The effect of inhaled hypertonic saline on lung structure in children aged 3–6 years with cystic fibrosis (SHIP-CT): a multicentre, randomised, double-blind, controlled trial

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Summary

Background In the Saline Hypertonic in Preschoolers (SHIP) study, inhaled 7% hypertonic saline improved the lung clearance index in children aged 3–6 years with cystic fibrosis, but it remained unclear whether improvement is also seen in structural lung disease. We aimed to assess the effect of inhaled hypertonic saline on chest CT imaging in children aged 3–6 years with cystic fibrosis.

Methods Children with cystic fibrosis were enrolled in this multicentre, randomised, double-blind, controlled study at 23 cystic fibrosis centres in Spain, Denmark, the Netherlands, Italy, France, Belgium, the USA, Canada, and Australia. Eligible participants were children aged 3–6 years who were able to cooperate with chest CT imaging and comply with daily nebuliser treatment. Participants were randomly assigned 1:1 to receive inhaled 2 puffs of 100 µg salbutamol followed by 4mL of either 7% hypertonic saline or 0·9% isotonic saline twice per day for 48 weeks. Randomisation was stratified by age in North America and Australia, and by age and country in Europe. Chest CTs were obtained at baseline and 48 weeks and scored using the Perth-Rotterdam Annotated Grid Morphometric Analysis for Cystic Fibrosis (PRAGMA-CF) method. The primary outcome was the difference between groups in the percentage of total lung volume occupied by abnormal airways (PRAGMA-CF %Disease) measured by chest CT at 48 weeks. Analysis was by intention-to-treat. This study is registered with Clinicaltrials.gov, NCT02950883.

Findings Between May 24, 2016, and Dec 18, 2019, 134 children were assessed for inclusion. 18 patients were excluded (nine had incomplete or unsuccessful chest CT at enrolment visit, two could not comply with CT training, two had acute respiratory infection, two withdrew consent, two for reasons unknown, and one was already on hypertonic saline). 116 participants were enrolled and randomly assigned to hypertonic saline (n=56) or isotonic saline (n=60). 12 patients dropped out of the study (seven in the hypertonic saline group and five in the isotonic saline group). Mean PRAGMA-CF %Disease at 48 weeks was 0·88% (95% CI 0·60–1·16) in the hypertonic saline group and 1·55% (1·25–1·84) in the isotonic saline group (mean difference 0·67%, 95% CI 0·26–1·08; p=0·0092) based on a linear regression model adjusted for baseline %Disease values and baseline age. Most adverse events in both groups were rated as mild, and the most common adverse event in both groups was cough.

Interpretation Inhaled hypertonic saline for 48 weeks had a positive effect on structural lung changes in children aged 3–6 years with cystic fibrosis relative to isotonic saline. This is the first demonstration of an intervention that alters structural lung disease in children aged 3–6 years with cystic fibrosis.

Funding Cystic Fibrosis Foundation.

Lancet Respir Med 2022

Published Online
March 11, 2022
https://doi.org/10.1016/S2213-2600(21)00546-4

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See Online for appendix

Introduction

Cystic Fibrosis lung disease is characterised by early impairment of mucociliary clearance, chronic airway infection, and inflammation. Structural lung disease has already developed in a large proportion of children with cystic fibrosis by the age of 3–6 years, and has a negative effect on prognosis and quality of life.[1,2] Effective and affordable therapies are needed to prevent or slow development of structural lung disease in children younger than 6 years.[3]

Chest CT is used to detect and monitor structural abnormalities in cystic fibrosis. Progressive airway wall thickening, bronchiectasis, and low attenuation regions often defined as trapped air have been reported in children aged 3–6 years with cystic fibrosis.[4] At 5 years of age, 55–84% of children with cystic fibrosis show bronchiectasis on inspiratory chest CT scans[2,5–7] and 45–76% have trapped air on expiratory scans.[5,7] Another method to detect and monitor cystic fibrosis lung disease in children younger than 6 years is the multiple breath washout test (MBW), which is a functional test that captures ventilation inhomogeneity quantified using the lung clearance index ($LCI_{2·5}$). Most infants with cystic fibrosis have a normal $LCI_{2·5}$ after diagnosis by newborn screening,[8,9] but $LCI_{2·5}$ values are abnormal in most children younger than 6 years with cystic fibrosis.[10] This

Research in context

Evidence before this study

We searched PubMed with the terms "hypertonic saline" and "cystic fibrosis" for reports published in English between Jan 19, 2006, and June 30, 2021. Previous studies have shown that inhaled hypertonic saline had a positive effect on the lung clearance index as a primary functional outcome measure in children younger than 6 years with cystic fibrosis. However, it remains unclear whether the improvement in lung clearance index also reflects improvement in structural lung disease.

Added value of this study

SHIP-CT is a unique study in children aged 3–6 years with cystic fibrosis using chest CT score as the primary outcome measure. We showed that treatment with inhaled hypertonic saline for 48 weeks resulted in less structural lung damage compared with children in the isotonic group. In addition, SHIP-CT combined CT outcomes and lung clearance index to improve our understanding of the relationship between structural and functional outcomes in this younger age group.

