Off-label Use of Recombinant Human DNase in Pediatric Lung Disease

Off-label gebruik van recombinant humaan DNase bij kinderlongziekten

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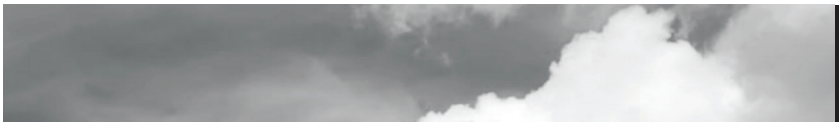
Contents



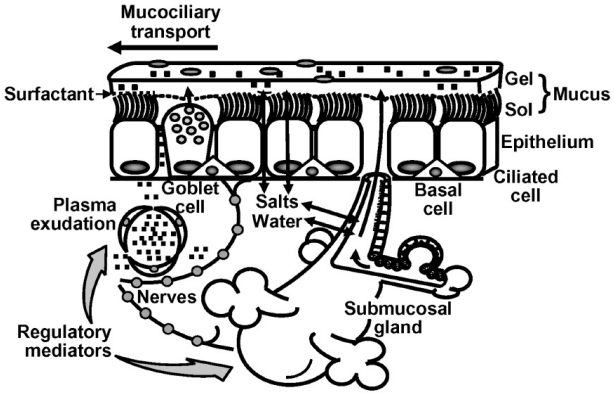
Chapter 1

General introduction and aims of study

Introduction

The ventilation of the human lung ranges between 1,000 and 21,000 liters per 24 h depending on body size and physical activity. This exposes the extensive epithelial surface of the respiratory tract between the nose and the alveoli to a large burden of potentially injurious materials, including inorganic and organic particulate and gaseous matter. A series of defense mechanisms have evolved to protect the airways. The mucociliary apparatus of the tracheobronchial tree is one of these defense mechanisms. It consists of a coordinated system of epithelial water and ion transport, mucin secretion, cilia action, and cough that results in a continuous flow of fluid and mucus over airway surfaces, collectively termed "mucus clearance" (Figure 1.1).[1]

Figure 1.1. Airway mucus secretion.[1]

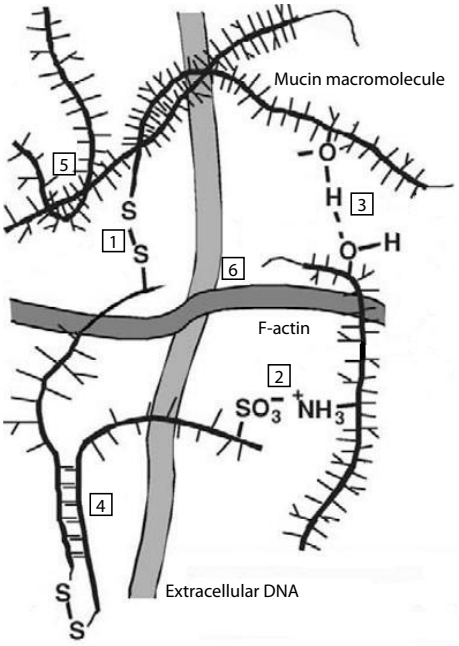


Mucus forms a bi-layer over the epithelium, with surfactant separating the gel and sol (periciliary fluid) layers. Mucins secreted by goblet cells and submucosal glands confer viscoelasticity on the mucus, which facilitates mucociliary clearance of inhaled particles. Mucus hydration is regulated by salt (and hence, water) flux across the epithelium. The glands also secrete water. During inflammation, plasma proteins leak from the tracheobronchial microvasculature, infiltrate the submucosa and contribute to the formation of mucus. These processes are under the control of nerves and regulatory mediators.

The mucociliary apparatus has three major functions.[2,3] First, it is a mechanical barrier that traps particulates in the surface liquid covering the airway epithelium and next clears them from the tracheobronchial tree by ciliary action, a process called mucociliary clearance. Second, the surface liquids act as a chemical screen; for example, airway mucus has antioxidant properties that protect the underlying epithelial surface from injurious effects of inhaled toxic gases as SO_2, O_3 and NO_2.[4] Third, the surface liquids provide a biological barrier by interacting with microorganisms and luminal inflammatory cells, thereby preventing them from adhering and migrating through the airway epithelium. This surface liquid is comprised of two layers: the periciliary fluid layer close to the cell surface and the mucus layer on top of this periciliary fluid layer. Both layers exhibit certain physical and chemical characteristics needed to fulfill their barrier function and to interact with cilia for efficient mucus clearance.[2]

Mucus is a heterogeneous, adhesive, viscoelastic gel that consists of glycoproteins forming an entangled three-dimensional network through many types of bonds (Figure 1.2).[5] The three-dimensional structure of airway mucus contributes to the viscoelastic properties of the gel. In some respiratory diseases, high concentrations of DNA released from degenerating polymorphonuclear leucocytes can be demonstrated in mucus,[6,7] and are associated with higher mucus viscosity.[8] Two characteristics that influence mucus clearance are depth of the pericili-

Figure 1.2. Schematic illustration of the types of bonds occurring in mucous gel, illustrating potential targets for mucoactive agents.[5]



Type of bond	Mucoactive agent
1. Covalent bonds: glycoprotein subunits are linked primarily by intramolecular disulfide bonds	N-acetylcysteine Dithiothreitol
2. Ionic bonds: mucin macromolecules have both positive and negative fixed charges capable of interacting	Hypertonic saline Dextran sulfate Heparin
3. Hydrogen bonds link neighboring oligosaccharide sidechains	Mannitol Dextran
4. Van der Waals' forces: bonds are due to van der Waals' attractive forces between complementary saccharide moieties on neighboring chains	
5. Intermingling: physical entanglements between mucins	High frequency oscillation
6. Extracellular DNA and F-actin: parallel network formation due to infection	rhDNase Gelsolin

ary fluid layer and amount of extracellular DNA present in mucus during airway inflammation or with bacterial infection. Cystic fibrosis is associated with a thinner periciliary fluid layer as a result of ion transport abnormalities.[9] This, then, will uncouple the normal interaction of cilia with mucus and result in impaired mucociliary clearance.[10]

Impaired mucociliary clearance in pediatric lung disease

Impaired mucociliary clearance or mucus hypersecretion are important clinical features in diseases such as cystic fibrosis (CF), recurrent bronchitis, asthma, primary ciliary dyskinesia (PCD), and in disorders and conditions with abnormal cough mechanics, such as airway malacia and neuromuscular diseases. Moreover, viral respiratory tract infections – which occur frequently during childhood – may cause epithelial damage and loss of cilia,[11] and thus lead to impaired mucociliary clearance through secondary ciliary dysfunction.

An imbalance between mucus secretion and clearance can occur if clearance is impaired because of cilial damage, inhibition of or uncoordinated cilial movement, or due to unfavorable rheological conditions of mucus. Such an imbalance causes mucus to accumulate in the airways and initiates cough and sputum production. Accumulated mucus may obstruct the airways, and it is assumed to promote bacterial colonization. Thus it is a risk factor for recurrent lower airways infections and hospital admissions, factors that negatively affect quality of life. By completely obstructing the peripheral airways it may lead to atelectasis and hypoxemia, and may contribute to a fatal outcome, such as in an acute asthma exacerbation.[12] Chronic mucus hypersecretion in adults with chronic obstructive pulmonary disease (COPD) is associated with lung function decline, higher likelihood of hospitalization and death from pulmonary infection.[13,14]

Specific features in the anatomy and physiology of the respiratory system in infants and children make them even more liable to respiratory symptoms when mucociliary clearance is impaired or mucus hypersecretion is present. Differences with adults include:

- Higher ratio of mucous glands in the airway epithelium and thicker airway walls[15];
- Smaller mucociliary clearance rate in newborns compared to young adults (studied in animals)[16,17];
- Relative absence of functional collateral ventilation (through pores of Kohn and canals of Lambert) that probably contributes to the occurrence of patchy atelectasis during airway disease[18];
- Smaller and more collapsible airways, and a more compliant chest wall in early childhood[19];

- Predominant supine position in infancy, leading to lower inflation level of the lungs and to lower airway patency.

These features underlie the high baseline airway resistance found in infancy,[20] as a result of which even small amounts of mucus in the airways can have a profound effect on the work of breathing.[21]

Treatment of impaired mucociliary clearance

Two different strategies are available for symptomatic treatment in pediatric lung disease patients with (symptoms suggestive of) impaired mucociliary clearance, can be used, namely airway clearance techniques and pharmacotherapy with mucoactive agents.

Airway clearance techniques

A wide range of airway clearance techniques and devices that aim to improve mucus clearance and to facilitate expectoration are used in clinical practice.[22-25] Techniques include conventional chest physiotherapy (e.g. manual percussion, vibration, and postural drainage), directed cough, forced expiratory technique or huff coughing, active cycle of breathing technique, and autogenic drainage. Techniques in which mechanical devices are used include positive expiratory pressure (PEP), manual hyperinflation, airway oscillation (using a flutter, or intrapulmonary percussive ventilation), high frequency chest compression, mechanical percussion, and mechanical in-exsufflation. Studies on the efficacy of these airway clearance techniques have been mainly conducted in CF patients.[22,24] Although there is widespread consensus among patients and health care professionals that airway clearance is an essential component of CF care, there is only some evidence from small, short-term trials of a beneficial effect on mucus transport. Moreover, evidence from long-term trials to support the efficacy of airway clearance techniques is lacking.[26] There is some evidence that either conventional chest physiotherapy or PEP are at least as effective as other forms of airway clearance in CF patients.[27,28] Studies on airway clearance techniques in non-CF patients, especially in children, are scarce and do not provide high-level evidence to support any technique.[23]

Mucoactive agents; recombinant human DNase (rhDNase)

Mucoactive agents are drugs designed to change the properties of airway secretions, so as to improve mucociliary clearance. These agents are targeted at the different types of bonds present in mucus (Figure 1.2).[5]

Of the available mucoactive agents, recombinant human DNase (rhDNase) has been studied most thoroughly in pediatric lung disease. It was developed to diminish viscosity of CF patients' sputum, which contains large amounts of free DNA.[29] Based on randomized clinical trials, rhDNase is currently licensed for use in CF patients older than 5 years with mild to moderate lung disease (FVC > 40% predicted). Phase 1,[30,31] phase 2,[32,33] and phase 3 studies[34] showed that rhDNase in a dose of 2.5 mg once or twice daily is safe, improves lung function and quality-of-life parameters, and reduces the risk of respiratory tract infections requiring parenteral antibiotics. Subsequent controlled and open studies have evaluated the efficacy of rhDNase in subgroups of CF patients, i.e. those with severe lung disease,[35,36] those undergoing exacerbation[37] and those aged 6-10 years.[38] Medium (6 months)[39] and long term (2 years)[40] efficacy has been studied in patients over 16 years of age in open extension studies. There is no evidence from randomized controlled trials (RCT) to indicate whether this positive effect is sustained over a period longer than 2 years, or whether rhDNase is associated with lower mortality rate. Although many pediatric CF patients improve on treatment with rhDNase, there is wide variation in individual responses.[41] The current recommended dose in children with stable CF lung disease of 2.5 mg once daily is as effective as two daily doses of 2.5 mg,[34] and alternate day rhDNase might also be an good alternative.[42] The use of rhDNase in CF has been reviewed extensively.[43-46]

In theory, rhDNase could also be effective in other childhood lung diseases involving mucus plugging or impaired mucociliary clearance, in which neutrophilic airway inflammation or increased DNA content of mucus is present.[47] Indeed, since 1994 several observational studies have been published on the off-label use of rhDNase in children with non-CF lung disease. At present, however, evidence from large RCTs to support off-label use of rhDNase in non-CF pediatric lung disease is lacking.

Aims of the study

The aims of the studies presented in this thesis are:

- To review the present literature on the mucoactive agents most frequently used in children with non-CF lung disease, that is, N-acetylcysteine (NAC) and other sulfhydryl compounds, rhDNase, and hypertonic saline.
- To evaluate the efficacy of rhDNase in infants with respiratory syncytial virus (RSV) bronchiolitis.
- To evaluate the efficacy of rhDNase in children with an asthma exacerbation.
- To assess the incidence and patient characteristics of tracheomalacia and bronchomalacia in children, and to evaluate the efficacy of rhDNase in children with airway malacia and lower respiratory tract infection.

- To evaluate indications for, outcomes of, and use of rhDNase lavage during bronchoscopies in pediatric CF patients.

The studies addressing these aims are described in this thesis. First, **chapter 2** presents an overview of the literature on the use of mucoactive agents in children. **Chapter 3** describes the results of a multicenter, randomized, placebo-controlled trial on the efficacy of rhDNase in oxygen-dependent infants hospitalized with RSV bronchiolitis. **Chapter 4** describes the results of a multicenter, randomized, placebo-controlled trial on the efficacy of rhDNase in addition to standard treatment at the emergency department for children with an asthma exacerbation. **Chapter 5** reports the results of a retrospective study that estimates the prevalence of primary airway malacia at birth, determines the predictive value of a clinical diagnosis of airway malacia compared with bronchoscopy results, and describes the presenting symptoms of children with airway malacia. In **chapter 6**, the results are reported of a randomized, placebo-controlled clinical trial on the efficacy of rhDNase in children with airway malacia and a lower respiratory tract infection. **Chapter 7** describes the results of a retrospective study on indications for, outcomes of, and use and safety of rhDNase lavage during bronchoscopies in pediatric CF patients. In **chapter 8** the results of the studies described in this thesis are summarized and directions for future research are discussed. In **chapter 9** the study results presented in this thesis are summarized in Dutch.

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Chapter 2

Pharmacotherapy of impaired mucociliary clearance in non-CF pediatric lung disease
A review of the literature

Ruben Boogaard
Johan C. de Jongste
Peter J.F.M. Merkus

Pediatr Pulmonol 2007;42(11):989-1001

Summary

Mucoactive agents are used to treat a variety of lung diseases involving impaired mucociliary clearance or mucus hypersecretion. The mucoactive agents studied most frequently are N-acetylcysteine (NAC), recombinant human DNase (rhDNase), and hypertonic saline. Studies on the efficacy of these have been mainly conducted in adults, and in patients with cystic fibrosis (CF). The exact role of mucoactive agents in children with non-CF lung disease is not well established. We present an overview of the current literature reporting clinical outcome measures of treatment with NAC, rhDNase, and hypertonic saline in children.

Introduction

Mucus clearance is an important primary innate airway defense mechanism, and our understanding of the key parameters underlying its function has grown rapidly in the last decade.[1,2] Impaired mucus clearance or mucus hypersecretion are important clinical features in diseases such as cystic fibrosis (CF), recurrent bronchitis, asthma, and primary ciliary dyskinesia (PCD). Moreover, viral respiratory tract infections – that occur frequently during childhood – may cause epithelial damage and loss of cilia,[3] and thus impaired mucus clearance through secondary ciliary dysfunction. Impaired clearance causes mucus to accumulate in the airways and to initiate cough and sputum production. Accumulated mucus can lead to airways obstruction, bacterial colonization, and recurrent infections, negatively affecting quality of life. By completely obstructing the peripheral airways it may lead to atelectasis and hypoxia, and may contribute to a fatal outcome, such as in an acute asthma exacerbation.[4]

Mucoactive agents are drugs that are meant to change the properties of airway secretions.[5] Many different mucoactive agents have been evaluated for their ability to either change the properties of airway mucus, or to decrease mucus secretion.[6,7] Classified by proposed mechanism of action, they encompass classical mucolytics, peptide mucolytics, nondestructive mucolytics, expectorants, mucokinetic agents, and mucoregulators (Table 2.1).

One possible means to evaluate a mucoactive agent is to assess its effect on mucociliary clearance (MCC) or cough clearance with the use of radiolabeled aerosol. Discussing this subject is outside the scope of this review. Moreover, studies on mucoactive agents in CF patients, and studies on physiotherapy or secretion clearance techniques in (pediatric) lung disease patients have been reviewed by others, and will therefore not be discussed in this review.

The interested reader is referred to existing reviews that cover:

- basic and clinical aspects of mucociliary clearance;[8]
- regulation of mucociliary clearance in health and disease;[9]
- different methodologies to measure mucociliary clearance;[10,11]
- effects of drugs on physiological properties of mucus,[12] and on MCC;[11,13]
- pharmacological approach to discovery and development of new mucolytic agents;[5]
- efficacy of N-acetylcysteine (NAC) in CF patients;[14]
- efficacy of recombinant human DNase (rhDNase) in CF patients;[15,16]
- efficacy of hypertonic saline in CF patients;[17]
- physiotherapy and secretion clearance techniques in (pediatric) lung disease.[18-20]

The aim of the present review is to summarize the published literature on the mucoactive agents most frequently used and studied in children with non-CF lung disease, that is, NAC and other sulfhydryl compounds, rhDNase, and hypertonic saline. We will focus on literature

reporting effects of mucoactive agents on clinical outcome measures, such as length of hospital stay, symptom severity and chest radiographs.

Table 2.1. Mucoactive agents (adapted from Rubin, 2002.[7])

Mucoactive Agent	Possible mechanism of action
Expectorants	
Hypertonic saline	Increases secretion volume and increases hydration
Mannitol	Increases hydration and may increase ciliary beat frequency by stimulating release of mediators; break hydrogen bonds between mucins; increase secretion volume
Classical mucolytics	
N-acetylcysteine	Breaks disulfide bond linking of mucin oligomers
Nacystelyn	Breaks disulfide bond linking of mucin oligomers and increases hydration (by increased chloride secretion)
Peptide mucolytics	
Dornase alfa	Hydrolyzes DNA polymer with reduction in DNA fragment length
Gelsolin or Thymosin β4	Depolymerizes F-actin
Non-destructive mucolytics	
Dextran	Breaks hydrogen bonds and increases hydration
Low molecular weight heparin	May break both hydrogen and ionic bonds
Mucoregulatory agents	
Anticholinergic agents	Decreases volume of stimulated secretions
Glucocorticoids	Decreases airway inflammation and mucin secretion
Indomethacin	Decreases airway inflammation
Macrolide antibiotics	Decreases airway inflammation and mucin secretion
Cough clearance promotors	
Bronchodilators	Improves cough clearance by increasing expiratory flow
Surfactants	Decreases sputum adhesivity

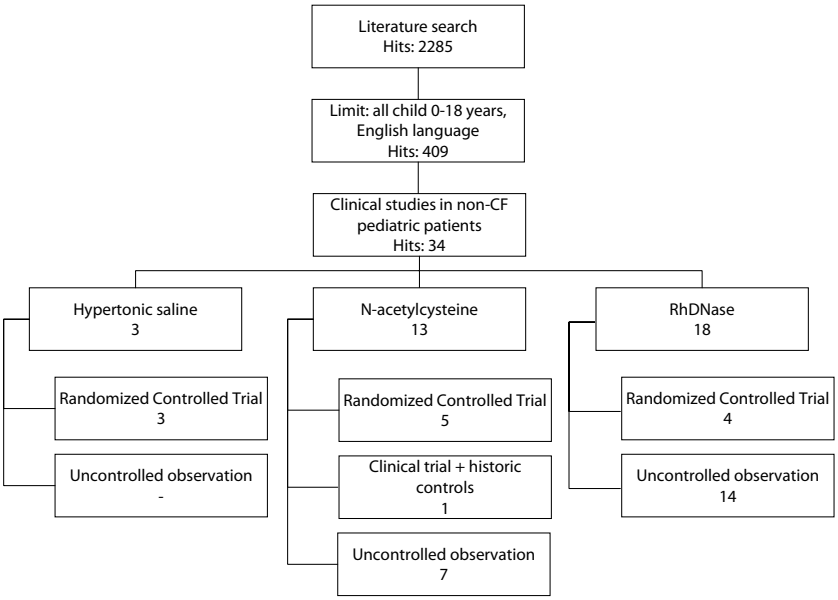
Methods

We searched MEDLINE (1966 to June 2007) with the medical subject headings (MESH): 'respiratory tract diseases', 'acetylcysteine', 'sulfhydryl compounds', 'hypertonic saline solution' and 'DNASE1 protein, human' – the latter is the MESH term for the drug 'recombinant human DNase (rhDNase, dornase alfa)'. Randomized controlled trials (RCTs) as well as uncontrolled, observational studies retrieved by MEDLINE, published in English and reporting clinical data

on children aged 0-18 years were looked up and their reference lists were scanned for relevant unretrieved material. The Cochrane library was checked for any additional clinical trials.

Figure 2.1

Results of the literature search in MEDLINE on mucoactive agents in non-CF pediatric lung disease, using the medical subject headings (MESH) 'respiratory tract diseases', 'acetylcysteine', 'sulfhydryl compounds', 'hypertonic saline solution' and 'DNASE1 protein, human' – the latter is the MESH term for the drug 'recombinant human DNase (rhDNase, dornase alfa)'.



Results of literature search

A total of 34 relevant articles were retrieved (Figure 2.1). Twelve articles reported RCTs, while 22 articles reported uncontrolled clinical observations.

N-acetylcysteine and other sulfhydryl compounds

Mode of action

N-acetylcysteine (NAC) and the other sulfhydryl compounds – that is, S-carboxymethylcysteine (carbocysteine) and 2-mercaptoethane sulphonate (Mesna) – depolimerize mucus in vitro by breaking disulfide bonds of the glycoproteins, thereby lowering viscosity and potentially improving expectoration.[21] Besides potential effects on MCC, sulfhydryl compounds have antioxidant effects that could be useful in preventing lung damage in chronic lung disease. NAC

is usually given orally, as inhalation can cause bronchospasm in patients with airway hyperresponsiveness,[22,23] releases an unpleasant sulphurous smell and is time consuming. However, for orally administered NAC to exert a clinical effect – that is, by changing the properties of mucus – it must pass into the mucus. There is no proof that orally administered NAC results in therapeutic concentrations in airway secretions.[24] In some studies in healthy adults or in chronic bronchitis patients, sulfhydryl compounds improved MCC, whereas in other such studies there was no effect.[13]

Randomized controlled trials of NAC in non-CF lung diseases

Most studies indicating efficacy of oral NAC or carbocysteine concern adults with chronic bronchitis or chronic obstructive pulmonary disease (COPD). A Cochrane review showed slightly fewer exacerbations and slightly shorter period of disability, but no difference in lung function, in patients using these agents.[25] These benefits of oral NAC might be due to its antioxidant properties rather than its mucolytic properties. Sulfhydryl compounds have been studied less intensely in pediatric patients (Table 2.2).[14,26-30]

Primary ciliary dyskinesia

Stafanger et al.[26] conducted a double blind, randomized crossover trial in 13 out-clinic patients with PCD. Oral NAC for 3 months had no effect on subjective clinical scores, lung function parameters and ciliary function.

Chronic lung disease of infancy

Bibi et al.[27] conducted a crossover trial in which they intratracheally administered NAC or placebo for seven days to premature infants ventilated for chronic lung disease. Airway resistance at day 3 worsened twofold in the NAC group. Dynamic compliance, clinical signs, ventilatory settings and chest X-ray scores did not change. Two patients treated with NAC showed more cyanotic spells and bradycardia. A large RCT (n=391) aimed to assess possible anti-oxidant benefits of intravenous NAC in premature infants with extremely low birth weight.[28,29] It appeared that a 6-day course had neither effect on mortality or the incidence of bronchopulmonary dysplasia (BPD), nor on lung function.[28,29]

Other diseases

Symptom scores for children admitted with pneumonia or asthma did not differ between groups receiving oral NAC or placebo.[30] This small study was described as "double blind", but seems of poor methodological quality as randomization and treatment allocation remain unexplained and the study population was very heterogeneous.

Observational studies of NAC in non-CF lung diseases

Several uncontrolled observations report beneficial effects of nebulized NAC in children with atelectasis, (acute) asthma, inhalation injury, and lower respiratory tract infections, but RCTs in these patient groups are lacking (Table 2.2).

Atelectasis

Amir et al.[31] reported a possible beneficial effect of nebulized NAC on chest X- ray appearance and ventilatory settings in 5 mechanically ventilated premature infants with severe recurrent atelectasis. Wiener et al.[32] administered nebulized acetylcysteine to a mechanically ventilated child with atelectasis caused by smoke inhalation. That done, sputum volume increased and the atelectasis cleared within 2 days.

Asthma

By liquefying mucous plugs, mucolytics could have potential benefit in acute asthma. On the other hand, NAC may cause bronchoconstriction when children have airways hyperresponsiveness.[22,23] Pretreatment with bronchodilators could prevent this. Uncontrolled observations in adults with acute severe asthma, unresponsive to regular therapy, nevertheless showed beneficial clinical effects of nebulized or bronchoscopically instilled NAC.[33,34] Kyncl et al.[35] reported improved blood gas values and chest X-ray pictures after bronchial lavage with Mesna in 14 children with status asthmaticus. However, RCTs are lacking and so are studies on NAC in stable asthmatic children.

Inhalation injury

Desai et al.[36] studied the combined, potential mucolytic and anti-oxidant properties of NAC in children mechanically ventilated for inhalation injury. The results were suggestive of a benefit, as inhalation of alternating NAC and heparin decreased mortality, reintubation rate and incidence of atelectasis. Nevertheless, as the control group was a historical one, these results have to be interpreted with caution.

