PEER REVIEW HISTORY

BMJ Paediatrics Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Normal saline for children with bronchiolitis: Study protocol for a Randomized Controlled Non-inferiority Trial
AUTHORS	Schmidt, Marika Nathalie Daugberg , Rie Nygaard, Ulrikka Nielsen , Xiaohui Chen Chawes, Bo Lung Krogsgaard Rytter, Maren Heilskov Schoos, Ann-Marie Malby

VERSION 1 – REVIEW

REVIEWER	Dr. Damian Roland University of Leicester, Health Sciences
REVIEW RETURNED	19-Sep-2023

GENERAL COMMENTS	Trials to determine the impact of saline (nebulised or otherwise) are needed in bronchiolitis as their persists in uncertainty about, especially nebulised, treatment. The study design appears reasonable but I would have liked to have seen more detail of previous studies examining saline and their limitations. I think a micro-scoping review in the form of a table would be useful to determine how this particular study will add to the literature. The inclusion criteria also doesn't mention timing of recruitment or severity. Could patients be recruited at any phase of the patient journey (perhaps having been in hospital for a couple of days). I think for this study it will be also important to record those who don't consent as this may be indicative of a particular parenting approach style which may impact on evaluation of outcomes.

REVIEWER	Dr. Peter Flom Peter Flom Consulting
REVIEW RETURNED	01-Oct-2023

GENERAL COMMENTS	.I confine my remarks to statistical and methodological aspects of this paper. Unfortunately, there are some fairly large problems that need to be addressed. But, since this is a protocol paper, fixing them should be relatively easy (It is good to read protocol papers and I commend BMJ for publishing them.)

	General Duration of hospitalization should not be tested with logistic regression. Given that it is measured in hours, OLS regression may be fine. For other outcomes: Symptom severity - probably ordinal logistic Ability to feed - either logistic or ordinal logistic (preferably, ability will be ordinally measured) Need for support and transfer to ICU - probably logistic The statistical plan section needs a lot more detail. You need to operationalize (that is, tell how every one is measured) all variables (including covariates listed at the top of p. 6) and to consider and say what method of analysis will be used. I listed some ideas for the outcomes in the abstract; other outcomes are listed in the text. Also, on p. 7 you say ANOVA will be used (presumably for the main outcome) but in the abstract you say logistic regression. (But it should almost certainly be OLS regression, which allows the inclusion of continuous and ordinal covariates). Specific p. 4 line 43 .... I'm not an expert, but I would think that staff other than the person administering the therapy could be blinded. I am guessing that a nurse would administer the drops, thus leaving the doctors blinded. p. 5 The outcomes listed here do not match those in the abstract. line 15 Since there are 3 treatments, there is no "opposite" and this should be "different". Peter Flom

REVIEWER	Dr. Paul McNamara University of Liverpool Department of Women's and Children's Health, Institute in the Park
REVIEW RETURNED	08-Oct-2023

GENERAL COMMENTS	In a proposed trial protocol, the authors outline a 3-armed randomised controlled non-inferiority trial of nebulised isotonic saline, isotonic saline nasal drops and no isotonic saline treatment in infants hospitalised with bronchiolitis. The proposed primary outcome measure is length of stay in hospital along with multiple other secondary outcome measures. The trial will take place in 6 paediatric centres in Denmark and aims to recruit 300 infants.

	Overall this is a well written protocol although lacking some important fine detail. My main comments include: 1. There is no mention of the new passive immunoprophylaxis treatments such as Nirsevimab, which are just about to be rolled out across Europe. The introduction should acknowledge their coming. Will the roll out impact on recruitment to this trial over the next couple of years? 2. The primary outcome measure needs to be defined in more detail. The Chung et al paper (reference 15) from which the estimate for length of stay (32 +/- 25 hours) is based examines hospital trends in bronchiolitis admissions over 15 years in Scotland betwen 2001-16. Nowhere in this paper could I find reference to length of stay in hospital in hours; were these figures obtained through personal communication with the Scotish authors or did they come from elsewhere? Would it not be more apposite to use recent figures from the Danish hospitals being used in the clinical trial? Length of stay in hospital for babies with bronchiolitis is difficult to accurately measure in clinical trials for multiple reasons. Will the authors use actual time to discharge from hospital or time when ready for discharge? How will this be standardised across sites? Could the authors clarify? Similarly, how will need for oxygen be measured? 3. What is the start date for the proposed trial, how many bronchiolitis seasons will recruitment take place over, who will collect the data? 4. Will data on risk factors such as parental smoking, mild prematurity, breast feeding be collected?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1 Dr. Damian Roland, University of Leicester, University Hospitals of Leicester NHS Trust