Implications of all the available evidence

The SHIP-CT study showed that hypertonic saline is a safe, cheap, and effective therapeutic option to reduce structural lung damage in clinically vulnerable children aged 3–6 years. We recommend that inhaled hypertonic saline be considered for all young children, particularly those without access to cystic fibrosis transmembrane regulator modulator therapy, in order to minimize progression of structural lung damage. For clinical trials of therapies to treat cystic fibrosis lung disease in children younger than 6 years, we recommend including outcome measures to evaluate both lung structure and function.

age period is therefore an important window of opportunity to initiate treatment for delay or prevention of irreversible lung damage.[11,12]

Inhaled 7% hypertonic saline enhances impaired mucociliary clearance[13] in the airways of children with cystic fibrosis by increasing the airway surface liquid and improving mucus rheology.[14,15] In the Infant Study of Inhaled Saline (ISIS) trial,[16] the efficacy of 7% hypertonic saline was assessed compared with 0·9% isotonic saline over 48 weeks in children aged 4–60 months with cystic fibrosis. No significant difference was found between groups for the primary outcome (rate of pulmonary exacerbations). However, results of two substudies[16,17] suggested that hypertonic saline improved physiological outcomes, $FEV_{0.5}$ measured through infant lung function testing, and $LCI_{2.5}$. Based on these results, the Saline Hypertonic in Preschoolers (SHIP) randomised controlled trial[18] was conducted in 150 children aged 3–6 years with cystic fibrosis to assess the efficacy of hypertonic saline on $LCI_{2.5}$ as the primary outcome measure. At 48 weeks, treatment with hypertonic saline was associated with a significant decrease (ie, improvement) in $LCI_{2.5}$ compared with isotonic saline. However, the study did not clarify whether hypertonic saline also affected structural lung disease, and it remains unclear whether the lung function changes alone justify the additional burden of twice daily inhalation therapy in young children with cystic fibrosis.[19]

To measure changes in early cystic fibrosis structural lung disease on chest CT in children with cystic fibrosis aged 3–6 years, the Perth-Rotterdam Annotated Grid Morphometric Analysis for Cystic Fibrosis (PRAGMA-CF) was developed.[4] PRAGMA-CF was developed because other available scoring systems were not sensitive or reproducible enough to quantify early and subtle structural lung changes in children with cystic fibrosis.[20] The main outcome measure of PRAGMA-CF is %Disease, the proportion of the lung volume occupied by diseased airways. %Disease was shown in several cohort studies to be a predictor for the development of later bronchiectasis.[2,6,21–24] Furthermore, inflammatory markers in bronchoalveolar lavage fluid are associated with the development of structural changes with %Disease as the outcome measure.[5,25–27]

We hypothesised that inhaled hypertonic saline, by improving mucociliary clearance, could not only improve lung function in children aged 3–6 years, but also affect structural lung disease.

Methods

Study design and participants

SHIP-CT study was a multicentre, randomised, double-blind, controlled, parallel group trial done in 23 centres in Spain, Denmark, the Netherlands, Italy, France, Belgium, the USA, Canada, and Australia. The Institutional Review Boards and Human Research Ethics committee at each participating centre approved the trial and written informed consent was obtained. Eligible patients were children aged 3–6 years (36–72 months) at enrolment, with diagnosis of cystic fibrosis, ability to cooperate with chest CT imaging, and ability to comply with daily treatment. Complete inclusion and exclusion criteria are in the appendix (p 7). The participant's legal representative gave written informed consent.

Randomisation and masking

Eligible participants were randomly assigned 1:1 to either hypertonic saline (treatment) or isotonic saline (control) based on random permuted blocks scheme through a secure website system developed by the central coordinating centre (Seattle, WA, USA). Randomisation was stratified by age (36–54 months and 55–72 months) in North America and Australia and by age (as above) and country in Europe. Participants, families, study coordinators, and investigators were masked to treatment assignment until data analysis was initiated. The study drug distributed to each centre was

identified only with a batch (Europe) or kit (Australia and North America) identifier. The only entities with access to the batch or kit-study treatment type were the manufacturer and the unblinded statistician at the data coordinating centre. After parental informed consent and inclusion and exclusion criteria were entered into the data entry system, the centre accessed an online randomisation programme, which provided the study batch or kit number directly to the site pharmacist. The study drug was then distributed to the patient. Centre pharmacists and study personnel were blinded to treatment. Centres and patients were informed of treatment assignment after the full analysis for the study was completed.

Procedures

Members of staff from each centre were trained and certified to perform chest CT and MBW before the start of the trial. For each centre, a specific scan protocol was developed by the Erasmus MC LungAnalysis Core Laboratory (Rotterdam, Netherlands) to standardise image quality (appendix p 11). At the screening visit, participants inhaled a test dose (4 mL) of hypertonic saline and were trained to perform breath hold manoeuvres for the chest CT procedure to reduce anxiety and optimise cooperation (CT training). Participants in some centres in Australia had CTs obtained under general anaesthesia as per their routine clinical protocol that combines chest CT with a bronchoalveolar lavage.[11] All other centres followed a technician or spirometry guided breath hold protocol without sedation. MBW testing was conducted according to a standardised protocol[28] defined by the MBW Resource Centre at the Hospital for Sick Children (Toronto, ON, Canada). Participants who did not qualify at the first CT training but met the other eligibility criteria could re-screen after additional training sessions. A detailed study schedule can be found in the appendix (p 29).

An enrolment visit was scheduled for 7–30 days after the screening visit. At the enrolment visit, MBW was performed and CT training was repeated. If the participant was considered sufficiently trained, the enrolment chest CT scan was performed. The aim was to obtain an inspiratory CT at a volume as close as possible to total lung capacity (TLC) and an expiratory scan as close as possible to functional residual capacity (FRC). If the participant had difficulties in following the volume specific manoeuvre, only an inspiratory chest CT was conducted at the best obtainable lung volume between FRC and TLC. Once the chest CT scan was obtained, the study participant was randomly assigned to one of the two study arms.

Participants inhaled a short-acting B2 bronchodilator (2 puffs 100 µg salbutamol) by metered dose inhaler with spacer followed by 4mL of either hypertonic saline or isotonic saline for approximately 15 minutes twice daily for 48 weeks through a PARI Sprint Junior nebuliser with a PARI Baby face mask or mouthpiece driven by a PARI compressor (PARI Vios Pro in USA and PARI BOYSX in Australia and Europe).

