ORIGINAL ARTICLE

Preventive Inhalation of Hypertonic Saline in Infants with Cystic Fibrosis (PRESIS)

A Randomized, Double-Blind, Controlled Study

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Abstract

Rationale: Cystic fibrosis (CF) lung disease starts in early infancy, suggesting that preventive treatment may be most beneficial. Lung clearance index (LCI) and chest magnetic resonance imaging (MRI) have emerged as promising endpoints of early CF lung disease; however, randomized controlled trials testing the safety and efficacy of preventive therapies in infants with CF are lacking.

Objectives: To determine the feasibility, safety, and efficacy of preventive inhalation with hypertonic saline (HS) compared with isotonic saline (IS) in infants with CF, including LCI and MRI as outcome measures.

Methods: In this randomized, double-blind, controlled trial, 42 infants with CF less than 4 months of age were randomized across five sites to twice-daily inhalation of 6% HS ($n = 21$) or 0.9% IS ($n = 21$) for 52 weeks.

Measurements and Main Results: Inhalation of HS and IS was generally well tolerated by infants with CF, and the number of adverse events did not differ between groups ($P = 0.49$). The change in LCI from baseline to Week 52 was larger in infants with CF treated with HS (-0.6) than in those treated with IS (-0.1; $P < 0.05$). In addition, weight gain was improved in infants with CF treated with HS ($P < 0.05$), whereas pulmonary exacerbations and chest MRI scores did not differ in the HS group versus the IS group.

Conclusions: Preventive inhalation with HS initiated in the first months of life was safe and well tolerated and resulted in improvements in LCI and weight gain in infants with CF. Our results support the feasibility of LCI as an endpoint in randomized controlled trials in infants with CF.

Clinical trial registered with www.clinicaltrials.gov (NCT01619657).

Keywords: cystic fibrosis; lung clearance index; lung disease; magnetic resonance imaging; preventive therapy

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At a Glance Commentary

Scientific Knowledge on the Subject: Cystic fibrosis (CF) lung disease starts in early infancy, suggesting that preventive therapy may be most effective. Implementation of CF newborn screening has created a unique window of opportunity to test this concept in clinical trials, and recent studies indicated that the lung clearance index and chest magnetic resonance imaging may be promising outcome measures of early CF lung disease. However, randomized controlled trials (RCTs) testing the feasibility, safety, and efficacy of preventive therapies in infants with CF are lacking.

What This Study Adds to the Field: This study demonstrates, for the first time, to our knowledge, that RCTs including lung clearance index and chest magnetic resonance imaging as quantitative outcome measures of lung disease are feasible in young infants with CF. In addition, this initial RCT supports preventive treatment with inhaled hypertonic saline as being safe and well tolerated and as having therapeutic benefits for lung function and thriving in the first year of life. These findings support the conduct of future RCTs to evaluate safety and efficacy of preventive treatment strategies that have the potential to delay or even prevent irreversible lung damage in patients with CF.

Previous observational studies in infants and preschool children with cystic fibrosis (CF) demonstrated early onset and progression of lung disease despite multidisciplinary treatment by experienced CF teams according to current standards of care (1–3). These studies showed that potentially reversible abnormalities such as airway mucus plugging, air trapping, neutrophilic inflammation, and bronchial wall thickening are already present in young infants in the first months of life and that presumably irreversible and progressive bronchiectasis are detected in many children with CF from preschool age (1, 2). These data suggested that preventive therapeutic intervention starting in early infancy may be a promising strategy to delay or even prevent irreversible lung damage in CF (3–5). This hypothesis was supported by preclinical studies showing that preventive therapeutic targeting of airway surface dehydration implicated in the pathogenesis of CF lung disease (6, 7), with both osmotically active hypertonic saline (HS) and the sodium channel blocker amiloride, prevented mucus plugging in mice with CF-like lung disease (8–10).

The implementation of CF newborn screening has created a unique window of opportunity to test this hypothesis in clinical trials (11). So far, trials testing the safety and efficacy of preventive therapies starting in the first months of life in infants with CF have been hampered by the lack of quantitative outcome measures of lung disease in this challenging age group. However, a series of recent studies suggested that the lung clearance index (LCI), a measure of ventilation homogeneity derived from multiple-breath washout (MBW), may be a suitable endpoint for this purpose. These studies showed that LCI is sensitive to detect abnormal lung function in infants and disease progression and response to therapy with inhaled HS or CFTR (cystic fibrosis transmembrane conductance regulator) modulators in older children with CF (12–20). Furthermore, it was shown that chest magnetic resonance imaging (MRI) is sensitive to detect early abnormalities in lung structure and perfusion in infants and preschool children with CF without radiation exposure (17, 21–27). Recent studies demonstrated feasibility of standardized measurements of LCI and chest MRI in infants and preschool children in a multicenter setting (28, 29).

The aim of this study was to explore the feasibility, safety, and initial efficacy of preventive inhalation of HS in young infants with CF using LCI and MRI as outcome measures. Inhaled HS was used as a preventive therapeutic intervention for the following reasons:

1. Inhaled HS counteracts airway surface dehydration and impaired mucociliary clearance constituting important abnormalities in CF airways (30).
2. A preclinical proof-of-concept study demonstrated that early treatment with HS prevented airway mucus plugging, mortality, and failure to thrive in mice with CF-like lung disease (8).
3. Inhaled HS was shown to be safe and had beneficial effects on LCI in older infants and preschool children with CF (14, 31–34).

To achieve this goal, we conducted an initial multicenter, randomized, double-blind, controlled study of inhaled HS versus isotonic saline (IS) in infants with CF starting in the first 4 months of life for a duration of 12 months and determined effects on LCI, chest MRI score, anthropometry, pulmonary exacerbation rates, and adverse events (AEs) between treatment groups. Some of these results have been previously reported in the form of an abstract (35).