Comment 1: Trials to determine the impact of saline (nebulized or otherwise) are needed in bronchiolitis as their persists in uncertainty about, especially nebulized, treatment. The study design appears reasonable, but I would have liked to have seen more detail of previous studies examining

saline and their limitations. I think a micro-scoping review in the form of a table would be useful to determine how this particular study will add to the literature.

Response 1: We have done a small literature review looking specifically at the effect of normal saline for children with bronchiolitis and identified the following studies listed in the table below. House et al. [11] investigates the placebo status of normal saline in children with bronchiolitis

conducting a systematic review and meta-analysis. Looking through several randomized controlled clinical trials using NS as placebo, they found that normal saline could be an active treatment in bronchiolitis, as respiratory symptoms improved relative to oral placebo. A limit to the review is the heterogeneity of the included trials, and that they only report short-term physiologic measures, and not clinically relevant endpoints like duration of hospitalization or escalation of treatment.

Two of the other studies, Pukai et al. [10] and Schreiber et al. [14], investigated treatment with NS compared to standard care through randomized controlled clinical trials.

Pukai et al. found reduced respiratory distress scores, improvement of hypoxemia and more patients discharged in the group receiving nebulized normal saline. Like our study, it is a not blinded which may introduce bias. However, using objective measurements as we seek to minimize the risk of bias. Also, Pukai et al. only measured for four hours and therefore cannot provide evidence whether children admitted to hospital can benefit from nebulized normal saline. They point out, that viral detection tests were not undertaken so the proportions with respiratory syncytial virus or other pathogens are not known, which we plan to do in our study. Schreiber et al., who included children under one year of age with an oxygen saturation between 88% and 94%, found that SpO2 increased rapidly, and that the improvement sustained for a 50-minute period. This study was restricted to children within a definite range of desaturation. Also like the previous studies, this study only evaluated short-term improvements and did not measure the possible discomfort caused by nasal lavages. Both studies are single-center studies, whereas we plan to perform a multicenter study.

The last studies are somehow different. Hassan et al [13] made an inventory of the use of nebulized normal saline before and after dissemination of educational material about treating patients with bronchiolitis and asthma, since nebulized normal saline is not mentioned in the guidelines for either bronchiolitis or asthma as potential treatment modality. Like we wish to investigate, their study suggests that nebulized normal saline treatments increase costs and perhaps the length of stay. However, causality cannot be determined since it was not the purpose of the study, and any variables influencing discharge such as severity scores was not accounted for.

At last, Sautter et al. [12] primarily investigated whether lung function measurements using the electromagnetic inductance plethysmography (EIP) was feasible in clinical setting. They conducted EIP before and after NS inhalation in children under six months of age admitted with acute bronchiolitis. Besides the fact that EIP was feasible they also found that inhalation with NS seemed

to increase airflow resistance. Results are limited, because it depended on the day it was measured, which could represent a possible source of error.

Two of the studies was already mentioned as reference in our protocol [11, 14], and the three not mentioned earlier have now been added [10, 12, 13] in the introduction on page 4, lines 22 + 31-33:

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"…, where treatment is generally supportive [9, 10]."

"A secondary analysis of studies using nebulized NS as placebo when testing other treatments suggested that nebulized NS could improve symptoms of respiratory distress, compared to an oral placebo [11]. In contrast, another study suggested that nebulized NS could cause airway obstruction [12]. A quality improvement study found that de-implementing the use of nebulized NS did not increase length of hospital stay for children with bronchiolitis [13]. The main limitation of these studies is the heterogeneity of the methodology which hinders comparison of the results. Further, they only report short-term physiologic measures, and not clinically relevant endpoints like duration of hospitalization or escalation of treatment."