Participants were followed up for 48 weeks with study visits at enrolment and weeks 12, 24, 36, and 48 (±2 weeks). Participants were trained in the chest CT-related breath hold manoeuvres at each visit with the aim to optimise chest CT acquisition at 48 weeks. Height and weight were measured at each visit and a MBW test was performed at screening, baseline, 24 weeks, and 48 weeks. If acceptable MBW measurements were not obtained at 48 weeks, the participant could attempt a repeat MBW measurement within the study visit window (2 weeks). Adverse events were assessed at each study visit until 14 days after administration of the last dose of study drug (detailed criteria are available in the appendix, p 9–10). Medication adherence was monitored by counting returned used and unused vials.

Outcomes

The primary outcome was the difference in PRAGMA-CF %Disease measured by chest CT at 48 weeks, adjusted for baseline %Disease values and baseline age between the hypertonic saline and isotonic saline groups. Secondary outcomes included differences in PRAGMA-CF subscores (%Bronchiectasis, %Mucus plugging, %Airway wall thickening, %Atelectasis, and %Trapped air) and airway-artery dimensions[29] at 48 weeks and changes in %Disease, %Bronchiectasis, %Trapped air and $LCI_{2.5}$ from baseline (the enrolment visit) to 48 weeks. We cannot include the analysis of the airway-artery dimensions in this Article because the development and validation of the automated software needed for this analysis is ongoing. The modified Cystic Fibrosis Questionnaire-Revised (CFQ-R)[30] was included as a secondary outcome measure to assess quality of life for participants in North America and Australia, but not in Europe as the questionnaire was not validated for all European languages. For this reason, we excluded CFQ-R results from further analyses. As a post-hoc analysis, we analysed the difference in $LCI_{2.5}$ at 48 weeks between treatment groups, adjusted for baseline $LCI_{2.5}$ values and baseline age similar to the analysis used for PRAGMA-CF outcomes.

All pseudoanonymised CTs were analysed in three batches in a random order using the PRAGMA-CF method by a certified and experienced masked observer (YC). In PRAGMA-CF, a grid is overlaid over ten equally spaced axial CT slices and bronchiectasis, mucus plugging, airway wall thickening, and atelectasis are scored in hierarchical order on the inspiratory scan and trapped air on the expiratory scan. For each of the annotated components, the total volume is computed and expressed as a percentage of the total lung volume. Furthermore, %Disease is computed, representing the percentage of total lung volume occupied by structural abnormalities of the airways by summing %Bronchiectasis, %Mucus plugging, and %Airway wall thickening. Intra-observer reliability and

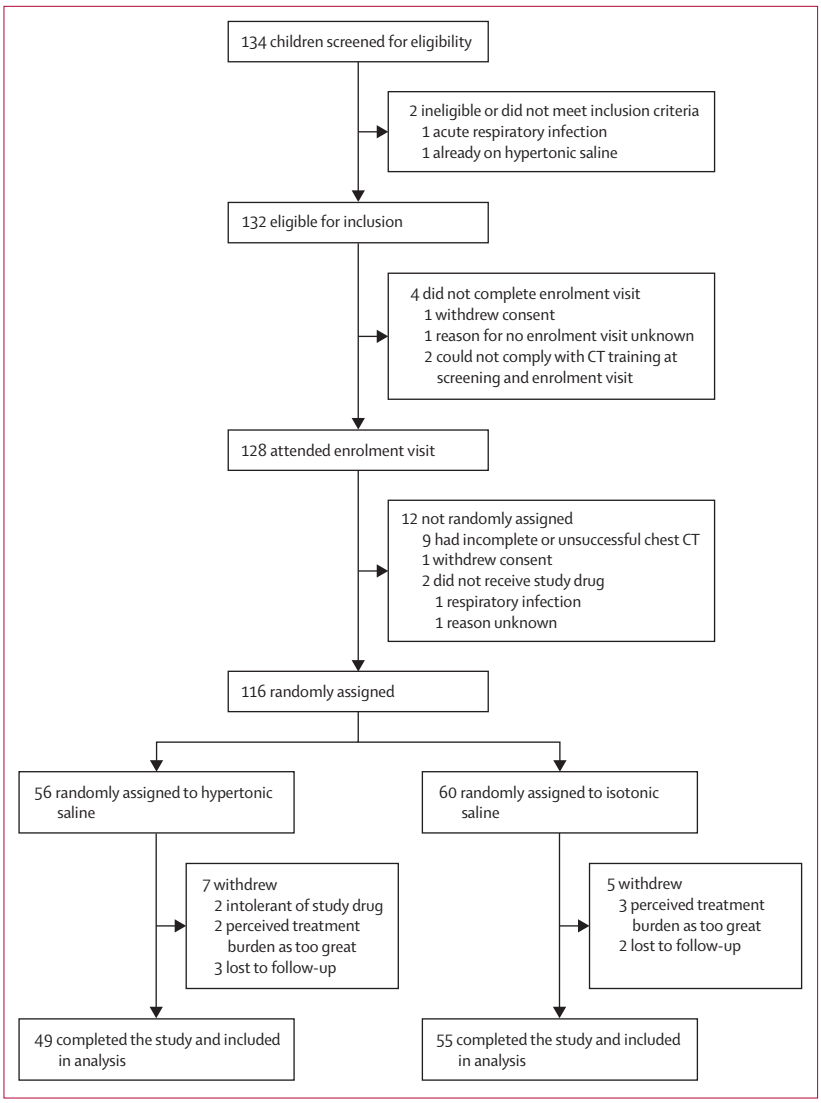

Figure 1: **Trial profile**

inter-observer variability were tested with standard operating procedures. Image and volume quality of CT scans were classified as good, moderate, and poor using example images with standard operating procedures (appendix p 8–9). A Data Safety Monitoring Board did the interim safety analysis.

