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Inhaled Hypertonic Saline in Infants and Children Younger Than 6 Years With Cystic Fibrosis

The ISIS Randomized Controlled Trial

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THE HALLMARK FEATURES OF cystic fibrosis (CF) lung disease include airway infection, inflammation, obstruction, and structural lung damage. These abnormalities begin in infancy, often prior to the onset of symptoms, and progress over the first years of life.¹⁻⁴ Thus, early initiation of effective chronic therapies, an opportunity afforded by newborn screening, could potentially delay or prevent progression of CF lung disease. There are no clinical trials of chronic nonantibiotic maintenance pulmonary therapies in infants and preschool-aged children with CF, even though this is the population with the greatest potential for long-term benefit.

Dysfunctional ion transport leads to reduced airway surface liquid volume in CF and reduction in mucociliary clearance.⁵ Retained mucus serves as a nidus for chronic infection and exaggerated, sustained neutrophilic inflammation, resulting in progressive air-

For editorial comment see p 2316.

Context Inhaled hypertonic saline is recommended as therapy for patients 6 years or older with cystic fibrosis (CF), but its efficacy has never been evaluated in patients younger than 6 years with CF.

Objective To determine if hypertonic saline reduces the rate of protocol-defined pulmonary exacerbations in patients younger than 6 years with CF.

Design, Setting, and Participants The Infant Study of Inhaled Saline in Cystic Fibrosis (ISIS), a multicenter, randomized, double-blind, placebo-controlled trial conducted from April 2009 to October 2011 at 30 CF care centers in the United States and Canada. Participants were aged 4 to 60 months and had an established diagnosis of CF. A total of 344 patients were assessed for eligibility; 321 participants were randomized; 29 (9%) withdrew prematurely.

Intervention The active treatment group (n=158) received 7% hypertonic saline and the control group (n=163) received 0.9% isotonic saline, nebulized twice daily for 48 weeks. Both groups received albuterol or levalbuterol prior to each study drug dose.

Main Outcome Measures Rate during the 48-week treatment period of protocol-defined pulmonary exacerbations treated with oral, inhaled, or intravenous antibiotics.

Results The mean pulmonary exacerbation rate (events per person-year) was 2.3 (95% CI, 2.0-2.5) in the active treatment group and 2.3 (95% CI, 2.1-2.6) in the control group; the adjusted rate ratio was 0.98 (95% CI, 0.84-1.15). Among participants with pulmonary exacerbations, the mean number of total antibiotic treatment days for a pulmonary exacerbation was 60 (95% CI, 49-70) in the active treatment group and 52 (95% CI, 43-61) in the control group. There was no significant difference in secondary end points including height, weight, respiratory rate, oxygen saturation, cough, or respiratory symptom scores. Infant pulmonary function testing performed as an exploratory outcome in a subgroup (n=73, with acceptable measurements at 2 visits in 45 participants) did not demonstrate significant differences between groups except for the mean change in forced expiratory volume in 0.5 seconds, which was 38 mL (95% CI, 1-76) greater in the active treatment group. Adherence determined by returned study drug ampoules was at least 75% in each group. Adverse event profiles were also similar, with the most common adverse event of moderate or severe severity in each group being cough (39% of active treatment group, 38% of control group).

Conclusion Among infants and children younger than 6 years with cystic fibrosis, the use of inhaled hypertonic saline compared with isotonic saline did not reduce the rate of pulmonary exacerbations over the course of 48 weeks of treatment.

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way obstruction and bronchiectasis.⁶ Hypertonic saline has been demonstrated to increase airway surface liquid in bronchial epithelial cells in vitro

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and to improve defective mucociliary clearance in patients with CF.^{7,8}

A clinical trial in older children and adults with CF demonstrated modest effects on lung function and a significant decrease in pulmonary exacerbations,⁹ resulting in widespread use of this therapy in patients older than 6 years. Given its mechanism of action, hypertonic saline is an attractive agent for early interventions. Although 3 short-term safety studies of 7% hypertonic saline have been conducted in patients younger than 6 years with CF,¹⁰⁻¹² its efficacy and long-term safety in this population have not been evaluated. Given that hypertonic saline use increased from 6% to 19% among US children with CF aged 2 to 5 years during the enrollment period (2007-2010),¹³ there was a window of opportunity to conduct a clinical trial before use of hypertonic saline became widespread in this age range.

We conducted a randomized controlled trial of 7% hypertonic saline use among children younger than 6 years with CF, to our knowledge the first multicenter clinical trial of a nonantimicrobial chronic CF therapy in this age range. We hypothesized that hypertonic saline would decrease the rate of pulmonary exacerbations and be safe in young children with CF.