10	Pukai G, Duke T. **Nebulised normal saline in moderate acute bronchiolitis and pneumonia in a low- to middle-income country: a randomised trial in Papua New Guinea.** Paediatr Int Child Health. 2020 Aug;40(3):171-176. doi: 10.1080/20469047.2020.1725338. Epub 2020 Feb 17. PMID: 32063157.	This study is single center study which limits generalizability. The study did not do viral detection.	Used as reference [10] See page 4, line 22
11	House SA, Gadomski AM, Ralston SL. **Evaluating the Placebo Status of Nebulized Normal Saline in Patients With Acute Viral Bronchiolitis: A Systematic Review and Meta-analysis.** JAMA Pediatr. 2020 Mar 1;174(3):250-259. doi: 10.1001/jamapediatrics.2019.5195. PMID: 31905239; PMCID: PMC6990821.	The studies differ a lot in evaluating the effect using different scores, HR, RR, LOS	Used as a reference [11]. See page 4, line 33

12	Sautter M, Halvorsen T, Engan M, Clemm H, Bentsen MHL. **Electromagnetic inductance plethysmography to study airflow after nebulized saline in bronchiolitis.** Pediatr Pulmonol. 2020 Dec;55(12):3437-3442. doi: 10.1002/ppul.25058. Epub 2020 Sep 15. PMID: 32897652.	The study primarily introduces EIP as lung function measuring in ill children, but it does show some signals of obstruction using saline.	Now listed as new reference [12] See page 4, line 35
13	Hassan S, Gonzalez A, Demissie S, Morawakkoralage K, James P. **Nebulized Normal Saline Solution for Treatment of Bronchial Asthma Exacerbations and Bronchiolitis: Not Standard of Care.** Clin	The study is exploring implementation of guidelines. Length of stay (LOS) in relation to saline treatment is not	Now listed as new reference [13] See page 4, line 38
	Pediatr (Phila). 2018 Nov;57(13):15821587. doi: 10.1177/0009922818796657. Epub 2018 Sep 6. PMID: 30188182.	directly measured, which is why causality is missing.	
14	Schreiber S, Ronfani L, Ghirardo S, Minen F, Taddio A, Jaber M, Rizzello E, Barbi E. **Nasal irrigation with saline solution significantly improves oxygen saturation in infants with bronchiolitis.** Acta Paediatr. 2016 Mar;105(3):292-6. doi: 10.1111/apa.13282. Epub 2016 Jan 8. PMID: 26607495.	The study is a randomized controlled study, but it only includes patient within a saturation 88 – 94 %. They are not measuring discomfort, the impact on LOS, and feeding.	Used as reference [14] See page 5, line 4

Comment 2: The inclusion criteria also doesn't mention timing of recruitment or severity. Could patients be recruited at any phase of the patient journey (perhaps having been in hospital for a couple of days).

Response 2: Thank you for this comment. This has made us discuss and revise our plans around the logistics of the study. Inclusion criteria have been specified in the protocol, so that timing and severity is clear, on page 5, lines 35-37:

"The child is preferably included immediately after admission, but may also be included later, for example if admitted at night and no saline treatment has been started yet."

And page 5, lines 39-42:

"Children with any disease severity may be included, however, children who require respiratory support with nasal continuous positive airway pressure (N-CPAP) or high flow oxygen therapy (HFOT) right from admission start will be excluded because this

makes delivery of nebulized NS difficult."

Comment 3: I think for this study it will be also important to record those who don't consent as this may be indicative of a particular parenting approach style which may impact on evaluation of outcomes.

Response 3: As per the Ethics Committee requirements, we cannot include clinical data from patients that have not provided consent to participate in the study. However, we will record the number of eligible children who did not consent to participate and report it in our results and discuss it as a potential limitation. This has been specified in the manuscript on page 5, line 42 - page 6, line 2:

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"For children admitted with bronchiolitis who are not included in the study we will record the age, sex, and the reason for non-inclusion."

