Supplementary Online Content


**eMethods.** Trial protocol

**eTable.** Respiratory Distress Assessment Instrument

This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods. Trial Protocol.

PROTOCOL NUMBER: CCI-06-00271

TITLE: Nebulized hypertonic saline for treatment of viral bronchiolitis

STUDY PHASE: Phase II and III

STUDY ARMS: 0.9% saline + albuterol vs. 3% saline + albuterol

IND OR IDE #: N/A

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### TABLE OF CONTENTS

**SCHEMA, SYNOPSIS, OR STUDY SUMMARY**

1.0 BACKGROUND AND HYPOTHESES  3

2.0 OBJECTIVES AND PURPOSE  6

3.0 STUDY DESIGN  8

4.0 DRUG/DEVICE INFORMATION  9

5.0 SELECTION AND WITHDRAWAL OF SUBJECTS  9

6.0 DESCRIPTIVE FACTORS/STRATIFICATION/RANDOMIZATION SCHEME  10

7.0 STUDY AGENT ADMINISTRATION OR INTERVENTION AND TOXICITY MANAGEMENT PLAN  10

8.0 ASSESSMENT OF EFFICACY AND SAFETY  11

9.0 CLINICAL AND LABORATORY EVALUATIONS  12

10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS  12

11.0 SPECIAL INSTRUCTIONS  12

12.0 DATA COLLECTION AND MONITORING  13

13.0 STATISTICAL CONSIDERATIONS  13

14.0 REGISTRATION GUIDELINES  16

15.0 BIOHAZARD CONTAINMENT  17

16.0 ETHICAL AND REGULATORY CONSIDERATIONS  17

17.0 REFERENCES  17

**APPENDICES**
1.0 BACKGROUND AND HYPOTHESES

1.1 Viral bronchiolitis in children. Bronchiolitis is the most common viral respiratory infection in young children and infants. It is responsible for hundreds of thousands of outpatient visits and an increasing proportion of hospitalizations each year.1,2 In children under 5 years of age, the rate of emergency department visits in 1999-2000 was 42.2/1000, and the rate of hospitalizations was 39.1/1000.3 In 2002, there were 209,000 admissions for bronchiolitis in the pediatric population, costing the U.S. healthcare system over $300 million annually.4 There have been many clinical trials testing various interventions including inhaled steroids, albuterol, epinephrine, mucolytics, and antiviral medications, but none have been proven consistently effective in meta-analyses.5,6,7,8 Inhaled steroids including budesonide and dexamethasone have been tried, with no clear benefit except possibly in premature infants.9,10 Systemic steroids have shown short term benefit in some studies, but no benefit in others.11,12,13,14,15 Albuterol may help a subset of patients, which may or may not be related to underlying asthma.16,17 Racemic epinephrine has shown promise in the outpatient setting, but has no clear benefit for inpatients.18,19,20 Surfactant, Ribavirin, and heliox have been studied, but only in critically ill infants.21,22,23,24 Recombinant deoxyribonuclease I has been shown in one study to improve CXR appearance, but had no effect on clinical outcome.25

1.2 Inhaled hypertonic saline in clinical context. Hypertonic saline has been studied extensively in patients with cystic fibrosis and COPD. More recently it has been used to treat infants with viral bronchiolitis, resulting in decreased clinical severity scores and decreased length of stay. Hypertonic saline may improve bronchiolitis via a variety of mechanisms:

1.2.1 Improvement in nasal congestion. A significant component of respiratory distress and poor feeding in infants with bronchiolitis is attributable to obstruction of the nasal passages by mucus. Routine hypertonic saline irrigation may improve symptoms in adults with chronic rhinosinusitis symptom.26 In addition, several studies have shown improvement in nasal ciliary motility following intranasal spray of hypertonic saline.27,28 The hyperosmolarity of the solution may also decrease viscosity of the nasal mucus, so it is more easily aspirated with a suction catheter or bulb syringe.

1.2.2 Improvement in pulmonary ciliary motility. Hypertonic saline nebulization has been studied extensively in cystic fibrosis patients. A Cochrane review found significant short term benefit in FEV1 and mucociliary clearance for patients with cystic fibrosis, compared to control.29 This effect seems to be proportional to saline concentration, with 7% and 12% saline showing greater improvement than 0.9% and 3% saline, although higher concentrations lead to more bronchoconstriction and local irritation.30 There is limited data on non-cystic fibrosis patients. Daviskas studied mucociliary clearance in asthmatic and control patients using radioisotope quantification, comparing no aerosol, 0.9% saline, and 14.4% saline nebulization. There was a profound increase in mucociliary clearance in both asthmatic and control subjects receiving 14.4% saline, compared to both no aerosol and 0.9% saline. The effect was relatively short, peaking during the inhalation, and lasting 20-60 minutes.31 However, other studies in cystic fibrosis patients show a sustained improvement (>8 hours) in mucus clearance, as measured by inhaled radio-isotope, which persisted after 2 weeks of QID treatment.32 Other effects may be even longer lasting- a recent double-blind randomized trial in cystic fibrosis patients who received 0.9% vs 7% saline inhalations only twice a day showed significant improvements in FVC and FEV1, and decreases in number of pulmonary exacerbations after 48 weeks of therapy.33

