Exploring the appropriate dose of nebulized hypertonic saline for bronchiolitis: a dose–response meta-analysis

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ABSTRACT

Nebulized hypertonic saline (HS) has gathered increasing attention in bronchiolitis. This study aims to evaluate the relationship between the dose of nebulized HS and the effects on bronchiolitis. Five electronic databases—PubMed, EMBASE, Cochrane Central Register of Controlled Trials, ClinicalTrials.gov, and ISRCTN—were searched until May 2021. Randomized controlled trials (RCTs) that investigated the effect of HS on bronchiolitis were included.

A total of 35 RCTs met the eligibility criteria. HS nebulization may shorten the length of stay (LOS) in hospital (mean difference −0.47, 95% CI −0.71 to −0.23) and improve the 24-hour, 48-hour, and 72-hour Clinical Severe Score (CSS) in children with bronchiolitis. The results showed that there was no significant difference between 3% HS and the higher doses (>3%) of HS in LOS and 24-hour CSS. Although the dose–response meta-analysis found that there may be a linear relationship between different doses and effects, the slope of the linear model changed with different included studies. Besides, HS nebulization could reduce the rate of hospitalization of children with bronchiolitis (risk ratio 0.88, 95% CI 0.78 to 0.98), while the trial sequential analysis indicated the evidence may be insufficient and potentially false positive. This study showed that nebulized HS is an effective and safe therapy for bronchiolitis. More studies are necessary to be conducted to evaluate the effects of different doses of HS on bronchiolitis.

Significance of this study

What is already known about this subject?

- In recent years, nebulized hypertonic saline (HS) has been applied in treating patients with bronchiolitis to help increase the mucociliary clearance.
- The results of previous systematic reviews showed that HS therapy could reduce the rate of hospitalization (ROH) and length of stay (LOS) in hospital in patients with bronchiolitis.
- There is no study evaluating the effect of HS doses on bronchiolitis.

What are the new findings?

- The trial sequential analysis of our study showed that the result that LOS could be shortened with nebulized HS treatment was conclusive with the growing number of studies.
- The results showed that there was no significant difference between 3% HS and the higher doses (>3%) of HS in LOS and 24-hour Clinical Severe Score.
- Although the dose–response meta-analysis found that there may be a linear relationship between different doses and effects, the slope of the linear model changed with different included studies.

How might these results change the focus of research or clinical practice?

- Nebulized HS could reduce LOS, ROH, and severity of disease in children with bronchiolitis.
- In clinical practice, 3% HS was widely chosen empirically, but more studies are needed to confirm the effect of different concentrations of HS on bronchiolitis.

INTRODUCTION

Bronchiolitis, presenting with cough and wheezing within a few days, is a leading cause of hospitalization in infants. Some patients even require intensive care.[1 2] Research showed the pathogenesis of bronchiolitis includes the mucosal inflammation, swelling at the bronchiolar level, and the overproduction of mucins.[3 4] Current managements of bronchiolitis for hospitalized children are limited to symptomatic treatment such as supplemental oxygen, respiratory support, and fluid replacement.[5]

Overproduction of mucins contributes to the pathogenesis of bronchiolitis.[6 7] In recent years, nebulized hypertonic saline (HS) has been applied in treating patients with bronchiolitis to help increase the mucociliary clearance. The possible mechanism may be as follows: HS may help rehydrate the surface liquid in the airway, reduce the viscosity and elasticity of the mucins, resulting in improving the mucociliary clearance.[8–10] Previous systematic reviews were conducted to summarize the effect of nebulized HS on bronchiolitis and provided evidence in clinical practice.[11–14] The results of these systematic reviews showed that HS therapy

could reduce the rate of hospitalization (ROH) and length of stay (LOS) in hospital in patients with bronchiolitis. However, obvious limitations should be noted in previous systematic reviews. First, different concentrations of HS may have different effects, but there is no dose–response meta-analysis evaluating the effect of HS dose on bronchiolitis. Second, few systematic reviews conducted trial sequential analysis (TSA) to assess if current evidence is enough to obtain a firm conclusion or further research on similar topic is necessary.[15]

In this study, we aim to incorporate current studies and perform a systematic review and dose–response meta-analysis to explore the therapeutic effect and safety of different concentrations of HS on bronchiolitis. Besides, we planned to conduct the TSA to evaluate the reliability of the results.

METHODS

This meta-analysis is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[16] The protocol of this systematic review and meta-analysis was registered in International Prospective Register of Systematic Reviews (CRD 42019143223).

