF centre at the Erasmus MC – Sophia Children's Hospital were eligible for the study when they met the following criteria: proven CF, age between 7- 13 years. CF was defined as clinical symptoms characteristic for CF plus an abnormal sweat test and/or the presence of two CF mutations. Patients with severe mental retardation were excluded from this study

Study design

The study had a randomized controlled design. Children who met the inclusion criteria were invited by letter to participate in this study. After informed consent by the parents and when applicable by the older children (>12 years) the subjects were randomized to either an intervention group (I) or to a control group (II). Children enrolled in the intervention group came from the south-western Netherlands, children enrolled in the control group from the area north of Rotterdam. Both areas are demographically comparable.

All subjects received the KQ by mail and were asked to return it within one week. An accompanying letter asked parents not to assist the children in completing the questions. Next, a pediatric physiotherapist (SOS) with CF experience scheduled appointments with the children assigned to the intervention group, intended to play *'Airway'*; these appointments were planned within one week after the KQ was completed. Children assigned to the control group did not play the game. Thereupon all subjects were asked to complete the KQ again within one week. The pediatric physiotherapist (SOS) who played the game was blinded to the responses to the first KQ. The time interval between the two KQ's was the same for intervention group and the control group.

The Erasmus MC Medical Ethical Review Board approved the protocol. The study was performed according to ICH-GCP guidelines.

Data analysis

Primary outcome variable was the change from baseline of the % correct answers to the questions in the KQ. The Mann-Whitney test was used to compare the difference of these changes between the study groups. Within groups analysis of the number of correct answers was done using Wilcoxon Signed Rank Test. Spearman correlation coefficients were used to investigate associations between lung function parameters and age versus the baseline KQ scores.
Statistical analysis was performed with SPSS version 11.0, and P=0.05 (two-sided) was considered the limit of significance in all analyses.

RESULTS

Of the 152 children followed by the CF centre, 47 met the inclusion criteria and were invited by letter to participate. Four children (8%) did not respond and six children (13%) declined to participate. Hence, the total study group included 37 children (79%), 17 of whom were enrolled in the intervention group and 20 in the control group
Baseline characteristics are summarized in Table 1. The groups did not differ significantly regarding distribution of age, lung function and sex.

Table 1. Patient characteristics
Characteristics of the study population (numbers of patients or median with range) for Group I and group 2. Group 1 played the board game *'Airway'*; group 2 did not play the game.

	Group 1	**Group 2**	**p-value***
N	17	20	NS
Sex (male/female)	9 / 8	10 / 10	NS
Age (year)	11.0 (8.0-12.8)	9.9 (7.9-12.9)	NS
FVC (% predicted)	88 (66-120)	88 (48-117)	NS
FEV_1 (% predicted)	85 (53-122)	87 (43-119)	NS
MEF_{25} (% predicted)	45 (10-105)	43 (12-131)	NS

* P-value according to Mann-Whitney test

The mean number of correct answers in the intervention group increased significantly (p=<0.001) from 12.9 (range 7-15), to 15.1 (range 13-16) after playing *'Airway'*. The mean number of correct answers in the control group did not increase significantly (p=0.08) from 13.4 (range 5-16), to 14.2 (89%; range 8-16). Hence, the mean percentage of correct answers increased significantly more in the intervention group as compared to the control group: 13% and 5%, respectively (p=0.03). Figure 2 shows individual outcomes of the study.

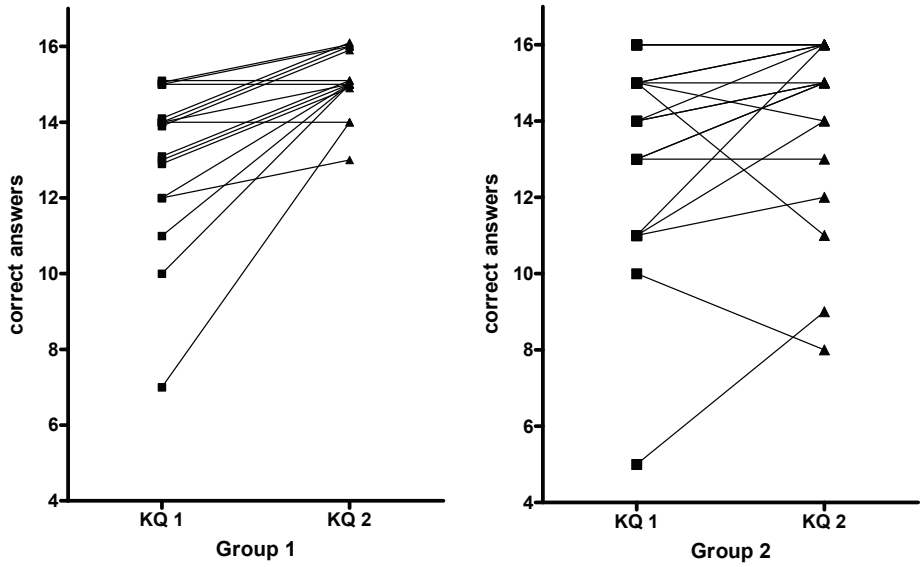

Figure 2. Numbers of correct answers to the 16 items of Knowledge Questionnaire (KQ) round 1 and 2. Children in group 1 played the board game *'Airway'* between the two KQ rounds. Lines connect individual data points. Bars represent mean values.

Children were generally knowledgeable about general items, such as location of the lungs, how to get as much air as possible in the lungs, function of cough and the presence of viscous sputum in CF. But also they showed major gaps in their understanding of the disease. For example, 18 (49%) children thought that swallowed sputum went back into the lungs, 19 (51%) were not aware that airways are smaller toward the periphery, 19 (51%) were not aware of the role of the abdominal muscles to cough up sputum effectively, and 5 (14%) children guessed that sputum in the lungs eventually would be eaten by bacteria.

No significant correlations were found between lung function parameters and age versus the percentage of correct answers.

DISCUSSION

In this study, children who were playing the board game *'Airway'* showed significantly improved knowledge about CF lung disease as compared to a control group. This finding confirms the results of another study that aimed to increase knowledge about CF lung disease within the framework of a self-management program.[14] In this study the higher knowledge level was maintained at follow-up 12 months later.