Lower respiratory tract infection

Several uncontrolled studies claim clinical benefits of NAC in children with lower respiratory tract infections or chronic lung diseases.[37-40] For one, Santangelo et al.[37] do so for combined treatment of cefuroxime and intramuscular NAC in children with lower respiratory tract infections. Another study also reported good "clinical and radiological results" of oral NAC in children with different lower respiratory tract diseases, such as atelectasis, "bronchiolopathology", or bronchiectasis.[38] Two patients with atelectasis and "bronchiolopathology", however, showed "asphyxia". The authors suggest this might have been due to excessively rapid liquefaction of retained secretions. Nevertheless, this explanation seems unlikely as there is no proof that orally administered NAC reaches therapeutic concentrations in airway secretions.[24]

Table 2.2. Literature on the clinical effects of sulfhydryl agents in non-cystic fibrosis pediatric patients*

Disease, patient characteristics	Study design	N	Age	Dose of sulfhydryl agent, mode of administration	Reported results
Primary ciliary dyskinesia	RCT, cross-over[26]	13	29.7 (2–47) years; 6 patients < 16 years	200 mg NAC 3 times a day (<30 kg), 400 mg NAC twice daily (>30 kg) for 3 months; oral	No effect on subjective clinical score, lung function, sputum bacteriology, blood leukocyte count, sedimentation rate, anti-microbial antibodies, and ciliary function
Chronic lung disease in premature infants, during mechanical ventilation	RCT, cross-over[27]	10	27 (25–33) weeks (gestational age); 22 (10–70) days (postnatal age)	0.5 ml NAC 5% every 4 hr for 1 week; endotracheal instillation	No effect on dynamic compliance, ventilator settings, blood gas value, clinical signs, and chest X-ray Negative effect on total airway resistance Side effects: bradycardia and cyanotic spells
Extremely low birth weight infants	RCT[28]	391	26 weeks (SD:1.8) (gestational age); 1 day (postnatal age)	16-32 mg/kg/day of NAC for 6 days; intravenously	NAC vs. placebo group: incidence of death or BPD: 51 vs. 49%; OR: 1.0 (CI: 0.7–1.6); incidence of BPD: 40 vs. 40%; OR: 1.0 (CI: 0.6–1.5); mean % of oxygen required at age of 28 days: 31.2 vs. 30.7% No effect on chest X-ray score and duration of respiratory support
Extremely low birth weight infants	RCT[29]	33	25 (24–29) weeks (gestational age); 1 day (postnatal age)	16-32 mg/kg/day of NAC for 6 days; intravenously	No effect on lung function at discharge from NICU (compliance, resistance, FRC, indices of gas mixing)
Bronchopneumonia or asthma in hospitalized children	"double blind" trial[30]	47	2 months–13 years	100 mg NAC 3 times a day; oral.	No effect on cough, dyspnea, auscultation, and chest X-ray
Severe recurrent atelectasis in premature infants during mechanical ventilation	CS[31]	5	23–34 weeks (gestational age); 3–26 weeks (postnatal age)	2 ml NAC 5% 4 times a day for 2-4 days; nebulization	Improved chest X-ray, and ventilator settings
Acute atelectasis following smoke inhalation in mechanically ventilated child	CS[32]	1	12.5 years	2 ml NAC 20% every 2 hr on 1st day, 4 times a day on 2nd to 5th day; nebulization	Improved chest X-ray, sputum volume, and cough

Disease, patient characteristics	Study design	N	Age	Dose of sulfhydryl agent, mode of administration	Reported results
Status asthmaticus	CS[35]	14	5.5 (1.5–19) years	1 ml/kg bodyweight of 4% Mesna solution; bronchoalveolar lavage	Improved blood gases, chest X-ray
Inhalation injury in mechanically ventilated children	historic control group[36]	90	7.7 years (SD: 5)	3 ml NAC 20%, alternated with 5000 units heparin, every 4 hr for 7 days; nebulization	Improved reintubation rate, mortality, and atelectasis
Lower respiratory tract infection in hospitalized children	CS[37]	103	2 months–11 years	20-30 mg/kg/day of NAC for 5-10 days; intra-muscular	Improved symptoms, chest x-ray, and sputum culture
Obstructive respiratory disease (atelectasis; bronchiectasis)	CS[38]	67	2.9 years (23 days–11 years)	10-50 mg/kg/day of NAC for 7-110 days; oral	Improved chest X-ray, and 'clinical results'
Acute (recurrent) bronchitis	CS[39]	20	3–14 years	100-200 mg NAC 3 times a day for 4 days; oral	Improved duration of cough, and lung function
Chronic lung diseases	CS[40]	52	2 months–12 years	200-600 mg/day of NAC for 4 weeks; oral	Improved auscultation. Doubtful effect on chest X-ray. No effect on lung function, and regional ventilation-perfusion

*Age is presented as mean and median (range) values. N, number of patients; RCT, randomized controlled trial; CS, case series or case report; NAC, N-acetylcysteine; Mesna, 2-mercaptoethane sulphonate; PEFR, peak expiratory flow rate; RV/TLC, residual volume to total lung capacity; FVC, forced vital capacity; Vmax50%VC, maximum flow at 50% of vital capacity; FRC, functional residual capacity; NICU, neonatal intensive care unit.

Nikolic and Korac[39] reported that oral NAC improved symptoms and lung function in children with recurrent bronchitis. Rudnik et al.[40] studied children with "chronic lung diseases" and reported clinical and radiological improvement after oral NAC treatment. There was no effect, however, on lung function and regional ventilation and perfusion. As none of these studies included control groups, it is impossible to conclude whether NAC indeed contributed to the reported improvements.

Recombinant human DNase (rhDNase)

Mode of action

Purulent sputum from patients with CF and several other respiratory diseases contains high concentrations of DNA released from degenerating polymorphonuclear leucocytes.[41,42] Higher DNA content in CF mucus is associated with higher mucus viscosity and mucus elastic modulus,[43] and adding exogenous DNA to sputum increases both viscosity and elasticity.[42] Purulent sputum contains both DNA and large amounts of broad-spectrum protease, both products of neutrophils. DNA prevents the protease from rapidly destroying mucins. If DNA is enzymatically removed, mucin becomes vulnerable to protease attack and is then rapidly hydrolyzed by the protease, leading to mucolysis.[44-46] rhDNase greatly reduces viscosity of purulent CF-sputum in a concentration-dependent manner. This reduction is associated with shortening of DNA-fragments in sputum.[47] rhDNase also improves surface properties of CF sputum, as demonstrated by a decrease in the contact angle.[43] In CF, rhDNase is also able to increase the free water content and alter the phospholipid profile of mucus, with a related improvement in mucus transportability.[48] Finally, while rhDNase improves ciliary and/or cough clearance in in vitro models[43,48,49] small in vivo studies in CF patients were unable to demonstrate such improvements in response to short courses of rhDNase.[50,51]

Rationale to use rhDNase in non-CF lung diseases

rhDNase was specifically developed for patients with CF. Yet it could also be a rational therapy for other childhood lung diseases involving mucus plugging or impaired mucociliary clearance, in which neutrophilic airway inflammation or increased DNA content of mucus is present.[52] Neutrophilic inflammation is seen in adults with stable and acute asthma,[53,54] in asthmatic children,[55,56] in infants with respiratory syncytial virus (RSV) bronchiolitis,[57] and in children with PCD.[58] Increased DNA content has been demonstrated in sputum of adults with stable[53] and acute asthma,[54] and in bronchoalveolar lavage fluid of infants with RSV bronchiolitis.[59]

Randomized controlled trials of rhDNase in non-CF lung diseases

Efficacy of rhDNase has mainly been studied in patients with CF.[15,60-62] No more than four RCTs have been performed in non-CF pediatric patients (Table 2.3).[59,63-65]

Table 2.3. Literature on the clinical effects of rhDNase in non-cystic fibrosis pediatric patients*

Disease, patient characteristics	Study design	N	Age	Dose of rhDNase, mode of administration	Reported results
RSV bronchiolitis, hospitalized infants	RCT[59]	75	5 (0.3–24) months	2.5 mg rhDNase once daily until discharge (max. 5 days); nebulization	rhDNase vs. placebo group: chest X-ray score: improvement of 0.46 vs. worsening of 0.60 points ($p<0.001$); length of stay: 3.3 vs.3.3 days ($p=0.97$) No effect on symptom score
RSV bronchiolitis, hospitalized infants	RCT[63]	225	2.2 (0.4–12.8) months	2.5 mg rhDNase twice daily until discharge; nebulization	rhDNase vs. placebo group: length of stay: 4.4 vs. 3.8 days ($p=0.19$); duration of oxygen supplementation: 2.6 vs. 2.0 days ($p=0.07$) No effect on symptom score and number of intensive care admissions
Mechanical ventilation after elective heart surgery	RCT[64]	100	median 3.6 months (5 days to 2.5 years)	0.2 mg/kg rhDNase (< 5 kg), 0.1 mg/kg rhDNase (> 5 kg) twice daily until extubation; endotracheal instillation	rhDNase vs. placebo group: reintubation rate: 7% vs. 9%; OR: 0.77 (CI: 0.11–4.9); incidence of atelectasis: 6 vs. 17; OR: 0.27 (CI: 0.08–0.84); length of PICU stay: 7 vs. 8 days; 25% reduction (CI: 4–42%); ventilation time: 52 vs. 82 hr; 24% reduction (CI: -3–44%); costs: ∈1,490 lower; 23% reduction (CI: 1–41%)
Moderate-to-severe acute asthma	RCT[65]	121	4.5 (2.0–16.3) years	5.0 mg rhDNase, single dose following the second dose of bronchodilators; nebulization	rhDNase vs. placebo group: Asthma score (scale 5–15) after 1 hr: mean improvement 1.0 vs. 0.7 points ($p=0.23$); asthma score over first 24 hr: mean improvement 4.1 vs. 3.9 points ($p=0.40$) No effect on time till discharge, duration of oxygen supplementa-tion and number of bronchodilator treatments in the first 24 hr
Asthma and chronic atelectasis	CS[66]	1	7 years	2.5 mg rhDNase twice daily for 3 weeks; nebulization	Improved chest X-ray

Disease, patient characteristics	Study design	N	Age	Dose of rhDNase, mode of administration	Reported results
Atelectasis in premature neonates during mechanical ventilation	CS[67]	3	27–30 weeks (post-conceptional age)	- 1mg/m^2 rhDNase, single dose, endotracheal instillation; - 1.25 mg rhDNase twice daily for 3 days; nebulization	Improved chest X-ray, oxygen need, ventilator settings, $PaCO_2$, and physical examination
Evolving BPD, pneumonia and/or mucus plugging in extremely low birth weight infants	CS[68]	7	27–35 weeks (post-conceptional age)	- 2.5 mg, single dose, bronchoscopic instillation; - 2.5 mg once-to-twice daily for 3 days; nebulization	Improved ventilator settings, FIO_2, and sputum thickness
Atelectasis in hospitalized patients	CS[69]	30	1.6 (0.1–11) years	- 2.5 mg rhDNase twice daily until improvement; nebulization, - 0.25 mg rhDNase in 5 ml saline twice daily until improvement; endotracheal instillation	Improved respiratory rate, $PaCO_2$, FIO_2, and chest X-ray score No effect on heart rate
Severe RSV bronchiolitis and atelectasis	CS[70]	5	5–54 weeks	2.5 mg rhDNase twice daily for 2 days; nebulization	Improved physical examination, $PaCO_2$, chest X-ray, oxygen need and respiratory rate No effect on PiP/FIO_2
Atelectasis during mechanical ventilation	CS[71]	7	7 months to 12 years	4mg/m^2 rhDNase twice daily for 2 days; endotracheal instillation	No effect on saturation, PaO2/FIO2, $PaCO_2$, PIP, volume of bronchial secretions, and chest X-ray
Recurrent atelectasis in premature neonate during mechanical ventilation	CS[72]	1	33 weeks (postconceptional age)	0.2 mg rhDNase once daily for 5 days; nebulization	No effect on chest X-ray
Status asthmaticus and atelectasis	CS[73]	1	7 years	10 mg rhDNase in 20 ml saline, two doses; endotracheal instillation	Improved chest X-ray, tidal volume, and gas exchange ratio
Life threatening asthma (without atelectasis)	CS[74]	1	3 years	2.5 mg rhDNase in 10 ml saline, single dose; endotracheal instillation	Improved peak airway pressure/expired tidal volume, and blood gas
Acute severe asthma	CS[75]	3	11–15 years	2.5 mg rhDNase, single dose; nebulization	Improved lung function, and efficacy of cough
Status asthmaticus and atelectasis	CS[76]	1	8 years	2.5 mg rhDNase in 10 ml saline, single dose; bronchoscopic instillation	Improved bronchoscopic view, and chest X-ray

Disease, patient characteristics	Study design	N	Age	Dose of rhDNase, mode of administration	Reported results
Kartagener's syndrome	CS[77]	1	14 years	2.5 mg rhDNase once daily for 4 months; nebulization	Improved lung function, cough, and sputum volume
Primary ciliary dyskinesia	CS[78]	1	3 weeks	2.5 mg rhDNase once daily for 7 months; nebulization	Improved respiratory rate, dyspnea, retractions, nocturnal pulse oximetry, and lung function
Plastic bronchitis in acute chest syndrome of sickle cell disease	CS[79]	1	7 years	2.5 mg rhDNase in 20 ml saline, single dose; bronchoscopic instillation	Improved oxygenation index, chest X-ray, and bronchoscopic removal of mucus plugs

*Age is presented as mean and median (range) values. N, number of patients; RCT, randomized controlled trial; CS, case series or case report; PICU, pediatric intensive care unit; $PaCO_2$, partial pressure of arterial CO_2; PaO_2, partial pressure of arterial O_2; FIO_2, fraction of inspired oxygen; PiP, positive inspiratory pressure; OR, odds ratio; CI= 95% confidence interval.

RSV bronchiolitis

Two RCTs have assessed efficacy of nebulized rhDNase in infants hospitalized with RSV bronchiolitis. The first is that by Nasr et al.[59] in 75 infants treated with 2.5 mg rhDNase or placebo once daily up to 5 days (or until discharge). Chest X-ray scores in the rhDNase group significantly improved, whereas those in the placebo group significantly worsened. Clinically relevant endpoints such as length of stay and respiratory symptoms did not differ between groups. Only 60% of patients received supplemental oxygen, indicating that a large proportion of children had only mild bronchiolitis, and perhaps limited room for improvement.

The second is a study by our group in which we assessed the efficacy of 2.5 mg rhDNase twice daily in 225 oxygen-dependent infants hospitalized with RSV bronchiolitis.[63] rhDNase did not reduce length of hospital stay, and did not influence clinical improvement or numbers of intensive care admissions. There was a trend, however, toward a longer duration of supplemental oxygen in the rhDNase treated group. We speculated that this might have been on account of these young infants' difficulty in expectorating liquefied mucus, as they cannot cough as forcefully as older children and do not receive airway clearance therapy.

Mechanically ventilated children

Riethmüller et al.[64] studied 100 children ventilated post-operatively after elective surgery for congenital heart disease. Prophylactic therapy with endotracheal rhDNase, twice daily in doses of 0.2 (children with body weight < 5 kg) or 0.1 mg/kg, or placebo was given until extubation. Numbers of children requiring reintubation, the primary endpoint in this study, did not differ significantly (3 vs. 4 children). However, rhDNase did reduce ventilation time, incidence of atelectasis, length of stay on the pediatric intensive care unit (PICU), and mean costs.

Asthma

Boogaard et al.[65] assessed the efficacy of adding a single dose of 5 mg rhDNase to standard treatment in the emergency room in 121 children with a moderate-to-severe acute asthma exacerbation. rhDNase had no effect on the asthma score over the study period of 24 hr, number of bronchodilator treatments, time until discharge, and duration of oxygen supplementation.

Observational studies of rhDNase in non-CF lung diseases

Several uncontrolled observations in non-CF patients report beneficial effects of rhDNase in children with atelectasis, (acute) asthma and PCD, but RCTs in these patient groups are lacking (Table 2.3).

Atelectasis/mucus plugging

Atelectasis is a common complication of different respiratory diseases. Impressive results of atelectasis treatment with rhDNase have been reported in the following observational studies. Gershan et al.[66] reported resolution of a therapy-resistant atelectasis that had been present for 21 months in an asthmatic child. El Hassan et al.[67] reported improved chest radiographs, oxygen requirement, ventilator settings, blood gas values, and findings at physical examination in mechanically ventilated premature neonates suffering from atelectasis. rhDNase treatment also improved ventilator settings, oxygen requirement, and sputum appearance in a series of ventilated, low birth weight infants with early BPD with acute pneumonia or mucus plugging.[68] It was also reported to have beneficial effects on chest radiographs, respiratory rate, blood gas values, and oxygen requirement in a large series (n=30) of hospitalized children with atelectasis.[69] Finally, in another series, rhDNase improved findings on physical examination, blood gas values, chest radiographs, and oxygen requirement in infants with severe RSV bronchiolitis and atelectasis.[70]

In contrast, others found no clinical or radiological improvement after rhDNase treatment in mechanically ventilated children with an atelectasis.[71] Küpeli et al.[72] reported even new atelectatic areas after rhDNase treatment in one premature infant with recurrent atelectasis, perhaps due to profuse secretions that could not be expectorated efficiently. Hendriks et al.[69] reported that 3 out of 30 children showed temporary clinical deterioration with desaturations due to increased airways obstruction immediately after endotracheal administration of rhDNase.

Asthma

Several case reports describe the effect of rhDNase administered bronchoscopically, endotracheally or by nebulization in children with severe acute asthma unresponsive to conventional therapy. Intervention with rhDNase was associated with improved ventilator settings,[73,74] improved arterial blood gas values,[74] improved lung function[75], more effective coughing,[75] and resolution of atelectasis.[73,76]

Primary ciliary dyskinesia

Two case reports suggest that rhDNase could be beneficial in children with PCD. The first describes a 14-year-old girl with suppurative lung disease secondary to PCD with worsening spirometry and intractable gastrointestinal symptoms despite treatment. During treatment with rhDNase she showed less cough and sputum volume, less gastrointestinal symptoms and improved lung function.[77] The second describes a 3-week-old neonate with PCD and severe persisting respiratory symptoms and oxygen dependency despite antibiotics, physiotherapy, bronchodilators, and nebulized isotone saline solution. Administration of nebulized rhDNase was associated with improved oxygenation, respiratory symptoms and lung function.[78] The

possible mode of action of rhDNase in PCD patients is unclear; their sputum clearance largely depends on effective cough, and one could argue that reduced sputum viscosity alone could even further impair sputum clearance. One possible explanation, as yet unproven, is that mucolysis would intensify antibiotic penetration and bacterial killing in the airways, thereby improving lung function and clinical condition.[78]

Other res piratory diseases

In a child with sickle cell disease with plastic bronchitis in acute chest syndrome, saline lavage, suction and physical therapy could not remove mucous plugs. Fragmented mucous plugs could be cleared away, though, following bronchoscopic instillation of rhDNase, and oxygenation index and chest X-ray improved immediately.[79]

Hypertonic Saline

Mode of action

Nebulized hypertonic saline has been used to facilitate mucus clearance mainly in CF patients. Different potential mechanisms of action have been postulated. By breaking the ionic bonds within the mucus gel, hypertonic saline reduces the degree of crosslinking and entanglements, resulting in lower viscosity and elasticity.[17,80] With chronic infection, mucin macromolecules develop fixed negative charges, which increases repulsion. Addition of hypertonic saline will raise the ionic concentration in mucus and bring about a conformational change by shielding the negative charges – thereby reducing repulsion. This would result in a more compact mucus macromolecule, and more effective clearance.[81] In addition, hypertonic saline induces an osmotic flow of water into the mucus layer, rehydrating secretions and thereby improving mucus rheology.[81] While the effect of hypertonic saline on airway surface liquid thickness, and consequently on mucus layer hydration, is short lived in healthy airways (10 min), it is much greater and longer lasting in CF-airways, perhaps because the dysfunctional cystic fibrosis transmembrane conductance regulator (CFTR) cannot transport excess salt and water from the airway surface.[82] Alternatively, Wills et al.[83] showed that viscoelasticity of sputum is markedly saline-dependent and that adding sodium chloride to sputum enhances its transportability. They concluded that it is low salinity and not underhydration that contributes to mucus retention in bronchiectasis due to CF and other disorders. Moreover, in infected mucus, hypertonic saline separates the DNA molecules from the mucoprotein, making the mucoprotein susceptible to proteolytic enzyme digestion.[45] In addition, it has been suggested that airway surface liquid hyperosmolarity will release mediators capable of enhancing ciliary activity.[84]

Mucociliary clearance studies in adults showed that inhalation of aerosolized hypertonic saline enhances mucociliary clearance in a concentration dependent fashion.[81] This was ob-

Table 2.4. Literature on the clinical effects of hypertonic saline in non-cystic fibrosis pediatric patients*

Disease, patient characteristics	Study design	N	Age	Dose of hypertonic saline solution, mode of administration	Reported results
Viral bronchiolitis (80% RSV +), ambulatory setting	RCT[89]	65	12.5 (3–24) months	2 ml 3% saline solution and 5 mg of terbutaline, 3 times a day for 5 days; nebulization	Hypertonic saline vs. placebo group: symptom score (scale 0–12) on 2nd day: mean improvement 2.7 vs. 1.2 points ($p<0.05$); symptom score on 3rd to 5th day: improvement greater in HS group ($p<0.05$) No effect on chest X-ray score, and hospitalization rate
Viral bronchiolitis (87% RSV +), hospitalized infants	RCT[90]	52	2.9 (0.5–12) months	4 ml 3% saline solution and 1.5 mg epinephrine; 3 times a day until discharge; nebulization	Hypertonic saline vs. placebo group: length of hospital stay: 3 (±1.2) vs. 4 (±1.9) days ($p<0.05$) Improved symptom score 30 min after inhalation No effect on chest X-ray score, and symptom score over time
Viral bronchiolitis, hospitalized infants	RCT[91]	42	2.6 (± 1) months	4 ml 3% saline solution and 1.5 mg epinephrine, 3 times a day until discharge; nebulization	Hypertonic saline vs. placebo group: length of hospital stay: 2.6 (±1.4) vs. 3.5 (±1.7) days ($p<0.05$) Improved symptom score 30 min after inhalation No effect on symptom score over time

*Age is presented as mean and median (range) values. N, number of patients; RSV, respiratory syncytial virus; RCT, randomized controlled trial; HS, hypertonic saline.

served in healthy subjects and asthmatics,[84] in chronic bronchitis,[85] and in CF patients.[81,82,86,87] King et al.[49] demonstrated that hypertonic saline significantly reduces CF sputum viscoelasticity in vitro and improves predicted cough clearability.

Randomized controlled trials of hypertonic saline in non-CF lung diseases

As with the other mucoactive agents, nebulized hypertonic saline has been mainly studied in CF patients.[17,82,88] Our literature search revealed only three small, RCTs assessing efficacy of hypertonic saline in children with respiratory disease other than CF (Table 2.4).[89-91]

Bronchiolitis

Sarrel et al.[89] compared regimens of nebulized terbutaline in normal saline and terbutaline in 3% hypertonic saline, thrice daily for 5 days in 65 ambulatory infants with mild to moderate RSV bronchiolitis. Symptom scores on each treatment day were lower (better) in the 3% saline

group, and on the first two treatment days this group also showed better improvement in symptom score 30 min after inhalation. These differences were small, however, and chest-X ray scores as well as hospitalization rates did not differ at all. Using the same design, Mandelberg et al.[90] compared the effect of epinephrine in 3% saline solution with that of epinephrine in normal saline solution in 52 infants hospitalized with RSV bronchiolitis. Treatment was thrice daily until discharge. The group receiving the 3% solution stayed significantly shorter in hospital than did the control group (4 vs. 3 days). Information on numbers of infants requiring supplemental oxygen and duration of oxygen supplementation is not provided. Pre-inhalation clinical scores did not differ between groups, but the 3% saline group showed greater improvement in clinical scores 30 min after inhalation. The same authors repeated this study in an additional 41 children, with similar results.[91]

Discussion, conclusions and recommendations

Our MEDLINE search revealed scarce literature on the efficacy of the mucoactive agents NAC, rhDNase and hypertonic saline in children with non-CF lung disease. There is a predominance of uncontrolled observations that suggest beneficial effects of mucoactive agents in different lung diseases. We expect there is a likely effect of publication bias, as uncontrolled observations of unfavorable findings are rarely published. For most of these diseases no RCTs have been conducted to confirm or refute these positive findings.

NAC and other sulfhydryl compounds

There is no solid evidence to support the use of inhaled or oral NAC or other sulfhydryl compounds in children with respiratory tract disease. The few published RCTs in non-CF pediatric patients were of crossover design and of short duration. They showed no effects at all or just futile effects of doubtful clinical significance. As oral NAC does not penetrate into airway secretions, it seems unlikely that any effects are the result of mucolysis.[24] A potential drawback of inhaled NAC in children with airway hyperresponsiveness is the risk of bronchospasm.[22] Observational studies also reported cyanotic spells and asphyxia after intratracheal and oral NAC, respectively.[27,38] Despite this lack of supporting literature, NAC is widely prescribed for children with various respiratory diseases.[92]

rhDNase

Two RCTs in infants with moderate-to-severe RSV bronchiolitis, [59,63] and one RCT in children with a moderate-to-severe asthma exacerbation demonstrated no clinical benefits of rhDNase.[65] One RCT showed shorter stay on the intensive care unit, and lower incidence of at-

electasis in children ventilated post-operatively.[64] Additional trials are needed to confirm these findings. Anecdotal evidence suggests that rhDNase could be beneficial in several childhood lung diseases with impaired MCC, such as acute severe life-threatening asthma, or atelectasis during mechanical ventilation. Still there is an obvious need for confirmation from well-designed RCTs with clinically relevant endpoints before rhDNase can be recommended in non-CF lung disease.

Hypertonic saline

Efficacy of hypertonic saline in non-CF patients has been studied in only 3 small RCTs in infants with RSV bronchiolitis, all conducted by the same research group. They reported a beneficial effect on length of hospital stay and symptoms. Although promising, these results need to be confirmed in larger trials.

Recommendations for future research

NAC and other sulfhydryl compounds

There is an evident imbalance between the widespread use of NAC as a mucolytic and the lack of data to support this practice. Based on the current literature any mucolytic effect of oral and inhaled NAC in patients with lung disease seems unlikely. Yet, its anti-oxidant properties could be therapeutically effective. Indeed, a recent, small, phase I study showed that high-dose oral NAC can modulate redox and inflammatory imbalances of CF airway disease.[93] Therefore, future studies would do well to focus on the efficacy of (high dose) oral or intravenous NAC in pediatric lung disease with major involvement of airway inflammation, such as CF and severe persistent asthma.

rhDNase

Many case reports have suggested a potential role for rhDNase in non-CF pediatric lung diseases with severe airways obstruction or mucus plugging. Future RCTs should establish whether rhDNase is effective in children with severe acute and severe persistent asthma or with persistent atelectasis, in mechanically ventilated children with atelectasis and in infants with severe RSV bronchiolitis requiring intensive care. Trials in children with severe acute asthma requiring intensive care obviously require multi-center collaboration for sufficient power. It might as well be worthwhile to assess the efficacy of rhDNase during airways infections in children with impaired mucociliary clearance due to an anatomical airway abnormality, such as malacia. Because rhDNase is an expensive drug, such studies should also take into account cost-effectiveness aspects of this treatment.

Hypertonic saline

Hypertonic saline reduced length of stay and relieved symptoms in infants with RSV in 3 small RCTs. Larger RCTs are warranted to confirm these findings. It would also be of interest to explore the effect of inhaled hypertonic saline on symptoms, admission rate or length of hospital stay in children with (viral) respiratory tract infections other than RSV, or with recurrent or chronic bronchitis.

In general, future studies should also report if physiotherapy was used as an adjunct of treatment with mucoactive agents. Although evidence on the efficacy of physiotherapy in (pediatric) lung disease is lacking,[20] it is theoretically plausible that an increase in mucus secretion or clearance should be accompanied by an effective cough and airway clearance.

In conclusion, although the pathophysiology of airway mucus secretion and clearance has been elucidated to some extent, and new mucoactive agents are being developed and tested in adults, there is also still an obvious need for clinical studies in pediatric lung disease using already available mucoactive agents. Of these, rhDNase and hypertonic saline seem to bear most promise. We propose future RCTs may answer the unresolved questions on the efficacy of mucoactive agents in pediatric lung disease. Until then, we must assume the widespread use of compounds such as NAC in children with respiratory illness is not evidence based, and should probably be abandoned.

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Chapter 3

Recombinant human deoxyribonuclease in infants with respiratory syncytial virus bronchiolitis

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Abstract

Background: Treatment of hospitalized infants with respiratory syncytial virus (RSV) bronchiolitis is mainly supportive. Bronchodilators and systemic steroids are often used but do not reduce the length of hospital stay. Because hypoxia and airways obstruction develop secondary to viscous mucus in infants with RSV bronchiolitis, and because free DNA is present in RSV mucus, we tested the efficacy of the mucolytic drug recombinant human deoxyribonuclease (rhDNase).

Methods: In a multicenter, randomized, double-blind, controlled clinical trial, 225 oxygen-dependent infants admitted to the hospital for RSV bronchiolitis were randomly assigned to receive 2.5 mg bid of nebulized rhDNase or placebo until discharge. The primary end point was length of hospital stay. Secondary end points were duration of supplemental oxygen, improvement in symptom score, and number of intensive care admissions.

Results: There were no significant differences between the groups with regard to the length of hospital stay (0.19) or the duration of supplemental oxygen ($p = 0.07$). The ratio (rhDNase/placebo) of geometric means of length of stay was 1.12 (95% confidence interval, 0.96 to 1.33); for the duration of supplemental oxygen, the ratio was 1.28 (95% confidence interval, 0.97 to 1.68). There were no significant differences in the rate of improvement of the symptom score or in the number of intensive care admissions.