Search strategy

A comprehensive search was conducted using the three databases: PubMed (1966 to May 2021), Cochrane Central Register of Controlled Trials (CENTRAL, through May 2021), EMBASE (1974 to May 2021), and two international trial registries (ClinicalTrials.gov and International Standard Randomized Controlled Trial Number Register (ISRCTN Registry)), from inception to May 2021. Details were shown in online supplemental table 1.

Study selection

Eligibility criteria are as follows: (1) randomized controlled studies (RCTs); (2) population: children with bronchiolitis who were under 2 years old; (3) intervention: the concentration of nebulized HS >0.9%; (4) comparison: the concentration of nebulized HS solution ≤0.9% or without nebulization; (5) outcomes: primary outcomes—length of stay in hospital (LOS), rate of hospitalization (ROH); secondary outcomes—Clinical Severity Scores (CSS) and adverse events (AEs).

Data extraction

Data of included studies were extracted by two reviewers, independently. The name of the first author, year of publication, study design, study location, number of participants, intervention, control, methods and outcomes were extracted.

Quality assessment

Cochrane Collaboration risk of bias tool was used for quality assessment in the included studies.[17] The generation of allocation sequence, allocation concealment, blinding of participants and researchers, blinding of outcome assessors, completeness of outcome data, selective outcome reporting, and other risk of bias were evaluated. Each item was marked by low, unclear, or high risk of bias.

Statistical analysis

We calculated mean difference (MD) for continuous variables, while risk ratio (RR) for dichotomous variables. The 95% CI was calculated for each effect size estimate. A random-effects model was used to pool the estimates from each study. The I^2 statistic was applied to assess the statistical heterogeneity within studies. A percentage no more than 50% ($I^2 \leq 50$) indicates a low statistical heterogeneity.[18]

We used three methods to explore which dose of HS is appropriate for bronchiolitis. First of all, we compared 3% HS with higher concentrations, and the meta-analysis was conducted. Second, we conducted the dose–response meta-analysis for the studies containing more than 2 doses of HS. Third, we conducted the dose–response meta-analysis for the all studies. Robust error meta-regression (REMR) proposed by Xu *et al*[19 20] was used to conduct the dose–response analysis. Considering the small samples of dose of HS, it is inappropriate to use a restricted cubic spline to fit the potential non-linear dose–response curve. Therefore, linear regression model was used to establish the 'average' trends between different doses of HS and estimate effects. A potential linear dose–response curve was generated in order to investigate the dose-specific effects.

To explore the association of different characteristics observed in prespecified subgroup analyses, univariable meta-regression analyses were conducted. We conducted sensitivity analysis to determine the stability of summary risk estimated by omitting one study in turn. Begg's test[21] and Egger's test[22] were conducted to assess the potential publication bias in primary outcomes. Data synthesis and analysis were performed with Stata V.15.0. A p value less than 0.05 was considered statistically significant unless otherwise specified, and all tests were two sided.

Trial sequential analysis

We performed TSA using TSA V.0.9.5.10 beta software.[15 23] Type I error (α) of 5%, a power (1-β) of 80%, and heterogeneity (I^2) calculated in the meta-analysis were considered for outcomes. The control event rates were calculated from the control groups, and other required information sizes (RIS) were calculated from studies with low bias risk. The cumulative Z-curve of each meta-analysis was constructed to assess its crossing of conventional boundary (Z=1.96) and the TSA monitoring boundary. The cumulative Z-curve crosses the TSA monitoring boundary, RIS line, or futility boundary, indicating a firm conclusion was reached and no more further trials are needed. On the contrary, evidence is insufficient for drawing a conclusion if the Z-curve does not cross any boundary or reach RIS line.

RESULTS

Search results

A total of 920 citations were obtained via electronic database searching. A total of 622 citations remained after removing duplicates, of which 93 citations were excluded after reading titles and abstracts. In the full-text reading process, 59 citations were excluded. One study was included in manual retrieval. Consequently, 35 published studies were included (online supplemental figure 1). We did not include unpublished studies, although there were

seven relevant ongoing studies searched in the international trial registries (online supplemental table 2).

Study characteristics and quality assessment

Thirty-five published RCTs researching on the nebulized HS for bronchiolitis were included in this review.[24–58] Studies were located in Asia, Europe, or North America. Ten studies were conducted in emergency or outpatient department. Twenty-four studies were conducted in wards. One study was conducted in both sites.[56] All children were under 24 months old, and the doses of HS ranged from 3% to 7%. The description of the included studies is shown in online supplemental table 3. A description of the quality assessment is presented in online supplemental figure 2. The bias of 5 studies were at low risk,[25 30 31 33 51] 24 studies at unclear risk,[24 26–29 32 34–43 45 47–50 52–55] while only 4 studies at high risk.[32 44 46 56]