Methods

Study Design and Participants

The PRESIS trial (Preventive Inhalation of Hypertonic Saline in Infants with Cystic Fibrosis Study; www.clinicaltrials.gov identifier NCT01619657) was a randomized, parallel-group, double-blind, controlled trial of inhalation of HS (6% NaCl) versus IS (0.9% NaCl) in young infants with CF. The study was conducted at five CF centers in Germany within the clinical trial network of the German Center for Lung Research (36) and approved by the ethics committee of the University of Heidelberg (approval S-397/2011) and the ethics committees of each participating site. Written informed consent was obtained from parents or legal guardians of all patients included in the study. Demographics and baseline characteristics of the study population are provided in Table 1. Exocrine pancreatic sufficiency was defined by fecal elastase greater than or equal to 200 μg/g stool, and information on pancreatic status and respective *CFTR* genotypes of study participants is provided in Table E1 in the online supplement. Key inclusion criteria were a confirmed diagnosis of CF and age younger than 4 months at inclusion. Additional information, including a complete list of inclusion and exclusion criteria, is provided in the online supplement.

Randomization and Outcome Measures

Study participants were randomized 1:1 to inhalation of 4 ml of HS or IS twice daily for a period of 12 months using a jet nebulizer

(LC sprint; PARI GmbH) and a baby face mask (size 0–1; PARI GmbH). The first inhalation of study solution was performed at the study site under supervision of a physician who assessed study participants for cough, wheezing, and drop in oxygen saturation, which did not occur in any infant. Key outcome measures were change in LCI, chest MRI score, weight, height, body mass index (BMI), and rate of pulmonary exacerbations. In addition, we determined change in respiratory rate, oxygen saturation, detection of pathogens, and safety and tolerability of inhaled HS, as determined from AEs and serious adverse events (SAEs). Adherence to treatment was assessed by a medication diary completed quarterly by the parents/legal guardians.

Table 1. Demographics and Baseline Characteristics of Study Population

	Isotonic Saline ($n = 21$)	Hypertonic Saline ($n = 21$)
Age, yr	0.26 (0.07)	0.26 (0.08)
Range, yr	0.09–0.35	0.10–0.41
Sex, M/F, *n*	10/11	10/11
CFTR genotype		
F508del/F508del	11 (52.4)	11 (52.4)
F508del/other	8 (38.1)	6 (28.6)
Other/other	2 (9.5)	4 (19.0)
Pancreatic insufficiency	20 (95.2)	17 (81.0)
Anthropometry		
Weight, kg	5.2 (1.1)	5.3 (1.1)
Weight, *z*-score	−0.7 (0.9)	−0.6 (1.1)
Height, cm	59.8 (4.0)	59.8 (5.1)
Height, *z*-score	−0.7 (1.2)	−0.6 (1.1)
BMI, kg/m^2	14.4 (1.6)	14.7 (1.6)
BMI, *z*-score	−1.0 (1.0)	−0.8 (1.1)
Mode of diagnosis*		
Positive CF newborn screening	10 (47.6)	10 (47.6)
Meconium ileus/atresia small intestine	3 (14.3)	6 (28.6)
Prenatal/positive family history	2 (9.5)	4 (19.0)
Failure to thrive	4 (19.0)	1 (4.8)
Respiratory symptoms	2 (9.5)	0 (0.0)
Positive respiratory culture†		
Staphylococcus aureus	6 (28.6)	4 (19.0)
Haemophilus influenzae	0 (0.0)	0 (0.0)
Pseudomonas aeruginosa	0 (0.0)	0 (0.0)
Streptococcus pneumoniae	0 (0.0)	0 (0.0)
Aspergillus species	0 (0.0)	1 (4.8)
Resting respiratory rate, breaths/min	41.3 (11.5)	39.2 (10.7)
Oximetry, %	98.8 (1.1)	98.5 (1.3)
LCI	7.2 (0.7)	7.5 (0.7)
Chest MRI		
Morphology		
Prevalence	95.2 (20/21)	95.2 (20/21)
Score	8.0 (4.0–13.5)	3.0 (2.0–12.0)
Wall thickening/bronchiectasis		
Prevalence	95.2 (20/21)	95.2 (20/21)
Subscore	4.0 (3.0–5.0)	2.0 (2.0–4.5)‡
Mucus plugging		
Prevalence	76.2 (16/21)	57.1 (12/21)
Subscore	2.0 (0.5–3.5)	1.0 (0.0–2.0)
Consolidation		
Prevalence	33.3 (7/21)	28.6 (6/21)
Subscore	0.0 (0.0–1.0)	0.0 (0.0–1.0)
Pleural reaction		
Prevalence	52.4 (11/21)	33.3 (7/21)
Subscore	1.0 (0.0–2.0)	0.0 (0.0–1.0)
Mosaic signal intensity		
Prevalence	47.6 (10/21)	33.3 (7/21)
Subscore	0.0 (0.0–3.5)	0.0 (0.0–2.0)

Definition of abbreviations: BMI = body mass index; CF = cystic fibrosis; *CFTR* = cystic fibrosis transmembrane conductance regulator; LCI = lung clearance index; MRI = magnetic resonance imaging.
MRI scores are presented as median (interquartile range) and prevalence of abnormal MRI scores as percentage (proportion); other data are presented as mean (SD) or number (percent).
*Multiple answers were possible; list reflects first abnormality leading to diagnosis.
†Isolated any time before enrollment.
‡$P < 0.05$ versus isotonic saline.