The baseline findings of our study showed that the children in our centre were relatively well informed about lung issues in relation to CF with a mean number of 13.2 correct answers out of 16 questions. Nevertheless some important misapprehensions were detected. The children in our centre seem to be much better informed about their disease than two decades ago. Our results thus clearly differ from those of a South African study in 1990 which concluded that patients' knowledge was poor.[15] In another study from 1996 adult patients had – comparable to our study – good general baseline knowledge about the lungs but misconceptions were observed.[16] A good baseline level of knowledge may be related to better possibilities to obtain information from the Internet, and the availability of other educational materials. In addition, CF teams today are likely to be more motivated to explain the pathophysiology of CF lung disease to their patients. Clearly, the results of this study are center specific. Both the KQ and the board game can be useful tools to systematically evaluate the level of education within different centres. As stated, the KQ and the board game have been designed in such a way that translation and validation into other languages will be relatively easy.

Importantly, playing *'Airway'* with the children allowed the physiotherapist to go over basic elements of CF lung disease in a systematic and joyful fashion. It proved to be a useful tool to facilitate interaction and discussion about the therapy. It gives the physiotherapist an excellent opportunity to teach the children the benefits and necessity of the therapy and to learn about specific gaps in the knowledge of individual patients. By educating individual patients about their current physical condition and progression of disease, the physiotherapist may stress the necessity of therapy compliance, without causing the patient to get overly concerned.[17] Generally, continuous educational dialogue with the patient about the treatment will empower the patient to take ownership for treatment.[18]

Another advantage of *'Airway'* is that relatively young children of seven years and older can play the game. We believe that it is important to teach young children about the importance of therapy compliance and to train them in taking some responsibility for their treatment before reaching adolescence. Throughout adolescence children should move towards complete control over their lives. The tendency in adolescents to rebel against authority often results in problems with compliance in this particular age group.[12,18] Playing *'Airway'* takes around 45 min per child. We feel strongly that this is time well spent.

Daily aerosol and ACT treatment regimens are recommended for most children with CF to assist the removal of excess sputum. These treatments are complex, and are associated with little immediate reward.[14] In situations like these, it has proven difficult for children to understand benefits and necessity of treatment compliance.[19] Therefore we believe that it is very important to ensure that children gain as much knowledge about CF lung disease as possible.

From this study it does not become clear how long the increased knowledge will persist and whether it results in improved compliance. There are studies, however, which support the general concept that the level of compliance among children with CF is related to their knowledge of the disease.[12, 13]

Our study showed that playing the board game *'Airway'* increases children's knowledge about CF lung disease and can help to identify specific knowledge gaps in the patient's understanding of the disease.

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REFERENCES

1. FitzSimmons SC. The changing epidemiology of cystic fibrosis. J Pediatr. 1993;122(1):1-9.
2. Morgan WJ, Butler SM, Johnson CA, et al. Epidemiologic study of cystic fibrosis: design and implementation of a prospective, multicenter, observational study of patients with cystic fibrosis in the U.S. and Canada. Pediatr Pulmonol. 1999;28(4):231-241.
3. Smith BA, Wood BL. Psychological factors affecting disease activity in children and adolescents with cystic fibrosis: medical adherence as a mediator. Curr Opin Pediatr. 2007;19(5):553-558.
4. La Greca A, Schuman WB. Adherence to prescribed medical regimens. In: Roberts MC, ed. Handbook of pediatric psychology. Vol 2nd. New York: Guilford Press; 1995:55-83.
5. Passero MA, Remor B, Salomon J. Patient-reported compliance with cystic fibrosis therapy. Clin Pediatr (Phila). 1981;20(4):264-268.
6. Modi AC, Lim CS, Yu N, et al. A multi-method assessment of treatment adherence for children with cystic fibrosis. J Cyst Fibros. 2006;5(3):177-185.
7. Myers LB, Horn SA. Adherence to chest physiotherapy in adults with cystic fibrosis. J Health Psychol. 2006;11(6):915-926.
8. Zindani GN, Streetman DD, Streetman DS, Nasr SZ. Adherence to treatment in children and adolescent patients with cystic fibrosis. J Adolesc Health. 2006;38(1):13-17.
9. Koocher GP, McGrath ML, Gudas LJ. Typologies of nonadherence in cystic fibrosis. J Dev Behav Pediatr. 1990;11(6):353-358.
10. Bernard RS, Cohen LL. Increasing adherence to cystic fibrosis treatment: a systematic review of behavioral techniques. Pediatr Pulmonol. 2004;37(1):8-16.
11. Modi AC, Quittner AL. Barriers to treatment adherence for children with cystic fibrosis and asthma: what gets in the way? J Pediatr Psychol. 2006;31(8):846-858.
12. Gudas LJ, Koocher GP, Wypij D. Perceptions of medical compliance in children and adolescents with cystic fibrosis. J Dev Behav Pediatr. 1991;12(4):236-242.
13. Ievers CE, Brown RT, Drotar D, et al. Knowledge of physician prescriptions and adherence to treatment among children with cystic fibrosis and their mothers. J Dev Behav Pediatr. 1999;20(5):335-343.
14. Downs JA, Roberts CM, Blackmore AM, Le Souef PN, Jenkins SC. Benefits of an education programme on the self-management of aerosol and airway clearance treatments for children with cystic fibrosis. Chron Respir Dis. 2006;3(1):19-27.
15. Henley LD, Hill ID. Global and specific disease-related information needs of cystic fibrosis patients and their families. Pediatrics. 1990;85(6):1015-1021.
16. Conway SP, Pond MN, Watson A, Hamnett T. Knowledge of adult patients with cystic fibrosis about their illness. Thorax. 1996;51(1):34-38.
17. Homnick DN. Making airway clearance successful. Paediatr Respir Rev. 2007;8(1):40-45.
18. Prasad SA, Cerny FJ. Factors that influence adherence to exercise and their effectiveness: application to cystic fibrosis. Pediatr Pulmonol. 2002;34(1):66-72.
19. Litt IF, Cuskey WR, Rudd S. Identifying adolescents at risk for noncompliance with contraceptive therapy. J Pediatr. 1980;96(4):742-745.