Meta-analysis for primary outcomes

LOS in hospital

Twenty-five studies reported the effects of HS on LOS of children with bronchiolitis between HS and control groups.[24 27–29 35 36 38–48 50–54 56–58] However, two studies were removed from meta-analysis due to the inappropriate data format and the original data could not be obtained.[40 51] Overall, the pooled MD of LOS for the HS versus non-HS of bronchiolitis was −0.47 (95% CI −0.71 to −0.23) days, with substantial heterogeneity (I^2=78.6%, $P_{heterogeneity}$ <0.001) (figure 1A). TSA of LOS showed that the trial sequential monitoring boundary for benefit has been crossed by Z-curve even if the required information size of 3406 had not been reached, indicating that the decrease of LOS with HS nebulization was conclusive (figure 1B).

There were three studies with three kinds of doses of HS.[24 35 53] The results showed that there was no significant difference between 3% HS and the higher dose (>3%) of HS (MD 0.17, 95% CI −0.2 to 0.53) with insignificant heterogeneity (I^2=0%, $P_{heterogeneity}$=0.62) (figure 2A). The random-effects dose–response meta-regression model with REMR approach showed there was a linear relationship between different doses of HS and LOS (figure 2B,C). However, when we included all the studies, the slope of the linear model changed. But the goodness of fit of the model was low.

In subgroup analysis on LOS, no evidence of modification effect by study location, number of participants, daily times of HS nebulization, and HS doses was obtained according to meta-regression analyses (all $P_{interaction}$ >0.05, figure 3A). However, a modification effect by nebulization combined with/without other medical solution was found ($P_{interaction}$=0.036). Sensitivity analysis was conducted by omitting one study in turn and recalculating the pooled MD of LOS, the results ranged from (MD −0.50, 95% CI −0.76 to −0.24) to (MD −0.40, 95% CI −0.65 to −0.14) (online supplemental figure 3). It showed that omitting one study in turn did not change the results of LOS significantly. No statistically significant publication bias was inferred by Egger's test (p>0.05) and Begg's test (p>0.05).

Rate of hospitalization (ROH)

Nine studies reported the effects of HS nebulization on the ROH between HS and non-HS groups.[25 26 30–33 37 49 56] The pooled data indicated the ROH was significantly lower in the HS group than in the non-HS group (RR 0.88, 95% CI 0.78 to 0.98) with insignificant heterogeneity (I^2=0%, $P_{heterogeneity}$=0.544) (figure 4A). Due to the limited variety of dose, we did not conduct the dose–response analysis. TSA showed that the trial sequential significance boundary for benefit had not been crossed and the RIS of 2987 also had not been reached, indicating evidence is insufficient for drawing a conclusion (figure 4B).

In subgroup analyses on ROH, no evidence of modification effect by study location, number of participants, daily times of HS nebulization, nebulization combined with/without other medical solution, and HS doses was obtained according to the meta-regression analysis (all $P_{interaction}$ >0.05, figure 3B). The results of sensitivity analyses by omitting one study in turn and recalculating the pooled RR of ROH ranged from (RR 0.81, 95% CI 0.68 to 0.96) to (RR 0.92, 95% CI 0.81 to 1.04) (online supplemental figure 4). No statistically significant publication bias was inferred by Egger's test (p>0.05) and Begg's test (p>0.05).

Meta-analysis for secondary outcomes

Clinical Severity Scores (CSS)

Twenty-eight studies reported the effects of HS on CCS between HS and non-HS groups in bronchiolitis.[24 27 29 32–35 37–54 56–58] Wang *et al*'s CCS[59] were used in most studies; therefore, we only pooled data of Wang *et al*'s CCS in bronchiolitis. The pooled data indicated that the 24-hour, 48-hour, and 72-hour CSS were significantly reduced in the HS group than in the non-HS group with substantial heterogeneity ((MD −0.65, 95% CI −0.93 to −0.37, I^2=72.6%, $P_{heterogeneity}$ <0.001), (MD −0.95, 95% CI −1.30 to −0.59, I^2=85.6%, $P_{heterogeneity}$=0.001), (MD −0.72, 95% CI −1.27 to −0.18, I^2=85.5%, $P_{heterogeneity}$ <0.001), respectively) (online supplemental figures 5–7). TSA showed that the trial sequential significance boundary for benefit had been all crossed for the 24-hour, 48-hour, and 72-hour CSS, indicating evidence is sufficient and further studies researching on the effect of HS on CSS are not necessary (online supplemental figures 8–10). There were four studies with three kinds of doses of HS.[24 35 40 53] The results showed that there was no significant difference between 3% HS and the higher doses (>3%) of HS (MD 0.02, 95% CI −0.26 to 0.3) with insignificant heterogeneity (I^2=31.4%, $P_{heterogeneity}$=0.224) (online supplemental figure 11A). The random-effects dose–response meta-regression model with REMR approach showed there was a linear relationship between different doses of HS and LOS (online supplemental figure 11B,C). But the goodness of fit of the model was low.