1.2.3 Decreased edema in pulmonary epithelium. Respiratory syncytial virus causes edema, necrosis and sloughing of the respiratory epithelium, leading to obstruction of the small and medium sized airways. Hypertonic saline may draw fluid from the submucosal and adventitial space, thereby decreasing airway edema. The fluid is then pulled into the lumen where it may soften up the thick mucus plugs, making it easier to expectorate. Studies in cystic fibrosis patients affirm a significant decrease in sputum viscosity, improvement in ease of expectoration, and increase in weight of expectorated sputum.34 In vitro studies of cystic fibrosis airway epithelium show increased airway surface liquid
height and increased transepithelial water transport after exposure to sodium chloride.\(^{32}\) However, studies with nebulized mannitol have not consistently shown a similar effect, which suggests that the mechanism of action is more specific to the NaCl ion transport than osmolality.\(^{35}\) Donaldson et al. tested this hypothesis in cystic fibrosis patients, by administering the sodium channel blocker amiloride prior to hypertonic saline treatments. Patients randomized to receive hypertonic saline with placebo showed a significant and sustained increase in mucus clearance and in lung function. Those who received amiloride did not show a significant response.\(^{32}\)

1.2.4 Immunomodulatory effect. In bronchiolitis, inflammatory cytokines and chemokines are responsible for much of the cell damage which occurs. Numerous in vitro and in vivo studies have shown that hypertonic saline may modulate the immune response. One mechanism may be deformation of the cell membrane due to hypertonicity, leading to decoupling of surface membrane receptors and loss of intracellular signaling cascades.\(^{36}\) In vitro human nasal epithelial cells have been shown to produce IL-6, IL-8, and RANTES in response to RSV infection; this leads to further potentiation of pro-inflammatory cytokine production, and further cell damage.\(^{37}\) Additionally, hypertonic saline in vitro causes decreased neutrophil CD11b/CD18 expression, decreased elastase release, decreased superoxide production, and decreased response to stimulation. This effect was reversible with return to normotonicity.\(^{38}\)

1.2.5 Use in bronchiolitis. There are three randomized controlled clinical trials from Israel evaluating nebulized hypertonic saline in children with bronchiolitis- one in the ambulatory setting and two in the inpatient setting. Sarrell et al. conducted a randomized, double-blind controlled trial of 65 infants < 24 months with viral bronchiolitis. Children were excluded if they had cardiac disease, chronic lung disease, previous history of wheezing, saturation < 96%, or required hospitalization. Group 1 received 0.5 ml terbutaline nebulized in 2 ml 0.9% saline three times a day for 5 days, and group 2 received 0.5 ml terbutaline nebulized in 2 ml 3% saline three times a day for 5 days. Clinical severity scores prior to treatments on days 2-5 were significantly better in group 2 (3.9, 2.1, 1.1, 0.9 vs 5.2, 4.8, 3.8, 2.9) (p<0.005), and clinical severity scores after treatments on days 1-5 were also significantly better in group 2 (p<0.005).\(^{39}\) A second study by the same group, Mandelberg et al. included 52 hospitalized infants age < 12 months who were hospitalized with bronchiolitis and fever > 38.0. Exclusion criteria included cardiac disease, chronic lung disease, previous history of wheeze, saturation< 85%, decreased level of consciousness, and respiratory failure. The patients were randomized into two groups- group 1 received nebulized racemic epinephrine mixed in 4 ml of 0.9% saline three times a day, and group 2 received nebulized racemic epinephrine mixed in 4 ml of 3% saline three times a day. Both groups were allowed to give prn doses as needed. The mean length of stay in the 0.9% saline group was 4 +/- 1.9 days, versus 3 +/- 1.2 days in group 2 (p<0.05). There was also a significant difference in the percent improvement of clinical severity scores recorded on each day (group 1 3.5%, 2%, 4%; group 2 7.3%, 8.9%, 10%) (p<0.001). There were no adverse events.\(^{40}\) An extension of this study was published in 2006, confirming previously reported improvements in clinical score and decrease in length of stay.\(^{41}\) Recently, a multi-centered trial of hospitalized bronchiolitis patients in Canada and the United Arab Emirates utilized 4 ml 3% saline vs 4 ml 0.9% saline, given initially q2h for 3 doses, then q4h for 5 doses, then q6h until discharge, showed a 26% decrease in average length of stay, with mean decrease of almost 1 day. Unlike previous studies, the Canadian trial allowed the saline to be nebulized alone, or if other prn aerosolized medications were ordered by the treating physician, mixed with other medications including albuterol, epinephrine, and inhaled corticosteroids.\(^{42}\)