Conclusions: Administration of rhDNase did not reduce the length of hospital stay or the duration of supplemental oxygen in oxygen-dependent infants with RSV bronchiolitis.

Introduction

Respiratory syncytial virus (RSV) is the most important cause of viral bronchiolitis in young children.[1] In general, approximately 0.5 to 2% of children with RSV disease need to be hospitalized.[2,3] It is estimated that each year approximately 50,000 to 80,000 children < 1 year old are hospitalized for RSV bronchiolitis in the United States,[4] and consequently RSV bronchiolitis constitutes a significant burden on patients, parents, and the health-care system.

Treatment of hospitalized children with RSV bronchiolitis is mainly supportive, with supplemental oxygen, nasal washings, tube feeding, or IV fluids, and mechanical ventilation where necessary. ß2-Agonists and systemic steroids are frequently used,[5] although their efficacy is disappointing. Several randomized studies[6-8] have demonstrated that neither nebulized ß2-agonists, epinephrine, nor systemic steroids reduce the length of hospital stay.

It has been well recognized that RSV bronchiolitis is associated with severe airways obstruction due to the presence of intraluminal secretions, loss of epithelial cilia, sloughing of epithelial cells, and cellular debris from accumulated inflammatory cells within the airway.[9] Because baseline airway resistance is high in infancy, even the presence of small amounts of mucus in the airways of infants can have a profound effect on the work of breathing.[10] Since airway obstruction due to intraluminal mucus plugs is an important pathophysiologic feature of RSV bronchiolitis, a logical approach to therapy might be to use a mucolytic agent. The DNA present in mucous plugs following lysis of inflammatory cells contributes to increased viscosity and adhesiveness of the mucus.[11,12] Such mucus can be liquefied by recombinant human deoxyribonuclease (rhDNase [dornase alfa]), which cleaves the extracellular DNA released by degenerating leukocytes.[13,14] The efficacy of rhDNase has been well documented in patients with cystic fibrosis,[15] and several publications suggest that rhDNase improves radiologic abnormalities in mild RSV bronchiolitis,[12] and is effective in infants with severe RSV bronchiolitis,[16] in pediatric patients with severe atelectasis or airways obstruction due to asthma,[17-19] and other respiratory diseases.[20-24]

It is unknown whether infants with RSV bronchiolitis admitted to the hospital because of hypoxemia benefit from rhDNase treatment. Therefore, we conducted a randomized, placebo-controlled trial to examine the effect of nebulized rhDNase in oxygen-dependent infants hospitalized with RSV bronchiolitis. The primary outcome measure was length of hospital stay. Secondary outcome measures included duration of supplemental oxygen, change in symptom scores, number of admissions to the ICU, and use of bronchodilators and antibiotics. The study was approved by the ethics committees of all 10 centers. Written parental informed consent was obtained for each infant.

Materials and methods

Setting and participants

For entry into this study, we considered infants < 12 months of age with proven RSV bronchiolitis requiring supplemental oxygen, who were admitted to 1 of the 10 participating hospitals between November 2002 and February 2006: Erasmus MC-Sophia Children's Hospital, Rotterdam; Amphia Hospital, Breda; Reinier de Graaf Gasthuis, Delft; Albert Schweitzer Hospital, Dordrecht; HagaTeaching Hospital/Juliana Children's Hospital, the Hague; Medisch Centrum Alkmaar, Alkmaar; Catharina Hospital, Eindhoven; Sint Franciscus Gasthuis, Rotterdam; Medisch Centrum Rijnmond Zuid, Rotterdam; and Isala Klinieken, Zwolle, the Netherlands. RSV infection was confirmed by a direct immunofluorescence assay of a nasopharyngeal aspiration sample. We did not include infants born at a gestational age < 32 weeks, infants with cardiopulmonary disease or an immunodeficiency. We also did not include infants who received or had been prescribed systemic steroids at hospital admission, and infants who required intensive care admission before parents could give consent for the study.

Randomization

After parental consent was given, patients were randomly assigned to receive 2.5 mg of rhDNase or 2.5 mg of placebo bid until discharge, until oxygen supplementation could be stopped for at least 12 h, or until the patient had to be transferred to an ICU. Randomization was performed in the hospital pharmacy of each participating hospital using a random table sample with blocks of four numbers made by the study statistician. Throughout the study, physicians, nurses, parents, and the trial coordinator remained unaware of the treatment assignment.

Study procedures and end points

After hospital admission, the admitting physician recorded the clinical history of the patient, including duration of symptoms before admission, use of medication, parental smoking history, and family history of atopy. Vital signs and the use of supplemental oxygen were recorded three times daily by the nurse responsible. At inclusion and subsequently each morning, respiratory symptoms were scored by the attending physician using a scoring system described previously.[25] The respiratory rate, presence of wheezing, and retractions were scored on a four-point scale (Table 3.1). A symptom score was calculated by adding up these three separate items, yielding a score ranging from 0 to 9.

During hospital admission, patients received supportive care according to the hospital guidelines. This included nasal washings, nasal decongestants, supplemental oxygen, and tube

Table 3.1. Symptom score

Variables	0 Points	1 Point	2 Points	3 Points
Respiratory rate, breaths/min	< 30	31 to 45	46 to 60	> 60
Wheezing	None	End-expiratory or only audible with stethoscope	Entire expiration or audible on expiration without stethoscope	In- and expiratory, audible without stethoscope
Retractions	None	Intercostal	Tracheosternal	Severe with nasal flaring

feeding or IV fluids when necessary. The decision to treat patients with antibiotics, bronchodilators, or systemic steroids was at the discretion of the attending physician, as was the decision on discharge. We calculated the length of hospital stay as the number of hours between the first dose of study medication and the moment of discharge. Because in real life, length of hospital stay can be affected by administrative and social factors unrelated to the clinical condition of a patient, we also assessed the duration that supplemental oxygen was required as a measure of efficacy. Supplemental oxygen was started when oxygen saturation was consistently < 93%, and stopped when saturation was consistently > 92%.

Twice daily, patients received either rhDNase (a 2.5-mL solution of 1 mg/mL rhDNase) or placebo (2.5 mL of sodium chloride 0.9%). A twice-daily dosing schedule was chosen to anticipate the poor lung deposition expected in young infants with airways obstruction due to bronchiolitis. Study medication was prepared by the hospital pharmacists. The first dose of study medication had to be administered within 32 h of hospital admission. Study medication was administered using a jet nebulizer through a firmly applied facemask with a constant oxygen supply of 6 to 8 L/min from a wall outlet. The same state-of-the-art nebulizing equipment (Sidestream; Romedic BV; Meersen, the Netherlands) was used in all participants.

Estimate of sample size

We aimed to detect a 25% difference in length of stay at the 5% significance level for a two-sided test with 80% power. Based on previous data,[26] this would require 180 patients, with 90 infants in each group. To anticipate possible dropouts, we aimed to include 220 patients.

Statistical analysis

The data from all randomized patients were analyzed on an intention-to-treat basis. A separate per-protocol analysis was conducted in which patients who violated the study protocol

were excluded (Figure 3.1). The differences between the treatment groups with regard to the baseline characteristics were assessed by χ^2 or Fisher exact test and the Mann-Whitney test. Main analyses of between-group comparisons regarding the length of hospital stay and duration of supplemental oxygen were performed by analysis of covariance (ANCOVA) after logarithmic transformation to obtain approximate normal distributions. The baseline covariates used in these analyses were the hospital of admission, the symptom score, sex and age. Birth weight was included as a covariate because it showed some imbalance between treatment groups. The analysis was performed using statistical software (SPSS version 11.5; SPSS; Chicago, IL). All reported p values are two sided.

After inclusion of the first 100 patients, an interim safety analysis was conducted by a committee of an independent statistician and two independent pediatricians, revealing no safety or ethical arguments to discontinue the study at that moment. Except for the interim safety analysis, the randomization code was not broken until the study was completed.

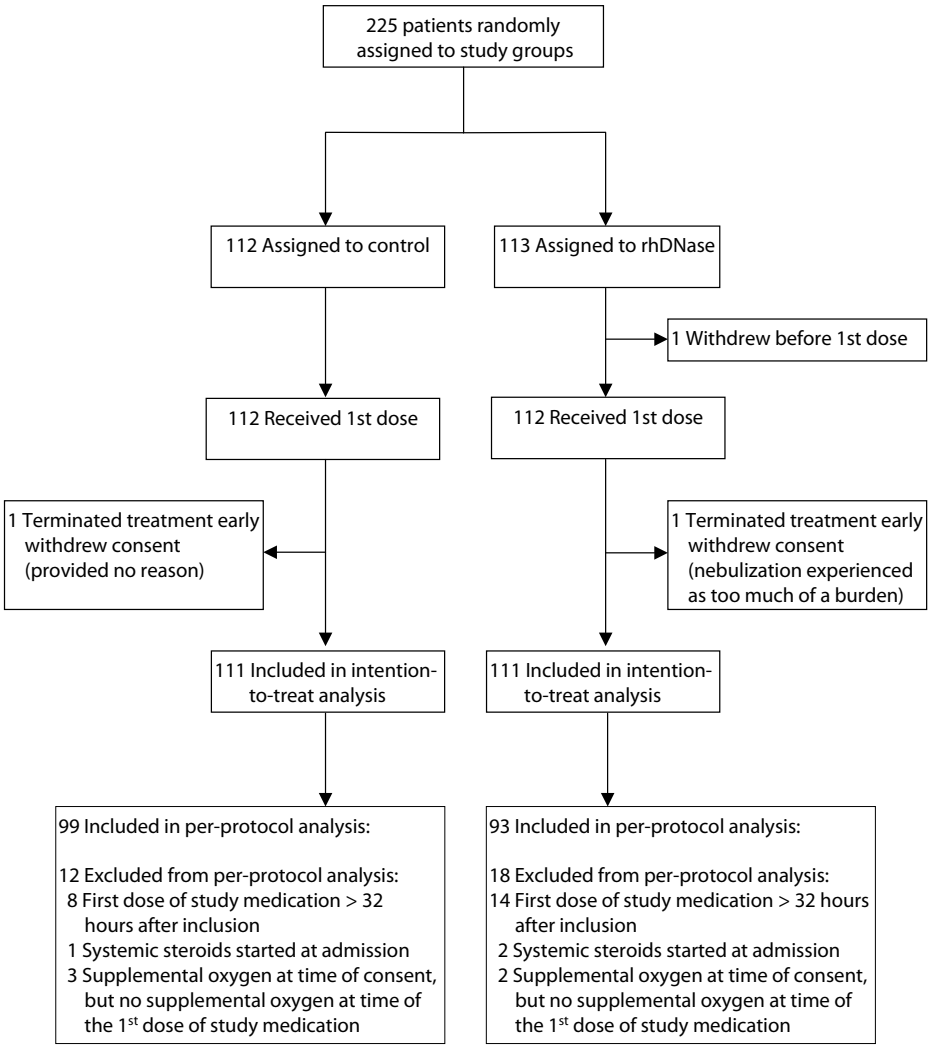
Results

A total of 225 infants were assigned to treatment: 113 infants to rhDNase and 112 infants to placebo (Figure 3.1). Two patients withdrew from the study after the first dose of study medication (one in each group) and consequently had no follow-up data available. During the first two RSV seasons of the study, patients were recruited in 4 hospitals; during the last two RSV seasons, patients were recruited from a total of 10 hospitals. In terms of demographic variables, there were no significant differences between the groups at randomization except for birth weight, which was slightly lower in the rhDNase group (Table 3.2). The duration of illness before hospital admission and the symptom score at randomization were comparable between the groups.

Primary end point

Overall, treatment with rhDNase had no significant effect on the length of hospital stay (Table 3.2). The duration of hospital stay is presented in Figure 3.2 . The length of hospital stay in the rhDNase group ranged from 1 to 32 days (interquartile range, 2.9 to 6.7 days); the range in the placebo group was 1 to 43 days (interquartile range, 2.7 to 5.7 days). ANCOVA, which allowed for center, age, sex, birth weight, and baseline symptom score, also showed that rhDNase had no significant effect on the length of hospital stay: the ratio of the length of hospital stay in the rhDNase group to that in the placebo group was 1.12 (95% confidence interval, 0.96 to 1.33; p = 0.19) (Table 3.4). At baseline, approximately one half of the infants had a symptom score 4 and one half of the infants had a symptom score 3. Irrespective of the severity of symptoms at inclusion, the length of hospital stay did not differ between treatment groups

Figure 3.1. Enrollment, random assignment, follow-up, and analysis



(Table 3.4). The baseline symptom score and age were significantly related to the length of hospital stay (Table 3.4, Figure 3.3). Analysis of the primary end point according to the per-protocol analysis yielded similar results (data not shown). The first dose of study medication was administered after a median of 21 h after hospital admission in the placebo group and after 22 h in the rhDNase group (p = 0.35).

Table 3.2. Baseline characteristics of the infants at admission to the hospital*

Characteristic	rhDNase n = 111	Placebo n = 111
Male/female gender, No.	53/58	56/55
Gestation, wk	39.5 (33-42)	40.0 (33-43)
Birth weight, kg	3.3 (1.5-4.8)	3.5 (1.4-5.0)
Actual weight, kg	5.1 (2.6-9.8)	5.3 (2.7-10.1)
Age, mo	2.1 (0.4-11.5)	2.3 (0.3-12.8)
Days sick		
0-2 days	35 (31.5)	33 (29.7)
3-5 days	55 (49.5)	62 (55.9)
≥ 6 days	21 (18.9)	16 (14.4)
Prenatal smoking mother	14/95 (15)	16/93 (17)
Parental smoking		
Neither parent	69/92 (75)	57/89 (64)
One or both parents	23/92 (25)	32/89 (36)
Atopy in first-degree relative	53/94 (56)	64/97 (66)
Mean symptom score	3.67 (± 1.68)	3.65 (± 1.79)
Symptom score ≤ 3	46/106 (43)	56/108 (52)
Symptom score ≥ 4	60/106 (57)	52/108 (48)
Drinking inadequate	52/109 (48)	61/111 (55)

* Data expressed as median (range), No. (%), No./total (%), or mean ± SD unless otherwise indicated.

Table 3.3. Length of hospital stay and time supplemental oxygen was required, according to the baseline symptom score*

Variables	rhDNase	No. of infants	Placebo	No. of infants	Ratio of geometric means of rhDNase and placebo groups (95% CI)	p value†
Length of hospital stay, d						
Overall	4.4 (3.9-4.9)	111	3.8 (3.4-4.3)	111	1.14 (0.97-1.35)	0.11
Baseline symptom score ≤ 3‡	3.9 (3.2-4.7)	46	3.4 (2.9-3.9)	56	1.15 (0.90-1.46)	0.27
Baseline symptom score ≥ 4	4.9 (4.2-5.7)	60	4.4 (3.6-5.2)	52	1.12 (0.89-1.42)	0.31
Time supplemental oxygen required, d§						
Overall	2.6 (2.2-3.1)	109	2.0 (1.6-2.4)	108	1.29 (0.99-1.67)	0.053
Baseline symptom score ≤ 3‡	2.0 (1.5-2.7)	45	1.6 (1.2-2.2)	54	1.27 (0.84-1.93)	0.26
Baseline symptom score ≥ 4	3.2 (2.6-3.9)	59	2.6 (2.0-3.3)	51	1.24 (0.91-1.70)	0.18

* Data are presented as geometric mean (95% confidence interval) unless otherwise indicated.

† Unadjusted p values (t test)

‡ In eight patients (five in the rhDNase group and three in the placebo group), no baseline symptom score was available.

§ Five children did not receive supplemental oxygen at inclusion (three in the placebo group and two in the rhDNase group), so the duration of supplemental oxygen could not be calculated for these children.

Table 3.4. Results of ANCOVA regarding length of hospital stay*

Variables	Effect estimate	95% confidence interval	p value
Treatment †	1.12	0.96 - 1 .33	0.19
Age ‡	0.96	0.93 - 0.99	0.01
Sex §	1.05	0.88 - 1.24	0.61
Birth weight ¶	0.90	0.80 - 1.03	0.12
Baseline symptom score#	1.08	1.03 - 1.14	0.003

* Data represent ratios of geometric means, adjusted for center.

† rhDNase versus placebo. ¶ Per increase of 1kg.

‡ Per increase of 1 month. # Per increase of 1 point.

§ Male vs female gender

Figure 3.2. Percentage of participants in each group remaining in the hospital

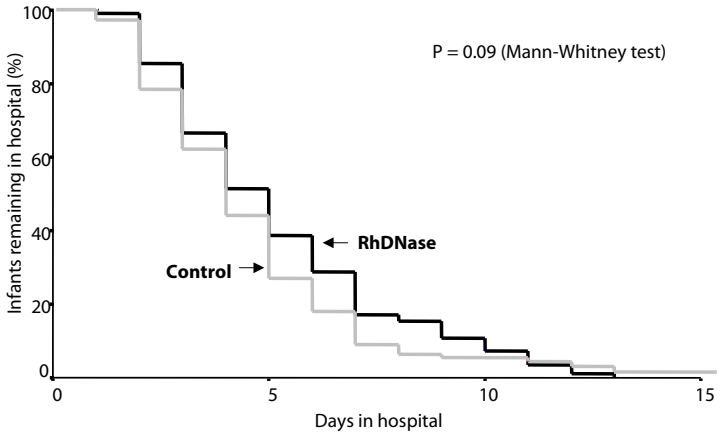
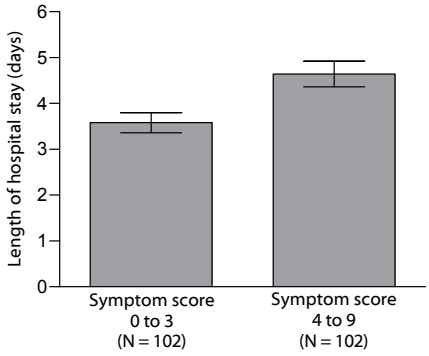


Figure 3.3. Geometric mean length of hospital stay, with SEs, according to the baseline symptom score for both treatment groups combined

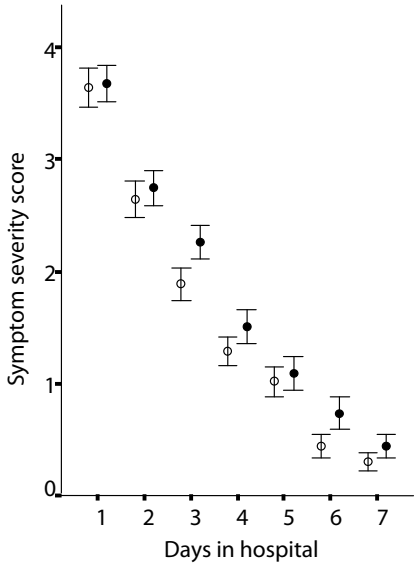


Secondary end points

There were five children who did not receive supplemental oxygen at inclusion (three in the placebo group and two in the rhDNase group). For the remaining children, the duration of supplemental oxygen ranged from 0.2 to 24 days in the rhDNase group (interquartile range, 1.8 to 4.7 days) and from 0.04 to 17 days in the placebo group (interquartile range, 1.2 to 3.8 days).

Overall, treatment with rhDNase had no significant effect on the duration of supplemental oxygen, irrespective of the baseline symptom score (Table 3.3). ANCOVA also showed that rhDNase had no significant effect on the duration of supplemental oxygen: the ratio of the duration of supplemental oxygen in the rhDNase group to that in the placebo group was 1.28 (95% confidence interval, 0.97 to 1.68; p = 0.07). This analysis further showed that the duration of supplemental oxygen was negatively correlated with the age of the child (p = 0.02) and positively correlated with the symptom score at baseline (p = 0.004). The mean symptom score on each study day, as well as the rate of improvement of this score, were not significantly different in the rhDNase group compared with the placebo group (Figure 3.4).

Figure 3.4. Mean symptom score, with SEs, during hospital stay



Day 1 corresponds to the baseline symptom score. In calculating mean values, the individual symptom score after discharge was arbitrarily set at 0 points. ● indicates rhDNase; ○ indicates placebo.

Seven infants (3.2%) required transfer to the ICU, and five infants required mechanical ventilation. There were no significant differences between the groups in proportions requiring intensive care (p = 1.0) or mechanical ventilation (p = 0.43). Twenty-four infants in the rhDNase group and 32 infants in the placebo group received antibiotics during hospital admission (p = 0.28). Fifty percent of the infants in the placebo group and 49% in the rhDNase group received bronchodilators on at least 1 day during hospital admission (p = 0.89). The number of adverse drug reactions (i.e., adverse events that in the opinion of the treating physician were directly and temporally related to the inhalation of the trial solution) did not differ between treatment groups (p = 0.21) (Table 3.5).

Table 3.5. Adverse reactions considered to be related to treatment*

Reactions	rhDNase	Placebo
Temporary desaturation	1	0
Increased coughing / increased mucus / difficulty coughing up thin mucus	1 / 0 / 2	0 / 1 / 0
Skin rash on face	1	1
Hoarseness	1	1
Bad taste	1	0
Dyspnea	1	0
Total	8	3†

* Data are presented as No.

† $p = 0.21$, χ^2.

Discussion

We report the first large, randomized, double-blind, controlled trial of rhDNase in infants with RSV bronchiolitis. This study demonstrates that the mucolytic rhDNase does not shorten length of hospital stay or duration of supplemental oxygen in hypoxemic infants with RSV bronchiolitis. Neither was the rate of clinical improvement better in infants treated with rhDNase than in those receiving a placebo.

Because mucus plugs play an important role in the pathophysiology of RSV bronchiolitis,[27] and because the DNA content is increased in mucus of these infants,[12] we hypothesized that rhDNase is an effective treatment for infants with RSV bronchiolitis. Anecdotal evidence indeed suggests that rhDNase treatment is effective in infants with severe RSV bronchiolitis.[16] Furthermore, one randomized study[12] in a small group of infants with mild bronchiolitis demonstrated that rhDNase improved radiologic abnormalities. In that study[12] no differences in length of hospital stay and symptom scores were observed, but as many of these patients were not oxygen dependent, they may have had only mild airway obstruction. In addition, the efficacy of rhDNase has also been reported in observational studies of pediatric patients with

atelectasis or severe airways obstruction due to asthma,[17-19] and other respiratory diseases[20-24]; and a recent randomized study[28] demonstrated that rhDNase effectively prevents the development of atelectasis in infants receiving postoperative mechanical ventilation.

There may be several explanations for the lack of effect of rhDNase in our study. One is that the infants probably had disease that was too mild, without large atelectasis, for rhDNase to be effective. Infants with risk factors for severe bronchiolitis, or who needed intensive care directly at hospital admission, were not included in this study. Although all infants required supplemental oxygen, reflecting the presence of clinically significant airways obstruction and mismatch of pulmonary ventilation and perfusion, only a small number required intensive care treatment. We cannot exclude that rhDNase could be effective in infants with more severe disease and/or atelectasis due to mucus plugging in the central airways that requires intensive care admission.

A second explanation could be a suboptimal lung deposition of rhDNase, resulting in deposition of rhDNase mainly in the more central airways, while in RSV bronchiolitis mucus predominantly blocks peripheral airways.[9] A third explanation might be that the number of neutrophils, and hence the amount of DNA released in the mucus, was too low for rhDNase to be effective in our study population of infants with mild-to-moderately severe bronchiolitis.[29]

A fourth explanation might be that mucus was liquefied but that infants were not able to clear their airways effectively. While rhDNase is combined with airway clearance therapy in order to evacuate mucus in children with cystic fibrosis, the infants in our study did not receive airway clearance therapy. Moreover, as young infants cannot cough as forcefully as older children, especially during an illness, it might be difficult for them to expectorate liquefied mucus. Indeed, the observed trend toward a longer duration of supplemental oxygen in the rhDNase group is consistent with such a mechanism.

Treatment with study medication started after a median of 21 h and 22 h in the placebo and rhDNase groups, respectively. It could be argued that starting study medication within a few hours of hospital admission might change length of stay when waiting almost 24 h does not. However, a separate ANCOVA adjusting for the time between hospital admission and the first dose of study medication did not influence the results (data not shown). Moreover, the symptom score during hospital admission showed no difference between the treatment groups. We therefore think it is unlikely that initiating therapy earlier after hospital admission would have changed the outcome of this study.

One half of the patients in our study received bronchodilators during their hospital stay. Despite the fact that current evidence suggests that bronchodilators are not effective,[6] the use of bronchodilators for hospitalized infants with RSV bronchiolitis is common practice.[5,30]

Although the risk of a secondary bacterial infection in infants with RSV bronchiolitis is minimal,[31] antibiotics were also prescribed frequently; in approximately one fourth of all patients, a course of antibiotics was started at hospital admission or during hospital stay. This probably reflects the fact that it can be difficult to rule out a bacterial pneumonia or sepsis solely on the basis of clinical signs.

Because our study population reflects bronchiolitis patients in district and tertiary care hospitals, our results can be generalized to the large majority of hospitalized, oxygen-dependent infants with RSV bronchiolitis with no complications other than hypoxemia. Studies on the effect of rhDNase in infants with more severe bronchiolitis might still be warranted. Another implication of this study, which echoes that of a previous study[8] with negative findings, is that we still have only supportive measures at our disposal. This emphasizes the need for effective and safe vaccination against RSV bronchiolitis. In conclusion, our study clearly shows that inhaled rhDNase does not reduce the length of hospital stay or the duration of supplemental oxygen in oxygen-dependent infants with RSV bronchiolitis.

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Chapter 4

Recombinant human deoxyribonuclease for the treatment of acute asthma in children

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Abstract

Background: Airway obstruction in acute asthma is the result of airway smooth muscle contraction, inflammation and mucus plugging. Case reports suggest that mucolytic therapy might be beneficial in acute asthma. The aim of this study was to determine the efficacy of the mucolytic drug recombinant human deoxyribonuclease (rhDNase) in addition to standard treatment at the emergency department in children with an asthma exacerbation.

Methods: In a multicentre randomized double-blind controlled clinical trial, 121 children brought to the emergency room for a moderate to severe asthma exacerbation were randomly assigned to receive either a single dose of 5 mg nebulized rhDNase or placebo following the second dose of bronchodilators. An asthma score (scale 5–15) was assessed at baseline and at 1, 2, 6, 12 and 24 h. The primary outcome variable was the asthma score 1 h after the study medication.

Results: One hour after the study medication the asthma score in the rhDNase group showed an adjusted mean decrease from baseline of 1.0 (95% CI 0.5 to 1.6) points compared with 0.7 (95% CI 0.3 to 1.2) points in the placebo group (mean difference 0.4 (95% CI –0.2 to 1.0) points; p = 0.23). The asthma score over the study period of 24 h also did not differ significantly between the rhDNase and placebo group (mean difference 0.2 (95% CI –0.3 to 0.7) points, p = 0.40). The duration of oxygen supplementation and number of bronchodilator treatments in the first 24 h were similar in both groups.

Conclusion: Adding a single dose of nebulized rhDNase to standard treatment in the emergency room has no beneficial effects in children with moderate to severe acute asthma.

Introduction

The standard treatment for children with acute asthma consists of frequent nebulized bronchodilators and early systemic corticosteroid therapy.[1] Since airway obstruction by viscous mucus is one of the pathophysiological features of acute asthma,[2-4] a logical approach to treatment might be to use a mucolytic agent. It is the DNA present in mucous plugs following lysis of inflammatory cells that contributes to increased viscosity and adhesiveness of the mucus,[5] and free DNA was indeed noted in the mucus of subjects with acute asthma.[6] Such mucus can be liquefied by recombinant human deoxyribonuclease (rhDNase; dornase alfa) which cleaves extracellular DNA.[7,8] The efficacy of rhDNase has been well documented in patients with cystic fibrosis,[9] and several publications suggest that it is also effective in children with severe acute asthma with[10-12]or without atelectasis.[11,13]

We performed a randomized controlled trial to determine whether nebulized rhDNase added to standard treatment would improve symptoms in children with moderate to severe acute asthma.