METHODS

Overview

The Infant Study of Inhaled Saline in Cystic Fibrosis (ISIS) was a 30-center, randomized, parallel-group, double-blind, controlled trial of 7% hypertonic saline (active drug) vs 0.9% isotonic saline (control agent) inhaled twice daily for 48 weeks among children with an established diagnosis of CF and aged 4 to 60 months at enrollment. The trial was monitored by a data and safety monitoring board established by the National Heart, Lung, and Blood Institute. Institutional review board approval and written informed consent from parents or guardians were obtained at each participating center. Participant inclusion and exclusion eligibility criteria are detailed in the

eMethods available at <http://www.jama.com>. The upper age limit of 60 months was chosen because previous clinical trials of hypertonic saline have enrolled children 6 years or older.⁹

Randomization, Blinding, and Treatment Regimen

Participants were randomized 1:1 to receive 7% hypertonic saline vs 0.9% isotonic saline, based on random permuted blocks stratified by age (4 to 29 months, 30 to 60 months) and site, via a secure website. Participants, their families, clinicians, and research personnel were blinded to treatment assignment. Seven-percent hypertonic saline (Hyper-Sal; PARI Respiratory Equipment) and 0.9% isotonic saline were supplied by Catalent Pharma Solutions as identically packaged 4-mL blow-fill-seal plastic ampoules. Each participant was supplied with a Proneb Ultra compressor with a Sprint Jr nebulizer equipped with a Baby face mask or mouthpiece (PARI Respiratory Equipment). Further details of hypertonic saline administration and other therapies are reported in the eMethods.

Clinical Evaluations

Study visits occurred at enrollment/randomization and 4, 12, 24, 36, and 48 weeks after randomization. At the enrollment visit, after pretreatment with albuterol or levalbuterol, all participants were evaluated for intolerance to a test dose of 7% hypertonic saline according to predefined criteria¹⁰ (eMethods). Participants who tolerated the test dose were randomized.

At the enrollment visit, medical history and demographic characteristics were recorded. Race/ethnicity information was obtained from parents or guardians according to predefined categories. This information was obtained to assess the representativeness of the study cohort relative to the general US population of patients with CF. At all visits, a physical examination was performed and information was recorded on interim history (including respiratory culture results), medications (including courses of antibiotics),

cough score, adverse events, and interim hospitalizations. Families also were contacted 2 weeks after enrollment and then quarterly between subsequent study visits to assess tolerability and adverse events. Parents or guardians completed a parent questionnaire weekly and the Cystic Fibrosis Questionnaire-Revised (CFQ-R)¹⁴ and the Treatment Adherence Questionnaire¹⁴ quarterly. Further details regarding clinical evaluations are reported in the eMethods.

Primary and Secondary Outcomes

The primary outcome was the rate of pulmonary exacerbations (events per person-year), defined as treatment with oral, inhaled, or intravenous antibiotics for 1 or more prespecified signs and symptoms within the period 3 days prior to antibiotic start date through antibiotic stop date (which could include 1 or more antibiotics prescribed for the same pulmonary exacerbation). The prespecified signs and symptoms included (1) oxygen saturation less than 90% on room air or a 5% or greater decline from previous baseline; (2) new lobar infiltrate(s) or atelectasis on chest radiograph; (3) hemoptysis; (4) increased work of breathing or respiratory rate; (5) increased cough; (6) working harder than usual to breathe during physical activity; (7) increased chest congestion or change in sputum; (8) new or increased adventitious sounds on lung examination; and (9) weight loss of 5% or more of body weight or decrease across 1 major percentile in weight percentile for age in the past 6 months.

Additional efficacy measures included change in weight, height, resting respiratory rate, room air oxygen saturation, and CFQ-R respiratory domain score¹⁴ over the course of the 48-week treatment period and parent report of daytime cough evaluated at the week 48 visit.¹⁵ Additional evaluations of pulmonary exacerbations included time to first pulmonary exacerbation as well as number of courses and total number of treatment days with oral, inhaled, and/or intravenous anti-

biotics for a pulmonary exacerbation or for any indication.