1.3 Safety of inhaled hypertonic saline. Hypertonic saline nebulization has been used widely in pediatric and adult populations with relative safety. There have already been two studies using nebulized 3% hypertonic saline with epinephrine and terbutaline at every 8 hour intervals for up to 5 days in infants with bronchiolitis (see above), showing no adverse effects.\(^{39,40}\) In addition, a third study comparing efficacy of nebulized hypertonic saline delivered via face mask versus hood, used
4 cc of 3% saline with epinephrine every 8 hours to infants 0-24 months old with bronchiolitis. Again, there were no adverse effects.43 Children with asthma have been shown to have decreased FEV1 after inhalation of hypertonic saline, so patients with any previous history of wheezing will be excluded. There may be patients with underlying asthma who have not had wheezing prior to their presentation with bronchiolitis. Even with asthmatic children, the amount required to cause bronchoconstriction is more than what we will be using (5.3-23 ml of 4.5% saline, vs. 4 ml of 3% saline in our protocol).44,45,46 Children without history of wheeze are much less likely to have hyperresponsiveness to hypertonic saline.45 A study of adults with moderate to severe asthma showed no significant drop in FEV1 after 4.5% saline nebulization when given concomitantly with salbutamol.47,48 Because of the risk of bronchoconstriction occurring with nebulized hypertonic saline alone in patients with underlying asthma, we will be pre-treating with albuterol. About a third of patients presenting with bronchiolitis may have underlying bronchial hyperreactivity, so even excluding patients with prior wheeze may not identify all patients at risk. Prior studies have demonstrated that the bronchoconstriction can be attenuated by pretreating with a bronchodilator such as albuterol, therefore we have chosen this approach.

1.4 Significance of current research. To date there have been no trials in the United States using hypertonic saline for treatment of bronchiolitis. All three published trials discussed above are from the same group in Israel. They use a clinical severity assessment tool, which has not been used in other bronchiolitis studies.14,15,16 We plan to use the Respiratory Distress Assessment Instrument (RDAI), which has been used in the majority of clinical trials.15,18,42,49,50 Also, the previous studies have small numbers of patients, and do not address hospitalization rate. Our study is powered to detect a difference in both hospitalization rate and length of stay. A key advantage to hypertonic saline is that it can be administered safely at home, and is a relatively inexpensive intervention. If patients can be given nebulized treatments at home they may be able to avoid hospitalization. The Israeli studies used epinephrine with saline, and Sarrell et al.39 used another beta-agonist, terbutaline. Our study would use albuterol, which is inexpensive and unlike epinephrine and terbutaline, can be safely given at home.

2.0 OBJECTIVES AND PURPOSE

2.1 Decreased length of stay. The average length of stay for patients admitted with viral bronchiolitis of mild-moderate severity is 3.55 days at CHLA and 3.13 at CHRCO. In the 2003-04 bronchiolitis season, there were 173 inpatient cases at CHLA and 424 cases at CHRCO. The weighted average length of stay is 3.25 days, standard deviation 1.4 days. Using a power of 80% to detect a difference in length of stay of 0.5 days with 0.05 significance (two-tailed), we need 124 admitted patients in each arm. We plan to enroll 150 patients in each arm, which would give us 87% power to detect a difference of 0.5 days, or 80% to detect a difference of 0.45 days. National Hospital Discharge Survey data from 2002 found 209,000 admissions for bronchiolitis, with an average length of stay of 3.2 days, costing the U.S. healthcare system more than $300 million.4 With a half day decrease in length of stay, the cost savings could potentially be $46,875,000 annually.

2.2 Decreased hospitalization rate. Hospitalization rates from previous studies range from 24-53% among children’s hospitals. Using MMWR data from 1999-2000, the rate of outpatient visits for bronchiolitis for children < 1 year old was 146.2/1,000. The rate of hospitalization was 39.1/1,000.3 This results in a 17.5% rate of admission among all outpatients, but likely severely underestimates emergency department admission rates because it includes patients who present to urgent care centers and outpatient clinics. Because of the very low admission rate from these settings, the sample size needed to detect a significant difference in admission rates would be prohibitively high. Therefore we will not be enrolling patients from ambulatory clinic or urgent care. The overall admission rate from both institutions is 620 out of 1850 visits, or 33.5%. To detect a 25% reduction in admission rate with a power of 0.8 and 2-sided significance level of 0.05, 472 patients need to be recruited in each group. To detect a 30% reduction in admission rate with the same power and significance levels, 335 patients would need to be recruited in each group. If we use 350 patients in each group, then the study would have a power of 0.8 to detect a 29% reduction in
rate of hospitalization. Again, using the 2002 National Hospital Discharge Survey data, a 25% decrease in admission rate could result in a $74,905,600 annual savings to the U.S. healthcare system.4

2.3 Decreased clinical severity. Because pulmonary function testing requires sedation and is prohibitively difficult and expensive in infants, we will use a clinical severity score to assess improvement. Clinical severity will be measured using the Respiratory Distress Assessment Instrument (RDAI), since this is the most widely used scoring instrument for bronchiolitis studies. Previous studies have shown inter-observer reliability to be very good (0.73-0.96 correlation coefficient). Mull et al. Arch Ped conducted a preliminary study which determined a clinically significant change in RDAI score to be 3 points. Their population at study entry included patients aged 6 weeks to 1 year who presented with RDAI score between 8-15. The mean baseline score was 10.1 in one group and 10.0 in the other group, with standard deviations of 1.4 and 2.0.20 Using an average RDAI score of 10 and standard deviation of 1.5, assuming a 3 point reduction in RDAI is significant, only 5 patients in each group would be necessary (power 0.8 and significance level 0.05, two-sided).

2.4 Decreased need for supportive therapy. Because clinical severity scoring is inherently somewhat subjective, we will measure objective outcomes which correlate with severity, including use of intravenous fluid, oxygen, and additional medications. Because the discharge time depends on many factors unrelated to medical readiness, these supportive measures will be an indirect measure of clinical improvement. Assessment will consist of number of hours requiring intravenous fluids, number of hours requiring oxygen, number of prn nebulizations given, and type of nebulizations given.