Methods

Patients

Eligible subjects for this study were children aged 2–18 years with symptoms of acute asthma whose asthma score (Table 4.1) at arrival in the emergency room was 8 and who required at least two treatments with nebulized bronchodilators. We did not include children with other causes of dyspnea, a chronic cardiopulmonary disease other than asthma or those with a neurological condition.

Study design

This was a multicentre double-blind parallel-group randomized study comparing the effect of inhaled rhDNase with placebo on the asthma score in children aged 2–18 years with symptoms of acute asthma. The trial was carried out in emergency rooms of eight participating hospitals in the Netherlands between September 2005 and October 2006. The study was approved by the ethics review boards of all eight centers and written parental informed consent was obtained for each child.

All children received a dose of nebulized bronchodilators on arrival (<4 years old: 2.5 mg salbutamol, 0.25 mg ipratropium; 4 years old: 5 mg salbutamol, 0.5 mg ipratropium). After

Table 4.1. Methods of calculating the asthma score and the severity of asthma*

	Asthma scoring		
Variable	1 point	2 points	3 points
Respiratory rate (breaths/min)			
2–3 yr	≤ 34	35–39	≥ 40
4–5 yr	≤ 30	31–35	≥ 36
6–12 yr	≤ 26	27–30	≥ 31
>12 yr	≤ 23	24–27	≥ 28
Haemoglobin saturation	> 95% with room air	90–95% with room air or ≥ 90% with supplemental oxygen	< 90% with room air or supplemental oxygen
Auscultation	Normal breathing or end-expiratory wheezing	Expiratory wheezing	Inspiratory and expiratory wheezing, diminished breath sounds, or both
Retractions	None or intercostal	Intercostal and substernal	Intercostal, substernal, and supraclavicular
Dyspnoea	Speaks in sentences or coos and babbles	Speaks in partial sentences or utters short cries	Speaks in single words or short phrases or grunts
	Severity of asthma		
	Mild	Moderate	Severe
Asthma score	5–7	8–11	12–15

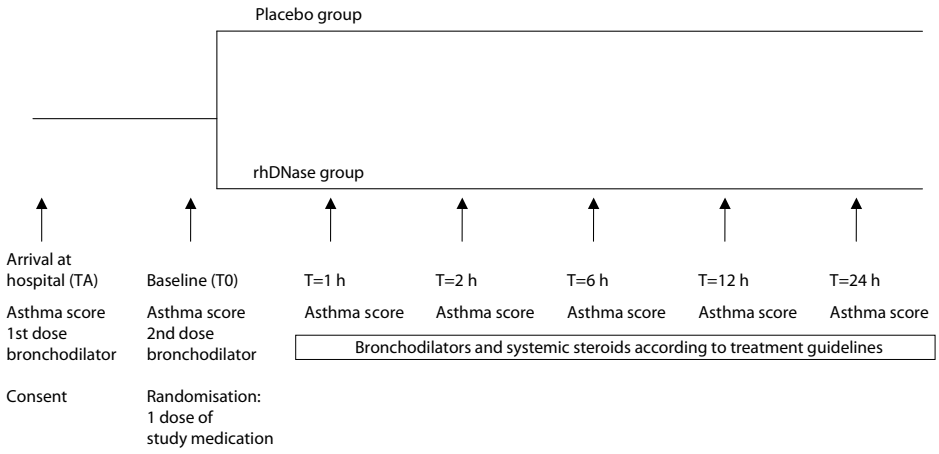
* The overall asthma score (scale 5–15 points) was calculated by adding the scores for each of the following five variables: respiratory rate, haemoglobin saturation, auscultation, retractions and dyspnoea.[14]

parental consent, patients were randomly assigned to receive a single nebulization of 5 mg rhDNase (5 ml solution of 1 mg/ml rhDNase; Roche, Basel, Switzerland) or 5 mg placebo (5 ml sodium chloride 0.9%) following the second nebulization of bronchodilators (Figure 4.1). We opted for a dose of 5 mg in anticipation of the expected suboptimal lung deposition in young children with airways obstruction due to asthma. Study medication was prepared by the hospital pharmacists and had identical appearance and aroma. The vials with study medication were stored in a refrigerator located in the emergency department.

Study medication was administered with the use of a jet nebulizer using a mouthpiece when possible or through a firmly applied facemask at a constant oxygen supply rate of 6–8 l/min from a wall outlet. The same nebulizing equipment (Pari LC Star, Pari GmbH, Germany) was used in all participants.

Randomization was carried out in the hospital pharmacies of the participating hospitals using a random table sample with blocks of four numbers prepared by the study statistician. Throughout the study, physicians, nurses, parents and the trial coordinator remained unaware of the treatment assignment.

Figure 4.1. Study design



The dosing interval of nebulized bronchodilators was determined by the attending physician based on symptom severity and clinical improvement rate. Systemic corticosteroids (1 mg/kg prednisolone as a starting dose and subsequently 1–2 mg/kg/day for 5–7 days to a maximal dose of 60 mg/day) were given after the second dose of bronchodilators according to Dutch national asthma guidelines.

On the child's arrival at the emergency department (TA) the attending physician recorded the clinical history (including previous admissions for asthma, duration and possible triggers of the current symptoms) and medication use. Vital signs, the need for supplemental oxygen and the asthma score were also assessed at TA and again just before nebulization of the second dose of bronchodilators that was followed by the single dose of study medication (T0). The asthma score was subsequently assessed at 1±0.25 h (T1), 2 ±0.5 h (T2), 6±1 h (T6), 12±2 h (T12) and 24±2 h (T24) after nebulization of the study medication (Figure 4.1). Supplemental oxygen was started when hemoglobin saturation was consistently lower than 93% and was stopped when saturation was consistently above 92%. The total number of nebulizer treatments in the first 24 h after the study medication, time until discharge and duration of oxygen supplementation were recorded.

The decision to admit or discharge the child was up to the discretion of the treating physician. If the child was discharged home from the emergency department or within 24 h after admission, the researcher reported 3–5 days later whether any subsequent visits had been made to a medical facility within 72 h after the initial presentation.

Efficacy end points

The primary outcome measure was the asthma score 1 h after the study medication. We used the asthma score developed by Qureshi and colleagues[14] in which respiratory rate, hemoglobin saturation, auscultatory findings, retractions and dyspnea are scored on a 3-point scale, yielding a total score ranging from 5 (mild) to 15 (severe) (Table 4.1). A previous study showed good inter-rater reliability of this asthma score (Pearson correlation statistic 0.92).[14] The asthma score had been introduced as a clinical tool in all participating centers before the start of the study, so all participating physicians were experienced in using the score.

The secondary outcome measures were the mean asthma scores at 2, 6, 12 and 24 h after the study medication, need for hospital admission, duration of admission, duration of supplemental oxygen and the number of nebulizer treatments in the first 24 h.

Estimate of sample size

In a pilot study of 26 children the mean (SD) asthma score decreased 0.8 (1.4) points between the time points T0 (before the second dose of bronchodilators) and T1 (1 h after the second dose of bronchodilators). To demonstrate an additional decrease of 0.8 points at T1 at a 5% significance level for a two-sided test with 80% power would require 100 patients (50 children in each group).

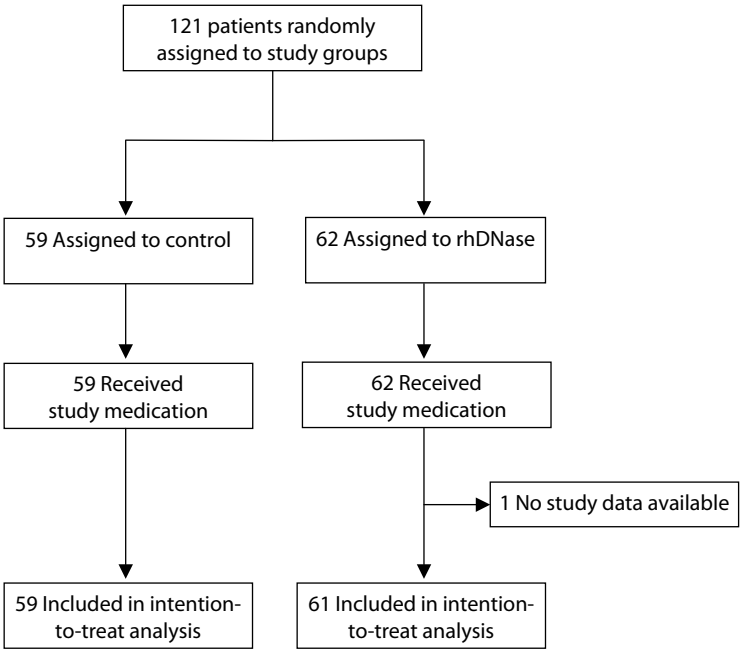
Statistical analysis

Data were analyzed on an intention-to-treat basis. Differences between baseline group characteristics and secondary outcome measures were assessed by χ^2 or Fisher exact tests and the Mann-Whitney test, as appropriate. Main analyses of between-group comparisons regarding asthma score changes were performed by repeated measures of analysis of variance (RmANOVA) with baseline (T0) asthma score, age, sex and study centre as covariates. In calculating mean values of the asthma score, the individual asthma score after discharge was arbitrarily set at 5 points. Linear interpolation of the asthma score was used if scores had been assessed outside the prespecified time range. Linear interpolation was also used when the item "dyspnea" could not be assessed accurately because the child was asleep at the time of observation. The analysis was performed with SPSS software Version 11.5 and SAS PROC MIXED. For all the analyses, a two-tailed p value of <0.05 was considered to indicate statistical significance.

Results

A total of 121 children were enrolled and randomly assigned to treatment groups: 62 to rhDNase and 59 to placebo (Figure 4.2). There was no difference in the demographic and baseline clinical characteristics of the two groups (Table 4.2).

Figure 4.2. Enrolment, random assignment, follow-up, and analysis



All children were treated with a dose of nebulized bronchodilators on arrival in the emergency department. Overall, the asthma score decreased after this first nebulizer treatment by a mean of 1.55 (95% CI 1.32 to 1.79) points (Figure 4.3). The study medication in the rhDNase group was given a median of 1.3 h (interquartile range (IQR) 1.0–2.0) after arrival and in the placebo group after 1.3 h (IQR 1.0–1.8) ($p = 0.95$).

Primary end point

Both groups showed a similar improvement in the asthma score during the first 24 h (Figure 4.3). At baseline, the mean asthma score was 10.2 in the rhDNase-treated group and 10.4 in the placebo group. One hour after nebulization of the study medication the asthma score in the rhDNase group showed an adjusted mean decrease of 1.0 (95% CI 0.5 to 1.6) points from

Table 4.2. Baseline characteristics of the children on arrival at the emergency department

Characteristic	rhDNase (N = 61)*	Placebo (N = 59)
Sex (M/F)	40/21	37/22
Age (years)	4.4 (2.0–16.3)	4.5 (2.1–15.4)
Duration of symptoms (n (%) of patients)		
< 12 h	19 (31)	19 (32)
12–24 h	29 (48)	29 (49)
>24 h	13 (21)	11 (19)
Current asthma medication (n (%)of patients)		
No medication	17 (28)	11 (19)
Short-acting β_2 agonist only	19 (31)	25 (42)
Corticosteroid	24 (39)	22 (37)
Combination (steroid + long-acting β_2 agonist)	7 (11)	6 (10)
Leukotriene antagonist	3 (5)	1 (2)
Systemic corticosteroid	2 (3)	2 (3)
Asthma score (scale 5–15)		
Arrival at hospital (TA)	12 (8–15)	12 (8–15)
Baseline (T0)	10 (5–14)	10 (7–15)
Severity of asthma (n (%) of patients)		
TA:		
Severe (score 12–15)	37 (61)	33 (57)#
Moderate (score 8–11)	24 (39)	25 (43)#
T0:		
Severe (score 12–15)	18 (30)	17 (29)
Moderate (score 8–11)	38 (62)	38 (64)
Mild (score 5–7)	5 (8)	4 (7)

Data expressed as median (range) unless otherwise specified.

TA, arrival at the emergency department; T0, before nebulization of the second dose of bronchodilators and the subsequent single dose of study medication.

* Study data were not available for one of 62 patients in the rhDNase group.

The asthma score at arrival (TA) was missing for one patient in the placebo group.

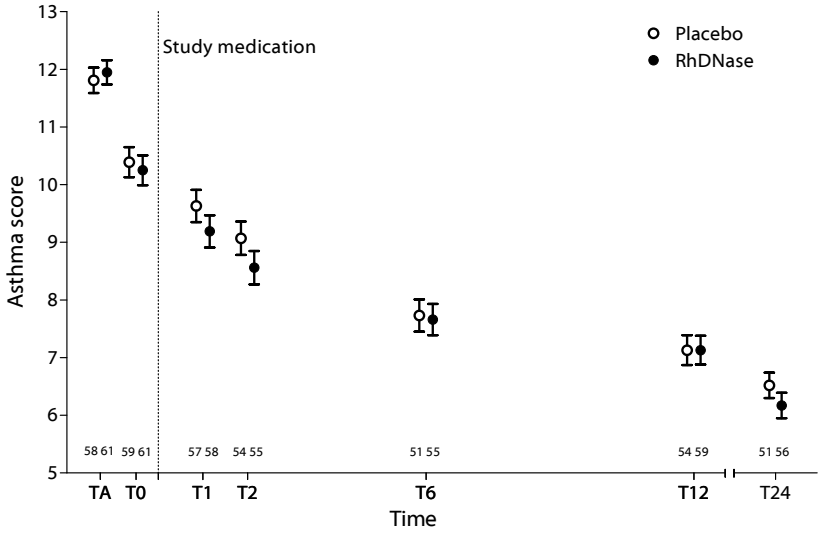
baseline compared with 0.7 (95% CI 0.3 to 1.2) points in the placebo group (mean difference 0.4 (95% CI –0.2 to 1.0) points; p = 0.23).

Overall, compared with baseline, the asthma score 1 h after the study medication had improved in 72 children (37 in the rhDNase group, 35 in the placebo group), had not changed in 21 (12 rhDNase, 9 placebo) and had worsened in 27 children (12 rhDNase, 15 placebo); p = 0.68.

Repeated measures analysis of variance showed no significant difference between the groups in the asthma score over the whole period of 24 h: the adjusted mean decrease was 4.1 (95% CI 3.6 to 4.6) points in the rhDNase group and 3.9 (95% CI 3.3 to 4.5) points in the placebo group (mean difference 0.2 (95% CI –0.3 to 0.7) points; p = 0.40).

The item "dyspnea" of the asthma score could not be assessed accurately in some children who were asleep at the time of the observation. In these cases, linear interpolation of the item "dyspnea" was used in order to obtain a total asthma score. An analysis in which the interpolated asthma scores of sleeping children were not included showed similar results (data not shown).

Figure 4.3. Mean asthma scores during study



TA, arrival at the emergency department; T0, before the second dose of bronchodilators and the subsequent single dose of study medication. Study medication was administered a median of 0.5 h after the assessment of the asthma score at T0. The asthma scores at T1, T2, T6, T12 and T24 were assessed at 1, 2, 6, 12 and 24 h after nebulization of the study medication, respectively. Data given are ANOVA estimates (with standard errors). At all time points the number of patients in each treatment group for whom an asthma score was available is noted above the horizontal axis. p Values for differences between study arms at the various time points are all >0.20.

Subgroup analyses

There was no significant effect modification by baseline asthma score, age or the use of anti-inflammatory medication prior to the asthma attack. A separate analysis of the subgroup of children with a severe asthma score (≥12) at baseline (n = 35) also showed no significant dif-

ference in the asthma score over time between the rhDNase group and the placebo group (mean difference –0.1 (95% CI –1.3 to 1.1); p = 0.85).

Secondary end points

Need for hospital admission

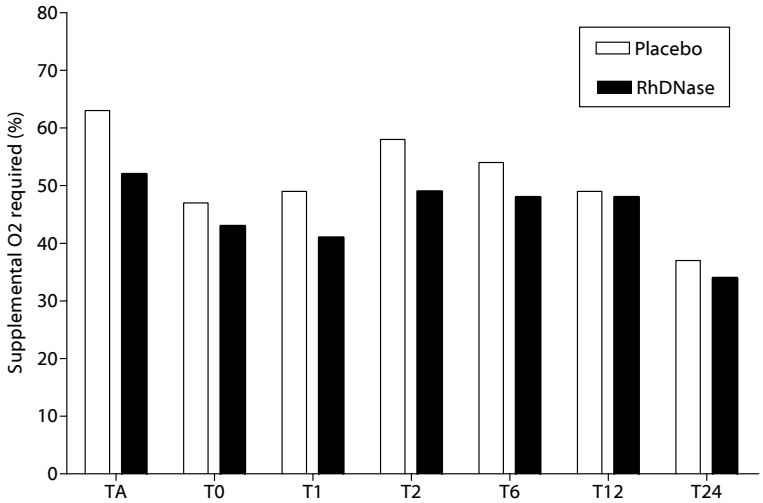
Most of the children (88%) were admitted to hospital. Only 14 children (6 in the rhDNase group, 8 in the placebo group) were discharged home from the emergency department. Four of the admitted children (4%) required intensive care (2 in the rhDNase group, 2 in the placebo group).

Thirty-one children were discharged within 24 h of study entry (14 in the rhDNase group, 17 in the placebo group). Three of those in the placebo group were readmitted to hospital within 72 h of discharge because symptoms had worsened (1 child after 1 day, 2 children within 1.5 h of discharge) compared with none in the rhDNase group (p = 0.23).

Time until discharge

The time until discharge did not differ between the rhDNase group and the placebo group (geometric mean (SE) 36.9 (1.2) vs. 33.9 (1.2) h; mean difference 0.92 (95% CI 0.55 to1.54), p = 0.75).

Figure 4.4. Need for supplemental oxygen during the first 24 h



Patients (% of total) requiring supplemental oxygen to maintain a haemoglobin saturation of ≥93%. TA, arrival at the emergency department; T0, before nebulization of the second dose of bronchodilators and the subsequent single dose of study medication; T1, T2, T6, T12 and T24, 1, 2, 6, 12 and 24 h after nebulization of the study medication, respectively.

Duration of oxygen supplementation

The proportions of children requiring oxygen supplementation to maintain a hemoglobin saturation ≥93% were similar in both groups over time (Figure 4.4). The geometric mean (SE) time of oxygen supplementation did not differ; for both groups it was 28.3 (1.2) h (p = 0.99).

Co-interventions

Overall, there was no difference between the two groups in the number of treatments with nebulized bronchodilators given in the first 24 h during the hospital stay. Children in the rhDNase group received a median number of 7.0 (IQR 5.5–11.0) nebulizer treatments compared with 8.0 (IQR 6.0–10.0) in the placebo group (p = 0.81). In the subgroup of children who were discharged within 24 h (n = 30), the number of nebulizer treatments did not differ between the rhDNase and placebo group (2.0 (IQR 0.0–5.3) and 2.5 (IQR 0.0–7.0), respectively, p = 0.40).

Prednisolone was administered to 90% of all children (55 of 59 in the placebo group and 53 of 61 in the rhDNase group). Twelve patients did not receive a course of systemic steroids (5 were discharged home from the emergency department after their symptoms had resolved following the dose of study medication).

Safety data

One child had a transient desaturation with an increase in dyspnea and tachypnea directly after the initiation of the nebulization with rhDNase, which resolved quickly after the nebulization was stopped. Hoarseness was reported in two children (one in each group).

Asthma scores for 27 children were higher (worsened) 1 h after nebulization of the study medication compared with baseline (12 children in the rhDNase group vs. 15 in the placebo group, p = 0.47).

Discussion

We report the first randomized double-blind controlled trial of nebulized rhDNase in children (aged 2–18 years) with an acute asthma exacerbation. The findings show no evidence to suggest that nebulization with the mucolytic rhDNase alleviates symptoms in children brought to the emergency room for moderate to severe acute asthma. We thus must reject our hypothesis that rhDNase is an effective additional treatment for children with acute asthma. This was based on the important role of mucus plugs in the pathophysiology of acute asthma,[2,3] and the finding that the DNA content is increased in the mucus of patients with acute asthma.[6]

To date, only case reports have suggested a benefit from rhDNase in children with status asthmaticus unresponsive to conventional treatment,[10-13] and in children with acute severe asthma treated at the emergency department.[11]Intervention with rhDNase, administered endotracheally or by means of a bronchoscope, was safe and improved ventilator settings[12,13] and arterial blood gas values[13] and resolved atelectasis[10,12] in children receiving intensive care. Nebulization of rhDNase in three children brought to the emergency department improved lung function parameters, the effectiveness of coughing and resolved atelectasis.[11]

There may be several explanations for the lack of effect of rhDNase in our study. First, the children might have had relatively mild disease with too little mucus plugging for rhDNase to be effective. Indeed, although all selected children had a moderate to severe asthma exacerbation and required at least two doses of bronchodilators, only four children required intensive care treatment. However, a subgroup analysis of children with an asthma score of at least 12 points after their first bronchodilator dose also could not demonstrate an effect of rhDNase. Because this analysis is underpowered (n = 35), definite conclusions about the effect of rhDNase in severe acute asthma cannot be drawn. We cannot exclude the possibility that rhDNase might have been effective in children with a more severe asthma exacerbation and/or atelectasis, or in those requiring admission to the intensive care unit. A separate study is needed to answer this question. In earlier case reports rhDNase was administered to children with a severe asthma exacerbation who also had atelectasis. We had no information about the presence or severity of atelectasis in our population because it was not considered necessary or ethical to perform two chest radiographs during treatment at the emergency department.

A second explanation might be that the amount of DNA present in the mucus was too low for rhDNase to be effective. The average DNA content of mucus in patients with stable asthma is higher than that in healthy controls (7.1 vs. 3.6 µg/ml).[15] Even higher levels were found in patients with an asthma exacerbation (0.5 mg/ml).[6] The DNA content of mucus in patients with asthma is much lower, however, than in those with cystic fibrosis (3–14 mg/ml)[16] in whom the beneficial effects of rhDNase have clearly been documented.[9]

A third explanation could be suboptimal lung deposition of rhDNase in children with bronchial obstruction, resulting in deposition of rhDNase mainly in the more central airways[17] and not reaching the peripheral airways. To compensate for suboptimal deposition, patients received a dose of 5 mg (twice the dose used as maintenance treatment in patients with cystic fibrosis). Arguably, it might have been more effective to administer the study medication immediately on arrival in the emergency department or following the first dose of bronchodilators rather than after the second dose, or to use repeated nebulizations of study medication instead of one. However, we think that other timing or dosing frequency would not change the results, since neither the symptom scores at any time points nor any of the secondary end points

differed between the groups. Moreover, a single dose of rhDNase has an effect lasting many hours.[18]

Finally, as diagnosing asthma in preschool children is difficult, part of our study population might have had "exclusive viral wheeze" and not asthma. The exact role of mucus plugging in the pathophysiology of airway obstruction in children with "exclusive viral wheeze" has not been investigated to our knowledge. Our study focused on current emergency clinical practice which does not take into account the child's asthma phenotype.

Most of the participating hospitals routinely admitted children with an acute asthma exacerbation who required at least two doses of nebulized bronchodilators. It was not therefore meaningful to assess the effect of rhDNase on the admission rate. Even if a longer period of observation in the emergency room had been possible, we probably would not have found a positive effect of rhDNase on the admission rate since the decision to admit a child is based on the symptoms and we found no significant effect of rhDNase on the asthma score over time.

In this study the administration of rhDNase in acute asthma appeared to be safe. It was stopped in one case of temporary desaturation with an increase in dyspnea and tachypnea directly after the start of nebulization. The mechanism of this desaturation is unclear, but we speculate that it might have been caused by the child's inability to clear mucus effectively after quick liquefaction by rhDNase.

Because our study population reflects the population of children with acute asthma treated in the emergency rooms of district and tertiary care hospitals, our results can be generalized to the large majority of children with a moderate to severe acute asthma exacerbation. Further studies on the effect of rhDNase in children with acute asthma requiring intensive care or with large atelectasis are still needed.

In conclusion, our study shows that a single dose of nebulized rhDNase in addition to nebulized bronchodilators and systemic steroids is not effective in the treatment of children with moderate to severe acute asthma.

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Chapter 5

Tracheomalacia and bronchomalacia in children
Incidence and patient characteristics

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Summary

Objective: Congenital airway malacia is one of the few causes of irreversible airways obstruction in children, but the incidence in the general population is unknown. Severe airway malacia or malacia associated with specific syndromes is usually recognized and diagnosed early in infancy, but information about clinical features of children with primary malacia, often diagnosed only later in childhood, is scarce.

Methods: We analyzed all flexible bronchoscopies performed between 1997 and 2004 in the Sophia Children's Hospital, summarized clinical features of children with primary airway malacia, estimated the incidence of primary airway malacia, and calculated the predictive value of a clinical diagnosis of airway malacia by pediatric pulmonologists.

Results: In a total of 512 bronchoscopies, airway malacia was diagnosed in 160 children (94 males) at a median age of 4.0 years (range, 0 to 17 years). Airway malacia was classified as primary in 136 children and secondary in 24 children. The incidence of primary airway malacia was estimated to be at least 1 in 2,100. When pediatric pulmonologists expected to find airway malacia (based on symptoms, history, and lung function) prior to bronchoscopy, this was correct in 74% of the cases. In 52% of the airway malacia diagnoses, the diagnosis was not suspected prior to bronchoscopy. Presenting clinical features of children with airway malacia were variable and atypical, showing considerable overlap with features of allergic asthma. Peak expiratory flow was more reduced than FEV_1.

Conclusion: Primary airway malacia is not rare in the general population, with an estimated incidence of at least 1 in 2,100 children. Airway malacia is difficult to recognize based on clinical features that show overlap with those of more common pulmonary diseases. We recommend bronchoscopy in patients with impaired exercise tolerance, recurrent lower airways infection, and therapy-resistant, irreversible, and/or atypical asthma to rule out airway malacia.

Introduction

Congenital malacia of the large airways is one of the few causes of irreversible airways obstruction in children, with symptoms varying from recurrent wheeze and recurrent lower airways infections to severe dyspnea and respiratory insufficiency.[1-4] Severe cases are usually detected in the neonatal period when children present with ventilator dependency or acute severe obstructive episodes with cyanosis. Airway malacia associated with specific syndromes or congenital heart disease may be detected in early life because of selective screening.[3,5,6]

Children with mild airway malacia often present after the neonatal period with nonspecific symptoms such as rattling, wheeze, stridor, exercise intolerance, cough, recurrent lower airway infections, and airways obstruction.[3,7] Because of the similarity in symptoms, the poor response to standard asthma treatment, and the irreversible nature of the airways obstruction, isolated airway malacia may be misdiagnosed for severe-persistent or therapy-resistant asthma.[8-10] Consequently, if airway malacia remains undetected in childhood, patients may be treated unnecessarily with high doses of inhaled corticosteroids into adulthood for many years, and may be undertreated for recurrent lower airways infections, with the risk of additional damage to the lung and airways. Based on percentages between 23% and 57% of observed airway malacia in pediatric bronchoscopic series, various authors[11-14] concluded that malacia of the central airways is more prevalent than previously thought, but general incidence data are lacking. This is partly due to the lack of an objective definition or classification of airway malacia[3,7,12,15] and the lack of noninvasive diagnostic tests.