MBW

MBW was performed with the child under sedation with chloral hydrate (100 mg/kg body weight; maximum dose, 2 g) using the EXHALYZER D system (Eco Medics AG) with 4% sulfur hexafluoride as tracer gas and a face mask as interface with the child lying in a supine position. The LCI was determined from acceptable washout curves as previously described (17, 28, 37–41). Details on quality criteria, data acquisition, and data analysis are provided in the online supplement.

Chest MRI

Chest MRI was performed using a clinical 1.5-T MRI scanner (MAGNETOM Avanto, Siemens Medical AG; Achieva, Philips Healthcare) using T1- and T2-weighted sequences as previously described (17, 22, 23, 29, 42). Images were assessed for morphological abnormalities (MRI morphology score and subscores) by an independent reader (M.O.W.) blinded to clinical and demographic data as well as treatment group using a dedicated MRI score (23, 42). The MRI protocol did not include perfusion studies, because administration of intravenous contrast material was not approved in Germany for children under 12 months of age during the conduct of this study. Details on MRI sequences and scoring are provided in the online supplement.

Sample Size Considerations and Statistical Analysis

No quantitative data on the effect of inhaled HS in infants with CF were available for sample size calculations before the initiation of this trial. On the basis of a previous study on the effect of inhaled HS on LCI in older children with CF with normal spirometry (43) and observational studies providing information on change in LCI in early CF lung disease (44–48), we estimated a mean effect size of inhaled HS on LCI of 0.5 ± 0.5 SD

in our study population of infants with CF. On the basis of these estimates, we expected that a minimum of 17 patients per arm would be required to assess the efficacy of inhaled HS using LCI as an endpoint in our trial. To account for possible dropouts, we aimed to include at least 20 patients per arm, resulting in a total sample size of 40 patients. This estimated sample size was met in our study and has been supported by the results of a pilot study of inhaled HS in older infants and preschool children with CF that became available after the initiation of our trial (14). Data were analyzed under the guidance of a statistician (J.H.) using IBM SPSS Statistics version 22.0 (IBM Corp.) and SAS version 9.4 (SAS Institute) software. Parametric data are presented as mean (±SD), and nonparametric data are presented as median (interquartile range). For categorical data, groups were compared with chi-square test or Fisher's exact test, and for continuous data, groups were compared with unpaired Student's *t* test or one-way analysis of variance with least significant difference Bonferroni *post hoc* test or Wilcoxon signed-rank test. Change in anthropometry was compared between trial arms using time, trial arm/time interaction, and age-specific median as fixed explanatory variables in a hierarchical linear mixed regression model (49, 50). AEs were coded according to MedDRA (Medical Dictionary for Regulatory Activities), grouped by system organ class, and compared between trial arms. The rates of AEs, SAEs, and pulmonary exacerbations were compared between trial arms using a Poisson model allowing for overdispersion, using the baseline radiological findings and trial arm as explanatory variables. The probability of remaining free of a pulmonary exacerbation was estimated using the Kaplan-Meier method and compared between groups by log-rank test. A *P* value less than 0.05 was accepted to indicate statistical significance.

Results

Participant Flow and Baseline Characteristics

A total of 42 infants with CF (mean age, 0.26 ± 0.08 yr; range, 0.09–0.41 yr) were enrolled between June 2012 and November 2015 at five sites and randomly assigned to receive inhaled HS ($n = 21$) or IS ($n = 21$) (Figure 1). Twenty of 21 patients in the HS group and 20 of 21 patients in the IS group completed 12 months of treatment. In the HS group, treatment was discontinued in one patient after 30 weeks owing to unblinding during a pulmonary exacerbation. In the IS group, treatment was discontinued in one patient after 34 weeks because the parents declined further sedation for study procedures (Figure 1). Adherence to inhalation with study solution was high and comparable in both groups, with 71.8–100% in the IS group and 77.2–100% in the HS group according to the medication diary completed quarterly by the parents ($P = 0.863$).

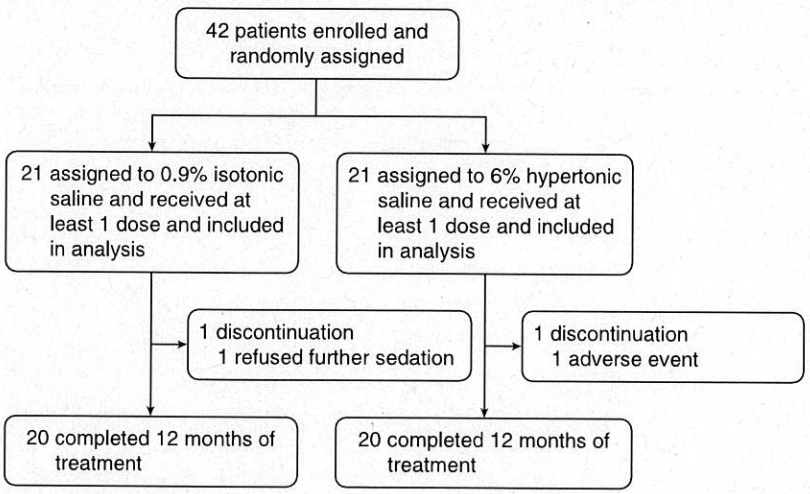

Figure 1. Progress of all participants through the PRESIS (Preventive Inhalation of Hypertonic Saline in Infants with Cystic Fibrosis) trial.