2.5 Relationship of response to hypertonic saline and cystic fibrosis mutation status. Patients with cystic fibrosis have improved clinical symptoms and pulmonary function tests with nebulized hypertonic saline.32,33 The postulated mechanism is abnormal sodium and chloride transport, leading to decreased airway surface liquid layer, which leads to poor mucus clearance. Therefore it is possible that patients who are CFTR mutation carriers may have a greater response to hypertonic saline treatments than non-carriers. Starting August 1 2007, the state of California began implementation of cystic fibrosis testing as part of the newborn screening program. The testing strategy involves screening blood spots for immunoreactive trypsinogen. Those found to have high levels will have a DNA mutation panel done, which includes 38 known mutations selected to have the highest yield in the California population. Those found to have one mutation will be referred for DNA sequencing. This strategy is expected to identify approximately 900 carriers and 92 cases per year, or about 90 of all CF cases in California. Consent will be obtained from the guardians to obtain California newborn screening results.51

3.0 STUDY DESIGN
This study is a double-blinded, randomized controlled parallel group trial. The two study arms are NS (control) and HT (experimental). Eligible patients will be identified in the emergency department. The investigator on call will obtain informed consent. A unique study ID number will be assigned to each enrolled patient. The pharmacy will be notified of enrollment, and the pharmacist will use a random number table to assign the patient to a study group. The NS group will receive albuterol 2.5 mg nebulized in 2 ml normal saline per routine protocol, followed by 4 ml of 0.9% normal saline nebulized via face mask, every 20 minutes, up to 3 inhalations. The HT group will receive albuterol 2.5 mg nebulized in 2 ml normal saline per routine protocol, followed by 4 ml of 3% saline nebulized via face mask, every 20 minutes, up to 3 inhalations. The study investigator will determine RDAI score on enrollment and 30 minutes after the first treatment. After up to 3 inhalations, the emergency department staff will determine whether the patient can be discharged or needs to be admitted. Patients who are discharged will be discharged from the study. Patients who are transferred will be discharged from the study. Patients who are admitted will proceed onto the inpatient phase. They will continue to receive the same treatments once every 8 hours on the floor. Study personnel will perform a focused assessment once per day, and assess the RDAI score within 1 hour after one of the nebulized treatments. Additional treatments can be ordered by the medical team per routine.

Patients who are not enrolled in the emergency department (due to direct admission, transfer from another facility, or no investigator available to enroll the patient in the emergency department) will be approached upon admission to the inpatient service. These patients will be randomized to either NS or HT. These patients will receive albuterol 2.5 mg inhaled per protocol or 2 puffs with valved holding chamber and mask, followed by either 4 ml of 0.9% normal saline or 4 ml of 3% hypertonic saline nebulized via face mask, every 8 hours until discharge. Just as above, study personnel will perform a focused assessment once per day, and assess the RDAI score within 1 hour after one of the nebulized treatments. Additional treatments can be ordered by the medical team per routine. In order to minimize risks of hypoxia, oxygen saturation will be maintained at 92% or above.

It is standard of care at CHLA to administer albuterol as a first line therapy for bronchiolitis. The current multidisciplinary action plan (MAP) recommends a trial of albuterol in the emergency department, up to 2 treatments (see Appendix I). If there is clinical improvement, prn albuterol is recommended. If there is no clinical improvement, a trial of racemic epinephrine is recommended. If there is clinical improvement, prn racemic epinephrine nebulizations are recommended. If there is no clinical improvement, supportive care only without further inhaled therapy is recommended. This differs from our protocol, where albuterol will be administered around the clock before study treatments without regard to clinical response. However, this should not differ too much from actual practice; during the 2004-05 bronchiolitis season, 80% of patients admitted to CHLA with mild to moderate bronchiolitis received albuterol treatments; over half of these patients received more than 3 doses during the course of their hospitalization.

4.0 DRUG/DEVICE INFORMATION

4.1 Albuterol sulfate, 0.5% solution for inhalation.
   4.1.1 Dose: 2.5 mg diluted in 2 ml normal saline nebulized Q8 hours
   4.1.2 Pharmacokinetics: Peak effect: 0.5-2 hours. Duration 2-5 hours. Halflife: 3.8 hours. Metabolism: liver. Elimination: urine, 30% unchanged drug.
   4.1.3 Storage: 2-25 degrees C, stable
   4.1.4 Side effects: tachycardia, hypertension, palpitations, tremor, insomnia, agitation, rash, xerostomia, vomiting, diarrhea, paradoxic bronchospasm, hyperglycemia, hypokalemia with prolonged frequent use
   4.1.5 Toxicity: Hypersensitivity; cardiac arrhythmias, cardiac arrest with overdose. No cardiotoxicity in children mean age 20.7 months receiving 3.3 mg/kg/h (+/- 2.2 mg/kg/hr) (Lexi-Comp)

4.2 Hypertonic saline, 3% solution for inhalation. Per verbal communication with Sandy Barnes at the Food and Drug Administration Center for Drug Evaluation and Research, no IND is necessary for use of hypertonic saline in this protocol.
4.2.1 Dose: 4 ml Q8 hours
4.2.2 Pharmacokinetics: Peak effect: 0.3 hours. Duration 1 hour. Halflife: Metabolism:
   Elimination: urine.
4.2.3 Storage: 15-30 degrees C, stable
4.2.4 Side effects: hypernatremia, bronchospasm, mucosal irritation
4.2.5 Toxicity: Hypersensitivity