Previous series[12,16,17] about children with airway malacia focused mainly on the association with other conditions and on airway malacia diagnosed in young infants. However, less is known about clinical features in children with primary airway malacia diagnosed during childhood. We therefore focused on children who underwent bronchoscopy after referral to our outpatient clinic with persistent pulmonary symptoms, but who were otherwise healthy. The aims of this study were to estimate the incidence of primary airway malacia in the general population, to estimate the predictive value of a clinical diagnosis of airway malacia by pediatric pulmonologists, and to characterize the presenting symptoms and findings in patients diagnosed with primary airway malacia.

Materials and Methods

The study protocol fulfilled the ethical standards of the Dutch Pediatric Society. Because of the retrospective and noninterventional nature of our study, ethical approval and patient consent were judged unnecessary.

Study subjects

In a descriptive, retrospective study the indication and outcome of all flexible bronchoscopies performed by pediatric pulmonologists in our hospital between 1997 and 2004 were evaluated. The Erasmus MC-Sophia Children's Hospital is a tertiary referral center for the southwest of the Netherlands with an adherence population of 4.4 million. Flexible pediatric bronchoscopies within this region are only carried out in this referral hospital.

Definitions

Primary airway malacia was defined as airway malacia in otherwise normal infants.[7] Secondary airway malacia was defined as airway malacia secondary to esophageal atresia, VATER/VACTERL association (condition with vertebral anomalies, anal atresia, congenital heart disease, tracheoesophageal fistula or esophageal atresia, renourinary anomalies, or radial limb defects), vascular or other external compression of the airways, or specific syndromes.

Incidence and clinical features

The incidence of primary airway malacia was calculated assuming that malacia is congenitally present and not acquired, and that the annual detection rate of isolated airway malacia was fairly constant and thus reflecting the annual incidence in the population. In outpatients who underwent bronchoscopy because of diagnostic or therapeutic workup, we calculated the predictive value of a clinical diagnosis of airway malacia made by pediatric pulmonologists prior to bronchoscopy. In otherwise healthy patients with a diagnosis of primary airway malacia, we conducted a detailed review of the clinical features, including presenting symptoms and signs, lung function data, bacterial cultures and the lipid-laden macrophage index (LLMI)—scored according to Corwin and Irwin[18]—from the BAL fluid. Clinical features of patients with a concurrent medical condition such as cystic fibrosis (CF) or an immunologic disorder were not analyzed because we wanted to be informed about symptoms that could be attributed to the airway malacia.

Data from in-hospital bronchoscopies were excluded from these analyses, because we wanted to gain insight in the clinical features of patients who underwent bronchoscopy during a diagnostic workup at the outpatient clinic; in-hospital bronchoscopies are often performed in children with different pathologic conditions.

Bronchoscopic examination

Bronchoscopies were carried out by a pediatric pulmonologist as part of diagnostic workup in routine patient care, using a flexible bronchoscope with external diameter 3.5 mm or 5.5 mm (Olympus; Tokyo, Japan) during general anesthesia. Airway malacia was diagnosed by visual inspection of airway shape and dynamics during spontaneous breathing without positive end-expiratory pressure, or during coughing. Malacia was defined as collapse of at least 50% of the airway lumen, during expiration, cough or spontaneous breathing, or a ratio of cartilage to membranous wall area of < 3:1.[7] Bronchoscopies were recorded on videotape and reevaluated by at least one independent experienced pediatric pulmonologist. Only when consensus between observers existed on the diagnosis airway malacia, children were labeled as such. BAL was carried out according to recommendations,[19] when lower airway infection and/or aspiration were expected on clinical grounds. Antibiotic treatment was discontinued 48 h in advance of bronchoscopy.

Statistics

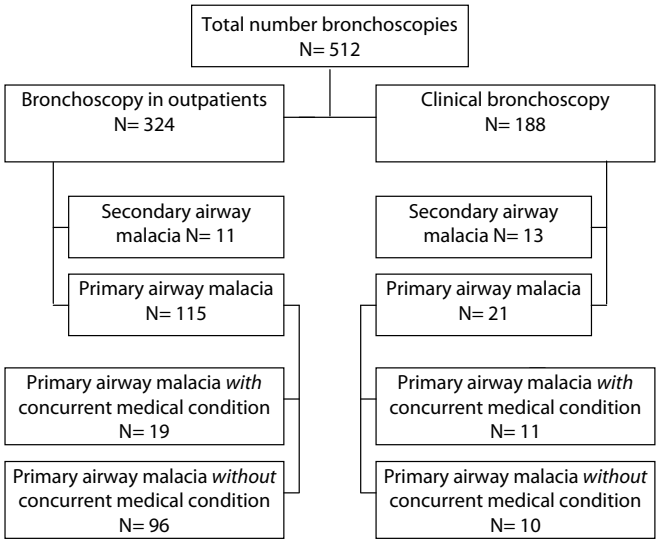
All data are summarized using descriptive statistics.

Results

Between 1997 and 2004, a total of 512 bronchoscopies were performed (Figure 5.1). In 324 children (193 male) with a median (range) age of 4.4 (0 to 18) years referred to our outpatient clinic, bronchoscopy was performed as part of a diagnostic workup. In 188 children, bronchoscopy was performed during a hospital admission. The indications for bronchoscopy are summarized in Table 5.1.

Primary airway malacia was diagnosed in 136 children (80 male) with a median (range) age of 4.3 (0 to 17) years (male/female ratio, 1.4:1). Tracheomalacia was present in 63 children (46%), tracheobronchomalacia in 49 children (36%), and bronchomalacia in 24 children (18%). The bronchomalacia was located on the left in 33 children, on the right in 21 children, and on both sides in 19 children. Secondary airway malacia was found in 24 children; airway malacia was secondary to esophageal atresia or VACTERL association (n = 11), a congenital heart defect with compression of the bronchial tree (n = 9), a vascular ring (n = 2), or a bronchogenic cyst with compression (n = 2).

Figure 5.1. Overview of bronchoscopies



Incidence estimate

Based on the annual birth rate of 50,000 in the region of adherence, assuming primary airway malacia as congenital, and a constant detection rate of 17 cases per year (136 cases in 7 years), it was estimated that the incidence of primary airway malacia was about 1 in every 2,600 newborns. An incidence estimate based on bronchoscopic findings from the last 4 years of the survey (from 2001 to 2004) was 1 in 2,100 children (95 cases in 4 years).

Predictive value of a clinically expected diagnosis of airway malacia prior to bronchoscopy

In the 324 out-of-clinic patients, airway malacia was found in 126 patients (115 cases of primary airway malacia). Prior to bronchoscopy, pediatric pulmonologists expected a malacia, based on history, physical examination, and/or lung function in 82 patients of whom 61 actually had malacia (positive predictive value, 74%). In 65 of 126 patients, airway malacia was not suspected prior to bronchoscopy (false-negative rate of 52%).

Primary airway malacia: clinical features

From the 115 patients with primary airway malacia diagnosed at the outpatient clinic, 96 patients (58 male) with a median age of 5.2 years (range, 0 to 16 years) had no concurrent medical conditions (isolated malacia). The presenting symptoms of those children are stated

Table 5.1. Indications for bronchoscopy*

Indications	Outpatients; total group	Outpatients; primary airway malacia without concurrent medical condition	Clinic patients; total group	Clinic patients; primary airway malacia without concurrent medical condition
Patients, No.	324	96	188	10
Total indications, No.	497	174	252	16
(Recurrent) lower airways infection	112 (35)	36 (38)		
Suspected airway malacia	82 (25)	54 (56)	5 (3)	1 (10)
Other suspected anatomical abnormality	38 (12)	14 (15)	8 (4)	1 (10)
(Recurrent) atelectasis or middle lobe syndrome	36 (11)	5 (5)	37 (20)	3 (33)
Persistent coughing	35 (11)	17 (18)	1 (1)	
Dyspnea	30 (9)	8 (8)	10 (5)	
Suspected aspiration	25 (8)	11 (12)	10 (5)	
Therapy resistant/atypical asthma	25 (8)	10 (10)	5 (3)	1 (10)
Stridor or persistent wheeze	25 (8)	7 (7)	2 (1)	
Acute life threatening event	3 (1)	2 (2)	4 (2)	3 (33)
Difficulty in weaning from mechanical ventilation			9 (5)	4 (40)
Difficulty in mechanical ventilation during ECMO			14 (7)	2 (20)
Infection in immunocompromised host	7 (2)		52 (28)	
Other (eg, CF, bronchiectasis, hemoptysis, foreign body)	79 (24)	12 (13)	95 (50)	

* For each bronchoscopy, one to three indications could be noted. Results presented as No. (%) unless otherwise indicated.

in Table 5.2 . Nineteen patients with primary airway malacia also had a concurrent medical condition (e.g., CF, immunodeficiency, Down syndrome), probably influencing their presenting clinical features, and were therefore not analyzed.

At time of referral to our outpatient clinic, 70 of 96 patients (73%) with primary airway malacia were using asthma medication. The pediatric pulmonologist diagnosed asthma or probable asthma in 30 patients (31%) with airway malacia.

Lung function was performed by 45 children (Table 5.3). Mean peak expiratory flow (PEF), FEV_1, and FEV_1/FVC were below predicted values. In most patients, lung function values did

Table 5.2. Clinical features in 96 outpatients with primary airway malacia and without a concurrent medical condition

Symptoms from patient history and signs at physical examination	Patients, No. (%)
Cough	
In general	80 (83)
Night time cough	40 (42)
Productive cough	58 (60)
Exercise induced cough	34 (35)
Typical cough (barking, seal-like)	41 (43)
(Recurrent) lower airways infection	60 (63)
Dyspnoea/ shortness of breath	57 (59)
Recurrent wheeze	47 (49)
Recurrent rattling	46 (48)
Reduced exercise tolerance	34 (35)
Symptoms of reflux	25 (26)
Retractions	18 (19)
Stridor	17 (18)
Funnel chest	10 (10)

not improve with bronchodilatation. A typical flow-volume curve of a child with tracheomalacia is presented in Figure 5.2 .

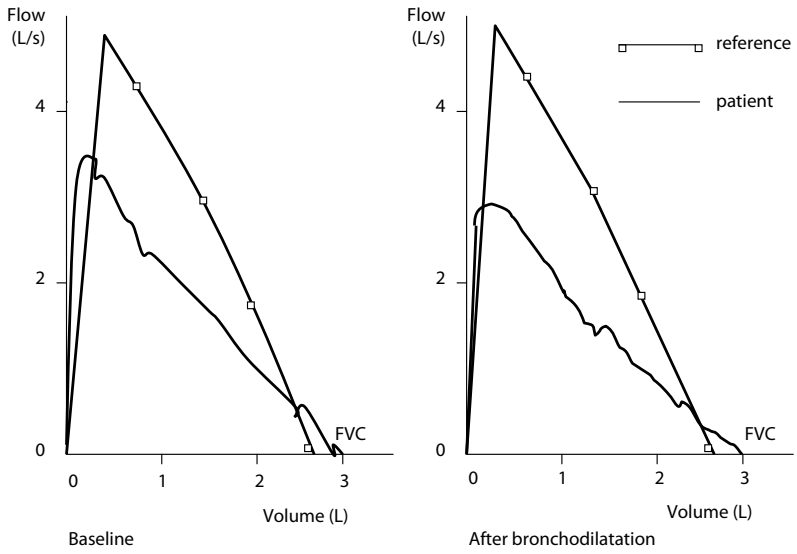
In 86 of 96 children with an airway malacia without a concurrent medical condition, BAL fluid was obtained. In 67 children, at least one pathogen was cultured. Haemophilus influenza B, Streptococcus pneumoniae, and Staphylococcus aureus were cultured in 34%, 30%, and 22% of the BALs, respectively. In 75 children, LLMI was determined in the BAL fluid. Median LLMI was 18 (range, 0 to 253). The LLMI did not correlate with age. In 14 children (20%), the LLMI was increased and thus indicative of aspiration.[18]

Table 5.3. Lung function data (percentage of predicted) from outpatients with primary airway malacia and without a concurrent medical condition*

Variables	Baseline (n = 45)	Before bronchodilatation (n = 35)	After bronchodilatation (n = 35)
FVC	99.3 (15.9)	98.3 (15.2)	100.1 (15.3)
FEV_1	91.5 (19.9)	88.7 (17.4)	92.5 (16.5)
FEV_1/FVC	87.7 (14.2)	85.6 (13.9)	88.0 (14.0)
PEF	74.7 (19.4)	74.7 (18.7)	76.8 (17.9)
MEF_{25}	62.2 (31.3)	59.0 (30.3)	66.9 (31.7)

*Data are presented as mean (SD). MEF_{25} = mean expiratory flow at 25% of vital capacity remaining in the lung.

Figure 5.2. A typical flow-volume curve of a child with tracheomalacia: a decreased PEF, with slight deterioration after bronchodilatation



Discussion

Based on retrospective bronchoscopic data from a 7-year period, we estimated the incidence of primary airway malacia in the general population, assessed the positive predictive value of a clinical diagnosis of malacia prior to bronchoscopy by pediatric pulmonologists, and evaluated the clinical features of children with primary airway malacia. Primary airway malacia was diagnosed in 136 patients in 7 years, implying an incidence of at least 1 in 2,600 newborns. When analyzing bronchoscopies from the last 4 years of the survey, the estimated incidence of primary airway malacia was even higher: 1 in 2,100 newborns. This increased incidence estimate probably does not reflect a true rise in incidence, but may reflect a greater awareness of airway malacia by pediatric pulmonologists, and a lower threshold to perform bronchoscopy in children with atypical respiratory problems. Moreover, our incidence estimate is a conservative one because of selection bias. Bronchoscopy was only conducted in referred patients, and it is likely that many patients with airway malacia and minor symptoms were not referred.

Other series[1,11-14] also suggest that airway malacia is a relatively common disorder, but incidence estimates are not provided and differences in patient selection make it difficult to compare with these series. Moreover, a uniformly accepted definition of airway malacia is lacking and the bronchoscopic diagnosis of airway malacia is based on subjective evaluation by the bronchoscopist, making comparisons between studies difficult.

When pediatric pulmonologists expected to find a malacia prior to bronchoscopy, this was correct in three fourths of the patients. However, in one half of the patients with airway malacia, the diagnosis was not suspected prior to bronchoscopy. Hence, experienced specialized clinicians have difficulty in recognizing primary airway malacia based on clinical features. Furthermore, the most frequent presenting symptoms were cough, dyspnea, recurrent lower airways infection, recurrent rattling and wheeze, and reduced exercise tolerance, showing considerable overlap with features of asthma and chronic bronchitis. These factors imply that children with isolated airway malacia are easily misdiagnosed and treated for the wrong disease, such as asthma. This is supported by the fact that a large majority of the patients with airway malacia were using asthma medication at time of referral, while a pediatric pulmonologist diagnosed asthma in only 40% of these children. A typical, barking, or seal-like cough is often described as an indicative symptom in airway malacia[2,4,16] but was only mentioned in less than one half of the patients with isolated airway malacia.

In the majority of patients, we obtained positive bacterial culture findings in the BAL fluid, consistent with the clinical impression of chronic bacterial bronchitis secondary to the expected impaired mucociliary clearance in children with airway malacia.[2,13]

The LLMI was routinely assessed in the BAL fluid as an additional step in the diagnostic workup for aspirations. The large majority of the patients with isolated airway malacia had no evidence of aspirations based on the LLMI. These data are not consistent with reports[3,11,20] that suggest that aspiration is a frequent complication of airway malacia. The older age at diagnosis and the exclusion of patients with associated syndromes and medical conditions (who are at greater risk of aspiration) in our series may explain this difference.

Lung function measurements in patients with isolated airway malacia demonstrated airways obstruction not improving after bronchodilatation, with a considerable reduction of PEF, being much more affected than FEV_1. The reduced PEF and FEV_1 are compatible with the increased central airway collapsibility during forced expiration.

The limitation of this study is the retrospective nature of the survey that could have affected the accuracy of the data collection of clinical features from the medical records. However, at our department a standardized medical record is kept, in which presenting signs and symptoms are collected uniformly. Comparisons with other retrospective studies on airway malacia may be difficult because of differences in population and diagnostic protocols, definition of airway malacia, and application and availability of flexible bronchoscopy.

Tracheomalacia and bronchomalacia were the topic of an excellent and thorough recent review in CHEST.[1] The present study provides some additional data and aspects of this disorder

that are clinically relevant. According to the review article,[1] mild-to-moderate airway malacia is a self-limiting disease and most infants outgrow the condition by the age of 2 years. In addition, it was stated that the delay from the onset of symptoms to diagnosis was not > 144 weeks.[1] Our study demonstrates that a substantial number of cases are diagnosed much later in childhood. Possibly, this difference between the studies is explained by differences in clinical severity of malacia. Nevertheless, our study suggests that there are a considerable number of children with airway malacia, in whom the diagnosis is made relatively late in life or not at all, and that airway malacia can be very difficult to recognize.

High-speed multidetector CT and specialized imaging provide novel diagnostic possibilities to diagnose airway malacia noninvasively.[21] This provides a diagnostic opportunity for children in whom invasive bronchoscopy cannot be performed. Nevertheless, in the majority of young children, bronchoscopy will be mandatory to diagnose airway malacia, because it is difficult to image the airway during dynamic maneuvers in young children due to their inability to perform breathing instructions.

What is the clinical relevance of this study? It suggests that the incidence of primary airway malacia is at least 1 in 2,100. Furthermore, it seems likely that a considerable proportion of pediatric (and probably adult) patients with atypical or recurrent respiratory symptoms, irreversible chronic airways obstruction labeled COPD or asthma,[10,22] or with persistent troublesome cough[23,24] do not have isolated airway malacia diagnosed. A correct diagnosis of airway malacia is important for several reasons: (1) the therapeutic approach is fundamentally different, being focused on treatment or prevention of lower airways infections and improvement of mucociliary clearance, sometimes even including surgery[2,3,25,26]; (2) the patient and his/her family benefit from a sound explanation for the exercise intolerance (caused by the dynamic airway collapse) that may be present lifelong; (3) for both pediatrician and chest physician, the irreversibility of airways obstruction is sufficiently explained and will lead to less prescription of asthma drugs that are not necessary in most cases and may have side effects. Children with both asthma and airway malacia pose a therapeutic challenge. Lung deposition of inhaled corticosteroids may be negatively affected by severe airways obstruction and anatomic abnormalities, resulting in less efficacy of asthma treatment and worse long-term outcome, as was found in asthmatic patients who start off with lower lung function in childhood.[22]

We conclude that primary airway malacia is a common disorder in the general population. Many of our patients had bacterial lower airways infections but no indications of aspirations based on the bronchoscopic findings. The most striking lung function abnormality in our population was a decreased PEF, which appeared more affected than FEV_1. Presenting symptoms of isolated airway malacia are atypical and also for pediatric pulmonologists difficult to recognize. As a correct diagnosis is important because of the therapeutic implications and per-

haps lifelong consequences, we recommend considering bronchoscopy in all patients with unexplained exercise intolerance, recurrent lower airways infection, and irreversible and/or atypical asthma.

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Chapter 6

Recombinant human DNase in children with airway malacia and lower respiratory tract infection

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Submitted

Abstract

Background: Children with airway malacia often have protracted courses of airway infections, because dynamic airway collapse during coughing results in impaired mucociliary clearance. The aim of this study was to determine the effect of the mucolytic drug recombinant human deoxyribonuclease (rhDNase) on the recovery of respiratory symptoms in children with airway malacia and lower respiratory tract infection.

Methods: In a randomized double-blind controlled clinical trial, 40 children with airway malacia and lower respiratory tract infection were randomly assigned to receive either 2.5 mg nebulized rhDNase or placebo twice daily for 2 weeks. The primary endpoint was the change in the cough diary score (scale 0-5) from baseline to the second week of treatment. Secondary endpoints were VAS symptom scores for cough, dyspnea, and difficulty in expectorating sputum, need for an antibiotic course, and lung function data (FVC, FEV_1, FEF_{75}, $Rint_{,e}$).

Results: There was no significant difference in the mean change in cough diary scores from baseline between the rhDNase group and the placebo group (mean difference for daytime 0.19 (95% CI -0.53 to 0.90); for nighttime 0.38 (95% CI -0.30 to 1.05). Proportions of patients requiring antibiotics, and the mean changes in symptom scores and lung function from baseline did not significantly differ between both groups.

Conclusion: Treatment with 2 weeks of nebulized rhDNase does not enhance recovery or reduce the need for antibiotics in children with airway malacia and lower respiratory tract infection.

Introduction

Malacia of the large airways is one of the causes of irreversible airways obstruction in children, with symptoms varying from recurrent wheeze and recurrent lower respiratory tract infections (LRTIs) to severe dyspnea and respiratory insufficiency.[1-5] Airway malacia is not rare in the general population, with an estimated incidence of at least one in 2,100 children.[1] Severe malacia, and malacia associated with or secondary to other congenital abnormalities (e.g. esophageal atresia), is usually detected in the neonatal period. Mild to moderately severe malacia is often diagnosed later in childhood. Patients usually present with recurrent cough, stridor, reduced exercise tolerance, and frequent LRTIs.[1,2] Moreover, children with malacia tend to have a tendency for delayed recovery from LRTIs in.[6] Current management of LRTIs in children with airway malacia is not evidence based, and includes liberal use of antibiotics and physiotherapy using positive expiratory pressure (PEP) devices.[1-3]

In airway malacia, there is a dynamic collapse of one or more central airways during forced expirations and coughing. This results in airway obstruction and impaired mucociliary clearance. It is likely that less mucociliary clearance negatively affects the resolution of a viral LRTI and increases the likelihood of a secondary bacterial infection.[3,4,7] The mucolytic recombinant human DNase (rhDNase) is effective in patients with cystic fibrosis,[8] and several publications suggest that nebulized rhDNase might also be effective in other childhood respiratory tract diseases with impaired mucociliary clearance, such as atelectasis,[9,10] severe acute asthma,[11] and primary ciliary dyskinesia.[12-14] We hypothesized that rhDNase could also be beneficial in children with malacia, and performed a randomized controlled trial to determine the effect of nebulized rhDNase on recovery of respiratory symptoms in children with airway malacia and LRTI.

Methods

Patients

Eligible subjects for this study were children aged 2-18 years with airway malacia and symptoms of a lower respiratory tract infection (LRTI). For lack of established criteria to diagnose airway malacia, we used the following criteria to diagnose airway malacia during bronchoscopy: ratio of cartilage to muscle less than 3:1,[15] and visible abnormal collapsibility during cough or quiet breathing in the absence of positive airway pressure. Flexible bronchoscopy was conducted during spontaneous breathing through a facemask. We defined LRTI as (an increase in) productive cough or dyspnea, and/or auscultatory abnormalities on physical examination, and/or increased bronchitic markings or consolidation on a chest radiograph.

We did not include children if an antibiotic course was deemed necessary by the attending pediatric pulmonologist, based on symptom severity and examination at initial presentation. Other exclusion criteria were chronic cardiopulmonary disease, such as bronchopulmonary dysplasia, cystic fibrosis, or primary ciliary dyskinesia, as well as esophageal atresia and neuromuscular disease. All these conditions will increase symptom severity and the course of LRTI.

Study design

We performed a prospective, double blind, parallel-group randomized study comparing the effect of inhaled rhDNase with placebo on respiratory symptoms, the need for antibiotics, and lung function in children aged 2-18 years with airway malacia and symptoms of LRTI. The study was carried out between September 2005 and March 2008 in the outpatient departments of a pediatric pulmonology department at a tertiary care clinic, and of a general hospital. The study was approved by the ethics review boards of the two centers and written parental informed consent was obtained for each child.

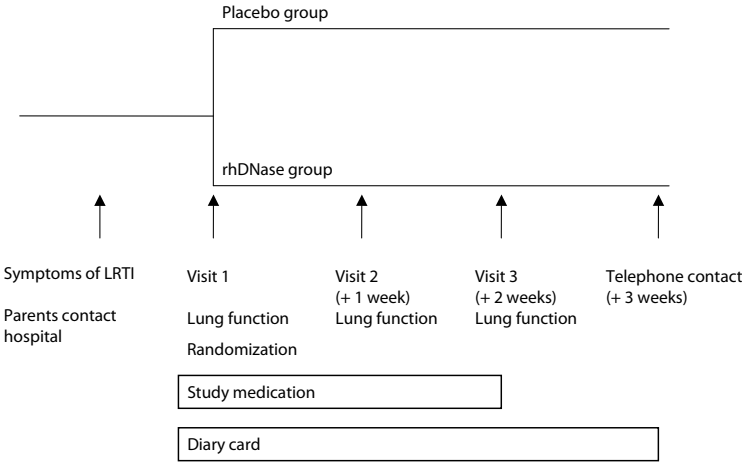
As evidence based therapies are not available, children with airway malacia at our outpatient department are treated according to an empirical treatment protocol that includes:

- use of a positive expiratory pressure (PEP)-device, aimed at improving mucociliary clearance, in children aged 5 years and older.
- liberal use of antibiotics for every severe or progressive LRTI, and for moderate to severe LRTIs that do not improve within one week.

This is based on the assumption that a (secondary) bacterial LRTI is likely if symptoms do not improve within one week, and that lung damage due to recurrent LRTIs might be prevented by initiating antibiotic treatment early.

Children with airway malacia and recurrent LRTIs who were followed regularly at the outpatient department, were instructed to contact the hospital when they developed symptoms suggestive of a LRTI. Within 2 days after this contact, the child was evaluated at the outpatient department (visit 1). The child was enrolled if inclusion criteria were met and parental consent was obtained. The decision to order a chest radiograph, sputum culture or laboratory tests was up to the pediatric pulmonologist. Patients were re-evaluated by a pediatric pulmonologist after one (visit 2) and two weeks (visit 3). The study coordinator contacted parents by telephone after three weeks (Figure 6.1). At each follow-up visit the pediatric pulmonologist again evaluated the need for an antibiotic course based on symptom severity and physical examination.

Figure 6.1. Study design*



* LRTI, lower respiratory tract infection.

Study medication

Children were randomly assigned to receive 2.5 mg of rhDNase (2.5 ml solution of 1 mg/ml rhDNase; Roche, Basel, Switzerland) or 2.5 mg placebo (2.5 ml sodium chloride 0.9%), twice daily for two weeks. Study medication was administered with a Sidestream jet nebulizer using a PortaNeb Compressor (Romedic, Meersen, the Netherlands) with a mouthpiece when possible or through a tightly applied facemask. The first dose of study medication was administered at the outpatient department.

The hospital pharmacist prepared the vials with study medication. Placebo and rhDNase had an identical appearance and smell. Randomization was carried out in the hospital pharmacy using a randomization schedule prepared by the study statistician. Patients were stratified by age (<6 years or ≥6 years) and use of maintenance antibiotics (yes or no). Throughout the study, pediatric pulmonologists, parents and children, and the trial coordinator remained unaware of the treatment assignment.

Efficacy endpoints

The primary outcome measure was the change in symptom severity from baseline to second week of treatment, assessed with a cough diary score (CDS). The CDS is one of the few validated instruments to assess severity of respiratory symptoms in children.[16] It assigns scores separately

to daytime and nighttime cough (Table 6.1). Changes from baseline were calculated by subtracting mean cough scores in the second study week (day 8 – 14) from those at baseline (day 1).