Reviewer: 2 Dr. Peter Flom, Peter Flom Consulting

General comment: I confine my remarks to statistical and methodological aspects of this paper. Unfortunately, there are some fairly large problems that need to be addressed. But, since this is a protocol paper, fixing them should be relatively easy (It is good to read protocol papers and I commend BMJ for publishing them.)

Comment 1: Duration of hospitalization should not be tested with logistic regression. Given that it is measured in hours, OLS regression may be fine.

Response 1: As the outcome is continuous (duration of hospitalization), we will use linear regression (or OLS regression) as suggested and Cox regression for survival analysis (time to discharge will be a good fit for this type of analysis, and it can be visualized using Kaplan Meyer kurve). This has been added to Page 9, lines 3-7:

"The three groups (no saline vs. nebulized NS, no saline vs. nasal irrigation with NS, and nebulized NS vs. nasal irrigation with NS) will be compared using linear regression and Cox regression analysis.

Comment 2: The statistical plan section needs a lot more detail. You need to operationalize (that is, tell how every one is measured) all variables (including covariates listed at the top of p. 6) and to consider and say what method of analysis will be used. I listed some ideas for the outcomes in the

abstract; other outcomes are listed in the text.

Response 2: We have now described in detail how all variables will be measured and included in the statistical method section. All continuous variables will be tested with linear regression, and binary outcomes will be tested with logistic regression.

Page 9, lines 14-15:

“Secondary outcomes will be tested using logistic regression. Exploratory outcomes are both binary (1-3) and continuous (4-8) and will be analyzed with linear and logistic regression respectively.

Comment 3: Also, on p. 7 you say ANOVA will be used (presumably for the main outcome) but in the abstract you say logistic regression. (But it should almost certainly be OLS regression, which allows the inclusion of continuous and ordinal covariates).

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Response 3: Please see response to comment 1. The mention of ANOVA in the manuscript has now been removed.

Comment 4: p. 4 line 43. I’m not an expert, but I would think that staff other than the person administering the therapy could be blinded. I am guessing that a nurse would administer the drops, thus leaving the doctors blinded.

Response 4: We like the idea of blinding doctors, but we believe that it will not practically be possible as the doctors too will be involved in the children’s care. The person analyzing the data will be blinded.

Comment 5: p. 5 line 15. The outcomes listed here do not match those in the abstract.

Response 5: We have aligned outcomes in both abstract and methods. See abstract, line 22-24:

“Secondary outcomes are need for respiratory support with nasal continuous positive airway pressure or high-flow oxygen therapy, and requirement of fluid supplements (either by nasogastric tube or intravenous).”

Comment 6: line 15. Since there are 3 treatments, there is no “opposite”; and this should be “different”.

Response 6: We have changed “opposite” to “different”.

Reviewer: 3 Dr. Paul McNamara, University of Liverpool Department of Women's and Children's

Health

Comment 1: There is no mention of the new passive immunoprophylaxis treatments such as Nirsevimab, which are just about to be rolled out across Europe. The introduction should acknowledge their coming. Will the roll out impact on recruitment to this trial over the next couple of years?

Response 1: This is a relevant consideration. We have added a section addressing this to the introduction.

At the moment only Palivizumab is used in Denmark, and only for high-risk infants. Nirsevimab is not yet used in Denmark, and the study will therefore be conducted in a predominantly unvaccinated population. Even if Nirsevimab is incorporated into the vaccination program for low risk infants in the future, we do not believe that it will eliminate bronchiolitis completely – and the results of this study may still benefit these patients. As an exploratory outcome we will also assess the effect of NS for children infected with specific pathogens – which may help us understand if the effect is different for bronchiolitis caused by other pathogens than RSV.

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The section is found on page 4, lines 20-22:

“Even though passive immunization strategies against RSV may change the disease pattern in the future, many children with bronchiolitis are still likely to require admission to hospital, where treatment is generally supportive [9, 10].