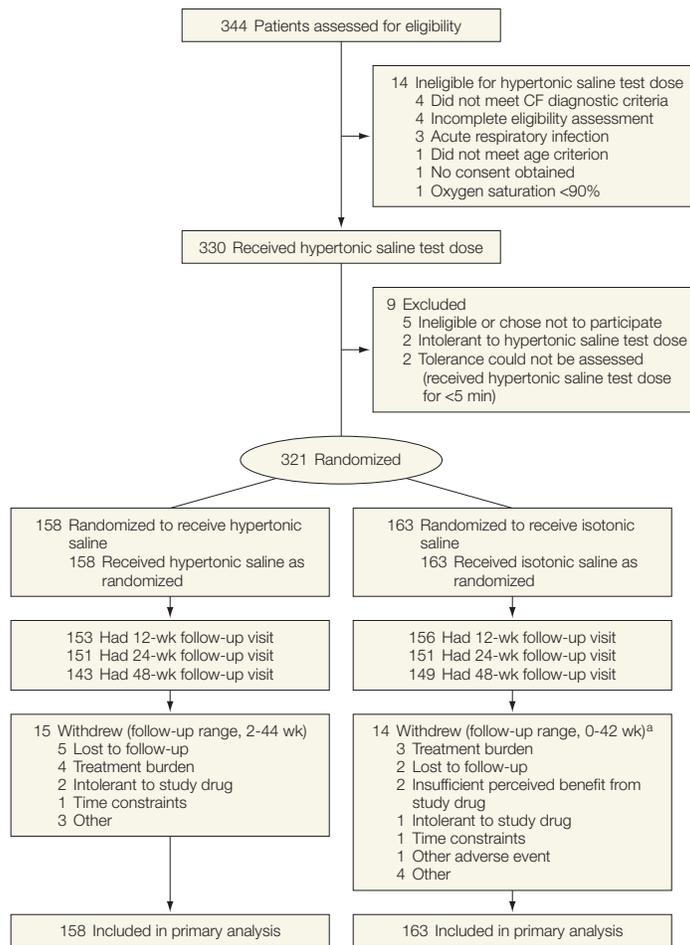
Safety outcomes included the rate of intolerance to the test dose of hypertonic saline at enrollment, adverse events and withdrawal rates, and treatment-emergent respiratory cultures positive for CF pathogens detected through clinical cultures performed at each site's microbiology laboratory. All serious adverse events were reviewed by the medical monitor and the data and safety monitoring board. Adherence to treatment was assessed by (1) the number of used study drug vials returned, (2) the Treatment Adherence Questionnaire¹⁴ completed quarterly, and (3) the weekly parent questionnaire.

Infant Pulmonary Function Testing Substudy

In a substudy at selected sites, infant pulmonary function tests were performed as an exploratory end point in participants 4 months or older and younger than 16 months at enrollment. Exclusion criteria are included in the eMethods. This substudy was performed at 15 sites certified to perform research-quality infant lung function testing. Participants underwent pulmonary function testing under sedation a minimum of 1 day and a maximum of 30 days after the enrollment visit and at the 48-week visit. For these participants, randomization was conducted and study drug was dispensed at the pulmonary function test visit rather than at the enrollment visit.

Pulmonary function assessments included functional residual capacity by body plethysmography^{16,17} and measurements of forced expiratory flows (forced expiratory flow at 75% of vital capacity and mid-maximal forced expiratory flow) and volumes (forced expiratory volume in 0.5 seconds [FEV_{0.5}] and forced vital capacity) by the raised-volume rapid thoracoabdominal compression technique.¹⁸ Additional lung volumes (residual volume and total lung capacity) were also calculated.¹⁷ Sites transferred all infant pulmonary function data to the Therapeutics Development Network Infant Pulmonary Function Resource Cen-

Figure 1. Profile of the Infant Study of Inhaled Saline in Cystic Fibrosis (ISIS) Randomized Trial



CF indicates cystic fibrosis.
^aOne participant had one-half day of follow-up.

ter at the University of North Carolina, which selected acceptable measurements according to published guidelines^{4,16,18} and provided quality control feedback to sites.

Sample Size Considerations and Statistical Analysis

For the design, we assumed the rate of pulmonary exacerbations in the isotonic saline (control) group would be 2.22 events/y, based on data from a recent large US observational study of children aged 0 to 6 years.²⁹ Using this control rate and an O'Brien-Fleming boundary function¹⁹ for early stopping with a .05-level 2-sided hypothesis test, we calculated that a sample size

of 150 per group would provide 80% power to detect a rate ratio (hypertonic saline to isotonic saline) less than or equal to 0.80 (or a relative reduction of at least 20%).

The primary outcome, pulmonary exacerbation rate, was compared between groups according to intent-to-treat principles using a Poisson log-linear regression model with the log of observation time as an offset. Observation time was defined as time since randomization to last in-clinic visit or follow-up telephone call. (One participant's observation time was defined to be one-half day, because he or she did not have an in-clinic visit or follow-up telephone call after randomization.) The

rate ratio was also analyzed with adjustment for age category and site.

The number of treatment days with oral, inhaled, or intravenous antibiotics was compared using a linear regression

model of the log of treatment days for participants with greater than 0 treatment days, and estimates were transformed back to the original scale. The probability of remaining free of a pul-

monary exacerbation was estimated using the Kaplan-Meier method and the hazard ratio for first pulmonary exacerbation with a proportional hazards regression model. The difference in mean change (week 48–randomization) in height, weight, respiratory rate, oxygen saturation, and CFQ-R respiratory domain score was estimated using a linear regression model with and without adjustment for age category, site, and baseline measure. The proportion of parental report of daytime cough at week 48 was estimated using a linear regression model with and without adjustment for age category and site.