Table 6.1. Cough diary score (CDS)

Score	Daytime	Nighttime
0	No cough	No cough
1	Cough for one or two short periods only	Cough on waking only/ cough on going to sleep only
2	Cough for more than two short periods	Awoken once or awoken early due to coughing
3	Frequent cough not interfering with school or other activities	Frequent waking due to coughing
4	Frequent cough interfering with school or other activities	Frequent coughs most of the night
5	Cannot perform most usual activities due to severe coughing	Distressing cough

The secondary outcome measures included the need for an antibiotic course during the 2-week treatment period, cough frequency, severity of dyspnea, and ease of expectorating sputum expressed on visual analogue scales (VAS), and lung function (FVC, FEV_1, FEF_{75}, $Rint_{,e}$). A VAS is a marked scale of 10 cm length, anchored by word descriptors at each end (for example, "absence of cough" and "continuous coughing"). The child or parent places a mark on this line in the position that best represents their perception. The VAS score is the distance in cm between the left anchor (= 0 cm) and the mark. Parents of younger children recorded the respiratory symptoms of their child daily in a diary for 3 weeks, starting at the first study visit with the recording of the previous night's symptoms. Children over 6 years of age were instructed to fill in the diary with parental assistance. Nighttime symptoms were scored the next morning, and daytime symptoms in the evening.

Spirometry and $Rint_{,e}$ measurements were performed at each study visit. Interrupter resistance ($Rint_{,e}$, $kPa \cdot L^{-1} \cdot s^{-1}$) was measured using the MicroRint (Micro Medical Ltd). $Rint_{,e}$ was always measured before spirometry and a minimum of five correct tracings (maximal 10) were obtained. The expiratory Rint ($Rint_{,e}$) was measured, as expiratory interruptions are more sensitive in detecting airway obstruction relative to inspiratory interruptions.[17] Rint values were expressed as Z-scores.[18] Spirometry was performed according to European Respiratory Society guidelines[19] and expressed as % of predicted. At the first study visit spirometry and Rint were performed just before and 1 hour after administration of study medication in order to assess possible short term effects on lung function.

Estimate of sample size

A formal power calculation was not possible, as detailed information on the course of respiratory symptoms during LRTI in children with airway malacia has not been published. We aimed to include 40 patients (20 children in each group).

Statistical analysis

Data were analyzed on an intention-to-treat basis. Differences between baseline group characteristics and between secondary outcome measures were assessed by chi-square or the Mann-Whitney test, as appropriate. Analyses of between-group comparisons regarding change in symptom scores (CDS and VAS scores) and lung function (FVC, FEV_1, FEF_{75}, Rint) were performed using analysis of covariance (ANCOVA) with adjustment for baseline values. Analyses were performed with SPSS software (version 11.5). For all analyses, a two-tailed p value of less than 0.05 was considered to indicate statistical significance.

Figure 6.2. Enrollment, random assignment, follow-up and analysis

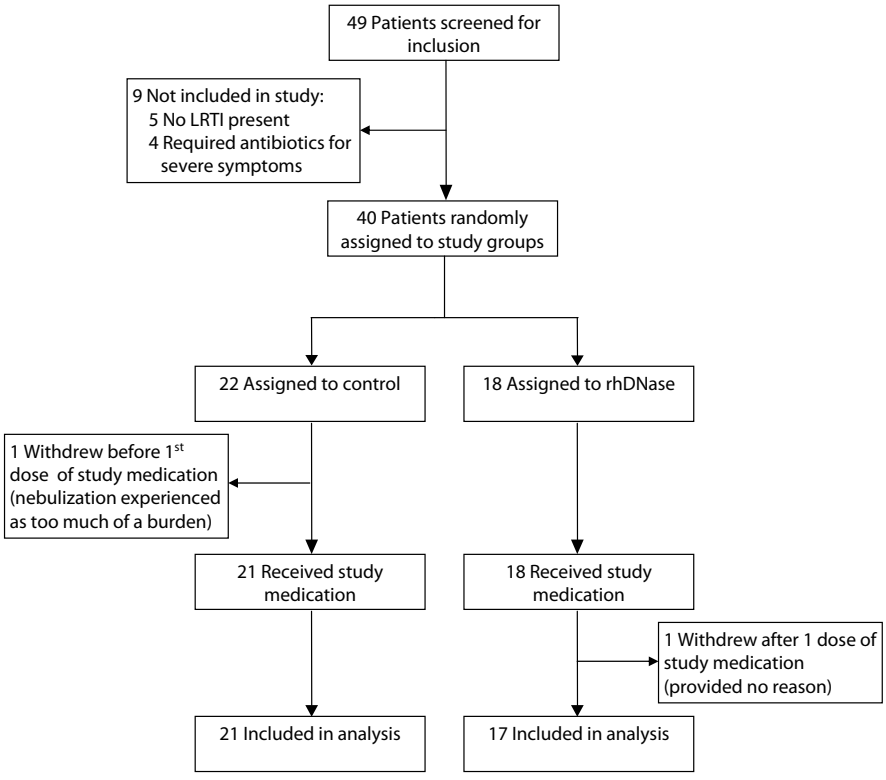


Table 6.2. Baseline characteristics*

Characteristic	rhDNase (n = 17)	Placebo (n = 21)
Male/female, No.	7 / 10	13 / 8
Age, years	6.7 (2.8, 15.0)	6.0 (3.0, 15.5)
Weight, kg	23 (13, 80)	28 (15, 71)
Location of malacia		
Tracheomalacia	7 (41)	10 (48)
Bronchomalacia	2 (12)	2 (9)
Tracheobronchomalacia	8 (47)	9 (43)
Duration of symptoms, days	7 (1, 21)	3 (1, 14)†
Atopy	6 (35)	7 (33)
Current treatment**		
None	7 (41)	9 (21)
Maintenance antibiotics	4 (24)	4 (19)
Positive expiratory pressure (PEP) device	8 (47)	10 (48)
Inhaled bronchodilators	4 (24)	1 (5)
Inhaled steroids	4 (24)	1 (5)
Daytime CDS	2.5 (1, 4)	3.0 (1, 5)
Nighttime CDS	1.5 (0, 4)	3.0 (0, 5)
Daytime cough, VAS	35 (3, 84)	53 (25, 83)
Daytime dyspnea, VAS	24 (0, 84)	23 (1, 88)
Nighttime cough, VAS	35 (0, 75)	57 (1, 89)
Nighttime dyspnea, VAS	1 (0, 73)	24 (0, 84)†
Difficulty expectorating sputum, VAS	45.5 (0, 94)	61.5 (0, 100)
FVC %pred#	101 (77, 115)	91 (47, 124)
FEV_1 %pred	99 (67, 117)	77 (48, 105)†
FEF_{75} % pred	80 (49, 114)	44 (21, 99)†
PEF %pred	78 (40, 102)	68 (43, 87)
Rint,e (Z-score)+	0.6 (-1.3, 2.6)	2.3 (-0.7, 4.2)†

*Data are expressed as median (range), No.(%) unless otherwise specified. CDS, cough diary score. VAS, visual analogue scale (in mm). †P value < 0.05.

** Patients could have more than one type of current treatment

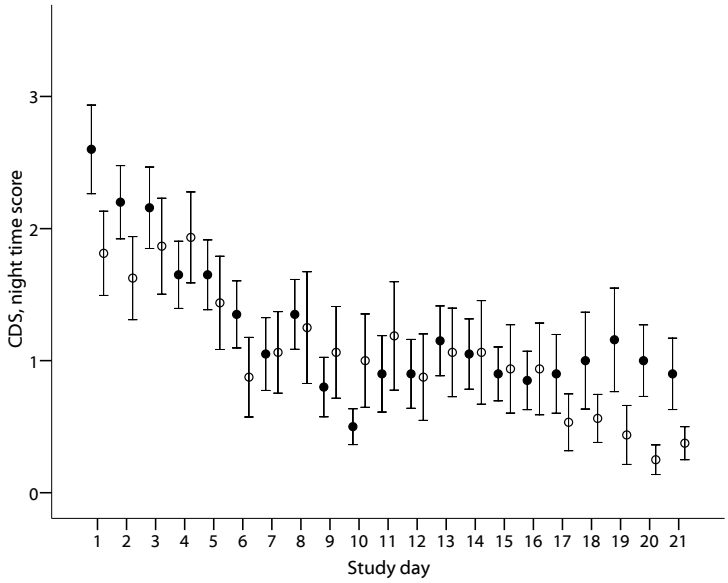
#Spirometry could be performed in 27 patients (13 placebo group; 14 rhDNase group)

+Rint measurements could be performed in 28 patients (16 placebo group; 12 rhDNase group)

Results

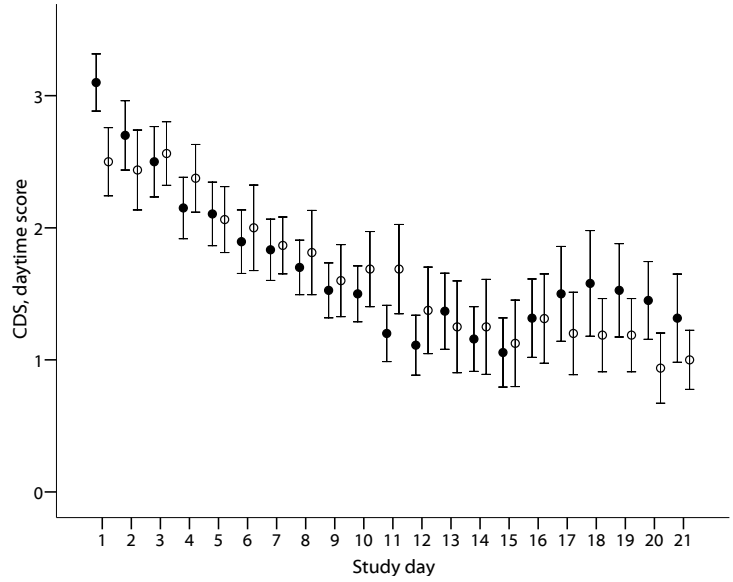
A total of 40 children were enrolled and assigned to treatment: 18 children to rhDNase and 22 children to placebo. For two patients no study data were available as they withdrew after randomization, had not used their study medication, and had not filled out their diaries (1 in each group; Figure 6.2.) Analysis therefore concerned 17 and 21 children, respectively. Baseline demographic characteristics did not differ between both treatment groups. Nevertheless,

Figure 6.3a. Mean nighttime cough diary scores (CDS) during study with standard errors



● indicates placebo; ○ indicates rhDNase

Figure 6.3b. Mean daytime cough diary scores (CDS) during study with standard errors



● indicates placebo; ○ indicates rhDNase

children in the rhDNase group had been symptomatic twice as long as children in the placebo group before they started the study. Despite randomization, at baseline the placebo group showed lower lung function, and higher VAS scores for nighttime cough and nighttime dyspnea than did the rhDNase group (Table 6.2).

Primary endpoint

In both groups the cough diary scores (CDS) improved over the study period (Figure 6.3). Mean changes in CDS from baseline did not significantly differ between the rhDNase group and the placebo group: mean difference for daytime CDS 0.19 (95% CI -0.53 to 0.90; p=0.60); for nighttime CDS 0.38 (95% CI -0.30 to 1.05; p=0.26); (Table 6.3).

Table 6.3. Primary endpoint, cough diary score (CDS)*

Variables		rhDNase (n = 17) #	Placebo (n = 21) #	Mean difference between rhDNase and placebo group (95% CI)
Daytime CDS,	Baseline	2.5 (1.0)	3.1 (1.0)	
	2nd week†	1.5 (1.2)	1.4 (0.8)	
	Change from baseline	-1.0 (1.6) †	-1.7 (1.1) †	0.2 (-0.5, 0.9)
Nighttime CDS,	Baseline	1.8 (1.3)	2.6 (1.5)	
	2nd week†	1.1 (1.3)	1.0 (0.7)	
	Change from baseline	-0.7 (1.1) †	-1.7 (1.5) †	0.4 (-0.3, 1.1)

* Data are presented as mean (SD), unless otherwise indicated. Mean CDS was calculated from CDSs at day 8 – 14. Mean difference was calculated using ANCOVA, adjusted for baseline CDS.

Symptom diaries were not available for 2 patients (1 in the rhDNase group and 1 in the placebo group)

† p value < 0.05 for change from baseline within treatment group using paired t-test.

Secondary endpoints

Antibiotics

Antibiotics were started in 5 of 17 (29%) children in the rhDNase group and in 8 of 21 (38%) children in the placebo group during the 2-week treatment period, mean difference 9% (95% CI -21% to 39%; p=0.58).

VAS scores

VAS scores for daytime cough, nighttime cough, daytime dyspnea, nighttime dyspnea, and for difficulty in expectorating sputum all improved significantly in the placebo group during the treatment period. In the rhDNase group VAS scores for daytime dyspnea and for difficulty

Table 6.4. VAS scores*

VAS score		rhDNase (n = 17)#	Placebo (n = 21) #	Mean difference between rhDNase and placebo group (95% CI)
Daytime cough	Baseline	40.1 (22.9)	51.3 (19.1)	
	2nd week+	23.8 (27.4)	19.4 (20.0)	
	Change from baseline	-16.2 (36.4)	-31.9 (18.5)†	7.2 (-9.3, 23.8)
Nighttime cough	Baseline	34.3 (25.5)	51.9 (28.1)	
	2nd week+	20.6 (29.2)	15.2 (20.0)	
	Change from baseline	-13.7 (30.6)	-36.7 (29.1)†	10.8 (-6.1, 27-6)
Daytime dyspnea	Baseline	27.6 (27.8)	30.8 (27.1)	
	2nd week+	8.4 (16.1)	14.9 (22.3)	
	Change from baseline	-19.2 (24.1)†	-15.8 (24.5)†	-5.4 (-17.2, 6.4)
Nighttime dyspnea	Baseline	14.1 (25.0)	34.3 (32.1)	
	2nd week+	7.0 (15.4)	15.3 (22.8)	
	Change from baseline	-7.1 (18.5)	-19.0 (29.1)†	-1.0 (-13.7, 11.7)
Difficulty expectorating sputum	Baseline	45.3 (31.8)	54.4 (27.5)	
	2nd week+	24.5 (30.7)	18.8 (23.8)	
	Change from baseline	-20.8 (29.8)†	-35.6 (33.1)†	8.8 (-8.8, 26.4)

* Data are presented as mean (SD) unless otherwise indicated. Mean difference was calculated using ANCOVA, adjusted for baseline VAS. VAS scores are presented in mm.

+ Mean symptom scores were calculated from CDSs at day 8 – 14.

Symptom diary not available for 2 patients (1 in the rhDNase group and 1 in the placebo group)

† p value < 0.05 for change from baseline within treatment group using paired t-test

Table 6.5. Lung function at visit 2, mean change from baseline*

Variables	rhDNase (n = 14)	Placebo (n = 13)	Mean difference between rhDNase and placebo group (95% CI)#
FVC %pred	-0.6 (13.8)	8.2 (9.4) †	-6.2 (-15.2, 2.8)
FEV_1 %pred	1.6 (14.1)	10.8 (11.7) †	-7.9 (-20.3, 4.4)
FEF_{75} % pred	0.1 (26.6)	8.1 (18.9)	-8.0 (-32.1, 16.1)
PEF %pred	1.5 (13.7)	9.9 (17.8)	-7.3 (-20.8, 6.2)
Rint,e Z-score	n = 11	n = 14	
	-0.02 (0.87)	-0.53 (1.24)	0.03 (-0.83, 0.89)

* Data are presented as mean ±SD unless otherwise indicated. At visit 2, eleven children were unable to perform spirometry (3 in rhDNase group, 8 in placebo group).

FEF_{75}, forced expiratory flow rate at the 75% point of FVC. Rint,e, expiratory interrupter resistance.

ANCOVA, adjusted for baseline lung function.

† p value < 0.05 for change from baseline within treatment group using paired t-test

expectorating sputum improved significantly. There were no significant differences in mean change from baseline in any of the VAS scores between both groups (Table 6.4).

Lung function

At the first study visit, lung function was assessed before and one hour after a dose of study medication. Nebulization of study medication had no effect on lung function within or between groups (data not shown). Within the placebo group, FVC and FEV_1 improved significantly during the 2-week study period. Lung function did not change significantly in the rhDNase group. After 2 weeks of treatment there was no difference in mean change in lung function from baseline between both groups (Table 6.5). Lung function measurements after 1 week of treatment showed similar results (data not shown).

Safety

We observed no serious adverse events. Two children reported coughing during nebulization, which then was discontinued temporarily (placebo group), and one child complained of coughing following nebulization (rhDNase group). Three children reported a sore throat during the study (2 in placebo group, 1 in rhDNase group).

Discussion

We report the first randomized double-blind controlled trial of nebulized rhDNase in children with airway malacia and a respiratory tract infection. The findings show no evidence that nebulization with rhDNase improves respiratory symptoms or reduces the need for antibiotics during LRTIs in children with airway malacia.

Based on the assumption that dynamic airway collapse during coughing in children with airway malacia results in airway obstruction and impaired mucociliary clearance, we expected a beneficial effect of a mucolytic during LRTIs. In previous (uncontrolled) observations in our outpatient department, children with airway malacia and severe symptoms and recurrent LRTI seemed to benefit from treatment with rhDNase. Moreover, case reports have suggested efficacy of rhDNase in several other pediatric lung diseases with impaired mucociliary clearance, such as primary ciliary dyskinesia,[12-14] atelectasis,[9,10] and severe acute asthma.[11]

There may be several explanations for the lack of effect of rhDNase in this study. One is that children had relatively mild disease at inclusion, as they were included soon after symptoms developed. Consequently, these patients might have had no, or too little airway mucus for rhDNase to be effective. Children with more severe symptoms at initial presentation, and perhaps more mucus plugging, received a course of antibiotics and were not included in our study. We focused on children with less severe symptoms, as one of the study aims was to assess a possible effect of rhDNase on the need for antibiotics during LRTIs. Further studies

are warranted to investigate whether rhDNase might be effective in addition to antibiotics in children with a more severe LRTI.

A second explanation might be that the amount of DNA present in mucus was too low for rhDNase to be effective. Although we have no information on DNA content of mucus in our patients with a respiratory tract infection, it is likely to be lower than in patients with cystic fibrosis.[20]

A third explanation might be that mucus was liquefied but that dynamic collapse of the central airways during coughing prevented patients from clearing their airways effectively. Half of the study patients routinely used a PEP device to facilitate mucus clearance. Because of small numbers, a subgroup analysis in patients using a PEP-device was not useful.

In this study we used a symptom score (CDS) that has been validated in children.[16] Some studies suggest that nighttime symptom scores might not accurately reflect objectively measured cough frequency,[21] although others did find good agreement.[22] Not only cough frequency, but also cough severity is important in determining disease burden, and measures of cough frequency alone cannot account for this. Nonetheless, nighttime and daytime symptom scores indicated that rhDNase had no beneficial effect compared to placebo.

Although the sample size in this study was relatively small, it is not to be expected that a possible beneficial effect of rhDNase has been missed. Based on the 95% CI of the mean difference in CDS it can be concluded that rhDNase could have had a beneficial effect of at most about 0.5 points (on the scale of 0-5).

Based on clinical grounds, one third of all study patients required antibiotics during the 2-week study period. We had no objective criteria, such as sputum culture results, inflammatory parameters, or chest radiographs available for every study patient to guide the decision to start antibiotics. Instead, our study reflected routine clinical practice in which antibiotics are initiated based on symptom severity and physical examination. Our results confirm that patients with airway malacia often are prescribed antibiotics for LRTIs.[1,2]

Lung function testing before and 1 hour after a dose of study medication showed that rhDNase can be safely used in children with airway malacia. By chance, baseline lung function in the placebo group was lower than in the rhDNase group. Unlike in the rhDNase group, lung function (FVC and FEV_1) in the placebo group improved during the two-week study period, possibly due to regression to the mean and more room for improvement. Corrected for baseline lung function, there was no difference in mean change from baseline between the rhDNase and placebo group.

Retention of mucus can theoretically reduce airway diameter and contribute to airflow obstruction, or completely obstruct some airways and influence static lung volumes and volumes of trapped gas. Spirometry data in patients with airway malacia will primarily be determined, however, by the degree of central airway collapse. Thus, measurements of airflow and simple lung volumes do not appear to reflect changes in mucus transport and are relatively insensitive to detect effects of airway clearance manoevres.[23] In future studies on mucolytics, measuring ventilation inhomogeneity using the lung clearance index (LCI) might be of additional value. LCI has been shown to be more sensitive than spirometry in detecting lung function abnormalities in young children with CF.[24,25]

In conclusion, our study shows that two weeks of nebulized rhDNase does not enhance recovery or reduce the need for antibiotics in children with airway malacia and lower respiratory tract infection.

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Chapter 7

Yield from flexible bronchoscopy in pediatric cystic fibrosis patients

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Abstract

Background: Recent data on the yield of bronchoscopy in pediatric CF patients are lacking. Therapeutic bronchoscopic lavage with the mucolytic rhDNase has been used during CF bronchoscopies, but efficacy data are scarce.

Methods: A retrospective review of all bronchoscopies performed in pediatric CF patients in our hospital in the past 16 years. Aims of the study: to evaluate indications for and safety of bronchoscopy in pediatric CF patients, to describe the findings of bronchoscopy and the contribution of these findings to clinical management, and to evaluate the application of bronchoscopic lavage with rhDNase.

Results: Between 1992 and 2007, 66 bronchoscopies were performed in 48 CF patients (25 males) at a median (range) age of 8.3 (0.1 to 20.4) years. Indications for bronchoscopy were persistent atelectasis (42%), refractory symptoms (29%), need for microbiologic culture (11%), suspected anatomical abnormality (11%), and bronchial toilet (7%). Relevant new information with therapeutic consequences was obtained in 28 (42%) bronchoscopies, including a first *Pseudomonas aeruginosa* infection (n=3), infection with atypical mycobacteria (n=3) or *Aspergillus fumigatus* (n=5), and severe tracheo(broncho)malacia (n=4). In patients with atelectasis, rhDNase lavage was associated with improved chest radiograph scores and a transient decline in forced vital capacity. In 7 of 11 patients with refractory symptoms, lung function tended to improve after rhDNase lavage. No serious complications were observed after bronchoscopy and rhDNase lavage.

Conclusions: Bronchoscopy provides clinically relevant information in about 40% of these pediatric CF patients. Lavage with rhDNase seemed safe, and was associated with improved chest radiographs in patients with therapy resistant atelectasis.

Introduction

Lung lavage was proposed in 1965 as a therapeutic option in cystic fibrosis (CF) patients. The idea was that lavage with N-acetylcysteine (NAC) could remove accumulated mucus, reduce airway resistance and restore pulmonary function.[1] Thereafter, several case reports were published on the use of lavage in CF patients using a variety of techniques.[1-13] After the introduction of the flexible bronchoscope, beneficial effects of lung lavage using smaller amounts of lavage fluid were reported.[6] The usefulness of bronchoscopy was questioned, however, as its efficacy in CF patients had not been evaluated in controlled studies.[14] Still, recent data on the clinical value of bronchoscopy in CF patients in clinical practice are lacking. Furthermore, since recombinant human deoxyribonuclease (rhDNase, dornase alfa) became available for CF treatment, only 2 small case series have been published on the use of rhDNase lavage during bronchoscopy. These concerned in total 5 pediatric CF patients with persistent atelectasis.[15,16] Yet, in our experience, rhDNase is often used during bronchoscopy in CF patients. Therefore, we conducted a retrospective review of all bronchoscopies performed in pediatric CF patients in our hospital in the past 15 years. The aims of this study were threefold. First, to evaluate indications for and safety of bronchoscopy in pediatric CF patients; second, to describe the findings of bronchoscopy and the contribution of these findings to clinical management; and third, to evaluate lung lavage with rhDNase during flexible bronchoscopy and its effects on lung function and chest radiographs.

Patients and methods

In a descriptive retrospective study we evaluated all flexible bronchoscopies performed in pediatric CF patients in our hospital between January 1992 and September 2007. The Erasmus MC - Sophia Children's Hospital is a tertiary referral centre with a 4.4 million referral area. Our hospital is the regional CF center, and currently 150 children with CF are in follow up. For each patient, we reviewed the indication for bronchoscopy, clinical characteristics, details of the lavage procedure, bronchoscopic findings, lung function data, and chest radiographs. One of the indications was 'persistent atelectasis'. This was defined as an atelectasis present for at least 3 weeks despite treatment with intravenous antibiotics, systemic steroids and vigorous physiotherapy. We defined 'refractory symptoms' as pulmonary symptoms or an acute deterioration in lung function that persisted despite oral or intravenous antibiotics for at least 2 weeks, or chronic symptoms with lung function and/or radiographic deterioration without improvement on antibiotics and/ or steroids. Because of the retrospective and noninterventional nature of our study, medical ethical review board approval and patient consent were considered unnecessary.

Bronchoscopies were performed using a flexible bronchoscope with external diameter 3.8 or 5.1 mm (Olympus, Tokyo, Japan), during general anesthesia. Bronchoalveolar lavage (BAL) was carried out according to recommendations[17] with 1 to 3 aliquots of 1 mL/kg warm isotonic saline in the relevant lung segment or in the right middle lobe, and retrieved in a sterile plastic bottle. For diagnostic bronchoscopies, antibiotic treatment was discontinued 48 h in advance. If clinically indicated, a mucolytic (NAC or rhDNase) was administered during bronchoscopy. The mucolytic was left in place for at least 5 min before suctioning. Often a physiotherapist assisted in this procedure.

Clinically relevant information obtained during bronchoscopy

We judged the information obtained during bronchoscopy to be clinically relevant and meaningful if bronchoscopy showed a:

- previously unknown relevant anatomical abnormality, such as tracheomalacia;
- previously unknown bacterial, fungal or mycobacterial infection for which treatment was initiated;
- bacterial culture and sensitivity results that led to a switch in antibiotic treatment;
- diagnosis of aspiration, for which treatment or further diagnostic procedures were initiated.

Chest radiographs and lung function

We retrieved all chest radiographs and lung function tests conducted around bronchoscopy. For analysis only those performed within 2 weeks prior to and 2 weeks after bronchoscopy were used. If multiple chest radiographs or lung function tests had been conducted in the 14 days after bronchoscopy, the first was used, as the indications for repeat investigations in such a short time span might have been biased by the outcome of the first. As BAL and anesthesia might negatively affect lung function after the procedure,[2,18,19] a second analysis was planned to assess lung function change in those patients for whom multiple lung function data after bronchoscopy were available. In this analysis, we used lung function data from the second week (≥7 days) instead of the first week (≤ 6 days) after bronchoscopy. If multiple lung function tests or chest radiographs in the 14 days prior to bronchoscopy were available, we used data from the most recent ones.

As a validated scoring system for atelectasis is lacking, we used a scoring system based on available literature[20-22] and personal experience of our radiologists.[23] Atelectasis was scored for each lobe separately as absent (0 points), partial (1 point) or complete (2 points). Furthermore, hyperinflation and mediastinal shift were scored as absent (0 points) or present (1 point), leading to a total score ranging from 0 to 12. Chest radiographs were anonymized, blinded, coded

and scored randomly by two independent pediatric radiologists (M.H.L, A.S.D). The mean of the total chest radiograph scores of both radiologists was used for analysis.

Statistics

Interobserver agreement of the chest radiograph scores was calculated using intraclass correlation coefficients. A value > 0.8 was considered to reflect good agreement. Change in lung function and chest radiograph scores was assessed with paired t test or Wilcoxon rank test.

Results

Of all 941 flexible bronchoscopies performed between January 1992 and September 2007, 66 (7%) were in 48 children (25 males) with CF at a median age of 8.3 years (range, 0.1 to 20.4). Median age at diagnosis was 0.4 years (range, 0.02 to 14.5) and bronchoscopy was performed at a median of 6.2 years (range, 0.05 to 18.6) after diagnosis. Thirty-six children underwent one, 9 underwent two, 2 underwent three, and one child underwent six bronchoscopies. Table 1 shows the main indications for bronchoscopy. In 7 patients, the main indication for bronchoscopy was to obtain cultures. In 5 of these 7 patients (age 0.1 to 3.1 years) it had not been possible to obtain a sputum sample by other means.