Chapter 9

Summary

SUMMARY

Cystic fibrosis (CF) is an autosomal recessive life-limiting disease. It is chronic and complex, involving organ systems such as the respiratory tract and the gastro-intestinal tract. Chronic lung infection and inflammation start early in life and result in airway wall thickening and plugging of bronchioles with purulent secretions.
The primary aim of therapy in CF lung disease is to preserve the normal architecture of the lung and to prevent any damage from occurring. Its management includes frequent treatment with antibiotics, mucoactive drugs and airway clearance techniques (ACT). Treatment is lifelong, and ensuring constant adherence to the often burdensome treatment regimens is a major challenge for the patient and the CF team alike. This thesis is focused on the timing of rhDNase inhalation and ACT, outcome measures in CF lung disease, as well as patient education about CF lung disease.

Chapter 1 contains a general introduction to the thesis; **Chapter 2** explains the outlines and aims of the studies.

In **Chapter 3** we describe a randomized, double blind, double dummy, cross over study performed to compare the effects of rhDNase before ACT versus rhDNase after ACT in 25 children with CF. All children were on maintenance therapy with rhDNase. We created two groups. Children in group I inhaled rhDNase 30 minutes before ACT, and placebo directly after ACT in weeks 1-3. The protocol was reversed during weeks 4-6. The other group performed the reversed sequence. All children continued their daily routine ACT. Primary endpoint was MEF_{25} %pred as an indicator of peripheral airway patency. We concluded that inhalation of rhDNase before ACT improves MEF_{25} %pred in children with CF.

In **Chapter 4** we present the results of a second randomized, double blind, double dummy, cross over study. This was aimed at comparing the effects of rhDNase inhaled before bedtime or after waking up in 25 children with CF. All children were on maintenance therapy with rhDNase. We created two groups. Children in Group I inhaled rhDNase before bedtime, and a placebo after waking up in weeks 1-2. The protocol was reversed during weeks 3-4. The other group performed the reversed sequence. Patients performed their routine ACT 30 minutes after the nebulisation in the morning. Primary endpoint was MEF_{25} %pred as an indicator of peripheral airway patency. We found no significant differences in lung function parameters between the study periods when rhDNase was inhaled before bedtime and those when it was inhaled after waking up. Neither did nocturnal cough frequency, oxygen saturation, or the other secondary endpoints significantly differ between the two study periods.

We concluded that in children with CF who are on maintenance treatment with rhDNase it is equally effective and safe to nebulise rhDNase before bedtime or after waking up.

In **Chapter 5** we examined the nocturnal cough frequency in 25 children with stable CF. During two nights cough was recorded with a digital audio recorder. In addition, oxygen saturation was measured. The day following the recording spirometry was carried out. To quantify the severity of CF lung disease we scored the most recent routine CT scan. Cough was expressed in cough seconds and in cough seconds per hour.
We found that the frequency of nocturnal cough in these children was higher than that described for normal children. Nocturnal cough tended to be more severe in children with more advanced CF lung disease. Cough was more severe in the first hour of sleep and was highly variable for individual patients from night to night.
We concluded that more studies are needed to assess whether cough frequency is sufficiently sensitive to be used as a surrogate endpoint in clinical studies aimed to improve mucociliary clearance. In addition, further studies should determine the optimal number of nights to be assessed for obtaining a reliable estimate of a patient's severity of nocturnal cough.

In **Chapter 6** we examined oxygen saturation during sleep in 25 children with CF. During two nights oxygen saturation was measured using pulse oximetry. The days following the recordings, lung function tests were carried out.
We found that the nocturnal oxygen saturation in children with stable CF was lower than that in healthy children, and was correlated with lung function parameters and CT scores. The mean oxygen saturation between the two nights correlated strongly. Lung function parameters did not predict nocturnal desaturation.
We concluded that monitoring oxygen saturation during one night is sufficient to get a representative recording for an individual patient.

In **Chapter 7** we assessed the feasibility and sensitivity of a method to detect changes in airway resistance in relation to ACT. This interrupter technique ($Rint_e$) was performed directly before and after ACT in 18 children with CF. The difference in $Rint_e$ was used as a measure of response to ACT.
Sputum production was estimated with a semi quantitative scoring system.
We found that $Rint_e$ was feasible in all children. Furthermore $Rint_e$ tended to improve after ACT and was negatively correlated to the sputum production. We concluded that $Rint_e$ is a feasible method but its clinical relevance for ACT in CF is questionable.

In **Chapter 8** we assessed whether we could improve CF patients' knowledge about CF lung disease. As an instrument to teach children about CF lung disease we developed

a dedicated board game named 'Airway'. Thirty-seven children aged 7-13 with CF were randomized into two groups. Both groups were tested twice with a written knowledge questionnaire that included questions about airways, CF and treatment. The children in the intervention group individually played 'Airway' with a pediatric physiotherapist with CF experience between the two questionnaire rounds.
This study showed that the mean percentage of correct answers to the questionnaire increased in the intervention group but not in the control group. We concluded that playing the board game 'Airway' increased these children's knowledge about CF lung disease.

Chapter 10

Discussion and Recommendations

DISCUSSION AND RECOMMENDATIONS

This chapter discusses the relevance for clinical practice of particularly the timing studies. In addition recommendations for future research are given.

10.1. Optimisation of timing of rhDNase inhalation

10.1.1. General discussion

The studies presented in this thesis focussed on an important knowledge gap concerning CF lung disease. These were the first studies to investigate the timing of the aerosol delivery of rhDNase in relation to ACT. Optimal timing is thought to be an important determinant for the effectiveness of this mucoactive drug.

Children who inhaled rhDNase before ACT showed improved peripheral airway patency. Furthermore, the impression that it is not safe to inhale rhDNase before sleep was not confirmed. Clearly, at the time of the development of rhDNase an opportunity was missed to identify the optimal relation between inhalation of the drug and ACT. Like in other studies investigating the effectiveness of mucoactive drugs, it was left to the patients to decide on the order of aerosol delivery and ACT. From our results it would appear that the effectiveness of rhDNase could have been underestimated in the phase III studies.