Statistical analysis

The sample size calculation for SHIP-CT was based on a random sample of 50 children aged 4–6 years from the AREST CF Perth cohort database. Mean PRAGMA-CF %Disease as measured on TLC CTs at follow-up was 5·18 (SD 2·54%, range 1·05–14·13). We estimated that a total of 120 participants (60 per group) would be sufficient to detect a treatment difference of 1·44% (relative difference between the two treatment groups 28%) in %Disease at 48 weeks with an alpha of 0·05 and a power of 80%, accounting for a maximum drop-out of 20% (including participants withdrawing from the study and patients without a CT scan at 48 weeks).

Data were included in an intention-to-treat analysis and summarised as mean (SD) or median (IQR). The primary outcome, the difference between the treatment groups in PRAGMA-CF %Disease at 48 weeks, was investigated using a multiple linear regression model. We used %Disease at 48 weeks as the outcome and included treatment group, mean baseline %Disease value, and mean baseline age as the covariates in the model. The differences in %Bronchiectasis, %Trapped air, and $LCI_{2.5}$ between the treatment groups at 48 weeks were assessed using the same approach.

To assess change in $LCI_{2.5}$ from baseline to 48 weeks, we used a multiple linear regression model assuming the difference in $LCI_{2.5}$ value between baseline and 48 weeks as the outcome and treatment, baseline $LCI_{2.5}$ values, and baseline age as the covariates. A mean $LCI_{2.5}$ value was calculated and used for analysis if participants had more than one $LCI_{2.5}$ measurement per visit. This statistical analysis method for $LCI_{2.5}$ was identical to that of the SHIP study.[18]

Changes in PRAGMA-CF %Disease, %Bronchiectasis, and %Trapped air from baseline to 48 weeks were assessed by linear mixed effects models accounting for the unbalanced nature of the data (missing values at 48 weeks). Time, treatment group, baseline age, sex, race, genotype, baseline height, baseline weight, and the interaction between time and treatment were treated as fixed effects, and a random intercept was assumed. For CT outcomes, we assumed the logarithmic scale [log(outcome) + 0·1] because the assumption that the error terms follow the normal distribution and that the variance of the error terms is constant were not satisfied in the original scale.

Missing values in CT and $LCI_{2.5}$ outcomes at 48 weeks are assumed missing at random and were imputed using the variables: baseline values for each CT and $LCI_{2.5}$ outcome, age, sex, genotype, race, height (visits 1–3), weight (visits 1–3), and treatment. We used multivariate imputation by chained equations assuming 10 imputed data sets and 5 iterations and using the predictive mean matching method.[31]

As sensitivity analysis, we repeated the previous analysis without imputation. Extra analysis was performed for PRAGMA-CF %Airway wall thickening and %Atelectasis outcomes assuming generalised linear models with a non-standard response distribution (Tweedie family[32]).

Intra-observer and inter-observer reliability for PRAGMA-CF scores were assessed by a two-way-mixed effects model. An ICC greater than 0·8 was rated as excellent, 0·6–0·8 was good, 0·4–0·6 was moderate, and lower than 0·4 was poor.

Statistical significance was accepted for p values less than 0·05. Statistical analyses were done with

	Isotonic saline group (n=60)	Hypertonic saline group (n=56)
Age (months)	54·6 (43·6–65·7)	55·2 (50·0–63·7)
Age group		
35–54 months	31 (52%)	27 (48%)
55–72 months	29 (48%)	29 (52%)
Sex		
Male	30 (50%)	29 (52%)
Female	30 (50%)	27 (48%)
CFTR genotype		
Homozygous ΔF508	21 (35%)	28 (50%)
Compound heterozygote ΔF508	30 (50%)	23 (41%)
Other	9 (15%)	5 (9%)
Race		
White	56 (93%)	54 (96%)
Asian	1 (2%)	0
Pacific	1 (2%)	0
Indian	1 (2%)	1 (2%)
Other	1 (2%)	1 (2%)
Weight (kg)	18·0 (SD 3·3)	17·9 (SD 2·9)
Height (cm)	105·6 (SD 7·7)	106·6 (SD 7·4)
CT scores		
%Disease	1·03 (0·40–1·93)	0·89 (0·41–2·00)
%Bronchiectasis	0·62 (0·20–1·56)	0·70 (0·22–1·83)
%Mucus plugging	0·0 (0·0–0·06)	0·0 (0·0–0·0)
%Airway wall thickening	0·03 (0·0–0·30)	0·0 (0·0–0·18)
%Atelectasis	0·0 (0·0–0·38)	0·0 (0·0–0·19)
%Trapped air*	7·70 (1·01–14·23)	0·57 (0·0–8·46)
CT scores		
%Disease	1·78 (SD 2·48)	2·21 (SD 3·82)
%Bronchiectasis	1·38 (SD 1·80)	1·56 (SD 2·16)
%Mucus plugging	0·22 (SD 1·13)	0·51 (SD 2·14)
%Airway wall thickening	0·18 (SD 0·27)	0·14 (SD 0·22)
%Atelectasis	0·73 (SD 1·86)	0·20 (SD 0·41)
%Trapped air*	11·05 (SD 13·04)	5·58 (SD 9·50)
$LCI_{2.5}$†	8·22 (SD 1·41)	7·88 (SD 1·56)
$LCI_{2.5}$†	7·88 (7·20–9·05)	7·61 (7·04–8·23)

Data are mean (SD), n (%), or median (IQR). CFTR=cystic fibrosis transmembrane conductance regulator. $LCI_{2.5}$=lung clearance index. %Disease=percentage of total lung volume occupied by abnormal airways. *Baseline %Trapped air was assessed from 53 participants in the isotonic saline group and 52 participants in the hypertonic saline group. †Baseline $LCI_{2.5}$ was assessed from 55 participants in the isotonic saline group and 52 participants in the hypertonic saline group.