Mixed-effects analysis was also used to model repeated measurements of height percentile, weight percentile, respiratory rate, and oxygen saturation from all visits. Among participants in the infant pulmonary function substudy, the differences between groups in mean change in lung function indices were evaluated using linear regression, with adjustment for baseline lung function, height, weight, age, and sex. Differences in proportions were evaluated by a normal approximation to the binomial distribution. A 2-sided significance level of $P < .05$ was used without adjustment for multiple comparisons.

Analyses were conducted by 2 investigators (L.B., R.K.) using R version 2.13.0 at the University of Washington Collaborative Health Studies Coordinating Center, Seattle.

RESULTS

Participant Flow and Baseline Characteristics

A total of 321 participants were randomized between April 2009 and October 2010 at 30 sites, 158 to the hypertonic saline (active treatment) group and 163 to the isotonic saline (control) group (FIGURE 1); these individuals comprised the intent-to-treat population. Fifteen participants (9%) withdrew from the hypertonic saline group and 14 (7%) from the isotonic saline group. Mean duration of study participation was 47 (95% CI, 45-48) weeks in the hypertonic saline group and 46 (95% CI, 45-48) weeks in the isotonic saline group. The baseline characteris-

Table 1. Baseline Characteristics of Participants by Treatment Group

Characteristic	Hypertonic Saline (n = 158)	Isotonic Saline (n = 163)
Age, mean (SD), y	2.2 (1.4)	2.3 (1.5)
Age category, mo		
<30	95 (60.1)	96 (58.9)
≥30	63 (39.9)	67 (41.1)
Male, No. (%)	84 (53)	92 (56)
CFTR genotype, No. (%)		
No. available	153	158
Homozygous ΔF508	82 (53.6)	88 (55.7)
Compound heterozygote ΔF508	34 (22.2)	36 (22.8)
Other	37 (24.2)	34 (21.5)
Race/ethnicity, No. (%)		
Non-Hispanic white	149 (94.3)	153 (93.9)
Hispanic	6 (3.8)	7 (4.3)
Other	3 (1.9)	3 (1.8)
Sweat chloride, mean (SD), mEq/L ^a	95.2 (18.0)	94.7 (18.9)
Weight, mean (SD), kg	12.2 (4.1)	12.5 (4.1)
Percentile, mean (SD)	39.7 (28.1)	43.0 (29.1)
Height, mean (SD), cm	84.8 (14.8)	85.7 (15.0)
Percentile, mean (SD)	36.9 (27.0)	39.9 (28.1)
Positive newborn screen, No. (%) ^b	101 (75)	92 (68)
Chronic medication use, No. (%)		
Dornase alfa	61 (39)	65 (40)
Albuterol/levalbuterol	115 (73)	120 (74)
Positive respiratory culture, No. (%) ^c		
<i>Pseudomonas aeruginosa</i>	60 (38.0)	69 (42.3)
<i>Staphylococcus aureus</i>	98 (62.0)	124 (76.1)
MRSA	5 (3.2)	11 (6.8)
<i>Stenotrophomonas maltophilia</i>	25 (15.8)	35 (21.5)
<i>Achromobacter xylosoxidans</i>	4 (2.5)	3 (1.8)
<i>Burkholderia cepacia</i>	0	0
Resting respiratory rate, mean (SD), breaths/min	31 (8.8)	30 (9.2)
Oximetry, mean (SD), %	98 (1.4)	98 (1.5)
Parent-reported daytime cough, No. (%) ^d	27 (17.1)	24 (14.7)
CFQ-R respiratory domain score, mean (SD) ^e	86.9 (13.9)	87.7 (12.3)
Pulmonary function substudy		
No. providing consent	36	37
Functional measure, mean (SD)		
FRC, mL	198 (50)	216 (59)
FEV _{0.5} , mL ^f	276 (68)	282 (66)
FEF ₇₅ , mL/s ^f	303 (132)	284 (105)
FEF ₂₅₋₇₅ , mL/s ^f	592 (212)	578 (172)
RV:TLC ^g	0.3 (0.1)	0.3 (0.1)

Abbreviations: CFQ-R, Cystic Fibrosis Questionnaire–Revised; FEV_{0.5}, forced expiratory volume in 0.5 seconds; FEF₇₅, forced expiratory flow at 75% of vital capacity; FEF₂₅₋₇₅, mid-maximal forced expiratory flow; FRC, functional residual capacity; MRSA, methicillin-resistant *Staphylococcus aureus*; RV:TLC, ratio of residual volume to total lung capacity.

^aData available from 125 participants in the hypertonic saline group and 126 in the isotonic saline group.

^bData available from 135 participants in the hypertonic saline group and 135 in the isotonic saline group.