Table 7.1. Main indications for and aims of bronchoscopy, and lavage fluid used during bronchoscopy in children with CF

	N	Aim			Lavage fluid		
Main indication for bronchoscopy		Diagnostic	Therapeutic	Both	Saline	rhDNase	NAC
Atelectasis	28	-	26	2	4	16	8
Culture	7	7	-	-	6	1	-
Refractory symptoms*	19	7	3	9	3	16	-
Suspected anatomical abnormality/ visual inspection	7	7	-	-	4	3	-
Bronchial toilet							
Pre-operative	2	-	2	-	1	1	-
During mechanical ventilation	3	-	3	-	-	3	-
Total	66	21	34	11	18	40	8

* This category includes patients with unexplained deterioration in lung function or chest CT scan imaging, or with frequent exacerbations.
CF indicates cystic fibrosis; CT, computed tomography; N, number of bronchoscopies; NAC, N-acetylcysteine; rhDNase, recombinant human deoxyribonuclease.

Table 7.2 Yield from flexible bronchoscopy*

Main indication for bronchoscopy	New information obtained		Clinical decision based on bronchoscopy findings
	No	Yes	
Atelectasis	18	10	rhDNase nebulization, twice instead of once daily (2) Culture results: Antibiotics, start (2); change (4) Antifungal therapy, start (3)
Culture	4	3	rhDNase nebulization, start (1) Culture results: antibiotics, start (1); stop (1); change (1)
Refractory symptoms#	8	11	Antigastroesophageal reflux therapy (1) rhDNase nebulization, start (1) rhDNase nebulization, targeting peripheral airways (1) Airway malacia, intensify airway clearance therapy (2) Culture results: Antibiotic therapy, start (4); stop (2); change (1) Therapy for atypical mycobacterial infection (3) Therapy for fungal infection (2)
Suspected anatomical abnormality/ visual inspection	4	3	Airway malacia, intensify airway clearance therapy (2) Culture results: antibiotics, start (2)
Bronchial toilet			
Preoperative	1	1	Culture results: antibiotics, start (1)
During mechanical ventilation	3	0	

*Data presented as No. More than 1 clinical decision per bronchoscopy could be reported.

This category includes patients with unexplained deterioration in lung function or chest CT scan imaging, or with frequent exacerbations.

Table 7.3. Complications of flexible bronchoscopy

Complication	n	Intervention
Decreased oxygen saturation during bronchoscopy		
Laryngospasm	1	Oxygen supplementation
Bronchospasm	1	Oxygen supplementation
At introduction of rigid scope*	1	Intubation, flexible bronchoscopy
Decreased oxygen saturation after bronchoscopy	4	Oxygen supplementation
Expiratory wheeze directly after bronchoscopy	1	Reevaluation with bronchoscope
Fever and increased CRP after bronchoscopy	1	Antibiotic course
Nose bleed	1	Conservative management

*Combined session with rigid bronchoscopy by otolaryngologist and flexible bronchoscopy by pediatric pulmonologist. CRP indicates C-reactive protein.

New information obtained during bronchoscopy

Relevant new information with therapeutic consequences emerged from 28 of all 66 (42%) bronchoscopies (Table 7.2), and from 16 of the 32 (50%) bronchoscopies with a diagnostic aim. In 3 children, BAL fluid cultures identified a first Pseudomonas aeruginosa infection, which in 2 cases could be eradicated by subsequent therapy. After bronchoscopy, treatment was initiated in 3 children with an atypical mycobacterial infection and in 5 with an *Aspergillus fumigatus* infection.

Complications

Few complications of flexible bronchoscopy were reported (Table 7.3). These could be resolved with minor interventions, and there were no long-term consequences. These complications had no effect on the moment of discharge or on length of stay.

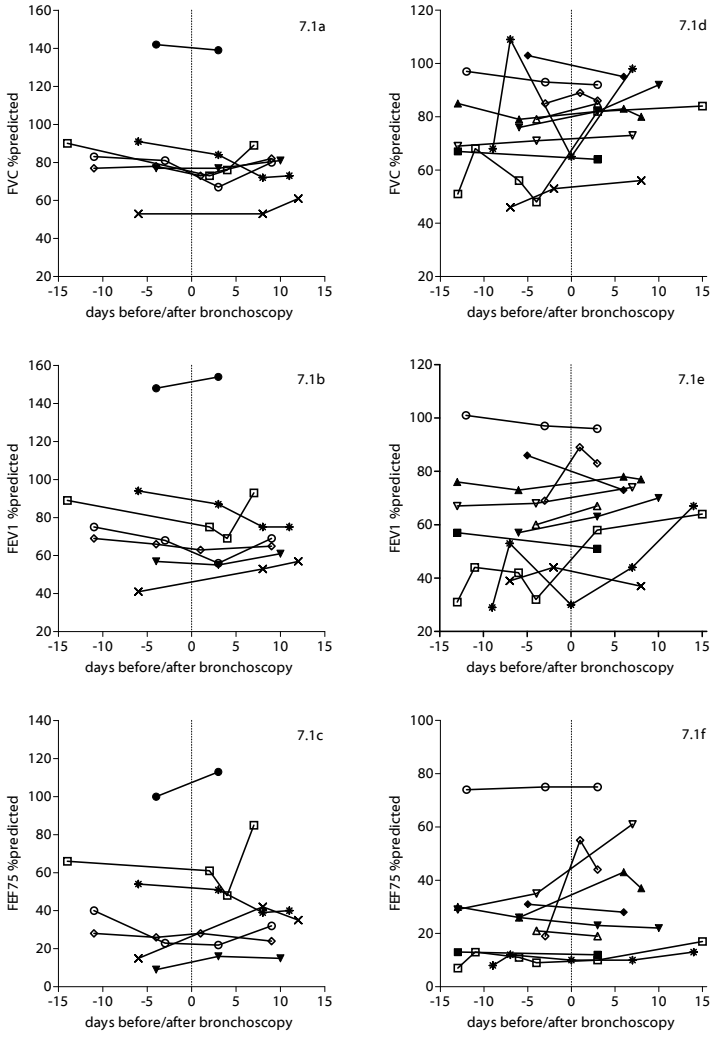
Lavage with NAC and rhDNase

In 1992 and 1993, 8 bronchoscopies using NAC in the lavage fluid were performed in 4 children with persistent atelectasis. Thereafter, NAC was no longer used. rhDNase was used for the first time during bronchoscopy in 1995, and it was subsequently used most frequently in patients with persistent atelectasis or with refractory symptoms (Table 7.1). Until 2000 the amounts of rhDNase were relatively small, ranging from 0.3 mg to 5 mg per session. From 2000 onward, amounts of rhDNase were larger, up to 20.0 mg per bronchoscopy (median 5.0 mg, interquartile range 2.5 to 10.0 mg). A 1:4 dilution with isotonic saline was used most often, with dilutions ranging from none to 1:100.

Lung function after bronchoscopy and rhDNase lavage

rhDNase was used in 16 bronchoscopies in 14 patients with an atelectasis and in 16 bronchoscopies in 14 patients with refractory symptoms. Paired lung function data – that is, within 2 weeks before and 2 weeks after bronchoscopy – were available for 7 and 11 of these patients, respectively (Table 7.4; Figure 7.1). The first lung function measurement after bronchoscopy was performed after a median (range) of 3 (0 to 8) days in patients with an atelectasis and after 3 (1 to 8) days in patients with refractory symptoms. Overall, mean changes in lung function (FVC, FEV_1 and FEF_{75}) were not statistically significant. A significant, transient deterioration in FVC was noted in the subgroup with atelectasis (Table 7.4; Figure 7.1); this decrease in FVC was no longer significant if lung function data from the second week after bronchoscopy were used. The bronchoscopist reported reopening of obstructed ostia and removal of mucus plugs in 15 of the 18 children for whom paired lung function data were available (7 in the

Figure 7.1. Lung function before and after bronchoscopy with rhDNase lavage; by indication for bronchoscopy: atelectasis (a-c) and refractory symptoms (d-f). Each line represents a single patient.



atelectasis group, 8 in the refractory symptoms group). Lung function improved in 7 of these 15 children (1 with atelectasis, 6 with refractory symptoms).

Chest radiographs

Paired chest radiographs were available for 12 of the 16 cases with atelectasis in whom rh-DNase was used, and for 7 of 8 cases in whom NAC was used (Table 7.5; Figure 7.2). The first chest radiograph after bronchoscopy was conducted after a median (range) of 2 (0 to 8) days

Table 7.4. Change in lung function after bronchoscopy during which rhDNase was used*

Indication	Atelectasis		Refractory symptoms	
Lung function	n	Mean change	n	Mean change
FVC % predicted	7	-6.6 (-12.7 – -0.4)†	11	7.3 (-1.9 – 16.4)
FEV_1 % predicted	7	0.4 (-11.7 – 12.5)	11	5.2 (-2.7 – 13.1)
FEF_{75} % predicted#	7	5.7 (-4.7 – 16.1)	10	7.1 (-3.0 – 17.2)

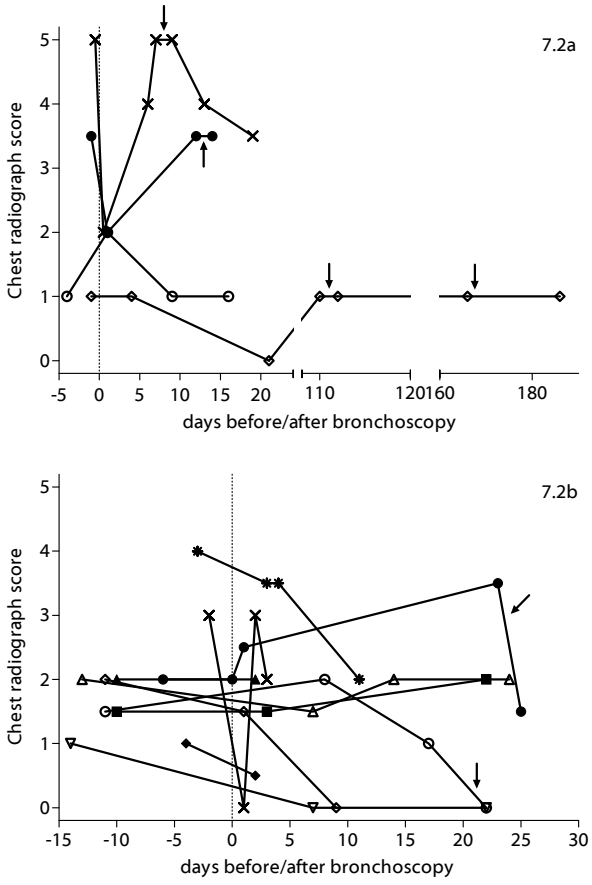
*Data presented as mean change (95% CI) unless otherwise indicated.

#FEF_{75} was not available for 1 patient

† $p = 0.04$ (paired t test)

CI indicates confidence interval; FEF_{75}, forced expiratory flow rate at 75%; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; rhDNase, recombinant human deoxyribonuclease.

Figure 7.2. Chest radiograph scores before and after bronchoscopy in patients with an atelectasis in whom NAC (a) or rhDNase (b) was used during bronchoscopy



*If a patient had a second or third bronchoscopy this is indicated with an arrow.

in patients with rhDNase lavage and after 1 (0 to 4) day in patients with NAC lavage. Overall, after bronchoscopy with rhDNase lavage there was a trend for improvement in chest radiograph scores (p=0.054). Scores improved in 6 out of 10 patients (Figure 7.2b). Improvement persisted in 3 patients, and was temporary in 2. For the sixth patient, no follow up was available.

Two of the 4 patients who underwent bronchoscopy with NAC lavage showed transiently improved scores (Figure 7.2a). For 12 of 14 patients with atelectasis who had paired chest radiographs, the bronchoscopist reported that mucus plugs had been removed after lavage with rhDNase or NAC and suctioning, and that obstructed ostia had been reopened. In 6 of these 12 patients, the observed improvement in airway patency was associated with an improved chest radiograph.

Table 7.5. Change in chest radiograph (CR) score after bronchoscopy for atelectasis *

Lavage fluid	n	CR score before bronchoscopy	CR score after bronchoscopy	p for change†
rhDNase	10	2.0 (1.4 – 2.3)	1.5 (0.4 – 2.0)	0.054
NAC	4	2.3 (1.0 – 4.6)	2.0 (1.3 – 2.0)	0.29

* Data presented as median (interquartile range). If multiple bronchoscopies were conducted for the same indication, only the first was used for analysis (in the rhDNase group, 2 patients had a second bronchoscopy within a few months. In the NAC group, one patient had 3 and one patient 2 bronchoscopies within a few weeks to months). If multiple chest radiographs were available after bronchoscopy, the first was used for analysis.
† Wilcoxon signed rank test.
NAC indicates N-acetylcysteine; rhDNase, recombinant human deoxyribonuclease.

Discussion

On the basis of retrospective data from our center over a 16-year period, we evaluated the indications for bronchoscopy in pediatric CF patients. In addition, we described the yield of bronchoscopy and the impact of findings on clinical management, and evaluated the application of rhDNase lavage during bronchoscopy.

The most frequent indications for bronchoscopy were persistent atelectasis, refractory or worsening symptoms, inability to obtain a sputum culture, and suspected anatomic abnormality. These indications are similar to those reported for other series in CF children[15,16] and consistent with current literature on pediatric bronchoscopy.[24-26] Bronchoscopy yielded clinically relevant information in 40% of the patients. This is much lower than reported for general pediatric populations, in which bronchoscopy, BAL and cultures contributed to management in up to 90% of cases.[27-29] Evidently, the yield of bronchoscopy depends on patient selection.

The first bronchoscopy series in CF patients provided little information on diagnostic yield as it summarized results of therapeutic bronchoscopies with large volume lavage.[14] In a small series in children with CF, useful information was obtained for 9 out of 16 patients, which is comparable with our series.[15]

We found previously unidentified pathogens – such as *P. aeruginosa, A. fumigatus*, and atypical mycobacteria – in most patients with refractory symptoms or persistent atelectasis. These findings had important therapeutic consequences. Furthermore, in one quarter of all patients antibiotic treatment was initiated or adapted based on BAL cultures. In a few children, the indication for bronchoscopy was to obtain a sputum culture. There is much to say for a more proactive approach to identify respiratory pathogens in CF patients: a retrospective study showed positive lavage cultures in 8 of 18 newly diagnosed, asymptomatic CF patients who underwent bronchoscopy after diagnosis.[30]

Nebulization of the mucolytic rhDNase improves lung function and quality of life parameters, and reduces the risk of respiratory tract infections in CF.[31] rhDNase lavage during bronchoscopy could be beneficial because peripheral lung segments can be reached. Moreover, obstructed lung segments can be targeted selectively with a higher dose of rhDNase than by nebulizer, and mucus can be actively removed by suctioning. A significant lung function improvement could not be demonstrated, however, after rhDNase lavage in children with persistent atelectasis or with refractory symptoms and there was even a transient decline in FVC in patients with atelectasis. This might be attributed to general anesthesia[2,18,19,32] or the lavage procedure.[2] Nevertheless, several patients showed clinically relevant lung function improvement after bronchoscopy. Because of the small number of paired observations, this analysis lacks the power to detect overall effects.

rhDNase lavage was often effective in reopening obstructed ostia or removing mucus plugs. Still, in many cases this did not result in a measurable improvement. In about half of the patients with persistent atelectasis or refractory symptoms, however, reopening the airways was associated with improved lung function. Improvements on chest radiographs were observed in half of all patients with an atelectasis in whom the bronchoscopist observed reopened ostia, or removed mucus plugs. The latter finding might partially be due to limited discriminative power of the chest radiograph score. Partial improvement does not necessarily lead to a better score. Finally, bronchoscopic examination is limited in its ability to visualize persistent obstruction in more peripheral airways. As lavage does not alter the underlying disease process, viscous mucus will reaccumulate after bronchoscopy. Consequently, the effect of bronchoscopy may be short-lived if a patient is unable to clear mucus efficiently from reopened lung segments.

Bronchoscopy has been used as a therapeutic intervention in different patient categories with persistent atelectasis.[33] Several series in premature infants, newborns, children and patients on extracorporeal membrane oxygenation (ECMO), reported resolution of atelectasis.[29,34-36] On the other hand, no resolution of atelectasis after bronchoscopy was found in a heterogeneous group of 11 children with chronic atelectasis.[37] This may have been due to the fact that intraluminal mucus obstruction was uncommon in this group. In one prospective study in patients with lobar atelectasis, restoration of volume loss on chest radiographs was similar for those treated with flexible bronchoscopy and those treated with airway clearance therapy alone.[38] That study was limited to adult non-CF patients (mean age 51 yrs), half of whom had post-operative atelectasis, and one third of whom was mechanically ventilated, making direct comparison with our findings difficult.

As our study was retrospective and lacked a control group, the results do not allow drawing conclusions on the efficacy of bronchoscopy and rhDNase lavage in pediatric CF patients with persistent atelectasis or refractory symptoms. Our data, however, do indicate that bronchoscopy was associated with clinically relevant improvements in lung function and chest radiograph scores in a subset of patients. In our opinion, using rhDNase during bronchoscopy is indicated for CF patients with persistent atelectasis or refractory symptoms despite previous intravenous antibiotics, systemic steroids and vigorous physiotherapy as these patients are at risk for progressive bronchiectasis. Bronchiectasis are reservoirs of bacteria and inflammatory mediators[39] that may further damage the adjacent healthy areas of the lung. Indeed, persistence of atelectasis in young children has been associated with a poor prognosis.[10]

Bronchoscopy is invasive and was performed under general anesthesia in our study. Yet our results confirm the short-term safety of bronchoscopy in this setting.[24] Even the use of large amounts of rhDNase appeared to be safe as no side effects occurred with doses of up to 20 mg of rhDNase. In non-CF pediatric lung disease doses ranging from 0.1 mg/kg to 10 mg rhDNase have been used safely during bronchoscopy or endotracheal instillation.[33]

In conclusion, bronchoscopy in pediatric CF patients provided us with new, clinically relevant information in about 40% of the procedures. Therapeutic bronchoscopy with rhDNase lavage is safe and is associated with improved chest radiographs in pediatric CF patients with persistent atelectasis.

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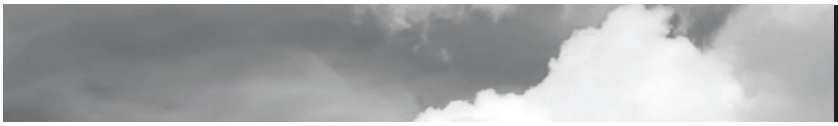

Chapter 8

Summary
General discussion
Future directions

Summary

Impaired mucociliary clearance or mucus hypersecretion are important clinical features in several pediatric lung diseases, such as cystic fibrosis (CF), recurrent bronchitis, (acute) asthma, and primary ciliary dyskinesia, as well as diseases with abnormal cough mechanics, such as airway malacia and neuromuscular diseases. Moreover, viral respiratory tract infections – which occur frequently during childhood – may cause epithelial damage and loss of cilia,[1] and thus impaired mucociliary clearance through secondary ciliary dysfunction. When mucus secretion outweighs clearance, mucus can accumulate in the airways and initiate cough and sputum production. Accumulated mucus promotes airways obstruction, bacterial colonization, and recurrent infections, negatively affecting quality of life. Therefore, various strategies, including pharmacotherapy with mucoactive agents, have been devised to treat children with (symptoms suggestive of) impaired mucociliary clearance.

One of the mucoactive agents is recombinant human DNase (rhDNase). Licensed for use in CF patients since more than a decade, rhDNase has also been used off-label for different pediatric lung diseases. Still there is little evidence from randomized controlled trials to support this off-label use.

Chapter 1 is a general introduction to the thesis that also provides the aims of the studies.

Chapter 2 presents an overview of the literature on the mucoactive agents most frequently used and studied in children with non-CF lung disease – NAC, rhDNase, and hypertonic saline. Uncontrolled observations that suggest beneficial effects of mucoactive agents in different lung diseases appear to predominate, but this may be due to publication bias, as uncontrolled observations of unfavorable findings are rarely published. In general, for most of the diseases reported in the uncontrolled observational studies, no RCTs had been conducted to confirm or refute the observed positive findings.

Evidence to support the use of inhaled or oral NAC in children with respiratory tract disease was lacking, despite the fact that NAC is widely prescribed.

One RCT with endotracheally administered rhDNase to children ventilated post-operatively demonstrated these children left the intensive care unit sooner, and had lower incidence rate of atelectasis. Two RCTs with nebulized rhDNase, discussed in detail in chapters 3 and 4, demonstrated no clinical benefit in infants with RSV bronchiolitis or in children with an asthma exacerbation. Anecdotal evidence suggests that rhDNase could be beneficial in acute severe life-threatening asthma, or atelectasis during mechanical ventilation, but RCTs in these patient groups were lacking.

Efficacy of hypertonic saline had been studied by one research group in three small RCTs in infants with RSV bronchiolitis. A beneficial effect on length of hospital stay and symptom severity was noted.

In conclusion, well-designed RCTs with clinically relevant endpoints are badly needed before rhDNase can be recommended in non-CF lung disease, and to confirm the promising effects of hypertonic saline in infants with bronchiolitis.

Chapter 3 describes a multicenter, randomized, placebo-controlled trial on the efficacy of rhDNase in 225 oxygen-dependent infants hospitalized for RSV bronchiolitis. They received either 2.5 mg of nebulized rhDNase or placebo twice daily until discharge. We demonstrated that rhDNase and placebo treated infants did not significantly differ in length of hospital stay, duration of supplemental oxygen, rate of improvement of symptom scores and number of intensive care admissions.

We therefore conclude that it is not effective to add nebulized rhDNase to standard, supportive care of infants with RSV bronchiolitis hospitalized for hypoxemia.

In **chapter 4** we present the results of a multicenter, randomized, placebo-controlled trial on the efficacy of rhDNase in addition to standard treatment at the emergency department in children with an asthma exacerbation. In this study, 121 children with a moderate-to-severe asthma exacerbation received either a single dose of 5 mg nebulized rhDNase or placebo following the second dose of bronchodilators. An asthma score was assessed at baseline and 1, 2, 6, 12 and 24 h after rhDNase treatment. We found neither a difference between the treatment groups in the asthma score over the study period of 24, nor in that at any of the separate time points. Duration of oxygen supplementation and number of bronchodilator treatments in the first 24 h were also similar in both groups.

We conclude that adding a single dose of nebulized rhDNase to standard treatment in the emergency room has no beneficial effects in children with moderate-to-severe acute asthma.

Chapter 5 reports a retrospective study that estimated the prevalence of primary airway malacia at birth, determined the predictive value of a clinical diagnosis of airway malacia compared with bronchoscopy results, and described the presenting symptoms of children with airway malacia. Airway malacia was diagnosed in 160 out of 512 bronchoscopies performed between 1997 and 2004. The prevalence of primary airway malacia at birth was estimated to be at least one in 2,100. When pediatric pulmonologists expected to find airway malacia (based on symptoms, history, and lung function) prior to bronchoscopy, this was correct in

three-quarters of the cases. Yet in half of all airway malacia cases, the diagnosis had not been suspected prior to bronchoscopy. Presenting clinical features of children with airway malacia were variable and atypical, showing considerable overlap with features of asthma.

We conclude that primary airway malacia is not rare in the general population, and is difficult to recognize even for pediatric pulmonologists as clinical features show overlap with those of more common pulmonary diseases. We recommend considering bronchoscopy in all patients with unexplained exercise intolerance, recurrent lower airways infection and irreversible and/or atypical asthma to rule out airway malacia, as a correct diagnosis has therapeutic implications and can have lifelong consequences.

In **chapter 6**, we present the results of a randomized, placebo-controlled clinical trial on the efficacy of rhDNase in children with airway malacia and lower respiratory tract infection. In this study, 40 children with airway malacia received either 2.5 mg nebulized rhDNase or placebo twice daily for 2 weeks during lower respiratory tract infections. We demonstrated that there was no significant difference between rhDNase and placebo treated children with regard to improvement of symptom scores, need of antibiotics, and change in lung function.

We therefore conclude that it is not effective to administer nebulized rhDNase to children with airway malacia and lower respiratory tract infection.

Chapter 7 describes the results of a retrospective review of all bronchoscopies performed in pediatric CF patients in our hospital in the past 16 years. Our aims were to evaluate the indications for and safety of bronchoscopy, to describe the findings of bronchoscopy and the contribution of these findings to clinical management, and to evaluate application of lavage with rhDNase during flexible bronchoscopy and its effect on lung function and chest radiographs. A total of 66 bronchoscopies were performed in 48 CF patients with indications ranging from persistent atelectasis, refractory symptoms, need for microbiological culture, suspected anatomical abnormality, to bronchial toilet. Relevant new information with therapeutic consequences was obtained in 42% of the bronchoscopies, including a first *P. aeruginosa* infection, infection with atypical mycobacteria or *A. fumigatus*, and severe tracheo(broncho)malacia. rhDNase lavage in patients with atelectasis was associated with improved chest radiograph scores and a transient decline in FVC. In 7 of 11 patients with persistent symptoms, lung function improved following rhDNase lavage, although overall lung function change was not statistically significant. No serious complications were observed after bronchoscopy and rhDNase lavage.

In conclusion, this study indicates that bronchoscopy provides clinically relevant information in about 40% of pediatric CF patients. Furthermore, lavage with rhDNase is safe and is associated with improved chest radiographs in patients with therapy-resistant atelectasis.

General discussion

Many drugs used in pediatric care are not licensed for their particular applications or are prescribed outside the terms of the product license (off-label use) in relation to target age group, indication, dose of frequency, route of administration, or formulation. Off-label use of drugs is widespread in specialized pediatric health care centers,[2] as well as in general pediatric medical wards.[3,4] Guidelines of the Dutch Medicines Evaluation Board (College ter Beoordeling van Geneesmiddelen) and the Netherlands Health Care Inspectorate (Inspectie voor de Gezondheidszorg) state that off-label use of a drug is acceptable, provided there is sound scientific evidence on its efficacy and safety. Such evidence is often lacking, however, especially in pediatrics.

The mucolytic rhDNase is licensed for use in CF patients ≥ 5 years of age with a FVC ≥ 40% of predicted, in a dose of 2.5 mg once daily. Although rhDNase was not specifically addressed in the above-mentioned studies reporting off-label drug use in children, published case reports and our own clinical experience indicate that off-label use of rhDNase is also common in non-CF patients.

In this thesis we reviewed the current literature on pharmacotherapy for impaired mucociliary clearance in non-CF pediatric patients, including off-label use of rhDNase. Furthermore, the studies described in this thesis expand our knowledge on the efficacy of off-label use of rhDNase in common non-CF pediatric lung diseases, namely RSV bronchiolitis, acute asthma and airway malacia. These studies also improve our understanding of a particular off-label application of rhDNase in CF patients, that is, the use of rhDNase lavage during bronchoscopy. This section discusses the most relevant findings of this thesis.

Pharmacotherapy of impaired mucociliary clearance in non-CF pediatric patients

An important finding in this thesis is that scientific evidence supporting the efficacy of the mucoactive agents NAC, rhDNase and hypertonic saline is scarce. Moreover, the available literature mainly consists of uncontrolled observations that suggest beneficial effects of mucoactive agents in different lung diseases. For most of these diseases, however, no RCTs have been conducted to confirm or refute the positive findings. Strikingly, NAC is often prescribed, especially in general practice,[5] while there is no literature supporting its efficacy. Over-the-counter pediatric cough and cold medications present a similar problem of even greater magnitude: these medications are purchased by 39% of the households (in the U.S). The few studies on these over-the-counter medications did not show any meaningful difference between active drug and placebo.[6] Nevertheless, products that contain various combinations

of antihistamine, antitussive, decongestant and expectorant, can have serious side-effects. For example, cardiac arrhythmias, hallucinations, and depressed levels of consciousness and encephalopathy, and even deaths have been linked to the use of such products.[7] The marketing of these preparations for young children does not reflect the risks or the lack of evidence of efficacy.