Table: **Demographic characteristics at baseline**

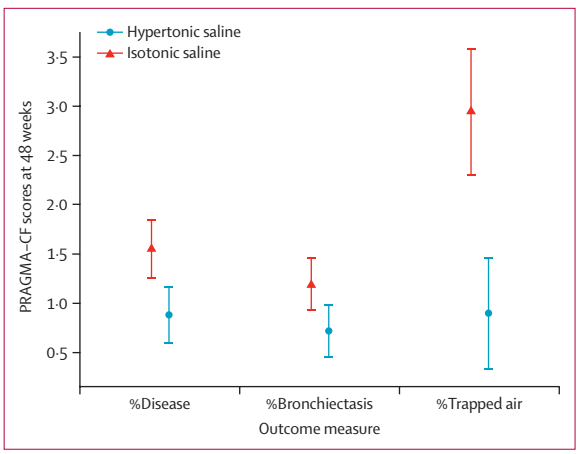

Figure 2: **Predicted PRAGMA-CF %Disease, %Bronchiectasis, and %Trapped air values at 48 weeks by treatment group, adjusted for mean baseline PRAGMA-CF values and mean baseline age**

Predicted mean values of PRAGMA-CF %Disease, %Bronchiectasis, and %Trapped air at 48 weeks were estimated from multiple regression models adjusted for mean baseline PRAGMA-CF values and mean baseline age. Data are the results for hypothetical participants whose baseline score was at the mean value. Error bars indicate 95% CI. For %Disease, the mean predicted values at 48 weeks were 1·55% (95% CI 1·25–1·84) in the isotonic saline group and 0·88% (0·60–1·16) in the hypertonic saline group (p=0·0092). %Disease=total volume of abnormal airways expressed as a percentage of total lung volume. %Bronchiectasis=total volume of bronchiectasis expressed as a percentage of the total lung volume. %Trapped air=total volume of trapped air expressed as a percentage of total lung volume.

R (version 4.0.5, packages: mice, ggeffects, estimatr, tweedie, nlme, effects, ggplot2, gghalves, dplyr) except for the ICC calculations for which we used IBM SPSS Statistics 21.

The study was monitored by a Data Safety Monitoring Board and was registered with ClinicalTrials.gov, NCT02950883.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. However, the funder and a funder-appointed data safety monitoring board oversaw study conduct and reviewed the final report.

Results

Between May 24, 2016 and Dec 18, 2019, 134 children were screened for inclusion. 18 patients were excluded (nine had incomplete or unsuccessful chest CT at enrolment visit, two could not comply with CT training, two had acute respiratory infection, two withdrew consent, two for reasons unknown, and one was already on hypertonic saline). 116 participants were enrolled from 23 cystic fibrosis centres in Europe (n=68; Spain, Denmark, the Netherlands, Italy, France, and Belgium), North America (n=37; USA and Canada) and Australia (n=11). Median age at screening was 55·0 months (range 36·2–71·7 months). Enrolment was stopped at 116 participants as we estimated based on the rate of completion at this point that we would be able to meet the required number of 50 participants per group completing the study. Eight participants from two North American centres participated concurrently in both the SHIP and SHIP-CT studies. 56 participants were assigned to the hypertonic saline group and 60 to the isotonic saline group (figure 1). Characteristics at baseline were generally well balanced between groups other than %Trapped air, which was higher in the