Hence, researchers investigating the effectiveness of mucoactive drugs or other inhaled drugs in CF should pay close attention to the optimal timing of inhalation in relation to ACT at an early stage of the drug development program. This is needed to optimise the costly, large-scale Phase III studies.

10.1.2. Recommendations for clinical practice

A practical implication of our findings is the recommendation that patients should inhale rhDNase at the most convenient time of day, provided that they wait at least 30 minutes before performing ACT.

Physicians and physiotherapists should be aware that our studies focussed on children of 5 years and older. Nevertheless, in anticipation of the results of a study in younger children we advise that younger children, too, nebulise the rhDNase at least 30 minutes before ACT.

10.1.3. Future research

Seeing that rhDNase is an established drug, it is striking that many avenues have been left unexplored that potentially can improve its effectiveness. The most important recommendation is to improve the efficiency of aerosol delivery to especially the peripheral airways. Our observation that the patency of the peripheral airways could be im-

proved, strongly suggests that the persistent peripheral airway obstruction is reversible – at least to some extent. Nebulisation of inhaled medications in CF such as rhDNase is commonly performed using highly inefficient nebulisers, resulting in poor deposition of the drug into the more peripheral airways. New smart nebulisers have been developed that are able to generate smaller particles with a limited size range that can be delivered more efficiently to the peripheral airways. It is likely that the use of these smart nebulisers will improve the effectiveness of rhDNase.[1] We are awaiting the results of ongoing studies in our centre aimed to improve peripheral targeting of rhDNase.

The second recommendation is that we should investigate the effects of rhDNase in early disease. Our studies focussed on children of 5 years and older. Little is known, however, on the use of rhDNase in younger children. The main reason for this is the lack of robust endpoints in these young children. It has been shown that substantial irreversible structural damage can develop even in infants with CF.[2] In only one randomized placebo controlled cross-over study in 9 infants it was shown that rhDNase improved the airway patency.[3] Clearly, there is great need to further investigate the potential role of rhDNase in young children. An additional challenge is that ACT techniques for young children are poorly developed.

The third recommendation is that patients with advanced to end stage lung disease should be studied more carefully. The CF population we investigated had relatively well preserved lung function. A recent study in patients who were transplanted for end stage lung disease showed that irreversible obstruction of peripheral airways plays a major role in the severe functional abnormalities in these patients.[4] This suggests that patients with more advanced disease might benefit from effective strategies to treat the peripheral airways. An option for patients with advanced disease who produce large amounts of sputum needs to be further studied: to perform ACT first, next to nebulise rhDNase, and finally to repeat ACT. Obviously this strategy is very time consuming and therefore only advisable for patients with severe disease that progresses in spite of intensive treatment.

Fourthly, in our CF centre the children are advised to intensively participate in sports as a method to increase their exercise tolerance and to mobilize sputum. The question whether it is more efficacious to nebulise rhDNase before or after exercise has not yet been addressed. Clearly, this needs to be further investigated in a crossover randomized controlled study.

Ideally all our findings should be further investigated in long term studies in larger cohorts, and with clinically relevant, sensitive endpoints. Running such studies with the current insensitive endpoints is extremely costly and would require a large number of patients. This would seem infeasible, therefore, as multicentre studies typically require up to 100 study sites. Yet, often no more than 5 patients per site can be included. Hence great effort should be made to develop clinically relevant, more sensitive endpoints.

10.2. ACT and Outcome measures

10.2.1. General discussion

The key surrogate endpoint in our studies was the MEF_{25}. We chose this endpoint because it is considered to be more sensitive to changes in the small airways relative to FVC and FEV_1.[5] It was no small feat being able to pick up a 6% difference in MEF_{25} between the two ACT-rhDNase regimens in a small cohort of patients, considering that they all were on maintenance rhDNase therapy. The importance of the MEF_{25} as a surrogate endpoint in CF is undervalued. It can be derived from routine spirometry, which is a well-standardized technique. The use of MEF_{25} is especially relevant and feasible in studies addressing early lung disease in children aged 6 years and above.[5]

The importance of the other endpoints used - nocturnal cough, oxygen saturation and $Rint_e$ – is less obvious.

Still, cough could be a feasible endpoint, as we found that it tended to be more severe in children with more advanced CF lung disease. Furthermore, cough is likely to be a clinically relevant endpoint since children cough more during pulmonary exacerbations.[6]

Likewise, our findings point at oxygen saturation as a promising endpoint. Oxygen saturation relates closely to the lung primary function as an organ for gas exchange. We found that a mean low saturation was associated with structural lung damage, as reflected by either a high total CT score or a high bronchiectasis subscore. Just like cough, oxygen saturation could be a clinically relevant endpoint too. All in all, one should be aware that sleep problems may have significant effects on daytime functioning: even milder levels of desaturation have been associated with lower IQ and Attention Deficit Hyperactivity Disorder symptoms.[7]

The third endpoint that we evaluated was $Rint_e$. Even though $Rint_e$ measurement was feasible in all eligible patients, its clinical relevance for ACT in CF is questionable. In our research ACT had only a borderline effect on $Rint_e$. In addition, we found a significant correlation with changes in $Rint_e$ with the reported amount of expectorated mucus, which cannot be considered a very sensitive and reproducible endpoint.

10.2.2. Recommendations for clinical practice

Seeing the results of our study, we recommend physicians and physiotherapists to pay close attention to the MEF_{25} as a sensitive indicator of peripheral airways disease. Furthermore, one should be alert to signs of desaturation, such as headache and tiredness, which are described to be associated with desaturation. Lung function parameters do not predict nocturnal desaturation events, unlike some other studies suggest. A nocturnal oxygen saturation measurement would be the assessment tool of choice to diagnose desaturation.

10.2.3. Future research

Clearly, the search for new sensitive and accurate endpoints for ACT studies is a highly appropriate though relatively unexplored territory. Before nocturnal cough and oxygen saturation can be used as endpoints in children with CF to evaluate the effectiveness of (ACT) studies, more validation research is needed in the following areas.