Baseline characteristics, including age, sex, *CFTR* genotype distribution, mode of diagnosis, and upper airway microbiology, were generally similar between the two treatment groups (Tables 1 and E1). In both groups, more than half of the patients were diagnosed preclinically on the basis of CF newborn screening or a positive family history. Mean weight, height, BMI, and respiratory rate were comparable between groups (Table 1). Previous MBW studies in healthy infants reported an upper limit of normal of the LCI of 8 (41, 51, 52). Mean LCI in our study in infants with CF was normal at baseline and did not differ between groups (IS, 7.2 ± 0.7; vs. HS, 7.5 ± 0.7; $P = 0.230$) (Table 1). Despite a normal mean LCI, the LCI was elevated above the upper limit of normal of 8 in a subgroup of patients in both groups (IS, range from 6.3 to 9.0 with three >8; HS, range from 6.0 to 8.7 with five >8; $P = 0.697$) (41, 51, 52). MRI detected morphological abnormalities of the lungs in most patients of both groups (40 of 42) (Table 1). Consistent with previous studies in infants and preschool children, bronchial wall thickening/bronchiectasis was the most prevalent finding, followed by mucus plugging, pleural reaction, mosaic signal intensity, and consolidation (Table 1) (17, 22, 23). The median MRI morphology score and MRI subscores for specific abnormalities were in the lower range of the maximum MRI scores possible and did not differ between groups (Table 1).

Efficacy of Preventive Inhalation of HS in Infants with CF

Infants with CF treated with inhaled HS showed a rapid and sustained decrease in mean LCI from 7.5 ± 0.7 at baseline to 6.9 ± 0.7 at 12 months ($P < 0.01$) (Figure 2 and Table 2). In the IS group, mean LCI was 7.2 ± 0.7 at baseline and remained at 7.2 ± 0.8 after 12 months of treatment ($P = 0.793$) (Figure 2 and Table 2). The change in LCI from baseline to 12 months was significantly larger in infants with CF treated with inhaled HS than in those treated with IS (HS, −0.6 ± 0.8; vs. IS, −0.1 ± 0.9; $P < 0.05$) (Figure 2 and Table 2).

Chest MRI demonstrated that the prevalence of morphological abnormalities remained high and the median MRI morphology score tended to increase slightly from baseline to 12 months in the HS group (1.9 ± 5.2; $P = 0.117$) and the IS

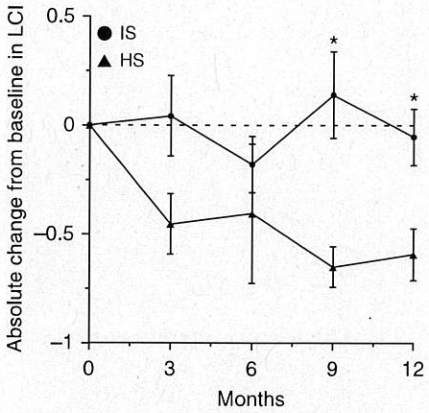

Figure 2. Effect of preventive inhalation of hypertonic saline (HS) on lung clearance index (LCI). Absolute change from baseline in LCI in infants with cystic fibrosis treated with inhaled HS (triangles) or isotonic saline (IS; circles). Decrease in LCI indicates improvement. Error bars are 95% confidence intervals. Dashed line indicates baseline level. *$P < 0.05$ between groups compared with baseline.

group (0.5 ± 6.7; $P = 0.740$) (Figure 3 and Table 2). This change in MRI morphology scores did not differ between the HS and IS groups ($P = 0.462$). Overall, MRI subscores for bronchial wall thickening/bronchiectasis, mucus plugging, and mosaic signal intensity tended to increase, whereas subscores for consolidation and pleural reaction tended to decrease, from baseline to 12 months in both groups (Table 2). These changes in MRI subscores did not differ between the HS and IS groups (Table 2).

Anthropometric measurements showed that weight gain was improved in infants with CF treated with inhaled HS compared with IS ($P < 0.05$) (Figure 4A and Table 2). Gains in height ($P = 0.175$) and BMI ($P = 0.919$), as well as changes in *z*-scores for weight, height, and BMI, tended to be increased in the HS group, but this trend did not reach statistical significance (Figures 4B and 4C and Table 2). Furthermore, no differences were detected between treatment groups in the change in oxygen saturation ($P = 0.602$) or respiratory rate ($P = 0.443$) (Table 2).

During the 12-month study period, 6 patients in the HS group and 9 patients in the IS group experienced a total of 21 versus 23 pulmonary exacerbations, resulting in a pulmonary exacerbation rate of 1.1 (95% confidence interval, 0.0–2.1) versus 1.2 (95% confidence interval, 0.4–1.9) per person-year for infants with CF randomized to receive HS versus IS ($P = 0.862$). Figure 4 displays the probability of remaining free of a pulmonary exacerbation in both treatment arms. Microbiology of nasal and pharyngeal samples showed that the prevalence and acquisition of upper airway infection with proinflammatory pathogens such as *Staphylococcus aureus*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, and *Aspergillus* species (53) were low in infants with CF and did not differ between treatment groups (Table E2).

Safety of Preventive Inhalation of HS in Infants with CF

Inhalation with HS and IS was generally well tolerated in infants with CF. AEs were reported in all patients from both groups and were mostly of mild (81.3%) or moderate (12.9%) severity, and 5.7% of AEs were rated as severe. The proportion of patients with reported AEs was similar in the HS and IS groups, with cough being the most common AE (Table 3). SAEs were reported in six (28.6%) patients in the HS group and seven (33.3%) patients in the IS group. A detailed summary of all AEs with an incidence greater than 5% in any treatment group and all SAEs reported during this study is provided in Table 3. In all cases, the rating as an SAE was due to necessity of hospitalization,

Table 2. Effects of Preventive Inhalation of Hypertonic Saline versus Isotonic Saline in Infants with Cystic Fibrosis