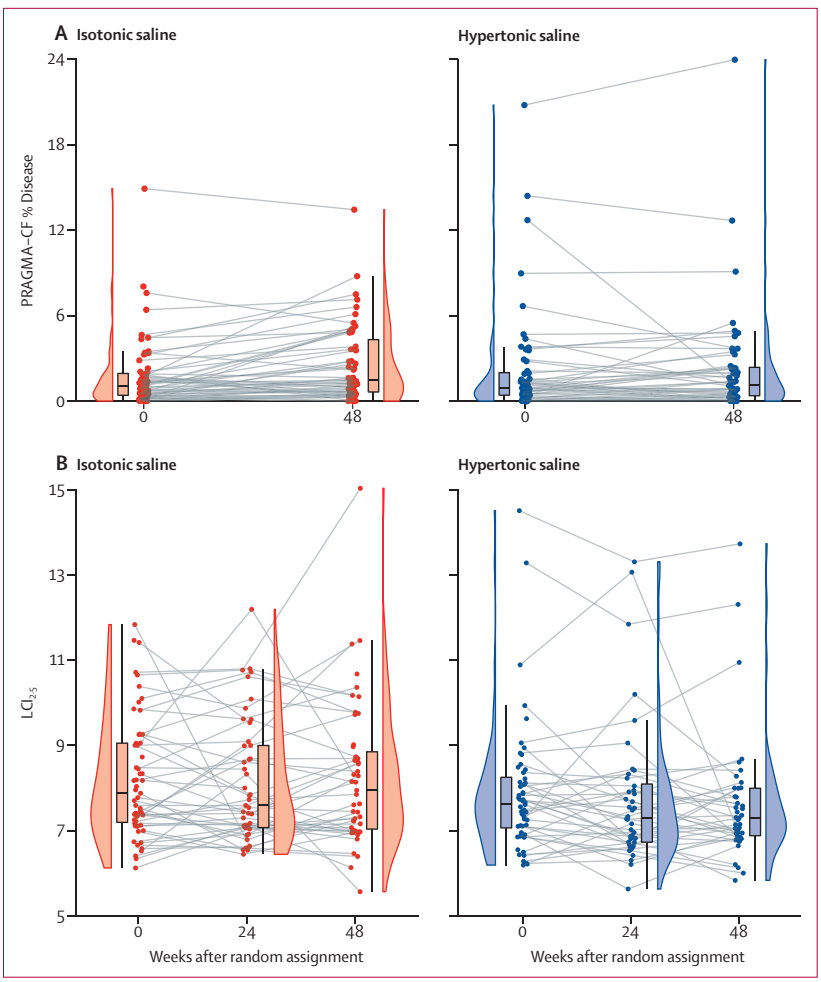

***Figure 3*: PRAGMA-CF %Disease and $LCI_{2.5}$ at baseline to 48 weeks between groups**
The raincloud plots[43] by treatment group at each time consists of one split-half violin plot showing data distribution, a boxplot including the median (IQR) and jitter data points with grey lines showing the trajectory of PRAGMA-CF %Disease and $LCI_{2.5}$ outcomes for individual participants from baseline to 48 weeks. (A) Change in PRAGMA-CF %Disease from baseline to 48 weeks. (B) Change of $LCI_{2.5}$ outcomes from baseline to 48 weeks.

isotonic saline group (table; appendix p 32–33). The mean adherence rate to treatment was 0·90 (95% CI 0·86–0·94) in the hypertonic saline group and 0·93 (0·90–0·95) in the isotonic saline group. 12 patients dropped out of the study (seven in the hypertonic saline group [two intolerant of study drug, two perceived treatment burden as too great, and three lost to follow-up] and five in the isotonic saline group [three perceived treatment burden as too great and two lost to follow-up]).

A total of 116 inspiratory and 105 (91%) expiratory CT scans were performed at baseline and 104 (90%) inspiratory and 102 (88%) expiratory scans were performed at 48 weeks. In Australia, five participants had seven CTs obtained under general anaesthesia. All scans were of sufficient quality to be scored, but image quality and volume quality scores of both inspiratory and expiratory scans were higher at 48 weeks compared with baseline (appendix p 12). Intra-observer reliability for the scoring was excellent for all CT outcomes. Inter-observer ICCs were excellent (>0·8) for the primary outcomes of %Disease and for %Bronchiectasis, %Mucus plugging, and %Atelectasis; and poor for %Airway wall thickening (0·27, 95% CI –0·08 to 0·58) and %Trapped air (0·23, –0·17 to 0·56). For %Trapped air, inter-observer ICC improved to 0·98 (0·95 to 0·99) after excluding one outlier that was interpreted incorrectly by the second observer as judged independently by two experts (appendix p 13).

Adjusted for the baseline values, the difference in log (%Disease+0·1) between the treatment groups at 48 weeks was 0·52% (95% CI 0·15 to 0·89; p=0·0092, appendix p 15). %Disease at 48 weeks was approximately 57% higher in the isotonic saline group than in the hypertonic saline group. To facilitate the interpretation of the results, we generated an effect plot to present the predicted %Disease values at 48 weeks per treatment group adjusted for the mean baseline %Disease values and baseline age (figure 2). In particular, mean predicted values at 48 weeks for participants with a mean baseline %Disease and a mean baseline age were 1·55% (95% CI 1·25–1·84) in the isotonic saline group and 0·88% (0·60–1·16) in the hypertonic saline group (mean difference 0·67%, 95% CI 0·26–1·08; p=0·0092). Mean %Bronchiectasis at 48 weeks was 1·20% (0·93–1·47) in the isotonic saline group and 0·72% (0·45–0·99) in the hypertonic saline group (p=0·021). Mean %Trapped air at 48 weeks was 2·94% (2·30–3·59) in the isotonic saline group and 0·90% (0·33–1·47) in the hypertonic saline group (p=0·0063). There was no significant difference in %Airway wall thickening (p=0·54) and %Atelectasis (p=0·34) at 48 weeks between groups (appendix p 16). We did not do an analysis for %Mucus plugging because of the large proportion of zero values.

At baseline, 107 (92%) participants provided acceptable MBW measurements; $LCI_{2.5}$ was above the upper limit of normal (ie, >8) in 44 (41%) participants.[10] The trajectories of $LCI_{2.5}$ for each treatment group are shown in figure 3B. Median $LCI_{2.5}$ in the isotonic saline group was 7·88 (7·20–9·05) at baseline (n=55), 7·59 (7·07–8·99) at 24 weeks (n=45), and 7·94 (7·04–8·85) at 48 weeks (n=47). Median $LCI_{2.5}$ in the hypertonic saline group was 7·61 (7·04–8·23) at baseline (n=52), 7·28 (6·70–8·07) at 24 weeks (n=40), and 7·26 (6·85–7·97) at 48 weeks (n=41). During the trial, mean $LCI_{2.5}$ tended to decrease in the hypertonic saline group (mean change –0·30, 95% CI –0·62 to –0·01) and increase in the isotonic saline group (0·19, –0·16 to 0·54) over 48 weeks (mean difference 0·49, –0·02 to 0·96; p=0·046, appendix p 20,34). Mean predicted $LCI_{2.5}$ values at 48 weeks for participants with a mean baseline $LCI_{2.5}$ and mean baseline age were 8·09 (95% CI 7·90–8·29) in the isotonic saline group and 7·62 (7·48–7·76) in the hypertonic saline group (mean difference 0·47, 95% CI 0·23–0·71; p=0·033; appendix p 25,37).