5.0 SELECTION AND WITHDRAWAL OF SUBJECTS

5.1 Inclusion Criteria. All patients between the ages of 0 and 24 months who present to the
   emergency department or non-intensive care inpatient service at Childrens Hospital Los Angeles
   (CHLA) or Children’s Hospital & Research Center at Oakland (CHRCO) between November and
   April with a primary clinical diagnosis of bronchiolitis are eligible. At the time of presentation,
   emergency department or ward staff will page the investigator on call. Each site will have a
   rotating pager for the investigator on call. Informed consent will be obtained from the child’s legal
   guardian by study personnel. Study information or other and consent forms will be translated into
   Spanish.

5.2 Exclusion Criteria. Patients with prior history of wheezing, asthma, or inhaled bronchodilator use
   are ineligible. History of asthma use is defined as a history of having a health care provider inform
   the parent that the child has or may have asthma. History of prior wheezing is defined as having a
   health care provider inform the parent during a previous illness that the child had wheezing.
   History of prior bronchodilator use is defined as a history of being given a prescription for inhaled
   albuterol, levalbuterol, or fomoterol during a previous illness. Patients with tracheostomy are
   excluded because the nebulization would not affect their upper airway. Patients born at less than
   34 weeks gestation, as well as those with chronic lung disease, or other significant pulmonary
   disease requiring chronic pulmonary medications or oxygen, are excluded. Chronic lung disease
   is defined as having a diagnosis of bronchopulmonary dysplasia or chronic lung disease, or history
   of being prescribed inhaled corticosteroids, diuretics, bronchodilator therapy, or oxygen. Patients
   with respiratory failure requiring assisted ventilation or intensive care stay are excluded. Patients
   with congenital heart disease are also excluded because they have significantly longer lengths of
   stay and may skew the results, and may be prone to arrhythmias triggered by albuterol.

5.3 Withdrawal Criteria. Patients and families may withdraw from the study at any time for any
   reason by notifying any member of the health care team. Patients will also be discontinued from
   the study if they need transfer to a different facility, transfer to an intensive care setting, or are
   found after study enrollment to meet any of the above exclusion criteria. Patients who experience
   serious adverse events (FDA Common Grading Scale of 3 or above) will be withdrawn from the
   study. In addition, patients with adverse events with FDA Common Grading Scale below 3 may be
   withdrawn from the study at the discretion of the treating physician and the principal investigator.

6.0 STRATIFICATION/DESCRIPTIVE FACTORS/RANDOMIZATION SCHEME

6.1 Stratification factors. The patients at each location will be randomized separately so as to accrue
   an equal number of patients from each site into each group. The control group will be called NS.
   The study group will be called HS.

6.2 Descriptive factors. Patient characteristics which will be collected include: age, gender, weight,
   gestational age at birth, ethnicity, duration of illness prior to presentation, tobacco exposure,
   atopy, and RSV status if known. These factors will not affect stratification, but will be analyzed to
   ensure equal randomization of these factors. In addition, univariate and multivariate analyses will
   be done looking at these factors at the end of the study.
6.3 **Randomization.** A random number table will be given to the pharmacy at each site. Patients will be enrolled sequentially. The patients will be assigned a unique study number. The pharmacist will then choose the next number down the column of the random number table.

7.0 **STUDY AGENT ADMINISTRATION OR INTERVENTION AND TOXICITY MANAGEMENT PLAN**

7.1 **Treatment plan.**

<table>
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<tr>
<th>AGENT</th>
<th>DOSE</th>
<th>ROUTE</th>
<th>FREQ</th>
<th>DURATION</th>
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<td>NS: 4 ml 0.9% normal saline</td>
<td>2.5 mg, or 2 puffs with valved chamber and mask</td>
<td>Inhalation (mask)</td>
<td>Q20 min x3 in ED, then Q8h</td>
<td>Duration of hospitalization</td>
<td>Additional prn treatments per medical team</td>
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<tr>
<td>HT: 4 ml 3% saline</td>
<td>2.5 mg, or 2 puffs with valved chamber and mask</td>
<td>Inhalation (mask)</td>
<td>Q20 min x3 in ED, then Q8h</td>
<td>Duration of hospitalization</td>
<td>Additional prn treatments per medical team</td>
</tr>
</tbody>
</table>

7.2 **Criteria for removal from treatment protocol.**

7.2.1 At the time of discharge, patients will be taken off treatments.
7.2.2 Treatment will be discontinued if symptoms become more severe and require a higher level of care (intensive care unit transfer, positive pressure ventilation).
7.2.3 Treatment will be discontinued if the patient is transferred to another institution.
7.2.4 Treatment will be discontinued if the patient is unable to tolerate the medication due to adverse effects as determined by the primary medical team.
7.2.5 A patient may always be removed from treatment whenever he/she wishes.

7.3 **Ancillary treatments.** There are no ancillary treatments that are contraindicated, except when such treatment results in a protocol violation (i.e., necessitating intensive care unit transfer, positive pressure ventilation). In this case, the patient will still receive the indicated treatment, but will be discharged from the study.