	Isotonic Saline ($n = 20$)	Hypertonic Saline ($n = 20$)	Treatment Difference vs. Isotonic Saline
Absolute change from baseline in			
LCI at Month 12	−0.1 (−0.5 to 0.4)	−0.6 (−1.0 to 0.2)*	−0.5 (−1.1 to 0.0)†
MRI morphology score at Month 12	0.5 (−3.0 to 3.6)	1.9 (−0.5 to 4.3)	1.4 (−2.4 to 5.3)
MRI wall thickening/bronchiectasis subscore at Month 12	0.8 (0.3 to 1.4)*	1.6 (0.9 to 2.3)‡	0.8 (−0.1 to 1.6)
MRI mucus plugging subscore at Month 12	0.3 (−0.6 to 1.3)	0.8 (0.0 to 1.5)†	0.5 (−0.7 to 1.6)
MRI consolidation subscore at Month 12	−0.5 (−1.0 to 0.0)	−0.6 (−1.1 to 0.0)	−0.1 (−0.8 to 0.6)
MRI pleural reaction subscore at Month 12	−0.2 (−0.7 to 0.3)	−0.2 (−0.6 to 0.3)	0.1 (−0.6 to 0.7)
MRI mosaic signal intensity subscore at Month 12	0.0 (−1.8 to 1.5)	0.3 (−0.7 to 1.2)	0.3 (−1.5 to 2.0)
Weight through Month 12, kg	4.3 (4.0 to 4.7)‡	4.8 (4.1 to 5.5)‡	0.5 (−0.3 to 1.2)†
Weight *z*-score through Month 12	−0.1 (−0.4 to 0.2)	0.3 (−0.4 to 0.9)	0.3 (−0.3 to 0.1)
Height through Month 12, cm	18.5 (17.4 to 19.5)‡	20.0 (17.3 to 22.6)‡	1.5 (−1.3 to 4.3)
Height *z*-score through Month 12	0.3 (−0.1 to 0.6)	0.9 (−0.3 to 2.1)	0.6 (−0.6 to 1.8)
BMI through Month 12, kg/m^2	1.1 (0.3 to 1.9)*	1.2 (0.5 to 1.9)*	0.2 (−0.9 to 1.2)
BMI *z*-score through Month 12	0.2 (−0.5 to 0.8)	0.3 (−0.2 to 0.8)	0.1 (−0.7 to 0.9)
Resting respiratory rate at Month 12, breaths/min	−9.9 (−15.7 to −3.9)‡	−10.3 (−16.6 to −4.0)*	−0.4 (−8.5 to 7.7)
Oximetry at Month 12, %	−1.6 (−2.4 to −0.9)‡	−1.3 (−2.3 to −0.2)†	0.4 (−0.9 to 1.7)

Definition of abbreviations: BMI = body mass index; LCI = lung clearance index; MRI = magnetic resonance imaging.
Values are mean (95% confidence interval).
*$P < 0.01$ within group or versus isotonic saline.
†$P < 0.05$ within group or versus isotonic saline.
‡$P < 0.001$ within group or versus isotonic saline.

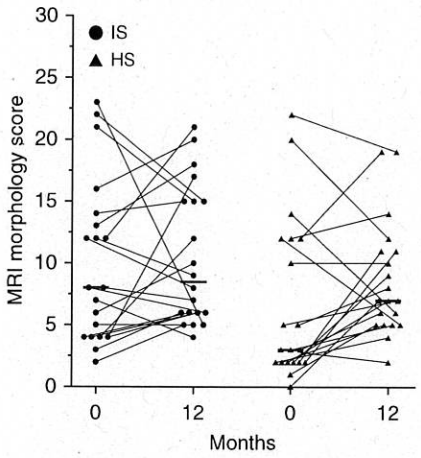

Figure 3. Effect of preventive inhalation of hypertonic saline (HS) on magnetic resonance imaging (MRI) morphology score. MRI morphology scores of individual infants with cystic fibrosis at baseline and after 12 months of treatment with inhaled HS (triangles) or isotonic saline (IS; circles). Horizontal lines represent group median.

and none was rated as related to study treatment.

Discussion

Randomized controlled trials (RCTs) are considered the gold standard for the assessment of safety and efficacy of therapeutic interventions, but none have so far been conducted to evaluate preventive treatment strategies in young infants with CF, in part owing to the lack of quantitative endpoints in this challenging age group. PRESIS is the first RCT testing feasibility, safety, and initial efficacy of preventive treatment of lung disease initiated in the first months of life in infants with CF using LCI and MRI as quantitative outcome measures of early lung disease. First, this study demonstrates that RCTs starting in early infancy that include regular treatment with inhalation solutions and repeated LCI and MRI measurements over a period of 12 months are feasible and well accepted by the parents (Figure 1). Second, this study shows that preventive inhalation of HS from early infancy was safe and well tolerated (Tables 3 and E2) and indicates that this early intervention had beneficial effects on lung function and thriving in infants with CF (Figures 2 and 4). Third, this study suggests that LCI is more sensitive to detect response to preventive treatment of CF lung disease than MRI morphology scores or clinical outcomes such as pulmonary exacerbations in infants with CF (Figures 2–4). The results of this initial RCT support the concept of preventive therapy and will inform future trials in young infants that are warranted to leverage the window of opportunity provided by CF newborn screening and to determine the most efficacious therapies to delay or even prevent irreversible lung damage in patients with CF.