The trajectories of PRAGMA-CF %Disease for each treatment group are shown in figure 3A. Median %Disease in the isotonic saline group was 1·03 (IQR 0·40–1·93) at baseline (n=60) and 1·45 (0·63–4·31) at 48 weeks (n=55). Median %Disease in the hypertonic saline group was 0·89 (0·41–2·00) at baseline (n=56) and 1·16 (0·37–2·37) at 48 weeks (n=49). Changes in PRAGMA-CF %Disease, %Bronchiectasis, and %Trapped air from baseline to 48 weeks were not significantly different between groups (linear mixed effects model, appendix p 22–23). In the hypertonic saline group, the mean difference in the progression of %Disease was 0·09 (95% CI –0·27 to 0·45) and the mean difference in progression of %Bronchiectasis was 0·06 (–0·25 to 0·37). In the isotonic saline group, the mean difference in the progression of %Disease was 0·59 (0·09 to 1·09) and the mean difference in progression of %Bronchiectasis was 0·54 (0·14 to 0·94). %Trapped air tended to decrease over time in the hypertonic saline group and increase in the isotonic saline group with a mean difference change of –0·06 (95% CI –0·67 to 0·55) in the hypertonic saline group and 0·15 (–1·94 to 0·24) in the isotonic saline group (appendix p 35–36).

Participants reported 622 adverse events in the isotonic saline group and 563 events in the hypertonic saline group. Most adverse events in both groups were rated as mild. The most common adverse events were cough, nasal congestion, fever, and rhinorrhoea. There were no reported adverse events related to anaesthesia for CT. 11 participants in the isotonic saline group reported 25 serious adverse events and 13 participants in the hypertonic saline group reported 18 serious adverse events; 42 (98%) of these events were considered unrelated to treatment and for which concomitant medications were needed. The most reported serious adverse events were pulmonary exacerbation, cough, and *Pseudomonas* infection. Four life-threatening adverse events were reported in three participants in the isotonic saline group, none of which were judged to be related to treatment. Reported life-threatening adverse events were cough, nasal congestion, pulmonary exacerbation, and stomach ache. Cough, nasal congestion and stomach ache were deemed mislabelled as they do not meet the definition of a serious adverse event (appendix p 24).

Discussion

In this 48-week randomised controlled trial, treatment with hypertonic saline had a positive effect relative to isotonic saline on lung structure as determined by chest CT imaging, with a significant difference in PRAGMA-CF %Disease at 48 weeks in children aged 3–6 years with cystic fibrosis. Significant differences between groups were also observed for %Bronchiectasis and %Trapped air at 48 weeks. Both treatments were well tolerated with an acceptable safety profile.

The mean absolute difference between treatment groups in PRAGMA-CF %Disease at 48 weeks was 0·67% of total lung volume. This is less than the anticipated 1·44% used for the power calculation based on the AREST CF cohort. However, the relative difference between study groups of 30% is consistent with the anticipated relative difference of 28% used to estimate sample size requirements. The baseline mean %Disease score for SHIP-CT was less than half of that observed in the AREST CF cohort. The absolute numerical discrepancy can be explained by systematic differences between the manual PRAGMA-CF scoring of each cohort and by the image acquisition protocol used for the different study groups. CTs were obtained from SHIP-CT participants using a technician or spirometry guided breath hold protocol without sedation, whereas the AREST CF cohort included CTs obtained under general anaesthesia, close to total lung capacity using pressure control that would have identified more abnormal airways relative to CTs obtained at a lower lung volume. To understand this difference in %Disease between treatment groups from a clinical perspective, it is in the range of the mean annual progression of %Disease observed in various cohorts. The mean annual change in %Disease in the AREST CF[25] cohort (mean age 3·3 years) was 0·5%, in the Gothenburg cohort[2] (mean age 6·8 years) 0·62%, and in the Ataluren study[23] (mean age 22·7 years) 1·23%. Secondly, this difference in %Disease reflects a substantial difference in the percentage of lung volume occupied by abnormal airways. Normal airways only occupy 0·10–0·25% of total lung volume in children aged 6·16 years with normal chest CTs (data not shown). The number of visible airways over the 1-year study period can increase due to increase in body size and age, but more importantly due to increased visibility of small airways in relation to airway disease.[33] The difference of 0·67% in PRAGMA-CF %Disease between treatment groups represents a substantial difference in the number of abnormal airways observed on the CTs.[21] As an example, a participant randomly assigned to the isotonic saline group progressed in %Disease over 48 weeks, from 0·57% to 1·46%. The number of diseased airways increased from 16 (22%) of 70 visible airways at baseline to 33 (32%) of 103 visible airways at 48 weeks. We therefore conclude that the difference in %Disease between treatment groups represents a clinically relevant difference in the severity of airways disease.

PRAGMA-CF %Disease is a composite score combining the most frequently observed structural changes of the airways related to cystic fibrosis lung disease: airway wall thickening, bronchiectasis and mucus plugging. Current understanding is that in the first 3 years of life, airway wall thickening and mucus plugging are the earliest structural changes that can be observed in association with impaired mucus clearance, airway infection and inflammation.[4,25,26] These early changes gradually lead to a diffuse widening of the airways defined as bronchiectasis when the outer diameter is wider than the diameter of the adjacent artery as shown in the AREST CF cohort.[29] Bronchiectasis is a

clinically important outcome measure as it is considered an irreversible structural change of the airways.[24] Importantly, hypertonic saline treatment also reduced progression of the secondary outcomes %Bronchiectasis and %Trapped air (a marker of small airways pathology).[34] Trapped air or low attenuation regions is thought to reflect a mix of trapped air and hypoperfusion of lung parenchyma as a consequence of small airway pathology.[35] These small airways cannot be observed directly on chest CT in children younger than 6 years due to the poor resolution of the CT scanners in relation to the small size of the airways.[21] Trapped air is present early in life, progressively increases throughout life in people with cystic fibrosis and makes up on average 50% of total lung volume on CTs of patients with cystic fibrosis and end-stage lung disease.[36,37] The positive effect of hypertonic saline on %Trapped air suggests a positive effect of hypertonic saline on small airways pathology.