8.0 **ASSESSMENT OF EFFICACY AND SAFETY**

8.1 **Side effects/Toxicities to be monitored.**

8.1.1 **Acute effects.** Heart rate, blood pressure, mental status, respiratory distress score will be monitored twice a day. The patient will be assessed for vomiting, diarrhea, and rash once a day. For prolonged frequent albuterol use as determined by the primary medical team, electrolytes will be monitored at their discretion.

8.1.2 **Long-term effects.** No long-term effects of albuterol or hypertonic saline are known. They are both very short acting medications. There will be no follow-up after discharge.

8.2 **Dosage change based on toxicity.** No dosage changes will be made. If a patient experiences toxicity from any of the treatment components, then he/she will be discharged from the study.

8.3 **Adverse Event Reporting.** This is a moderate risk study involving evaluation of a low-risk well-established drug in pediatric patients. Safety monitoring will be done by the principal investigator and co-investigators and external DSMB (see Appendix III). Adverse events will be evaluated by the principal investigator and assigned a score using the Common Grading Scale 0-5. The relationship between the event and saline nebulizations will also be determined as “not related,” “possibly related,” “related/probably related,” or “unknown.” The principal investigator will review the patients’ physical exam, vital signs, symptoms, medications or interventions, adverse event, and interventions or treatments given due to the adverse event. Any event with Common
Grading Scale score of 3 or higher will be reported to the respective site IRB per timeline established below.

8.31 Unexpected and serious side effects will be reported to the principal investigator within 24 hours. Transfer to higher level of care, patient death, withdrawal from the study will be reported to the principal investigator within 24 hours. Life-threatening events or death will be reported to the CCI within 24 hours. Other events will be reported to the CCI within 5 days.

9.0 CLINICAL AND LABORATORY EVALUATIONS AND STUDY CALENDAR

Study calendar

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre Treatment</th>
<th>ED</th>
<th>Hospital Day 1-D/C</th>
<th>End of Treatment</th>
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<td>Targeted history and physical exam</td>
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10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

The outcome status (ie not approached, declined enrollment, enrolled but dropped out due to toxicity or other reasons, transferred to higher care, missing data, violated protocol, discharged, etc.) of all eligible patients will be reported. All eligible patients who begin treatment will be included in the analysis of survival and time-to-failure.

Discharge time is defined as the time a discharge order is written. This approximates the time at which the patient achieved medical readiness for discharge. Because both institutions use computerized physician order entry, the admission time and discharge time can be easily extracted from the medical record.

Length of stay is defined as the date of discharge (as defined above) minus the date of admission, giving an integer representing the number of nights spent in the hospital. This method of determining length of stay is the industry standard.

11.0 SPECIAL INSTRUCTIONS:

Not applicable.

12.0 DATA COLLECTION AND MONITORING

12.1 Case Report Forms. See Appendix II for Case Report Forms. Case Report forms will be submitted within 72 hours of a patient’s discharge from the hospital.

12.2 Retention of Study Record. Study records will be kept in a locked file in a locked office of the principal investigator and the CHRCO site sponsor. Records will be overnight mailed from CHRCO to
the principal investigator every month. These will be kept for a minimum of 3 years after completion of the study.

12.3 **Data Management.** De-identified data will be entered into an excel database by the research assistant and principal investigator (see Appendix IV). Paper study records with primary data will be maintained as above. The randomization key will be maintained by the pharmacy and will be deposited with the statistician. Emergency decoding procedures are included in Appendix XI.

12.4 **Data Monitoring.** Data monitoring will be done by an external DSMB (see Appendix III).

13.0 **STATISTICAL CONSIDERATIONS**

13.1 **General Study Design.** This study is a multi-site, randomized, stratified, double-blind, controlled trial comparing a standard treatment to a new treatment in a large population. The patient population will be drawn from the Emergency Departments and inpatient services of two large academic tertiary care free-standing children’s hospitals.

13.2 **Study objectives.** Demonstrating a decreased length of stay and decreased hospitalization rate in the experimental group are the primary objectives. Secondary outcomes include decreased need for supportive therapies such as hours of oxygen saturation, supplemental intravenous fluid use, and use of additional medications. Tertiary outcomes include decreased clinical severity score as measured using the RDAI.

13.2.1 **Decreased length of stay.** The average length of stay for patients admitted with viral bronchiolitis of mild-moderate severity is 3.55 days at CHLA and 3.13 at CHRCO. In the 2003-04 bronchiolitis season, there were 173 inpatient cases at CHLA and 424 cases at CHRCO. The weighted average length of stay is 3.25 days, standard deviation 1.4 days. Using a power of 0.8 to detect a difference in length of stay of 0.5 days with 0.05 significance (two-tailed student’s t-test), we need 124 admitted patients in each arm. We plan to enroll 150 admitted patients in each arm, which will give us 87% power to detect a difference in length of stay of 0.5 days. Length of stay analysis will be based on the general linear model with length of stay or possibly log length of stay as the dependent variable, and demographic covariates as possible independent variables, including treatment. Evaluation of residuals, influence points, and leverage points will be used to evaluate the goodness of fit of the model and identify and exclude any outliers.