In subgroup analysis on LOS, no evidence of modification effect by number of participants, daily times of HS nebulization, and HS doses was obtained according to meta-regression analyses (all $P_{interaction}$ >0.05, figure 3C). However, a modification effect by study location ($P_{interaction}$=0.015) and nebulization combined with/without other medical solution was found ($P_{interaction}$=0.001). The results of sensitivity analyses showed that the RR of the

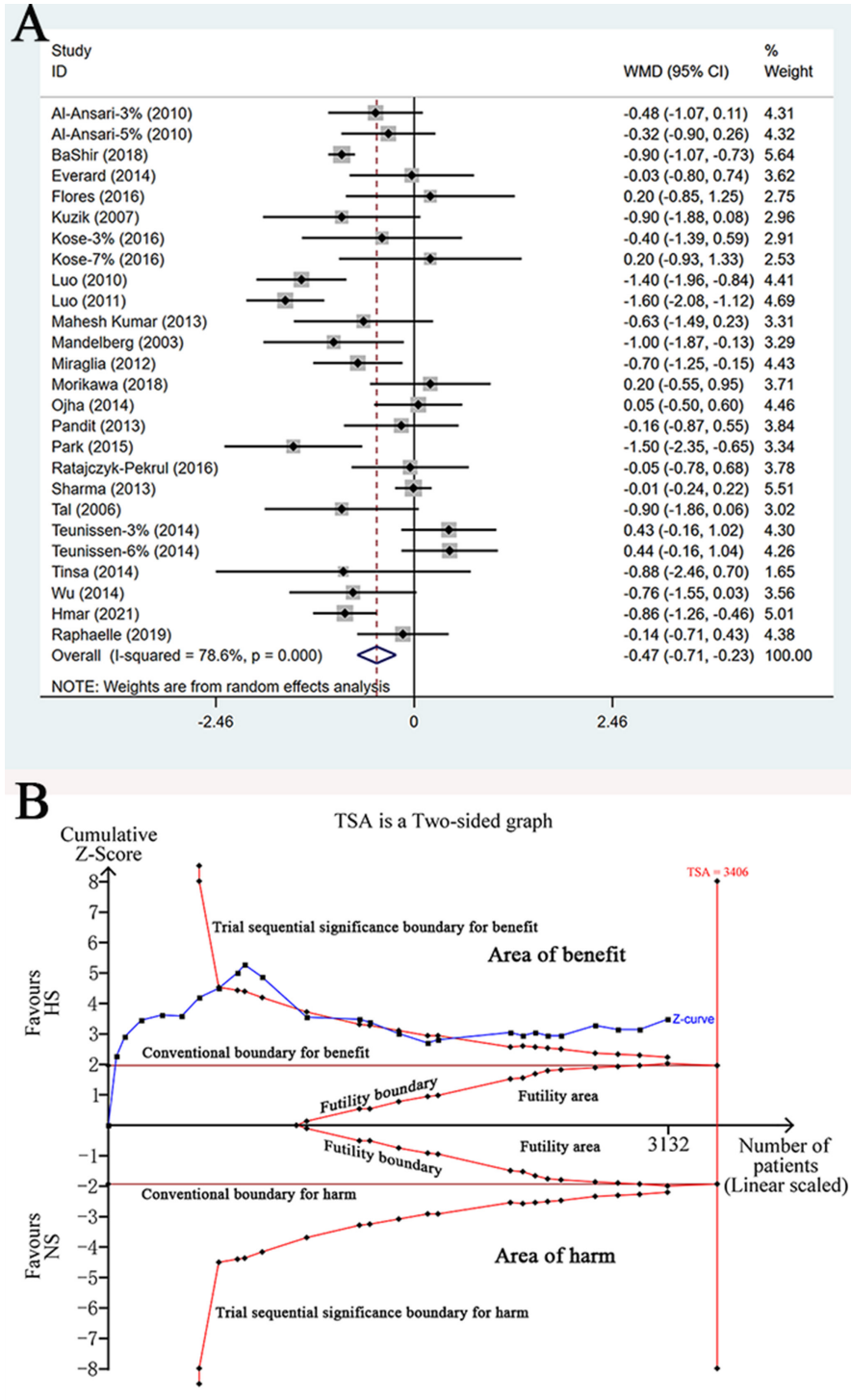

Figure 1 (A) The forest plot of the effect of nebulized hypertonic saline (HS) on length of stay (LOS) in bronchiolitis. (B) Trial sequential analysis (TSA) of the effect of nebulized HS on LOS in bronchiolitis, α of 5% (two-sided), β of 20%. The cumulative Z-curve (bold solid line) was constructed using a random-effects model. The horizontal line at cumulative Z=−1.96 indicates a conventional level of statistical significance. These trial sequential significance boundary and futility boundary were constructed based on the O'Brien–Fleming method. Although the required information size had not been reached, Z-curve has crossed the trial sequential monitoring boundary for benefit, indicating that the decrease of LOS with HS nebulization was conclusive. NS, normal saline; WMD, weighted mean difference.