Inhaled HS was previously tested in the ISIS trial (Infant Study of Inhaled Saline in Cystic Fibrosis) in older infants and preschool children with CF (4 to 60 mo of age; mean age at baseline, 2.2 yr) who received inhaled HS versus IS for 48 weeks (34). The ISIS trial did not show differences between treatment groups for the rate of pulmonary exacerbations as a primary endpoint, nor did it demonstrate differences for secondary endpoints, including height, weight, respiratory rate, or oxygen saturation (34). In the PRESIS trial, the age range and mean age were substantially lower (0 to 4 mo of age; mean age at baseline, 0.28 yr) (Table 1). Similar to the ISIS trial, the pulmonary exacerbation rate, respiratory rate, or oxygen saturation did not differ between treatment groups in our study (Figure 4 and Table 2). However, unlike the ISIS trial, the absolute change in weight was significantly increased (500 g higher mean weight at Month 12), and absolute change in height tended to be greater (1.5 cm higher mean height at Month 12), in infants with CF treated with inhaled HS versus IS (Figure 4). This observation is reminiscent of previous studies in mice with CF-like lung disease showing beneficial effects of inhaled HS on growth (8). Because thriving is a global parameter of well-being in infants, we speculate that this finding may reflect therapeutic benefits of preventive HS inhalation in early CF lung disease. Of note, it is an established phenomenon that when children with chronic diseases associated with failure to thrive are started on an effective therapy, they typically gain weight before they start showing catch-up growth in height (54–59). We therefore speculate that this known delay in catch-up growth in height versus weight may explain why weight diverged from 3 months onward, whereas height started diverging after 9 months, between the two treatment arms (Figure 4). This difference between our study and the ISIS trial suggests that there may be a unique window of opportunity for preventive treatment of CF lung disease initiated in the first months of life. However, future RCTs comparing benefits of therapeutic interventions initiated in different age groups from early infancy to preschool ages are required to substantiate this hypothesis. Similar to results from ISIS in older infants and preschool children, detection of proinflammatory pathogens was rare and did not differ between treatment groups (34). Also consistent with the ISIS study, adherence to inhalation therapy was high (70–100%), and dropout rates were low, with only 1 of 21 patients discontinuing the study early in each group and the remainder completing 12 months of treatment and all endpoint measurements (Figure 1 and Table 2). AE profiles in our study were also consistent with those previously observed in older children in the ISIS trial, with the most common treatment-emergent AEs being cough, rhinorrhea, obstructive bronchitis, and nasal congestion with frequencies that did not differ between groups (Table 3). These data support preventive treatment with inhaled HS as safe and feasible from early infancy and suggest that more sensitive outcome measures are needed to assess therapeutic benefits on early lung disease in young infants with CF.

In this context, the LCI has previously been shown to detect abnormal ventilation in infants and progression of lung disease in preschool children with CF (13, 15, 41, 60). Furthermore, a single-center substudy of the ISIS trial demonstrated that, on average, LCI decreased in the HS group and remained stable in the IS group, with an overall significant treatment effect observed for the LCI z-score in older infants and preschool children with CF (14). On the basis of these findings, we included longitudinal measurements of LCI as an outcome measure in the PRESIS trial. In young infants with CF included in our study, LCI at baseline was normal in both treatment groups. Initiation of inhaled HS in the first four months of life resulted in a rapid and sustained decrease in LCI in the range of 0.5 units that was already observed at the first measurement after 3 months of HS inhalation and persisted throughout the study, whereas LCI in the IS group remained unchanged during the course of the study (Figure 2 and Table 2). Notably,

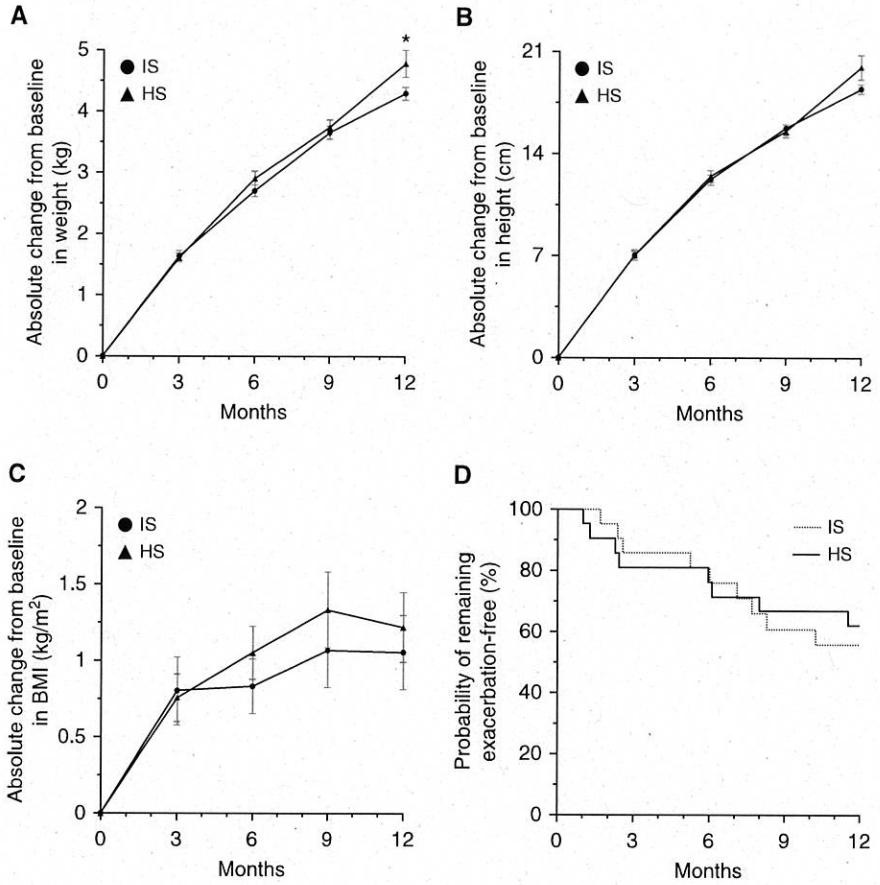

Figure 4. Effect of preventive inhalation of hypertonic saline (HS) on anthropometry and pulmonary exacerbations in infants with cystic fibrosis. (*A–C*) Absolute change from baseline in (*A*) weight, (*B*) height, and (*C*) body mass index (BMI) at 3, 6, 9, and 12 months of treatment with inhaled HS (triangles) or isotonic saline (IS; circles). Data are shown as mean with 95% confidence intervals. (*D*) Kaplan-Meier plot of time to first pulmonary exacerbation, by treatment group. Probability of remaining free of pulmonary exacerbation in the HS group (continuous line) and in the IS group (dotted line) ($P = 0.78$). *$P < 0.05$ between groups from baseline through Month 12.