13.2.2 **Hospitalization rate.** Rates of hospitalization differ depending on hospital type (children’s hospital vs. general hospital) and setting (emergency department vs. clinic). Among children’s hospitals, the rate of hospitalization ranges from 24-53%. Most hospitalizations originate from the respective Emergency Departments; a very small number come from direct admissions and transfers. Only very rarely are patients admitted from the ambulatory clinic or the urgent care clinic (non-emergency acute care: Kids Care at CHLA, ED Too at CHRCO). Because of the very low admission rate from these settings, the sample size needed to detect a significant difference in admission rates would be prohibitively high. Therefore we will not be enrolling patients from ambulatory clinic or urgent care. At CHLA in 2004, there were 854 visits to the Emergency Department and KidsCare with primary diagnosis of bronchiolitis. There is no CHLA data available which separates Emergency Department visits for bronchiolitis from KidsCare; however, using admitting physician as a proxy for patient location, overall about 60% of patients are seen in the Emergency Department and 40% in KidsCare. This means approximately 512 of the 854 bronchiolitis visits would present to the Emergency Department. The same year, 173 patients were hospitalized with a primary diagnosis of bronchiolitis. At CHRCO in 2003-04, there were 996 visits to the Emergency Department (excluding ED Too, the KidsCare equivalent). The same season (July 2003 through June 2004), there were 447 admissions for bronchiolitis to CHRCO. The overall admission rate from the emergency departments of both institutions is 620 out of 1508 visits, or
41.1%. To detect a 25% reduction in admission rate with a power of 80% and 2-sided significance level of 0.05, 350 patients need to be recruited in each group. Recruiting 350 patients in each group would give us 91.7% power to detect at 30% difference in admission rate. Each patient who is enrolled from the ED will be randomized to the treatment or control group, and patients in each group will be categorized as hospitalized or not hospitalized. Logistic regression will be used to evaluate the odds of admission to the hospital following ER evaluation. Covariates (age, gender, ethnicity, gestational age at birth, birth weight, day of illness, tobacco smoke exposure, history of eczema, RDAI at admission, oxygen saturation) will be considered. The area under the receiver operating curve will be used to evaluate the strength of the logistic model, and the Hosmer-Lemeshow test will evaluate the model fit.

13.2.3 Clinical score. We will be using the RDAI as our clinical scoring tool. This scoring tool is the most frequently used in bronchiolitis treatment trials. Several studies have shown very high inter-rater reliability, with excellent kappa and intraclass correlation coefficient scores. Since the scores are ordinal but not necessarily ratio values, we will need to use the chi-square test to analyze the scores. We will also convert the RDAI score to respiratory assessment change score (RACS), which is the difference between pre-treatment score and post-treatment score. We plan to analyze the change in RDAI score using the general linear model, including covariates (as above).13,20

13.2.4 Oxygen use. Because there is no gold standard to quantify clinical improvement in patients with bronchiolitis, and any clinical scoring tool is difficult to validate, we will be using several other outcomes as a proxy for clinical improvement. Patients who are in respiratory distress are given additional oxygen, and oxygen is titrated and weaned every 30-60 minutes with improving clinical status. Therefore amount of oxygen use should be related to clinical severity. We will review charts after discharge and document how many hours of supplemental oxygen use was needed per day of admission, as well as calculate how many hours of oxygen used in total per admission. We will compare the mean hours of oxygen use using a student’s t-test. We plan to use linear regression models to compare the amount of additional supportive treatments after adjusting for severity based on RDAI score. Although the standard of care for otherwise healthy patients with bronchiolitis is to accept oxygen saturations above 90%, study patients will be maintained at 92% or above, in order to minimize the risks of hypoxia.

13.2.5 Supplemental treatment use. Supplemental medications and nebulizations are another proxy measure for clinical severity. The frequency of nebulized medication use is proportional to clinical response to treatments and clinical severity. In practice, other medications such as steroids and diuretics are given when patients remain ill, despite maximizing nebulized medications. We will review charts after discharge and document how many nebulized treatments in addition to the schedule q8h study treatments are given. Treatments that will be included in analysis include racemic albuterol, levoalbuterol, racemic epinephrine, terbutaline, corticosteroids, and furosemide. We will compare number of additional nebulized treatments given (albuterol only, epinephrine only, albuterol + epinephrine). We plan to use linear regression models to compare the amount of additional supportive treatments after adjusting for severity based on RDAI score.

13.2.6 Intravenous fluid use. Increasing clinical severity often results in decreased ability to maintain oral intake. Therefore more ill infants need supplemental intravenous maintenance fluids to compensate for decreased oral intake. Patients with severe symptoms are made NPO and started on maintenance fluids. We will calculate volume per kg of intravenous fluid administration during the entire admission and compare both groups.

13.2.7 CFTR carrier status. Consent will be obtained from the guardians to obtain California newborn screening results. This is included in the general consent form for the study. We will perform analysis of co-variance to examine the relationship between cystic fibrosis carrier status with treatment effect. Patients who did not receive screening, have screening results unavailable, or who were born outside California, will be excluded from this portion of the analysis.

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13.2.8 **Other co-variates.** We will collect descriptive information about each enrollee, including patient age, gestational age, birth weight, ethnicity, days of symptoms prior to presentation, tobacco smoke exposure, and history of eczema or allergies. Site of enrollment will also be included as a co-variante. Indicator variables to adjust for individual rater’s RDAI scores will also be used in the analysis. Analysis of co-variance will be done to see if any of these characteristics contribute to the treatment effect.