^c*Pseudomonas aeruginosa* isolated from respiratory culture at or at any time prior to randomization. For other organisms, positive culture at or within 24 mo prior to randomization.

^dPer West et al.¹⁵

^eScores range from 0 to 100, with a higher score indicating milder symptoms. Data available from 156 participants in the hypertonic saline group and 157 in the isotonic saline group.

^fData available from 29 participants in the hypertonic saline group and 32 in the isotonic saline group.

^gData available from 27 participants in the hypertonic saline group and 29 in the isotonic saline group.

Table 2. Comparison of Pulmonary Exacerbation Rates and Related End Points

End Point	Hypertonic Saline	Isotonic Saline	Hypertonic Saline to Isotonic Saline Ratio (95% CI)	
			Unadjusted	Adjusted ^a
Pulmonary exacerbations rate, events/person-year (95% CI) ^b	2.3 (2.0-2.5)	2.3 (2.1-2.6)	0.97 (0.83-1.13)	0.98 (0.84-1.15)
Total No. of treatment days for a pulmonary exacerbation, mean (95% CI) ^c	60 (49-70)	52 (63-71)	1.13 (0.91-1.40)	1.11 (0.89-1.37)
First pulmonary exacerbation, hypertonic saline/isotonic saline, HR (95% CI)			0.94 (0.74-1.21)	0.94 (0.73-1.22)

Abbreviation: HR, hazard ratio.

^aAdjusted for age category and site.

^bIncludes log of observation time as an offset in the Poisson regression model. One hundred twenty-four participants randomized to receive hypertonic saline experienced 321 pulmonary exacerbations during 142 person-years of follow-up; 129 participants randomized to receive isotonic saline experienced 338 pulmonary exacerbations during 145 person-years of follow-up.

^cAmong participants with pulmonary exacerbations.

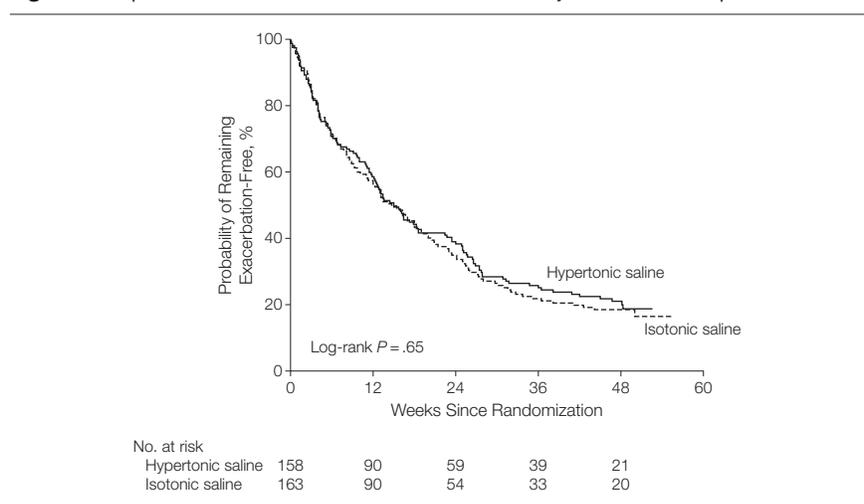
tics of participants were similar in the 2 groups (TABLE 1). About 60% were younger than 30 months at enrollment.

Pulmonary Exacerbations and Secondary Efficacy End Points

The pulmonary exacerbation rate was 2.3 (95% CI, 2.0-2.5) per person-year among participants randomized to receive hypertonic saline and 2.3 (95% CI, 2.1-2.6) per person-year among participants randomized to receive isotonic saline. The ratio of the mean pulmonary exacerbation rate in the hypertonic saline group compared with the isotonic saline group was 0.97 (95% CI, 0.83-1.13) (TABLE 2). A Kaplan-Meier plot of time to first pulmonary exacerbation for both groups is shown in FIGURE 2. The hazard ratio for time to first pulmonary exacerbation in the hypertonic saline group compared with the isotonic saline group was 0.94 (95% CI, 0.74-1.21) (Table 2). Among participants with pulmonary exacerbations, the mean number of total antibiotic treatment days for pulmonary exacerbations was 60 (95% CI, 49-70) in the hypertonic saline group and 52 (95% CI, 43-61) in the isotonic saline group; the median was 41 (interquartile range, 24-71) for the hypertonic saline group and 35 (interquartile range, 21-56) for the isotonic saline group. The ratio of mean total number of antibiotic treatment days for a pulmonary exacerbation in the hypertonic saline group compared with the isotonic saline group was 1.13 (95% CI, 0.91-1.40).