SHIP-CT is the first study in children aged 3–6 years with cystic fibrosis using chest CT as a primary outcome measure. Only four participants could not be randomly assigned as they were not able to cooperate with the chest CT protocol. All participants had technician guided chest CT training. When designing the study, we took into account that lung volume at baseline would be less well controlled in these children relative to 48 weeks. For this reason, we selected as the primary outcome measure %Disease adjusted for baseline %Disease values and baseline age. The lung volume is important for the correct diagnosis of bronchiectasis, as the ratio between the airway diameter and artery diameter is highly dependent on lung volume.[29] For this reason, great effort was put into training breathing manoeuvres throughout the study to optimise the volume at 48 weeks. As expected, image and lung volume quality scores improved between the baseline and the 48-week chest CT scan. SHIP-CT results add to the external validation of chest CT as a feasible modality and to PRAGMA-CF as a sensitive image analysis system to evaluate structural lung changes for clinical studies in children aged 3–6 years with cystic fibrosis.

The mean $LCI_{2.5}$ decreased in the hypertonic saline group and increased in the isotonic saline group, findings that are in concordance with the SHIP study.[18] Importantly, the SHIP-CT trial was powered to detect differences in CT outcomes whereas SHIP was powered to detect changes in $LCI_{2.5}$. For SHIP, 150 children aged 3–6 years were randomly assigned versus 116 for SHIP-CT; therefore, it is likely that our study was underpowered to detect differences in $LCI_{2.5}$. A post-hoc analysis computing the difference in $LCI_{2.5}$ at 48 weeks adjusted for baseline $LCI_{2.5}$ values and baseline age similar to the analysis for CT outcomes supported that the $LCI_{2.5}$ results of our study are in line with the SHIP results. Furthermore, $LCI_{2.5}$ is thought to be an outcome measure especially sensitive to changes in the small airways.[38] Hence, the $LCI_{2.5}$ results support the positive effect of hypertonic saline on trapped air observed on chest CT.[39]

SHIP-CT supports that hypertonic saline therapy is safe in children aged 3–6 years. Importantly, this profile was like that of SHIP[18] and ISIS.[16] Most adverse events were considered mild and unrelated to the treatment. Cough was the most common adverse event in each group.[40]

The SHIP-CT study has some limitations. Firstly, the comparator isotonic saline might also have some effects on mucociliary clearance. However, inhaled isotonic saline has been considered an appropriate comparator in previous clinical trials investigating the effectiveness of hypertonic saline.[16,18] We can only speculate whether isotonic saline might improve CT outcomes, in which case the true effect of hypertonic saline in this study might have been underestimated. Secondly, the use of chest CT as an outcome measure exposes the study participants to ionising radiation. For this reason, a site-specific low-dose CT protocol was defined for each scanner used in the study, optimising the balance between radiation and image quality in accordance with the guidelines for research studies.[41] The exposure in the SHIP-CT study is similar to 15 months of background radiation and is considered to be low.[42] Thirdly, we might have somewhat underestimated the volume of trapped air as expiratory scans were acquired at FRC and not at residual volume. We considered an expiratory scan at residual volume to be challenging for children aged 3–6 years. However, this is unlikely to have affected the comparison between treatment groups.

In conclusion, our results indicate that maintenance treatment with hypertonic saline for 48 weeks improve trajectories of structural airway changes as measured by chest CT outcomes relative to isotonic saline in children aged 3–6 years with cystic fibrosis. Although cystic fibrosis transmembrane regulator (CFTR) modulator therapy has been approved in most jurisdictions for children aged 6 years and older, in most countries, infants and children younger than 6 years do not yet have access to these transformational therapies. Therefore, SHIP-CT adds to the evidence base that twice daily nebulised hypertonic saline is an inexpensive and safe intervention with a positive effect on both respiratory function and on progression of structural lung disease. We recommend that inhaled hypertonic saline be considered for all young children, particularly those without access to CFTR modulator therapy, to minimise progression of structural lung damage. For clinical trials of therapies to treat cystic fibrosis lung disease in children younger than 6 years, we recommend including outcome measures to evaluate both lung structure and function.

Contributors

HAWMT, SDD, MR, FR, and SMS designed the study. YC, AD and KDHS gathered and managed the data. E-RA and YC did the analyses. RAK and KDHS rerun the primary outcome analysis. HAWMT, YC, E-RA, RAK, and KDHS have accessed and verified the data. YC and HAWMT wrote the first draft of the manuscript. All authors contributed to data interpretation and critical review and revision of the manuscript. HAWMT and SMS are responsible for the submission. The corresponding author had full access to all the data and had final responsibility for the decision to submit for publication.

Declaration of interests
HAWMT reports grants from the Cystic Fibrosis Foundation and Health Holland, is director of the Erasmus MC- LungAnalysis laboratory, which also acts as the ECFS-CTN CT expert centre, and serves as consultant for Thirona on image analysis. Erasmus MC is expected to receive future license royalties for PRAGMA-CF. SDD reports grants from Cystic Fibrosis Foundation. MR reports grants from Cystic Fibrosis Foundation. FR served as consultant for Vertex, Bayer, Roche, Genentech, and Proteostasis. RAK reports grants from Cystic Fibrosis Foundation. KDHS reports grants from Cystic Fibrosis Foundation. SMS reports grants from the Cystic Fibrosis Foundation and National Health and Medical Research Foundation, and Telethon Kids Institute is expected to receive future license royalties for PRAGMA-CF. All other authors declare no competing interests.

Data sharing
De-identified individual participant data that underlie the results reported in this article will be immediately available after publication (note: chest CT images can only be accessed on site in the Erasmus MC). The study protocol will be available online. The data will be shared with researchers who provide a methodologically sound proposal to achieve aims in the approved proposal. Proposals should be directed to h.tiddens@erasmusmc.nl to gain access, data requestors will need to sign a data access agreement.

Acknowledgments
This study was funded by Cystic Fibrosis Foundation Therapeutics (grants TIDDEN15K0).

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