Clearly, pediatricians and general practitioners have an important role in educating parents of coughing children on the natural course of airway infections, and on the (lack of) usefulness of mucoactive agents like NAC, and over the counter cough and cold medications.

The past few decades have seen a huge gain in our understanding of the composition of mucus and regulation of mucus production in health and disease, and of the basic defects of CF lung disease.[8] Based on this knowledge, new targets for mucoactive agents have been developed. Although research on the development of new mucoactive agents mainly focuses on CF patients, it can be expected that new insights and treatment options will also be valuable for other pediatric lung diseases. For example, $P2Y_2$ receptor agonists stimulate ciliary beat frequency, mucin secretion, and specific ion channels in the respiratory epithelium, thereby increasing the hydration of the periciliary fluid, and enhancing airway clearance.[9,10] These agonists are presently being developed for clinical use. Another potential target for mucoactive therapy is filamentous (F-) actin. With airway inflammation and inflammatory cell necrosis a secondary polymer network of F-actin and DNA develops in purulent secretions, probably contributing to the viscoelasticity of sputum.[11] In vitro studies suggest that F-actin depolymerizing agents (such as thymosin β4 or gelsolin) used in conjunction with dornase alfa may reduce sputum viscoelasticity and cohesivity more effectively than either used alone.[12]

So the pathophysiology of airway mucus secretion and clearance has been elucidated to some extent, and new mucoactive agents are being developed and tested in adults and CF patients. Nevertheless we must no overlook the still obvious need for clinical studies in pediatric lung disease on already available mucoactive agents.

Off-label use of rhDNase in pediatric lung disease

Mucolytic therapy is a rational approach in acute asthma, RSV bronchiolitis, and airway malacia. Furthermore, anecdotal evidence suggests that rhDNase treatment is effective in infants with severe RSV bronchiolitis,[13] or severe acute asthma.[14-17] However, our RCTs have clearly demonstrated that adding rhDNase to standard treatment has no beneficial effects in oxygen-dependent infants with RSV bronchiolitis, children with moderate to severe acute asthma, and children with airway malacia and a lower respiratory tract infection. Several factors could have contributed to the observed lack of efficacy in these patient groups, including too mild dis-

ease, absence of large atelectasis, suboptimal lung deposition and timing of rhDNase administration, too low mucus DNA content, and an inability to clear liquefied mucus efficiently. The major differences between our RCTs demonstrating no beneficial effect, and case series suggesting a beneficial effect of rhDNase, lie in disease severity and the presence of atelectasis. These case series included children with life threatening asthma or severe bronchiolitis and atelectasis who failed to respond to routine therapy. Patients included in our RCTs had less severe symptoms and also probably less severe mucus plugging. Furthermore, there is a likelihood of publication bias, as case series reporting unfavorable findings are rarely published.

Based on our studies, we cannot exclude that rhDNase might be effective in patients with RSV bronchiolitis or acute asthma with more severe disease and/or atelectasis, in those requiring intensive care admission, or in those patients with asthma who were not receiving inhaled corticosteroids and had longstanding active inflammation. Furthermore, we do not know whether children with airway malacia and severe lower respiratory tract infection requiring antibiotics might benefit from rhDNase. Answering these questions would require additional studies.

Regrettably, rhDNase has not provided a breakthrough in the treatment of hospitalized children with RSV bronchiolitis. Providing infants with supportive care is the main thing clinicians can do, as other treatments strategies, including nebulized ß2-agonists, epinephrine, and systemic steroids, also failed to reduce length of hospital stay.[18-21] Moreover, despite much research, the complex immunopathology of RSV has prevented the development of a safe and effective vaccine.[22] Current studies, however, are evaluating subunit vaccines[23] and medicines that can block its replication.[24]

Still, there are recent promising results on the use of nebulized hypertonic saline in bronchiolitis patients.[25-27] Hypertonic saline treatment was safe, reduced respiratory symptoms, and decreased length of hospital stay. It is to be hoped that these findings will be confirmed in larger RCTs, so that clinicians can provide bronchiolitis patients with a cheap and effective intervention.

rhDNase might be of clinical value in children receiving intensive care. One RCT[28] demonstrated that prophylactic therapy with endotracheal rhDNase reduced ventilation time, length of stay on the intensive care, and incidence of atelectasis in children ventilated post-operatively after elective surgery for congenital heart disease. However, children are not always routinely ventilated post-operatively, like in our hospital. If they are then only a short period, so prophylactic therapy will then be of little value. A more practical approach would be to treat only those mechanically ventilated patients with atelectasis. Case reports indeed suggest that rhDNase might be effective in mechanically ventilated infants and children with atelecta-

sis.[14,15,29-31] We have designed a RCT to assess the efficacy of rhDNase for this indication, and expect that results will be available within a few years.

Bronchoscopy in pediatric CF patients

A difficult clinical problem for pediatric pulmonologists is the management of CF patients with refractory symptoms or persistent atelectasis who fail to respond to standard therapy. There are not any evidence based treatment guidelines for these patients. Our retrospective study indicates that bronchoscopy with rhDNase lavage can be performed without serious side effects, and can be of diagnostic and some therapeutic value. Whether (repeated) therapeutic or elective bronchoscopy can prevent future deterioration and protect against more airways obstruction remains speculative. There are arguments, though, for a more proactive approach to identify respiratory pathogens in CF patients. These come from a retrospective study that showed positive lavage cultures in more than half of newly diagnosed, asymptomatic CF patients who underwent bronchoscopy following diagnosis.[32] Randomized studies would have to be performed for a definite answer on the short-term and long-term efficacy of bronchoscopy with rhDNase lavage in CF patients, and on the efficacy of routine bronchoscopy following diagnosis.

Airway malacia in children

Children with nonspecific respiratory symptoms such as rattling, wheeze, stridor, exercise intolerance, cough, recurrent lower airway infections, and airway obstruction are a diagnostic and therapeutic challenge for general practitioners and pediatricians. These symptoms mostly will be due to recurrent viral respiratory tract infections or asthma. An important finding in one of our studies was that airway malacia is a common disorder in the general population, and that children with malacia present with nonspecific symptoms that can be difficult to recognize even for pediatric pulmonologists. A review of the literature[33] has shown that mild-to-moderate airway malacia is a self-limiting disease and that most patients outgrow the condition by the age of 2 years. We demonstrated that a substantial number of children is diagnosed much later in childhood, however, and that symptoms can very well persist for many years. Clinicians therefore should consider airway malacia in all children with unexplained exercise intolerance, recurrent lower airways infection, and irreversible and/or atypical asthma.

Current management of lower respiratory tract infections in children with airway malacia is not evidence based. It is characterized by liberal use of antibiotics and physiotherapy, using positive expiratory pressure (PEP) devices. Based on the assumption that dynamic airway collapse during coughing in children with airway malacia results in airway obstruction and impaired mucociliary clearance, mucolytic therapy was considered to be potentially beneficial.

We were, however, unable to demonstrate a beneficial effect of the mucolytic rhDNase during respiratory tract infections in children with airway malacia.

The present criteria to diagnose airway malacia are somewhat subjective. They are twofold: a less than 3:1 ratio of cartilaginous vs. membranous part,[34] and visible abnormal collapsibility during cough or quiet breathing observed during bronchoscopy. A more objective method has been developed for identification and measurement of airway lumen in bronchoscopy, using a color histogram mode technique.[35] This method advantageously allows for quantitative comparisons of airway lesions with symptom severity, as well as for longitudinal quantification of airway growth and change in airway malacia sites.[36] It is not yet clear whether quantitative assessment will provide the clinician with sufficient information to estimate prognosis, or to guide treatment, as neither malacia type nor severity seemed to influence growth pattern of malacia sites or illness profile in children with airway malacia.[36]

Directions for future research

Mucolytics are of potential therapeutic benefit in many pediatric lung diseases. rhDNase has been applied off-label for different lung diseases, but until now, mainly uncontrolled observations have been published. In this thesis we described results that extend our knowledge on the efficacy of off-label use of rhDNase.

In general, we need to understand the disease being treated, understand the mechanism of action of an intervention, and recognize relevant outcomes that can be accurately measured. There are some relevant, clinically important research questions that need to be addressed in future studies.

Basic knowledge on pathophysiology of respiratory disease

Currently available mucoactive agents have been developed based on research on the pathophysiology of respiratory diseases, regulation of mucus production and clearance, and on sputum characteristics. Future studies will need to improve our understanding of disease specific:

- biochemical and structural composition of sputum;
- rheology, surface properties, and in vitro transportability of sputum;
- regulation of ion- and water transport, mucin secretion, and inflammation in the respiratory epithelium.

Based on this research, new targets might be found for which mucoactive agents can be developed.

Endpoints in studies on mucoactive agents

One of the difficulties in assessing the effectiveness of mucus clearance therapy is selecting relevant outcomes for clinical trials that accurately reflect therapeutic effects. Various outcomes have been suggested, such as mucus transport, or secondary effects of changes in mucus transport, e.g. duration, severity and frequency of illness exacerbation, days in hospital, change in pulmonary function, and quality of life.[37] The natural variability of the outcome measure in the population being studied must be known in order to design an appropriately powered study. However, in pediatric lung diseases other than CF and asthma, baseline and longitudinal data on these outcome measures are often scarce or not available. An important area for future research, therefore, is the development and validation of endpoints for clinical studies on mucoactive agents.

Pulmonary function

Retention of mucus can theoretically reduce airway diameter and contribute to airflow obstruction, or completely obstruct some airways and influence static lung volumes and volumes of trapped gas. Measurements of airflow and simple lung volumes do not appear to reflect changes in mucus transport and are relatively insensitive to detect effects of airway clearance manoevres.[38] Measurements of gas trapping (e.g. RV/TLC ratio) may better reflect mucus transport than do measurements of FVC or FEV_1.[39] A promising, relatively new pulmonary function parameter for future studies is the lung clearance index (LCI). This index is a measure of ventilation inhomogeneity derived from the multiple-breath inert gas washout technique. LCI has been shown to be more sensitive than spirometry in detecting lung function abnormalities in infants[40] and young children with CF.[41,42] Therefore, LCI might be of interest as a study endpoint in studies on new mucoactive drugs in childhood CF and in other respiratory diseases with peripheral airways obstruction. More studies are needed to assess reference LCI values in non-CF pediatric lung disease patients and to obtain insight in the course of LCI values during disease exacerbations.

Imaging

Interventions with mucoactive agents aimed at the removal of mucus plugs are often evaluated with chest radiographs. Nevertheless, radiography can be insensitive for detecting mucus plugging and is therefore insensitive for identifying improvement of mucus plugging.[43,44] High-resolution CT scanning can provide more detailed information on the extent of mucus plugging and on ventilation inhomogeneity, but is not an attractive outcome measure for short term intervention studies as it exposes the children to radiation.

Hyperpolarized gas MRI (HP MRI) is a new, noninvasive technique to assess lung function, using imaging without exposure to ionizing radiation.[45] Providing three dimensional regional

information and quantitative or semi-quantitative measures of ventilation and perfusion, HP MRI can be used to detect and quantify changes in ventilation defects in respiratory disease following therapy.[46] This imaging technique might be useful for proof of concept studies evaluating efficacy of (new) mucoactive drugs. Still it is expensive and available in specialized research centers only.

How valuable it would be if one were able to assess short term effects of an intervention in patients with mucus plugging or atelectasis, especially in pilot studies with new mucoactive agents, or in patient populations that are difficult to study, like those receiving intensive care. New techniques are being developed that could aid the clinician. An example is electrical impedance tomography (EIT), a non-invasive and radiation-free technique based on the measurement of electric potentials at the chest wall surface. This technique produces a two-dimensional image of the electric impedance distribution within the thorax. EIT can measure both gas and fluid volume changes in the lungs with limited but clinical useful accuracy,[47] and can reliably assess ventilation distribution during mechanical ventilation.[48] Its performance has been reported to be good enough for bedside adjustments of mechanical ventilation with immediate feedback, and offers the potential to detect regional atelectasis.[49] There is good hope that EIT can also be applied as an endpoint in studies on the efficacy of atelectasis treatment in (mechanically ventilated) children.

Sputum markers

In future studies, markers in sputum associated with neutrophilic inflammation (e.g. interleukin-8, DNA, myeloperoxidase) might be used as surrogate endpoints, as they correlate with temporal changes in pulmonary function, at least in CF.[50,51] Further research is needed before these markers can be recommended as reliable outcomes for clinical trials of mucoactive agents.

Future studies on the use of off-label rhDNase

In theory, rhDNase could be effective in any childhood lung diseases involving mucus plugging or impaired mucociliary clearance, in which neutrophilic airway inflammation or increased DNA content of mucus is present.[52] Future RCTs should establish whether rhDNase is effective in those non-CF pediatric lung disease patients that were reported to benefit from rhDNase in many case reports,[53] namely:

- children with severe acute and severe persistent asthma;
- children with persistent atelectasis;
- mechanically ventilated children with atelectasis;
- infants with severe respiratory syncytial virus bronchiolitis requiring intensive care;

- premature infants with infant respiratory distress syndrome who cannot be weaned off the ventilator;
- children with primary ciliary dyskinesia and persistent symptoms despite regular therapy.[54]

RCTs in children with severe respiratory symptoms requiring intensive care will have to be multi-center collaborations to ensure sufficient power.

Off-label use of rhDNase might also be of value for otolaryngologists. For example, an ongoing study assesses the effect of off-label rhDNase in patients with clogged tympanostomy tubes (www.clinicaltrials.gov; NCT00419380). Furthermore, improved drainage of secretions from sinuses might speed up recovery in sinusitis patients.

The field of pediatric drug research has seen important developments over the past few years. To improve and accelerate the development of new, safe and effective medicines for children, a European regulation on medicines for children came into force in 2007. This legislation demands drug companies to evaluate new compounds in children before they can license and label these new drugs. Furthermore, one of the goals of the recently founded Netherlands National Expertise Centre on Pharmacotherapy in Children (Nederlands Kenniscentrum Farmacotherapie bij Kinderen), is to improve quality of pediatric drug research. The Expertise Centre provides an infrastructure and a national network of clinicians and researchers and will coordinate Dutch pediatric drug research and drug development activities.

It is to be hoped that greater efforts in the field of pediatric drug research will provide us with drugs that have been properly studied and licensed for use in children, or at least will provide clinicians with sufficient efficacy and safety data for a sound application of off-label drugs.

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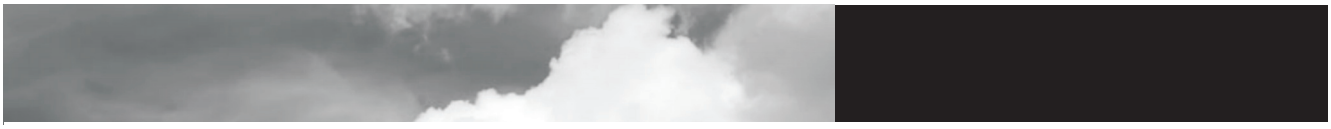

Samenvatting
Dankwoord
Curriculum vitae
List of publications
List of abbreviations

Samenvatting

Verstoorde mucociliaire klaring of mucus hypersecretie zijn belangrijke klinische kenmerken van verschillende kinderlongziekten, zoals cystic fibrose (CF), recidiverende bronchitis, (acuut) astma en primaire ciliaire dyskinesie, evenals van ziekten met een abnormale hoestfunctie, zoals luchtwegmalacie en neuromusculaire ziekten. Bovendien kunnen virale luchtweginfecties, die frequent optreden op de kinderleeftijd, epitheelschade en verlies van trilhaarcellen veroorzaken[1] en daardoor een verstoorde mucociliaire klaring vanwege de secundaire trilhaarceldisfunctie. Als mucus secretie domineert boven mucus klaring kan mucus accumuleren in de luchtwegen en hoest en sputumproductie initiëren. Geaccumuleerd mucus bevordert luchtwegobstructie, bacteriële kolonisatie en recidiverende infecties, wat de kwaliteit van leven negatief beïnvloedt. Daarom zijn er verschillende strategieën ontwikkeld, waaronder farmacotherapie met mucoactieve middelen, om kinderen te behandelen met (klachten passend bij) gestoorde mucociliaire klaring. Een van de mucoactive middelen is recombinant humaan DNase (rhDNase). rhDNase is al meer dan 10 jaar geregistreerd voor gebruik bij CF patiënten, maar wordt hiernaast ook off-label gebruikt bij verschillende kinderlongziekten. Er is echter weinig bewijs uit gerandomiseerde, gecontroleerde studies om dit off-label gebruik te ondersteunen.

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

Hoofdstuk 2 geeft een literatuuroverzicht van studies over de meest gebruikte en bestudeerde mucoactieve middelen bij kinderen met non-CF longziekten, namelijk NAC, rhDNase en hypertoon zout. Ongecontroleerde observationele studies die een gunstig effect van mucoactive middelen suggereren bij verschillende longziekten lijken te domineren. Dit zou door publicatiebias kunnen zijn veroorzaakt, aangezien ongecontroleerde observaties van ongunstige bevindingen zelden worden gepubliceerd. Voor de meeste ziekten waarover werd gerapporteerd in de ongecontroleerde observationele studies, zijn geen gerandomiseerde gecontroleerde studies (RCTs) verricht die de geobserveerde positieve bevindingen kunnen bevestigen of weerleggen.

Er ontbrak bewijs om het gebruik van oraal of verneveld NAC bij kinderen met longziekten te ondersteunen, ondanks het feit dat NAC veelvuldig wordt voorgeschreven.

Een RCT bij kinderen die postoperatief werden beademd, liet zien dat kinderen behandeld met endotracheaal toegediend rhDNase sneller van de intensive care konden worden ontslagen en minder atelectasen hadden. Twee RCTs, die in detail worden besproken in hoofdstukken 3 en 4, toonden geen klinisch effect van verneveld rhDNase bij zuigelingen met RSV

bronchiolitis en bij kinderen met een astma exacerbatie. Observationele studies suggereren dat rhDNase werkzaam kan zijn bij acuut ernstig, levensbedreigend astma, of bij atelectasen tijdens kunstmatige beademing, maar RCTs bij deze patiëntgroepen ontbraken.

De effectiviteit van hypertoon zout werd bestudeerd door één onderzoeksgroep in drie kleine RCTs bij zuigelingen met RSV bronchiolitis. Er werd een gunstig effect gevonden op de opnameduur en op de ernst van symptomen.

Concluderend, goed opgezette RCTs met klinisch relevante eindpunten zijn dringend nodig voordat rhDNase kan worden aanbevolen bij non-CF longziekten, en om de veelbelovende effecten van hypertoon zout bij zuigelingen met RSV bronchiolitis te bevestigen.

Hoofdstuk 3 beschrijft een multicenter, gerandomiseerde, placebogecontroleerde studie naar de effectiviteit van rhDNase bij 225 zuurstofafhankelijke zuigelingen opgenomen in het ziekenhuis met RSV bronchiolitis. Zij kregen tot ontslag twee keer per dag 2,5 mg verneveld rhDNase of placebo. Wij toonden aan dat er tussen de rhDNase en placebo behandelde kinderen geen significant verschil was in opnameduur, duur van zuurstofsuppletie, verbetering van symptoomscores en aantal intensive care opnames.

Wij concluderen daarom dat het niet effectief is om verneveld rhDNase toe te voegen aan de standaard, ondersteunende zorg van zuigelingen met RSV bronchiolitis opgenomen in het ziekenhuis vanwege hypoxemie.

In **hoofdstuk 4** presenteren we de resultaten van een multicenter, gerandomiseerde, placebogecontroleerde studie naar de effectiviteit van rhDNase als aanvulling op de standaard behandeling op de spoedeisende hulpafdeling van kinderen met een astma exacerbatie. In deze studie kregen 121 kinderen met een matig-tot-ernstige astma exacerbatie een eenmalige gift van 5 mg verneveld rhDNase of placebo aansluitend aan een tweede gift luchtwegverwijders. Een astmascore werd bepaald bij baseline en vervolgens na 1, 2, 6, 12 en 24 uur. We vonden noch een verschil tussen de behandelgroepen in de astmascore over de gehele studieperiode van 24 uur, noch in die op een van de afzonderlijke tijdpunten. De duur van zuurstofsuppletie en het aantal behandelingen met luchtwegverwijders in de eerste 24 uur waren ook gelijk in beide groepen.

Wij concluderen dat toevoegen van een enkele gift verneveld rhDNase aan de standaardbehandeling op de spoedeisende hulpafdeling geen gunstig effect heeft bij kinderen met matig-tot-ernstig acuut astma.

Hoofdstuk 5 beschrijft een retrospectieve studie waarin een schatting wordt gemaakt van de prevalentie bij geboorte van primaire luchtwegmalacie en waarin de voorspellende waarde wordt bepaald van een klinische diagnose luchtwegmalacie vergeleken met bronchoscopie resultaten. Ook worden de symptomen bij presentatie van kinderen met luchtwegmalacie beschreven. Luchtwegmalacie werd gediagnosticeerd in 160 van de 512 bronchoscopieën uitgevoerd tussen 1997 en 2004. De prevalentie bij geboorte van primaire luchtwegmalacie werd geschat op tenminste 1 in 2100. Als kinderlongartsen een luchtwegmalacie verwachten te vinden (gebaseerd op symptomen, voorgeschiedenis en longfunctie) voorafgaand aan de bronchoscopie, dan was dit correct in driekwart van de gevallen. Echter in de helft van alle gediagnosticeerde gevallen werd de diagnose niet vermoed voorafgaand aan de bronchoscopie. Klinische kenmerken bij presentatie van kinderen met luchtwegmalacie waren variabel en atypisch en vertoonden aanzienlijke overlap met die van kinderen met astma.

Wij concluderen dat primaire luchtwegmalacie niet zeldzaam is in de algemene populatie en moeilijk te herkennen kan zijn, zelfs voor kinderlongartsen, aangezien de klinische kenmerken overlappen met die van vaker voorkomende kinderlongziekten. Een diagnostische bronchoscopie moet worden overwogen bij alle patiënten met onbegrepen inspanningsintolerantie, recidiverende lagere luchtweginfecties, en 'atypisch' of 'therapieresistent' astma om een luchtwegmalacie uit te sluiten, omdat een correcte diagnose mogelijk langdurig consequenties heeft voor de therapie.

In **hoofdstuk 6** presenteren we de resultaten van een gerandomiseerde, placebogecontroleerde studie naar de effectiviteit van rhDNase bij kinderen met luchtwegmalacie en een lagere luchtweginfectie. In deze studie kregen 40 kinderen met luchtwegmalacie gedurende 2 weken 2 keer per dag 2,5 mg verneveld rhDNase of placebo tijdens een lagere luchtweginfectie. We toonden aan dat er geen significant verschil was tussen de rhDNase en placebo behandelde kinderen in verbetering van symptoomscores, noodzaak van antibiotica, en verandering in longfunctie.

We concluderen daarom dat het niet effectief is om rhDNase te vernevelen bij kinderen met een luchtwegmalacie tijdens een lagere luchtweginfectie.

Hoofdstuk 7 beschrijft de resultaten van een retrospectieve studie van alle uitgevoerde bronchoscopieën bij pediatrische CF patiënten in ons ziekenhuis in de laatste 16 jaar. Onze doelen waren om de indicatie voor en veiligheid van bronchoscopie te evalueren, de bevindingen bij bronchoscopie en de bijdrage van deze bevindingen aan de klinische behandeling te beschrijven, en om het gebruik van lavage met rhDNase tijdens flexibele bronchoscopie en de effecten hiervan op longfunctie en thoraxfoto's te evalueren. Er werden in totaal 66 bronchoscopieën uitgevoerd bij 48 CF patiënten met indicaties variërend van persisterende atelecta-

se, aanhoudende symptomen, noodzaak tot verkrijgen van microbiële kweken en vermoeden van een anatomische afwijking tot bronchiaal toilet. In 47% van de bronchoscopieën werd relevante, nieuwe informatie met therapeutische consequenties verkregen, zoals een eerste *P. aeruginosa* infectie, infectie met atypische mycobacteriën of *Aspergillus fumigatus* en ernstige tracheo(broncho)malacie. Lavage met rhDNase bij patiënten met atelectase was geassocieerd met een verbetering van de thoraxfoto-score en een tijdelijke vermindering van de FVC. Bij 7 van de 11 patiënten met aanhoudende symptomen verbeterde de longfunctie na rhDNase lavage, hoewel er over de hele groep geen significante verandering in longfunctie was. Er werden geen ernstige complicaties gezien na bronchoscopie en rhDNase lavage.

Concluderend, deze studie geeft aan dat er bij ongeveer 40% van de pediatrische CF patienten klinisch relevante informatie wordt verkregen bij bronchoscopie. Bovendien is lavage met rhDNase veilig en geassocieerd met een verbetering van thoraxfoto's bij patiënten met therapieresistente atelectase.

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Curriculum vitae

Ruben Boogaard was born in Sliedrecht on April 21st, 1978. He passed his secondary school exam (VWO) at the "christelijke scholengemeenschap de Lage Waard" in Papendrecht in 1996. In the same year he started his medical training at the Medical Faculty of the Erasmus University of Rotterdam (1996-2002). During his study he assisted at the practical courses of the department of Anatomy, and performed a research project on 'quality of aerosol therapy in a university children's hospital' at the Pediatric Pulmonology department, Erasmus MC-Sophia Children's Hospital, Rotterdam (supervisor: Dr. H.A.W.M. Tiddens, head: Prof.dr. J.C. de Jongste). He did an optional internship at the department of Clinical Genetics at the "Stichting klinische genetica" in Rotterdam (Drs. A.J.M. Hoogeboom) and at the department of Pediatrics at the "Albert Schweitzer Hospital" in Dordrecht (Drs. R. Schornagel). After obtaining his medical degree (cum laude) in September 2002, he worked as a resident at the department of Pediatrics at the "Albert Schweitzer Hospital" in Dordrecht (Drs. R. Schornagel). In February 2004 he started a research fellowship at the department of Pediatric Pulmonology of the Erasmus MC-Sophia Children's Hospital (supervisor: Dr. P.J.F.M. Merkus, head: Prof.dr. J.C. de Jongste). The research performed during this period is presented in this thesis. In March 2008 he enrolled in the residency program in Pediatrics at the Erasmus MC-Sophia Children's Hospital, Rotterdam (Prof.dr. A.J. van der Heijden, Dr. de Hoog).

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Abbreviations

ANCOVA	Analysis of covariance
BAL	Bronchoalveolar lavage
BPD	Bronchopulmonary dysplasia
CDS	Cough diary score
CF	Cystic fibrosis
COPD	Chronic obstructive pulmonary disease
ECMO	Extracorporeal membrane oxygenation
EIT	Electrical impedance tomography
FEF_{75}	Forced expiratory flow rate at the 75% point of FVC
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
LLMI	Lipid-laden macrophage index
LRTI	Lower respiratory tract infection
NAC	N-acetylcysteine
MCC	Mucociliary clearance
Mesna	2-mercaptoethane sulphonate
PEP	Positive expiratory pressure
PCD	Primary ciliary dyskinesia
RCT	Randomized controlled trial
rhDNase	Recombinant human DNase
Rint, e	Expiratory interrupter resistance
RSV	Respiratory syncytial virus
VAS	Visual analogue scale