Of the 659 total pulmonary exacerbations, 636 (96.6%) were treated with

Figure 2. Kaplan-Meier Plot of Time to First Exacerbation by Treatment Group



oral, 50 (7.6%) with inhaled, and 45 (6.8%) with intravenous antibiotics (not mutually exclusive) (eTable 1). There was no difference between groups in the rates of pulmonary exacerbations treated by oral, inhaled, or intravenous antibiotics as separate categories or in the number of courses of oral, inhaled, or intravenous antibiotics administered for any indication (eTable 1). Similarly, the rates of pulmonary exacerbations were similar in the hypertonic saline and isotonic saline groups among participants younger than 30 months and 30 months or older at enrollment (eTable 1).

Significant differences were not detected between groups in weight, height, respiratory rate, room air oxygen saturation during the study, or the CFQ-R respiratory domain score or parent report of daytime cough at the final study visit (TABLE 3, eTable 2).

Consent to participate was obtained for 73 participants in the infant pulmonary function substudy. The baseline lung function measures of the substudy participants were similar in the hypertonic saline and isotonic saline groups (Table 1). Acceptable measurements at the enrollment and final study visit were obtained in 62 participants (85%) for functional residual capacity, 45 (62%) for raised-volume forced expiratory flows and volumes, and 36 (49%) for residual volume. No significant differences between the hypertonic saline and isotonic saline groups were detected in the raw change from baseline to week 48 in any of the pulmonary function measures (TABLE 4). After adjustment for baseline lung function, sex, age, height, and weight, the mean change in FEV_{0.5} was 38 mL greater (95% CI, 1-76) in the hypertonic saline group compared with the isotonic saline group (Table 4).

Table 3. Summary of Secondary End Points

End Point	Hypertonic Saline		Isotonic Saline		Difference (95% CI) ^a	
	No.	Mean or % (95% CI)	No.	Mean or % (95% CI)	Unadjusted	Adjusted
Change in weight percentile, mean ^b	143	5.7 (3.1 to 8.2)	149	3.2 (0.7 to 5.7)	2.5 (-1.1 to 6.1)	1.6 (-1.9 to 5.0)
Change in height percentile, mean	143	8.2 (5.2 to 11.2)	149	4.5 (2.0 to 7.0)	3.7 (-0.2 to 7.5)	2.3 (-1.4 to 6.0)
Change in resting respiratory rate, mean, breaths/min	143	-3.8 (-5.1 to -2.4)	146	-3.6 (-5.2 to -2.1)	-0.2 (-2.2 to 1.9)	0.6 (-0.5 to 1.8)
Change in room air oximetry, %	143	-0.1 (-0.4 to 0.2)	148	0.1 (-0.2 to 0.3)	-0.2 (-0.6 to 0.2)	-0.1 (-0.4 to 0.2)
Change in CFQ-R respiratory score, mean	133	0.5 (-2.1 to 3.1)	139	-3.2 (-6.3 to 0)	3.7 (-0.5 to 7.8)	3.3 (0.0 to 6.7)
Parent report of cough at wk 48, %	143	18 (12 to 25)	149	25 (18 to 32)	-7 (-16 to 3)	-6 (-15 to 4)

Abbreviation: CFQ-R, Cystic Fibrosis Questionnaire-Revised.

^aDifference in mean change or proportion, estimated from linear regression model with and without adjustment for age category, site, and measure at randomization (cough is adjusted for age category and site only).

^bChange = measurement at week 48 - measurement at randomization.

Table 4. Summary of Infant Pulmonary Function Measures

Measure ^a	Hypertonic Saline		Isotonic Saline		Difference (95% CI) ^b
	No.	Mean or % (95% CI)	No.	Mean or % (95% CI)	
Change in FEV _{0.5} , mean, mL ^c	22	162 (132 to 193)	23	121 (91 to 152)	38 (1 to 76)
Change in FEF ₇₅ , mean, mL/s	22	163 (105 to 220)	23	111 (46 to 175)	46 (-29 to 121)
Change in FEF ₂₅₋₇₅ , mean, mL/s	22	269 (191 to 348)	23	186 (91 to 281)	99 (-7 to 204)
Change in FRC, mean, mL	35	106 (90 to 122)	27	105 (79 to 131)	-2 (-30 to 26)
Change in RV:TLC, %	18	0.7 (-3.4 to 4.9)	18	1.5 (-2.7 to 5.6)	-1.1 (-4.8 to 2.6)

Abbreviations: FEV_{0.5}, forced expiratory volume in 0.5 seconds; FEF₇₅, forced expiratory flow at 75% of vital capacity; FEF₂₅₋₇₅, mid-maximal forced expiratory flow; FRC, functional residual capacity; RV:TLC, ratio of residual volume to total lung capacity.

^aFor FEV_{0.5}, FEF₇₅ and FEF₂₅₋₇₅, a positive treatment effect would manifest as a larger change; for FRC and RV:TLC, a positive treatment effect would manifest as a smaller change.