the change in LCI from baseline was significantly different between the HS and IS groups from 9 months onward, supporting therapeutic benefits of inhaled HS for lung function in the first year of life. Of note, this pattern of improvement in LCI observed with inhaled HS in our study in young infants with CF (<4 mo) differs from the pattern observed in a subgroup of 10 older infants with CF (aged 4 to 16 mo) who were included in the ISIS MBW pilot study that showed no change in LCI in the HS group but worsening in the IS group (14). On the basis of LCI trajectories in healthy infants, as well as infants with CF during the first years of life (52, 61), we speculate that this difference is related to the timing of the intervention. The decrease in LCI observed in our study in infants of the HS group was also observed in healthy infants (52). Furthermore, in longitudinal MBW studies in the AREST CF (Australian Respiratory Early Surveillance Team for Cystic Fibrosis) cohort, the LCI dropped (improved) by approximately 0.5 units between ages 3 months and 2 years in infants with CF who were never infected, whereas LCI did not change substantially in infants ever infected with proinflammatory pathogens (61). We speculate that when viewed in combination with LCI trajectories in healthy infants, the AREST CF study, and the ISIS pilot study (14, 52, 61), early initiation of inhaled HS in the first months of life may prevent or delay the onset of early CF airway disease and thus may result in a physiological drop in LCI during infancy, whereas initiation of HS in older infants who may already have early airway obstruction may prevent or delay early disease progression but may not reverse early CF lung disease. Collectively, these data support LCI as a sensitive outcome measure in interventional studies testing effects of preventive therapies for CF from early infancy.

In previous cross-sectional studies, we demonstrated that chest MRI is sensitive to detect abnormalities in lung structure and perfusion in early CF lung disease (17, 22). These studies identified bronchial wall thickening/bronchiectasis, mucus plugging, and abnormal lung perfusion as the most prevalent changes in clinically stable preschool children with CF (17, 22). The present study shows, for the first time, to our knowledge, that MRI-defined morphological abnormalities such as bronchial wall thickening and mucus plugging are already prevalent in the first four months of life (Figure 3 and Tables 1 and 2). However, the MRI morphology score did not differ between the HS and IS groups after 12 months of inhalation therapy. We speculate that several reasons may explain why MRI was less sensitive than LCI to detect response to treatment in the PRESIS trial. First, we were not able to perform MRI perfusion studies, because the use of contrast material was not approved in Germany for infants under the age of 12 months at the time of the study. Our previous studies indicated that abnormal lung perfusion due to hypoxic pulmonary vasoconstriction in areas of abnormal ventilation may be a sensitive surrogate parameter for small airway mucus plugging (17, 22). Furthermore, contrast material enhanced the signal of morphological abnormalities. Therefore, the lack of perfusion studies may have reduced the sensitivity of MRI in the present study. Second, whereas the current semiquantitative MRI morphology score was sensitive to detect response to antibiotic therapy for acute pulmonary exacerbation in older children with CF, this three-grade scoring system may still be too coarse to detect more moderate changes in clinically stable infants with CF, because these changes may not exceed the limit of 50% of lobar involvement that differentiates scoring between grades 1 and 2 (17, 22, 42). Thus, additional studies will be required to determine the role of MRI perfusion studies