13.3 **Patient accrual.** Of the patients who present with a primary diagnosis of bronchiolitis, we anticipate 80% of these to be eligible by inclusion/exclusion criteria. Of those eligible we anticipate 80% will consent to enroll. With total ED visits 996 at CHRCO and approximately 512 at CHLA, this means approximately 637 and 328 patients at each site respectively. For the inpatient part of the study: with total 424 admissions at CHRCO and 173 admissions at CHLA, estimated eligibility at least 80% and of those eligible estimated enrollment of 80%, we should have a minimum of 271 inpatients from CHRCO and 111 inpatients from CHLA each year. This is a total of 965 expected subjects per year (965 emergency department patients and 382 inpatients). We plan to have study personnel available to enroll patients for 12 hours each day, seven days a week, which will capture about 50% of the patients, or 482 emergency and 191 inpatients per year, or 241 and 95 in each group. Over two years we will be able to enroll twice that, or 482 emergency and 191 inpatients in each group, which is more than enough power to answer the study questions. If halfway through the first year, enrollment is below target, we will increase number of hours study personnel are available for enrolling patients.

13.4 **Reliability of clinical scoring tool.** The RDAI scoring tool has been previously validated in several studies, and has also been shown to have very high inter-rater reliability. The principal investigators at each site have been trained together to use the RDAI scoring tool, and have trained the staff at each site. Inter-rater reliability will be established before allowing additional scorers prior to each season. Prior to allowing investigators to participate in scoring, he/she will score at least 5 study patients with the key investigator. The weighted kappa score for the investigator must be greater than 0.6 for that investigator to use the RDAI scoring instrument.

13.5 **Interim analysis.** Interim analysis will be done at the end of the first study year by the independent statistician and will include:

13.5.1 **Adverse event data.** Blinded aggregate adverse events data will be available to the study team, while unblinded comparative data will only be available to DSMB members. The DSMB biostatistician will determine whether there is a difference in frequencies of adverse events of any type and of each common grading scale score, between control and study groups.

13.5.2 **Efficacy data.** This data will only be available to DSMB members. See DSMB charter and Data Analysis Plan. If a statistically significant difference between control and treatment groups (p < 0.02) for both of the primary outcomes (length of stay and admission rate), the DSMB may consider early termination, if they determine the risk-benefit profile has changed significantly enough that study continuation is not justified.

13.6 **Special considerations for minorities and women.** Our study will include all eligible minorities and women. Consent forms and study information will be available in English and Spanish. No patients will be excluded due to minority or gender. All efforts will be made to use interpreters for patients whose primary language is not English. There will likely not be enough Asian patients enrolled for the results to be valid in that population. Previous studies have shown that patients who present to the emergency department and who are admitted with bronchiolitis are more likely to be male, but there are more than adequate numbers of females to make the results valid for both males and females.

14.0 **REGISTRATION GUIDELINE**

14.1 **Registration contact.** Flyers describing the study, enrollment criteria, and contact information for enrollment will be posted in the emergency departments and distributed among medical and
support staff (see Appendix V). A pager number will be set up at each site to register patients for the study. The pager will rotate among study personnel who are available for enrolling patients. In addition, the principal investigator and the CHRCO site lead investigator will be available by pager for questions or event reports. Patients will be stratified by site then randomized by the pharmacist using a random number table.

14.2 **Registration materials.** At the time of registration, the patient’s guardian will be given the Informed Consent form and Bill of Rights/ HIPAA Authorization form (Appendices VI and VII), as well as the Release of Information form. Copies will be made for the patient and the principal investigator’s file, and the original will be placed in the medical record. A Registration/Eligibility Worksheet, Emergency Department Flowsheet, and Inpatient Flowsheet will be used (see Appendices VIII, IX, X, XI).

15.0 **BIOHAZARD CONTAINMENT**

Not applicable.

16.0 **ETHICAL AND REGULATORY CONSIDERATIONS**

All institutional and Federal regulations concerning the Informed Consent form will be fulfilled. The study will be conducted in adherence to ICH Good Clinical Practice.

17.0 **REFERENCES**


23. Wambnit DAM, Wormald P-J. A blinded, randomized, controlled study on the effect of buffered 0.9% and 3% sodium chloride intranasal sprays on ciliary beat frequency. Laryngoscope 115:803-05, 2005.


46. Leuppi JD, Anderson SD, Brannan JD, Belousova E, Reddel HK, Rodwell LT. Questionnaire responses that predict airway response to hypertonic saline. Respiration 72;52-60, 2005.

APPENDICES

Appendix I: CHLA bronchiolitis Multidisciplinary Action Plan (MAP)
Appendix II: Case report form
Appendix III: Data safety monitoring plan
Appendix IV: Database for de-identified data (Excel)
Appendix V: Subject recruitment flyer
Appendix VI: Informed consent, Parental permission form
Appendix VII: Patient Bill of Rights, HIPPA Authorization form
Appendix VIII: Registration and eligibility worksheet
Appendix IX: Data collection form- ED flowsheet

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Appendix X: Data collection form- inpatient flowsheet
Appendix XI: Emergency decoding procedures
eTable. Respiratory Distress Assessment Instrument

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