^bDifference in mean change between hypertonic saline and isotonic saline groups, estimated from linear regression model with adjustment for baseline lung function, sex, age, height, and weight.

^cChange = measurement at week 48 - measurement at randomization.

Adherence

Mean adherence to study medications was 75.2% (95% CI, 72.2%-78.2%), based on returned study drug vials among 311 participants. Based on the weekly parent questionnaire (available for 309 participants), mean adherence to twice-daily dosing was 91% (95% CI, 89%-93%) and to at least once-daily dosing was 96% (95% CI, 95%-98%). Based on the quarterly treatment adherence questionnaire (available for 312 participants), mean adherence to twice-daily dosing was 88% (95% CI, 85%-90%), to using treatment at least 6 days per week was 86% (95% CI, 83%-89%), and to nebulizing 10 or more minutes per treatment was 89% (95% CI, 86%-92%). Adherence was similar between the 2 groups (eTable 3).

Safety

Of the participants who received the test dose of 7% hypertonic saline at enroll-

ment, 2 were found to be intolerant and were not randomized. Serious adverse events are shown in TABLE 5. In the hypertonic saline group, there were 56 serious adverse events among 33 participants. In the isotonic saline group, there were 74 events among 43 participants. No significant differences between groups in the proportion of participants with serious adverse events of each category were detected. The most common serious adverse event in both groups was cough or increased cough, occurring in 8% of participants in the hypertonic saline group and 10% of participants in the isotonic saline group.

A significant difference between groups was not detected in the proportion of adverse events of moderate or severe severity occurring in more than 10% of participants in either group (eTable 4). The most common adverse event of moderate or severe severity was cough (39% of hypertonic sa-

line group, 38% of isotonic saline group). New isolation of bacteria, including *Burkholderia cepacia*, from respiratory cultures during the study period is presented in the eResults and in eTable 5; statistically significant differences between groups were not detected (eTable 5).

COMMENT

This is to our knowledge the first clinical trial assessing a chronic nonantimicrobial pulmonary therapy in children younger than 6 years with CF. Hypertonic saline did not reduce the rate of pulmonary exacerbations in these young children. In addition, hypertonic saline did not demonstrate any significant effects on secondary end points including weight, height, respiratory rate, oxygen saturation, antibiotic use, or parent report of respiratory signs and symptoms.

Previous studies in older children and adults with CF have documented benefits of inhaled hypertonic saline.^{7-9,20,21} In a multicenter Australian study in patients older than 6 years, treatment with hypertonic saline did not demonstrate a significant effect on the primary outcome measure, the rate of change of lung function, but was associated with a significant reduction in the rate of pulmonary exacerbations.⁹ Pulmonary exacerbation rate was chosen as the primary outcome in the current trial because of the important effect observed in the Australian trial of hypertonic saline⁹ and because pulmonary exacerbations are a clinical end point

(affecting how a person feels, functions, or survives²²) that have been associated with survival in CF.^{23,24} The definition of pulmonary exacerbation in the current study differed from that in the Australian study, in which pulmonary exacerbations were defined as treatment with intravenous antibiotics for predefined signs and symptoms or the occurrence of those signs and symptoms independent of treatment. Our definition, similar to that used in 2 prior studies in young patients with CF,^{25,26} was designed to capture all events in which several days of new respiratory signs or symptoms triggered treatment with oral, inhaled, or intravenous antibiotics, the standard clinical practice for patients in this age range with CF. In the current study, although the majority of pulmonary exacerbations were treated with oral antibiotics, there was no difference between the 2 groups in the rate of exacerbations, even if limited to patients treated with intravenous antibiotics, or in respiratory symptoms. Thus, it is unlikely that the difference in our results is attributable to a different definition of pulmonary exacerbation.

Unlike in older patients, pulmonary exacerbations in infants and young children are frequently triggered by viral infections. It is thus possible that hypertonic saline has less ability to prevent exacerbations in children younger than 6 years than in older patients with CF. Previous studies have demonstrated that viral infections occur at similar rates in infants with and without CF but that the severity and duration of symptoms is increased in those with CF.²⁷ Thus, even if hypertonic saline does not affect the rate of pulmonary exacerbations in young children with CF, it might reduce the severity and duration of symptoms, similar to its observed effect in infants without CF but with bronchiolitis.²⁸ However, the current study provides no evidence that the severity or duration of pulmonary exacerbations was influenced by hypertonic saline, because parent-reported respiratory signs and symptoms and days of antibiotic therapy did not differ between groups.