24-hour CSS ranged from (MD −0.71, 95% CI −0.98 to −0.44) to (RR −0.59, 95% CI −0.86 to −0.31) (online supplemental figure 12); 48-hour CSS ranged from (MD −1.02, 95% CI −1.38 to −0.67) to (RR −0.82, 95% CI −1.12 to −0.51) (online supplemental figure 13); and 72-hour CSS ranged from (MD −0.92, 95% CI −1.42 to −0.41) to (RR −0.59, 95% CI −1.3 to 0.12) (online supplemental figure 14). No statistically significant

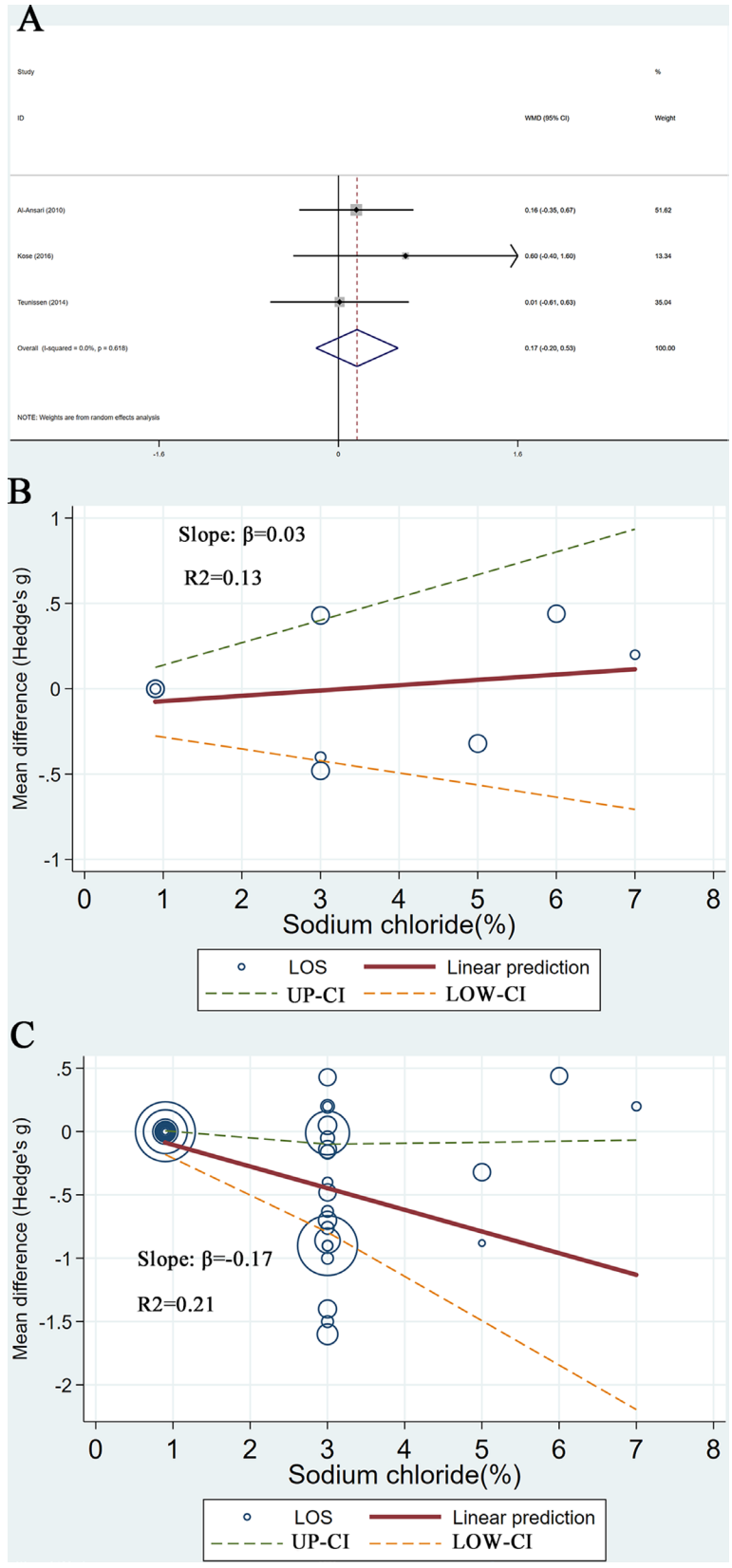

Figure 2 Exploring the appropriate dose of nebulized hypertonic saline (HS) for bronchiolitis in length of strength (LOS). (A) There was no significant difference between 3% HS and the higher dose (>3%) of HS. (B) The random-effects dose–response meta-regression model with robust error meta-regression (REMR) approach of studies containing more than 2 doses of HS. (C) The random-effects dose–response meta-regression model with REMR approach of all studies.