Comment 2: The primary outcome measure needs to be defined in more detail. The Chung et al paper (reference 15) BMJ Paediatrics Open bmjpo-2023-002273 from which the estimate for length of stay (32 +/- 25 hours) is based examines hospital trends in bronchiolitis admissions over 15 years in Scotland betwen 2001-16. Nowhere in this paper could I find reference to length of stay in hospital in hours; were these figures obtained through personal communication with the Scotish authors or did they come from elsewhere? Would it not be more apposite to use recent figures from the Danish hospitals being used in the clinical trial? Length of stay in hospital for babies with bronchiolitis is difficult to accurately measure in clinical trials for multiple reasons. Will the authors use actual time to discharge from hospital or time when ready for discharge? How will this be standardised across sites? Could the authors clarify? Similarly, how will need for oxygen be

measured?

Response 2: Unfortunately, no recent studies from Denmark have reported the average length of hospitalization for a general population of children with bronchiolitis. Accordingly, the referenced paper by Chung et al. is the best available, to our knowledge. We recognize that it can be hard to find the reference to length of stay in the paper. We found it in online supplements figure 4: "temporal trends in the number of bronchiolitis admissions by length to stay". We looked at the latest trends in admissions from the season 2015/2016. We took the median, which represents LOS 1 day, that is between 0 and 2 days. Therefore, it was estimated to 1,5 days, which equals 36 hours, which we used in our power analysis.

Time of discharge will be when the child is ready to discharge examined by a doctor. This will be noted in a similar fashion in all sites and has been specified in the text.

Need for oxygen will be evaluated by the doctor, supported by local guidelines suggesting an oxygen saturation limit < 90%, in combination with work of breathing and general condition.

To standardize this across sites we will make sure that the cause of using oxygen therapy will be noted. Also, we will run training sessions on each of the involved departments to optimize adherence to guidelines, and to standardize practices across sites. This has now been elaborated on page 6, lines 29-31+35-36:

"Duration of hospitalization is defined as number hours from admission until a doctor
has evaluated that the child is ready for discharge.

(…)

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Exploratory outcomes include: (1) need for oxygen therapy according to local
guidelines (usually oxygen saturation <90%) and doctor's discretion"

Also, we have clarified how we will standardize data collection across sites page 8, line 7:

"Data collection will be standardized across sites using a standardized electronic
patient record.

Comment 3: What is the start date for the proposed trial, how many bronchiolitis seasons will recruitment take place over, who will collect the data?

Response 3: We expect the trial to start in January 2024, but we have now specified that it will be

January 1

st, 2024, in the protocol on page 7, lines 9-10:

“Recruitment of participants will start January 1st, 2024, and recruitment is expected

to last for one and a half year through two seasons of bronchiolitis.”

Data collection will take place for 1.5 years recruiting through 2 seasons of bronchiolitis. We estimated that this will be enough according to our power analysis. All 6 sites included in this study will be recruiting and collecting data.

Comment 4: Will data on risk factors such as parental smoking, mild prematurity, breast feeding be collected?

Response 4: Yes, we have arranged to record multiple risk factors including the ones suggested. They have all now been added to the manuscript on page 7, lines 38-41:

“While interviewing the parents and examining the child upon admission, information

will be collected about symptoms and treatment given at home, baseline health data

including feeding practice, medical history including factors related to pregnancy and

birth, gestational age and neonatal course, comorbidities, medications, risk factors,

including family history of respiratory disease and allergies, smoking exposure, home

environment, socio-economic status, clinical presentation, and vital parameter

VERSION 2 – REVIEW

REVIEWER	Dr. Damian Roland University of Leicester, Health Sciences
REVIEW RETURNED	11-Dec-2023

GENERAL COMMENTS	Many thanks for taking the time to consider previous feedback. I think, along with the others reviewers comments, this is a far more robust protocol. Personally I think it would be useful to have a table of previous papers (given there are not many) as this is the easiest way to quickly see limitations from previous work and why this work is needed.

VERSION 2 – AUTHOR RESPONSE

None