For cough it is important to determine its night-to-night variability and its response to changes in established treatments such as intravenous antibiotics. Furthermore the optimal number of nights needed to assess this variability should be established. In addition better software should be developed to improve analysis of the recordings. Currently, cough frequency analysis is too time consuming. Automated cough detection and analysis could well overcome this problem. To this end we have already started to develop such software in collaboration with a specialized centre.

Similarly, further validation studies are needed for oxygen saturation. Its response to established treatments such as intravenous antibiotics needs to be further defined. In addition it needs to be determined what changes of oxygen desaturation are clinically relevant.[8] Finally, it is evident that the relation between subnormal oxygen saturation profiles in CF on sleep quality, daytime functioning, IQ and behaviour should be further studied. The conclusions from such studies could be helpful in making decisions on when to start oxygen supplementation in CF.

Because $Rint_e$ measurement is one of the easiest ways to assess respiratory resistance during tidal breathing with minimal subject cooperation, this could be an excellent endpoint to use in infants. Further research in infants but also in adults is needed to further explore the potential of $Rint_e$ for ACT studies.

There are other new developments that are of interest in the search for new sensitive and accurate endpoints: CT and Magnetic Resonance Imaging (MRI).

CT has been shown to be more sensitive than pulmonary function tests to detect progression of CF lung disease. CT is the gold standard to detect bronchiectasis which is probably currently the most accurate and sensitive surrogate endpoint of CF lung disease. In addition, CT can detect and quantify trapped air, indicating small airways disease using ultra low radiation dose. [9] CT has now been used in several clinical studies and could play a role in future short and long term ACT studies.

MRI has recently been used for ACT studies. Proton MRI enabled to visualise mucus and hyperpolarized 3-He MRI can detect ventilation defects. In a small interesting study (n=8) the researchers performed hyperpolarized 3-He MRI before and after ACT; they concluded that the total volume of ventilation defects had not changed.[10] Further studies using proton and hyperpolarized 3-He MRI in larger groups of patients are needed to obtain more conclusive answers on the effect of ACT on mucus clearance. A major disadvantage of MRI is that the technique is expensive and relatively poorly standardized. Yet it can be of great help to improve our understanding of the effects of ACT in CF.

In conclusion, the currently used endpoints in ACT studies are not well developed. It is key that one should come up with more accurate, precise and easy to use endpoints for use in ACT studies.

10.3. Patient education and CF lung disease

10.3.1. General discussion

Since CF therapy is time consuming and complicated, we feel that it is important to teach young children about the basics of CF lung disease and the importance of therapy compliance. In addition they should learn to take (some) responsibility for their treatment before they reach adolescence. Throughout adolescence children should move towards complete control over their lives. We showed that with the help of a purpose-made board game, *'Airway'*, we could improve 7 to 12-year-old children's knowledge about CF lung disease. It became clear from our study that a substantial number of children had important misapprehensions about their CF lung disease.

10.3.2. Recommendations for clinical practice

It is worthwhile to incorporate the *'Airway'* board game instrument into the regular teaching program for each child with CF. The game is played by a physiotherapist and a child with CF, in the hospital or at the home of the patient. It takes approximately 45 minutes to play the game. Obviously the physiotherapist needs to have sufficient knowledge about CF lung disease and its treatment.

10.3.3. Future research

Clearly there are many unresolved questions related the use of the *'Airway'* board game that need to be answered in future studies. Firstly, we do not know how long the children will hold on to the acquired knowledge. Hence, other studies are needed to establish the long-term effect of playing the *'Airway'* board game on the children's knowledge level. In addition we do not know whether better knowledge about CF lung disease truly results in better treatment compliance.

In our study we administered a knowledge test to measure the increase of knowledge about CF lung disease after playing *'Airway'*. This test disclosed some important misapprehensions, which we could then address on an individual basis. Once knowledge gaps have been uncovered, patients and parents can be taught individually. So, it would seem useful to construct a new questionnaire with (more) questions about the lung disease, treatment, medication, gastro-intestinal problems, etc. This questionnaire has to be adapted for various age ranges. Separate parent and child versions should also be available.

Continuous educational dialogue about the treatment is expected to stimulate the patient to take ownership for his treatment.[11]

10.4. General conclusions

This thesis presents several studies performed to optimize airway clearance in children with CF; focussing on timing, assessment and compliance related to ACT.
The first part of this thesis shows better patency of the airways when rhDNase is nebulised before the child performs ACT. We suggest that established drug therapies can be made more effective by optimizing the time relation between ACT and nebulisation of the drug. It is not unthinkable that alternative, even more effective regimens can be identified in the future. Clearly when designing new studies the timing of medication and of other therapies like ACT must be taken into account.
In the second part of this thesis alternative endpoints were explored. The currently used endpoints do not suffice for today's pediatric CF population. There is a definite need for adequate surrogate endpoints that can be used in (ACT) studies. This thesis supports the use of peripheral airway flows. Nocturnal cough and saturation could well be feasible endpoints in children with CF, but more studies are needed to identify, for example, the sensitivity to treatment changes.
The last part of this thesis shows that the children's knowledge of their CF lung disease can be improved by playing the board game *'Airway'*. This may have a positive effect on treatment compliance. One should be alert to children's misapprehensions about their disease and its treatment. By designing a knowledge questionnaire, which can detect the gaps in their understanding, children can be educated individually in the future.

In conclusion, ACT forms an essential but time consuming part of CF treatment and therefore represents a great burden for most patients. It is in these children's interest that we continue to optimise ACT. Currently used endpoints are inadequate for today's pediatric CF population. Developing adequate surrogate endpoints is of prime importance in future research in patients with CF for further advancement of evidence based ACT practice.