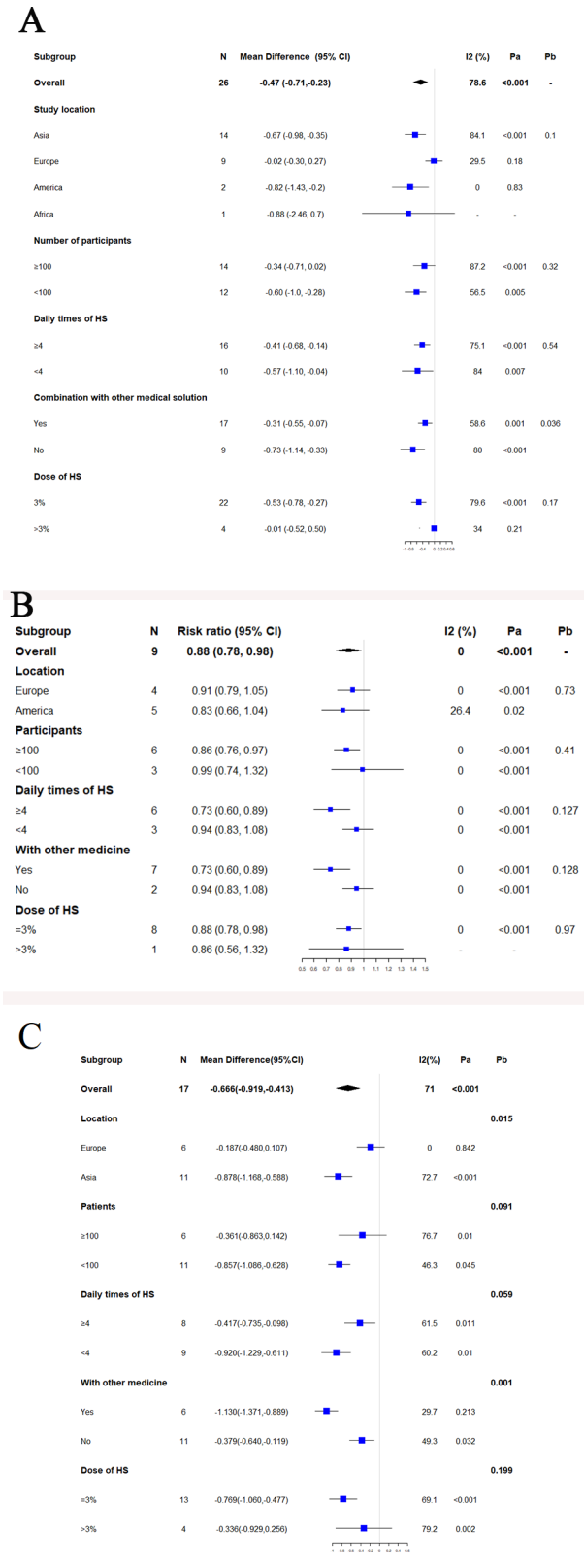

Figure 3 Subgroup analysis and meta-regression of the effect of hypertonic saline (HS) on length of stay (LOS) and rate of hospitalization (ROH). (A) HS for LOS in bronchiolitis; (B) HS for ROH in bronchiolitis; (C) HS for 24-hour Clinical Severe Score in bronchiolitis.

publication bias was inferred by Egger's test (p>0.05) and Begg's test (p>0.05).

Adverse events (AEs)

Most of the included studies reported AE of nebulized HS in bronchiolitis. Studies reported many potential AEs, such as cough, vomiting, bronchial constriction or bronchospasm, diarrhea, etc. Cough may be the most frequent AE of nebulized HS in bronchiolitis, but there was no difference in severe AE between HS and control groups. One study reported a case that developed bradycardia and desaturation during HS nebulization but resolved the next day.[28] No death was caused by nebulized HS directly.