Table 5. Serious Adverse Events by Treatment Group

Adverse Event Category	Hypertonic Saline (n = 158)		Isotonic Saline (n = 163)	
	No. (%)	No. of Events	No. (%)	No. of Events
All	33 (21)	56	43 (26)	74
Abdominal distension	2 (1)	2	1 (1)	1
Abdominal pain or stomachache	3 (2)	3	4 (2)	4
<i>Burkholderia cepacia</i>	2 (1)	2	2 (1)	2
<i>Clostridium difficile</i> colitis	2 (1)	3	0	0
Constipation	1 (1)	1	2 (1)	2
Cough or increased cough	13 (8)	16	16 (10)	19
Decreased appetite	0	0	2 (1)	2
Diarrhea	0	0	1 (1)	1
Distal intestinal obstructive syndrome	1 (1)	1	2 (1)	2
Fever	1 (1)	2	4 (2)	4
Nasal congestion or stuffy nose	2 (1)	2	3 (2)	3
Poor growth and/or need for gastrostomy	5 (3)	6	5 (3)	5
<i>Pseudomonas aeruginosa</i> eradication	4 (3)	4	2 (1)	2
Pulmonary congestion or chest congestion	0	0	2 (1)	2
Rectal prolapse	0	0	1 (1)	2
Respiratory syncytial virus	1 (1)	1	1 (1)	1
Vomiting or emesis	3 (2)	5	6 (4)	6
Wheezing	4 (3)	4	2 (1)	2
Other	4 (3)	4	11 (7)	13

We estimated the expected pulmonary exacerbation rate based on data from an ongoing US observational study of early CF lung disease, the Early Pseudomonas Infection Control (EPIC) Observational Study.²⁹ The rate of pulmonary exacerbations in the current study (mean, 2.3 events per person-year) was very similar to that observed in the EPIC Observational Study (2.22 per person-year), indicating that our trial was adequately designed to observe the predefined treatment effect. In addition, the participants in the current study had baseline characteristics similar to those of the overall patient population in the US Cystic Fibrosis National Patient Registry in this age range, suggesting that our findings are generalizable to the overall CF population younger than 6 years.

This study was designed to primarily demonstrate an effect on clinically meaningful events rather than on prevention of lung disease progression. Our choice of end points was limited by the fact that validated outcome measures commonly used in older patients are lacking for very young children with CF. It could be argued that an inter-

vention targeting mucociliary clearance in a population with limited clinical lung disease is unlikely to improve any short-term clinical outcome measure and that a more realistic goal would be to slow the progression of structural airway damage or to improve lung function. We conducted a substudy of infant pulmonary function tests as an exploratory end point at selected sites to gain information to adequately power future studies using this end point. Interestingly, the mean change in FEV_{0.5} during the treatment period was significantly greater in the hypertonic saline group compared with the isotonic saline group. Although these findings may be attributable to chance, they also may reflect improvement in airflow limitation in the hypertonic saline group that was not detectable with our primary or secondary outcome measures. Because of the relatively silent nature of early CF lung disease, sensitive end points are critical.

When the current study was being planned, protocols for chest computed tomography and multiple breath wash-out for multiple age ranges were not ad-

equately developed, and multicenter experience in infants and young children with these techniques was limited. The availability of appropriate multicenter protocols and networks as well as increased expertise in these techniques suggests that adequately powered trials using physiologic measures as outcomes may be conducted. Future studies of hypertonic saline in young children using these or other end points will allow evaluation of the effects of this treatment on early structural airway damage and lung function, including ventilation inhomogeneity.

In both the current study and the Australian hypertonic saline trial, isotonic saline served as the control agent. It is possible that isotonic saline has a more pronounced effect on mucus hydration in very young patients than in older patients. In addition, participants in both groups received albuterol prior to each dose of study drug. Both of these factors might have limited our ability to detect a difference in outcomes between the 2 groups. That the exacerbation rate in the control group was very similar to the rate in an untreated historical cohort would suggest that there was not an important effect of isotonic saline on the primary end point. However, it is not feasible to perform a true placebo-controlled study of hypertonic saline, because no inhaled agent is completely inert.

Treatment with hypertonic saline was well tolerated, and adherence to therapy was high overall. Chronic inhaled therapy could pose a risk of new acquisition of bacterial pathogens if nebulizers are not properly cleaned and disinfected.³⁰ Because this study did not include an untreated control group, this potential adverse effect of inhalation therapy cannot be excluded. However, the rate of new acquisition of organisms did not differ significantly from that reported in the CF Registry or in the EPIC Observational Study.²⁹ Therefore, while not showing a decrease in pulmonary exacerbation rate, this study supports previous smaller series demonstrating that inhalation of hypertonic saline is safe in infants and young children.

In conclusion, among infants and children younger than 6 years with CF, the use of inhaled hypertonic saline compared with isotonic saline did not reduce the rate of pulmonary exacerbations over the course of 48 weeks of treatment. Further study with physiologic end points is warranted to better understand how this drug may slow progression of structural airway damage or improve lung function in the youngest population.

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Online-Only Material: The eMethods, eResults, and eTables 1-5 are available at <http://www.jama.com>.

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