REFERENCES

1. Bakker EM, Tiddens HA. Pharmacoloy, clinical efficacy and safety of recombinant human DNase in cystic fibrosis. Expert Rev Resp Med 2007;1(3):317-329.
2. Rosenfeld M, Gibson RL, McNamara S, et al. Early pulmonary infection, inflammation, and clinical outcomes in infants with cystic fibrosis. Pediatr Pulmonol 2001;32(5):356-366.
3. ten Berge, E vdW, Tiddens HA, Merkus PJ, Hop WC, de Jongste JC. DNase improves lung function in stable infants with Cystic Fibrosis. 2002.
4. Burgel PR, Montani D, Danel C, Dusser DJ, Nadel JA. A morphometric study of mucins and small airway plugging in cystic fibrosis. Thorax 2007;62(2):153-161.
5. Tiddens HA. Detecting early structural lung damage in cystic fibrosis. Pediatr Pulmonol 2002;34(3):228-231.
6. Rosenfeld M, Emerson J, Williams-Warren J, et al. Defining a pulmonary exacerbation in cystic fibrosis. J Pediatr 2001;139(3):359-365.
7. Bass JL, Corwin M, Gozal D, et al. The effect of chronic or intermittent hypoxia on cognition in childhood: a review of the evidence. Pediatrics 2004;114(3):805-816.
8. Urquhart DS, Montgomery H, Jaffe A. Assessment of hypoxia in children with cystic fibrosis. Arch Dis Child 2005;90(11):1138-1143.
9. Tiddens HA, de Jong PA. Imaging and clinical trials in cystic fibrosis. Proc Am Thorac Soc 2007;4(4):343-346.
10. Mentore K, Froh DK, de Lange EE, et al. Hyperpolarized HHe 3 MRI of the lung in cystic fibrosis: assessment at baseline and after bronchodilator and airway clearance treatment. Acad Radiol 2005;12(11):1423-1429.
11. Prasad SA, Cerny FJ. Factors that influence adherence to exercise and their effectiveness: application to cystic fibrosis. Pediatr Pulmonol 2002;34(1):66-72.

Samenvatting

SAMENVATTING

Cystic fibrosis (CF) is een autosomaal recessieve aandoening met een verkorte levensverwachting. Het is een chronisch, complex ziektebeeld waarbij verschillende organen, zoals longen, alvleesklier en lever zijn aangedaan. Chronische longinfectie en -ontsteking beginnen al op de zuigelingenleeftijd en resulteren in verdikking van de luchtwegwand en verstopping van de kleine luchtwegen met purulente secreties.
Het belangrijkste doel van de CF therapie op het gebied van de longen is om zo lang mogelijk een normale structuur van de longen te behouden en schade te voorkomen. De behandeling behelst regelmatig gebruik van anti-biotica, muco-actieve medicatie en airway clearance technieken (ACT). Behandeling duurt het gehele leven en vergt constante therapietrouw aan een complexe en tijdrovende therapie. Het is een enorme uitdaging voor de patiënt en het CF team om de therapietrouw zo groot mogelijk te laten zijn.
Dit proefschrift richt zich op timing van rhDNase inhalatie en ACT, eindpunten voor wetenschappelijk onderzoek bij CF en op educatie aan kinderen met CF over de behandeling en kennis van de longen.

Hoofdstuk 1 is een algemene introductie van dit proefschrift; **Hoofdstuk 2** verklaart de opzet en het doel van de studies.

In **Hoofdstuk 3** wordt een gerandomiseerde, dubbel blind, dubbel dummy, cross-over studie beschreven, waarin bij 25 kinderen met CF het effect van inhalatie van rhDNase voor ACT is vergeleken met inhalatie van rhDNase na ACT. Alle kinderen gebruikten al langere tijd rhDNase. De kinderen werden verdeeld in twee groepen. Kinderen in de eerste groep inhaleerden rhDNase 30 minuten voor ACT, en een placebo direct na ACT in de weken 1-3. In de weken 4-6 werd het protocol omgedraaid. De andere groep volgde het protocol in precies de omgekeerde volgorde. Alle kinderen voerden dagelijkse ACT uit zoals zij gewend waren. Het primaire eindpunt was de MEF_{25} %pred, gebruikt als indicator van perifere luchtwegdoorgankelijkheid.

Wij concludeerden dat door inhalatie van rhDNase voor ACT de MEF_{25} %pred verbeterde bij kinderen met CF.

In **Hoofdstuk 4** wordt de tweede gerandomiseerde, dubbel blind, dubbel dummy, cross-over studie gepresenteerd. Deze studie had tot doel om de effecten te vergelijken als rhDNase voor het slapen gaan wordt geïnhaleerd ten opzichte van inhalatie na het wakker worden. Deze studie werd uitgevoerd bij 25 kinderen met CF. Alle kinderen gebruikten al langere tijd rhDNase. Ook hier werden de kinderen verdeeld in twee groe-

pen. Kinderen in groep 1 inhaleerden rhDNase voor het slapen gaan, en een placebo na het opstaan gedurende week 1-2. Het protocol werd in de weken 3-4 omgedraaid. De andere groep volgde precies de andere volgorde gedurende de 4 weken. In de ochtend voerden de kinderen hun dagelijkse ACT 30 minuten na de inhalatie uit. Het primaire eindpunt was de MEF_{25} %pred, een indicator van de perifere luchtwegdoorgankelijkheid. Er werd geen significant verschil in longfunctie parameters gevonden, als rhDNase voor het slapen gaan of in de ochtend werd geïnhaleerd. Tevens was er geen verschil in nachtelijke zuurstofsaturatie, hoesten of de andere secundaire eindpunten.

Vastgesteld kan worden dat bij kinderen met CF, die rhDNase als onderhoudsmedicatie hebben, het even effectief en veilig is om rhDNase voor het slapen gaan of in de ochtend te inhaleren.

In **hoofdstuk 5** onderzochten we nachtelijk hoesten bij 25 kinderen met stabiel CF. Gedurende twee nachten werden de hoestgeluiden opgenomen met een digitale geluidsrecorder. Tevens werd tijdens deze nachten de zuurstofsaturatie gemeten. De dag na deze nachtelijke metingen werd longfunctie gemeten. Om de ernst van de longschade te kwantificeren werd de meest recente CT scan gebruikt. Hoesten werd uitgedrukt in hoestsecondes en in hoestsecondes per uur.
We vonden dat de frequentie van het nachtelijk hoesten bij kinderen met CF hoger was dan welke beschreven is bij gezonde kinderen. Nachtelijk hoesten lijkt bij kinderen met meer longschade meer op te treden. Nachtelijke hoesten treedt in het eerste uur van de nacht meer op dan in de daaropvolgende uren en varieert sterk voor de individuele patiënt van nacht tot nacht.