DISCUSSION

The main finding of this comprehensive systematic review and dose–response meta-analysis was that nebulized HS could reduce LOS, ROH, and severity of disease in children with bronchiolitis, and the most optimal dose may be 3%. The results of the TSA showed the evidence was conclusive for the LOS and CSS.

Previous meta-analyses on the bronchiolitis were performed in the past decades.[11–14] The main view was that the nebulized HS significantly reduced LOS and ROH in bronchiolitis. One study based on TSA indicated that the evidence that the HS could reduce LOS was insufficient.[60] However, the TSA of our study showed that the result that LOS could be shortened with nebulized HS treatment was conclusive with the growing number of studies. Moreover, the main finding of this study adds more clinical endpoints and further extends the finding of previous meta-analyses. Noted to that, although the results of TSA showed a confirmed conclusion of LOS, this does not mean that the future studies will not substantially increase the precision of estimating the overall effect or the dose–response curve.

Our results showed that there was no significant difference between 3% HS and the higher doses (>3%) of HS in LOS and 24-hour CSS. The meta-analysis of different high doses of HS was not a reasonable method, so we used the dose–response meta-analysis for further analysis. Although the dose–response meta-analysis found that there may be a linear relationship between different doses and effects, the slope of the linear model changed with different included studies. Besides, the goodness of fit of the model was low. Therefore, it is possible that the relationship between different doses of HS is not a simple linear relationship. Due to the lack of kinds of doses, we could not use restricted cubic spline, which can better fit the relationship between them. It is necessary to study the effects of more different doses of HS on bronchiolitis in the future. In the subgroup analysis, children treated with mere HS nebulization without any other drugs had shorter LOS than those in the control group, while the significance between two groups was not obtained when the intervention was HS nebulization with beta-agonists such as epinephrine, salbutamol, terbutaline or fenoterol. Bronchodilators could prevent bronchospasm and edema of airway mucosa and improve mucociliary clearance of the epithelial cilia cells. However, their effects may lead to an indistinct different effect of HS nebulization on bronchiolitis between two groups (intervention and control).