Table 3. Treatment-Emergent Adverse Events

	Isotonic Saline ($n = 21$)		Hypertonic Saline ($n = 21$)	
	Affected Infants	Events	Affected Infants	Events
All adverse events	21 (100)	240 (100)	21 (100)	219 (100)
All adverse events with incidence >5% in any treatment group				
Infection of upper respiratory tract without fever	17 (81.0)	52 (21.7)	16 (76.2)	41 (18.7)
Rhinorrhea	10 (47.6)	17 (7.1)	17 (81.0)	31 (14.2)
Cough	12 (57.1)	34 (14.2)	14 (66.7)	25 (11.4)
Infection of upper and lower respiratory tract without fever	7 (33.3)	9 (3.8)	9 (42.9)	13 (5.9)
Infection of upper respiratory tract with fever	8 (38.1)	13 (5.4)	7 (33.3)	9 (4.1)
Infection of upper and lower respiratory tract with fever	7 (33.3)	8 (3.3)	6 (28.6)	10 (4.6)
Abdominal distention/flatulence	4 (19.0)	4 (1.7)	8 (38.1)	8 (3.7)
Fever	6 (28.6)	7 (2.9)	5 (23.8)	10 (4.6)
Diarrhea	6 (28.6)	7 (2.9)	5 (23.8)	9 (4.1)
Infection of lower respiratory tract without fever	6 (28.6)	7 (2.9)	3 (14.3)	10 (4.6)
Conjunctivitis	5 (23.8)	9 (3.8)	2 (9.5)	3 (1.4)
Gastroenteritis	3 (14.3)	4 (1.7)	4 (19.0)	7 (3.2)
Otitis media	4 (19.0)	7 (2.9)	3 (14.3)	3 (1.4)
Obstructive bronchitis	4 (19.0)	9 (3.8)	1 (4.8)	1 (0.5)
Abdominal pain	3 (14.3)	5 (2.1)	2 (9.5)	2 (0.9)
Constipation	3 (14.3)	4 (1.7)	2 (9.5)	3 (1.4)
Dyspnea	2 (9.5)	4 (1.7)	3 (14.3)	3 (1.4)
First detection of *Pseudomonas aeruginosa*	2 (9.5)	2 (0.8)	3 (14.3)	3 (1.4)
Nasal congestion	2 (9.5)	2 (0.8)	3 (14.3)	3 (1.4)
Candida diaper rash	1 (4.8)	1 (0.4)	3 (14.3)	3 (1.4)
Exanthema subitum	2 (9.5)	2 (0.8)	2 (9.5)	2 (0.9)
Iron deficiency anemia	3 (14.3)	3 (1.3)	1 (4.8)	1 (0.5)
Salt loss syndrome	2 (9.5)	3 (1.3)	1 (4.8)	1 (0.5)
Urticaria	2 (9.5)	2 (0.8)	1 (4.8)	1 (0.5)
Bronchopulmonary secretion	1 (4.8)	1 (0.4)	2 (9.5)	2 (0.9)
Vomiting or emesis	1 (4.8)	2 (0.8)	2 (9.5)	2 (0.9)
Poor growth	2 (9.5)	3 (1.3)	0 (0.0)	0 (0.0)
Refusal to eat or drink	0 (0.0)	0 (0.0)	2 (9.5)	2 (0.9)
Steatorrhea	2 (9.5)	2 (0.8)	0 (0.0)	0 (0.0)
Tympanic effusion	2 (9.5)	2 (0.8)	0 (0.0)	0 (0.0)
All serious adverse events	7 (33.3)	12 (100)	6 (28.6)	21 (100)
First detection of *P. aeruginosa*	2 (9.5)	2 (16.7)	3 (14.3)	3 (14.3)
Obstructive bronchitis	2 (9.5)	3 (25.0)	1 (4.8)	1 (4.8)
Salt loss syndrome	2 (9.5)	2 (16.7)	1 (4.8)	1 (4.8)
Infection of upper and lower respiratory tract with or without fever	0 (0.0)	0 (0.0)	2 (9.5)	7 (33.3)
Gastroenteritis	0 (0.0)	0 (0.0)	2 (9.5)	4 (19.0)
Infection of lower respiratory tract without fever	0 (0.0)	0 (0.0)	2 (9.5)	2 (9.5)
Brain concussion	1 (4.8)	1 (8.3)	0 (0.0)	0 (0.0)
Cholangitis	0 (0.0)	0 (0.0)	1 (4.8)	1 (4.8)
Refusal to eat or drink	0 (0.0)	0 (0.0)	1 (4.8)	1 (4.8)
Hypoxemia	0 (0.0)	0 (0.0)	1 (4.8)	1 (4.8)
Partial bowel obstruction	1 (4.8)	1 (8.3)	0 (0.0)	0 (0.0)
Poor growth	1 (4.8)	1 (8.3)	0 (0.0)	0 (0.0)
First detection of *Pseudomonas oryzihabitans*	1 (4.8)	1 (8.3)	0 (0.0)	0 (0.0)
Pyelonephritis	1 (4.8)	1 (8.3)	0 (0.0)	0 (0.0)

Values are *n* (%).

and the granularity of the scoring system regarding the sensitivity of MRI as an outcome measure in early intervention trials in infants with CF. These studies should also include longitudinal assessment of clinically stable infants and preschool children with CF to establish the trajectories and variability of abnormalities detected by chest MRI in early CF lung disease over time.

This study has limitations. First, IS is an active comparator rather than true placebo that may have therapeutic benefits itself, which may lead to underestimation of treatment effects of inhaled HS in our study. However, the use of an active comparator was necessary for blinding of inhalation solutions in this RCT. Second, because this was the first RCT conducted in young infants with CF, it was not possible to perform formal sample size calculations to power the study for the outcome measures used. However, our estimated sample size for LCI as an outcome measure was based

on a previous HS study in older children with CF and observational studies on changes in LCI in infants and young children with CF (43–48) and is supported by the results of a pilot study of inhaled HS in older infants and preschool children with CF (14), as well as the data obtained in our study. Furthermore, our data provide estimates for sample size calculations for the endpoints included in our study for the design of future trials in young infants with CF. Third, our trial was underpowered to determine effects of inhaled HS on pulmonary exacerbations. Moreover, the applicability of current definitions of pulmonary exacerbations, including the modified Fuchs definition used in our study, as well as the EPIC trial (Comparison of Two Treatment Regimens to Reduce PA Infection in Children with Cystic Fibrosis) definition put forward for preschool- and school-aged children, remains limited for studies in infants with CF owing to inclusion of criteria such as spirometry and hemoptysis (62, 63). Fourth, the duration of this initial efficacy trial was limited to 12 months, and longer observation times will be necessary to substantiate therapeutic benefits of preventive treatment with inhaled HS and other emerging therapies including CFTR modulators in early CF lung disease (64). In this context, it will be of interest to determine to what extent preventive therapies can delay the onset and long-term progression of irreversible structural lung damage in patients with CF.

In summary, this study shows, for the first time, to our knowledge, that RCTs including LCI and MRI as quantitative outcome measures of early lung disease are feasible in young infants with CF. Furthermore, this initial RCT supports preventive treatment with inhaled HS starting in the first months of life as being safe and having therapeutic benefits for lung function and thriving of infants with CF. These data support the conduct of future RCTs to determine safety and efficacy of preventive treatment strategies that have the potential to delay or prevent progressive lung damage in patients with CF. ■

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