Concluderend zijn er meer studies nodig om te onderzoek of hoesten sensitief genoeg is om te worden gebruikt als eindpunt in CF studies, welke als doel hebben het effect van mucociliaire klaring te verbeteren. Tevens is meer onderzoek nodig om het optimale aantal metingen vast te stellen dat nodig is om een betrouwbare schatting te verkrijgen van de ernst van het nachtelijk hoesten bij kinderen met CF.

Hoofdstuk 6 beschrijft de studie waarin de nachtelijke zuurstofsaturatie bij 25 kinderen met CF gedurende twee nachten werd onderzocht. De zuurstofsaturatie werd gemeten middels pulse oximetrie. De dag na de nachtelijke metingen werd de longfunctie gemeten. Om de ernst van de longschade te kwantificeren werd de meest recente CT scan gebruikt.
Wij vonden dat nachtelijke zuurstofsaturatie bij kinderen met stabiel CF lager was dan beschreven bij gezonde kinderen. Er was een goede correlatie tussen de twee nachten wat betreft de zuurstofsaturatie. De nachtelijke zuurstofsaturatie was gecorreleerd met

longfunctie parameters en CT scores, maar de longfunctie kon geen voorspelling geven ten aanzien van nachtelijke desaturaties.

Vastgesteld kan worden dat het monitoren van zuurstofsaturatie bij kinderen met CF gedurende één nacht genoeg is om een representatieve opname te krijgen. De longfunctie kan nachtelijke desaturaties niet voorspellen.

In **Hoofdstuk 7** wordt de toepasbaarheid en sensitiviteit van een methode om luchtwegweerstand middels de interruptie techniek ($Rint_e$) te meten in relatie tot ACT beschreven. De $Rint_e$ werd direct voor en direct na ACT gemeten bij 18 kinderen met CF. Het verschil in $Rint_e$ werd gebruikt als eindpunt om het effect van ACT te meten. Sputum productie werd geschat met een semi-kwantitatief scoringssysteem.
We vonden dat $Rint_e$ toepasbaar was bij alle kinderen. $Rint_e$ lijkt te verbeteren na ACT en was negatief gecorreleerd met de sputumproductie tijdens ACT. Concluderend is $Rint_e$ een toepasbare methode en kan mogelijk gaan dienen als eindpunt in kortdurende ACT studies.

In **Hoofdstuk 8** wordt de studie beschreven of de kennis over CF longziekte kon verbeteren bij kinderen met CF. Om deze kinderen meer te leren over CF longziekte is een bordspel ontwikkeld, genaamd 'Vluchtweg'. Zevenendertig kinderen met CF in de leeftijd van 7-13 jaar werden verdeeld over twee groepen, een interventie en controle groep. Beide groepen kregen twee maal een kennistest met vragen over luchtwegen, CF en de behandeling daarvan. De kinderen in de interventiegroep speelden tussen de twee kennistesten het spel 'Vluchtweg' met een kinderfysiotherapeut, welke ervaring had met CF.
De studie liet zien dat het gemiddelde percentage goede vragen op de kennistest toenam bij de interventiegroep.

Vastgesteld kan worden dat door het spelen van het spel 'Vluchtweg' de kennis van de kinderen over CF longziekte toenam.

Dankwoord

DANKWOORD

Na 14000 kilometer met een 2CV, 27 koortslippen, 225 huisbezoeken, 3700 ampullen studiemedicatie en minstens 75 handtekeningen is mijn proefschrift klaar.
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vanuit hun verdriet zoveel positieve energie te halen. Hiermee worden nu vele kinderen in India geholpen.

Lieve Koen, toen ik met het onderzoek begon was je vier jaar. Toen ging je trots naar school om te leren schrijven met een pen. Inmiddels ben je 11 jaar. Je hebt je ontpopt als een stoere zeiler, een kanjer met wrikken, een natuurkenner, een zelfstandige jongen, een goede kok, een filosofisch kind etc. Met andere woorden, ik ben enorm trots op je. Soms heb je mij vergezeld op mijn huisbezoeken. Wat een gezelligheid was dat in de auto. Enige tijd geleden heb ik je verteld dat ik ging promoveren. Het enige antwoord wat dat ontlokte was: Ja, en dan? Zulke antwoorden zetten je weer even met beide benen op de grond. Gelukkig was je laatst wel trots toen je zag dat ik alles in het Engels had geschreven. Koen, ik hoop dat het nog jaren zo gezellig blijft met jou aan boord. Dat je mag opgroeien tot een goed mens met oog voor de ander en de natuur.

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En als laatste wil ik iedereen bedanken die naast mijn dankwoord en stellingen ook andere stukken uit dit proefschrift leest.

Curriculum Vitae

CURRICULUM VITAE

Lianne van der Giessen werd geboren op 3 januari 1967 in Dordrecht. Zij groeide op in Dubbeldam. In 1985 legde zij met succes het VWO examen af aan het Christelijk Lyceum te Dordrecht. In datzelfde jaar begon zij met de studie fysiotherapie aan de Internationale Academie voor Fysiotherapie Thim van der Laan te Utrecht. Als student was zij actief in het begeleiden van mensen met een verstandelijke of lichamelijke beperking bij de Stichting Watersport met Gehandicapten in Loosdrecht.
Na het behalen van haar diploma fysiotherapie in 1989 begon zij als fysiotherapeut in Nieuw Buitenzorg, een zorginstelling voor ernstig meervoudig beperkte kinderen in Leiderdorp. In 1990 begon zij met de postacademiale opleiding kinderfysiotherapie in Utrecht. Voor deze opleiding heeft zij onder andere stage gelopen in het Sophia Kinderziekenhuis in Rotterdam, alwaar zij in oktober 1993 een vaste aanstelling kreeg.
Sinds 1998 is zij bestuurslid van de Lisette Vroege Stichting. Deze stichting is opgericht ter nagedachtenis aan Lisette Vroege, een hartsvriendin van Lianne die sinds 1992 is vermist en wordt gemist. De stichting ondersteunt beperkte en kansarme kinderen in ontwikkelingslanden, die kinderfysiotherapeutische hulp nodig hebben.
Naast haar werk in het Sophia Kinderziekenhuis is zij betrokken bij de Masteropleiding Kinderfysiotherapie van de Transfergroep Rotterdam waar zij met name verantwoordelijk is voor het blok onderzoek en behandeling bij kinderen met een pulmonale aandoening.
Van 2000-2007 is zij eerst lid en later voorzitter geweest van de congrescommissie van de Nederlandse Vereniging voor Kinderfysiotherapie.
In 2002 is zij in deeltijd gestart met haar onderzoek onder supervisie van Prof Dr HAWM Tiddens. Dit onderzoek wordt in dit proefschrift gepresenteerd.
Lianne is getrouwd met Jeroen Onck. Zij hebben samen een zoon, Koen (1998). Zij wonen sinds 2008 in historisch Delfshaven op de Rien Sans Dieu, een Hasselteraak uit 1907.

List of publications

LIST OF PUBLICATIONS

- Giessen LJ van der, Hartman A, Bouman NH. Somatoforme aandoeningen bij kinderen: indicatie kinderfysiotherapie? Tijdschr NVFK 1997;17:12-16.
- Giessen LJ van der, Liekens D, Rutgers KJM, Hartman A, Mulder PGH, Oranje AP. Validation of Beighton Score and Prevalence of connective tissue signs in 773 Dutch children. J Rheumatol 2001;28:2726-30.
- Jacobs-van der Bruggen M, Baan C, Hendriks E, Giessen LJ van der. Verslag pilotonderzoek "Fysiotherapie bij kinderen met astma". Tjidschr NVFK 2000;1:10-12.
- Ketelaar M, Schie P van, Gorter JW, Visser J, Veenhof C, Voet F, Giessen LJ van der. Actuele ontwikkelingen in de Gross Motor Function Measure (GMFM). Kinderfysiotherapie 2003;15: 5-7.
- Giessen LJ van der, Pelt C van. Pas op, besmettingsgevaar! Kinderfysiotherapie 2003; 15: 14-15.
- Giessen LJ van der, Theunissen C. Protocollering van het klinisch fysiotherapeutisch onderzoek bij patiënten met Cystic Fibrosis. Kinderfysiotherapie 2005; 17: 3-7.
- Giessen LJ van der, Gulmans VAM, Lucas T, Lugt M van der, Hendriks HJM. KNGF Richtlijn Astma bij kinderen. Ned Tijdsch Fys 2005;115(5). Supplement
- Giessen LJ van der. In de beperking toont zich de meester. Kinderfysiotherapeutische behandelmogelijkheden van lage luchtweginfecties bij kinderen met een ernstig meervoudige beperking. TAVG 2007;25(3): 64-67.
- Giessen LJ van der, Gosselink R, Jongste JC de, Hop WCJ, Tiddens HAWM. RhDNase before airway clearance therapy improves airway patency in children with CF. Pediatr Pulmonol. 2007 Jul;42(7):624-30.
- Giessen LJ van der, Gosselink R, Jongste JC de, Hop WCJ, Tiddens HAWM. Recombinant human DNase nebulisation in children with cystic fibrosis: before bedtime or after waking up? Eur Respir J. 2007 Oct;30(4):763-8.
- Giessen LJ van der. Does the timing of inhaled dornase alfa matter? J Cystic fibrosis. 2009;8: S6-S9.

Chapters in books

- Giessen LJ van der, Takken T, Vulmans VAM. Kinderfysiotherapie bij cardiopulmonale problemen. In: Empelen R, Nijhuis-van der Sanden R, Hartman A.(red) Kinderfysiotherapie. Door: Elsevier Gezondheidszorg Maarsen. 2000.
- Giessen LJ van der, Gulmans VAM, Laag J van der. Fysiotherapie. In: Brand PLP, Hoekstra MO, Merkus PJFM, Schite PPM. Werkboek Kinderlongziekten. VU Uitgeverij. Amsterdam. 2001.

- Giesen LJ van der. Fysiotherapie bij ademhalingsproblematiek. In: Kock- van Beerendonk I, Ridder I den, Westmaas A. Specialistische kinderverpleegkunde. Elsevier gezondheidszorg, Maarsen. 2006.
- Giessen LJ van der, Custers J, Douma D. Kinderfysiotherapie bij cardiopulmonale problemen. In: Empelen R, Nijhuis-van der Sanden R, Hartman A.(red) Kinderfysiotherapie. Door: Elsevier Gezondheidszorg Maarsen. Tweede druk, 2006.

ABBREVIATIONS

ACBT	Active cycle breathing techniques
ACT	Airway clearance therapy
AFRT	Artifact free recording time
ASE	ACT related sputum expectoration score
CF	Cystic fibrosis
CFQ	Cystic Fibrosis Questionnaire
CFTR	Cystic fibrosis transmembrane regulator
CFRD	Cystic fibrosis related diabetes
Cs/h	Cough seconds per hour
CSS	Cough symptom score
D_4	Fall in SO_2 by ≥4%
D_{90}	Fall in SO_2 to ≤90%
DSE	Daily sputum expectoration score
FEV_1	Forced expiratory volume in one second
FEF_{75}	Maximal expiratory flow when 75% of FVC is expired
FVC	Forced vital capacity
IQR	Inter quartile range
HRCT	High resolution computed tomography
KQ	Knowledge questionnaire
MEF_{25}	Expiratory flow at 25% of the actual forced vital capacity
MCC	Mucociliairy clearance
MRI	Magnetic resonance imaging
ODI_4	Oxygenation desaturation index per hour of D_4
ODI_{90}	Oxygenation desaturation index per hour of D_{90}
PCL	Periciliary liquid
PFT	Pulmonary function test
Qol	Quality of Life
Rint	Interrupter resistance
rhDNase	Recombinant human deoxyribonuclease
SO_2	Oxygen saturation
T_4	Total time in seconds of D_4
T_{90}	Total time in seconds of D_{90}
TOBI	Tobramycin solution for inhalation
TRT	Total recording time
TSI	Tobramycin solution for inhalation
sGaw	Specific airway conductance
VAS	Visual analogue scale