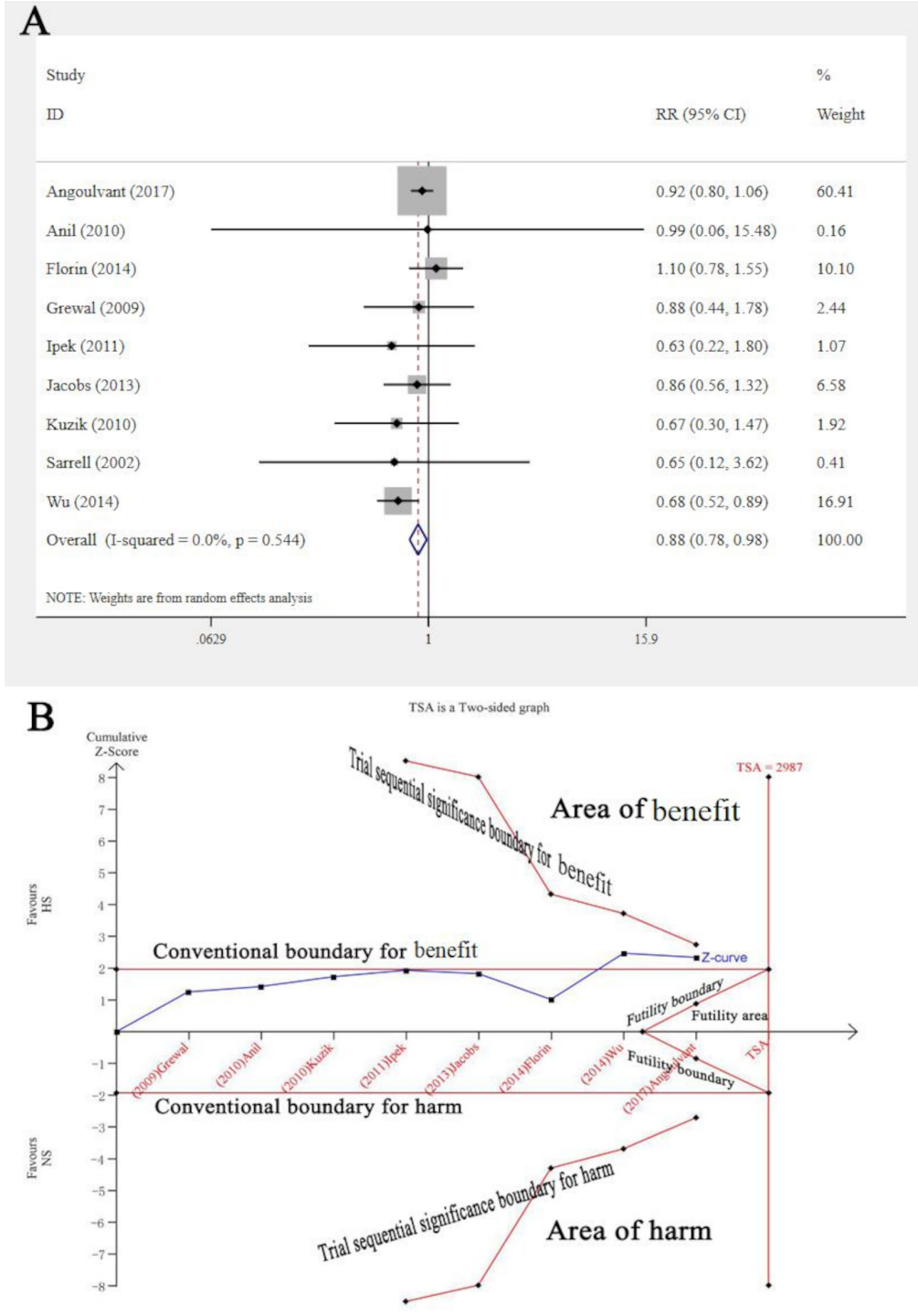

Figure 4 (A) The forest plot of the effect of nebulized hypertonic saline (HS) on rate of hospitalization (ROH) in bronchiolitis. (B) Trial sequential analysis (TSA) of the effect of nebulized HS on ROH in bronchiolitis, α of 5% (two sided), β of 20%. The cumulative Z-curve (bold solid line) was constructed using a random-effects model. The horizontal line at cumulative Z=−1.96 indicates a conventional level of statistical significance. These trial sequential significance boundary and futility boundary were constructed based on the O'Brien–Fleming method. The trial sequential significance boundary for benefit had not been crossed and the required information size also had not been reached, indicating evidence is insufficient for drawing a conclusion. NS, normal saline.

The optimal dose of HS needs to be explored for treating bronchiolitis, although 3% HS was commonly used in clinical practice. In this review, the results of dose–response meta-analysis showed that 3% may be the most effective for shortening LOS and decreasing CSS in bronchiolitis. To our knowledge, higher doses of HS may cause more AEs; however, an interesting finding is that 5% or 7% HS did not increase the AE compared with the control group. Therefore, less stimulation for airway may not be the only reason that 3% HS has higher effect than higher doses of

HS. Previous research suggested that there is extracellular ATP which reaches a high concentration in vivo and regulates the airway surface liquid water content. Thus, 3% HS could be sufficient to cause significance in milder bronchiolitis in which no further improvement achievable by higher concentration of HS over 3% HS. However, this might not be the case in severe cases because the high load of respiratory syncytial virus probably causes a considerable extracellular ATP reduction, which is crucial for maintaining airway surface liquid hydration. Thus, the generalization of this meta-analysis might not hold for severely affected infants and more experimental studies are needed to investigate the mechanism inside.[61] The results of TSA showed that current evidence was enough to obtain a reliable conclusion; therefore, more studies are not necessary to be conducted to evaluate the effect of HS on LOS and CSS in children with bronchiolitis.

There are strengths which should be noted. All included studies were RCTs with high quality, which provides stronger evidence. Our meta-analysis further extends the finding of previous meta-analyses in more clinical endpoints. Also, this is the first review to explore the relationship between doses of HS and the effect size of outcomes with a novel statistical method. Meta-regression analysis helps to find out factors which influence the outcomes. Moreover, TSA helps us to judge the reliability of results and whether more researches are needed in the future.

This systematic review also has several potential limitations. First, great heterogeneity existed among studies and it could not be eliminated completely by conducting subgroup analysis and meta-regression. Furthermore, meta-regression and dose–response analysis were not available in some cases due to the limited number of studies or substantial heterogeneity among studies.

In the future, more studies need to be performed to evaluate the effect of HS on ROH in children with bronchiolitis because the results of TSA showed that the evidence was insufficient. In this study, 3% was found the most effective concentration in treating children with bronchiolitis than other levels of concentration with unclear reasons. Therefore, more experimental studies should be performed to investigate the possible mechanisms.

CONCLUSION

To conclude, the current available evidence suggests that nebulized HS is an effective and safe therapy for bronchiolitis. It could reduce LOS, ROH, and severity of disease in children with bronchiolitis. The positive effect of HS on ROH could benefit from more studies to confirm the current observation. Due to the poor goodness of fit of the model, the most appropriate dose of HS could not be determined by dose–response meta-analysis in this study. More studies are necessary to be conducted to evaluate the effects of different doses of HS on bronchiolitis.

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Contributors JL is responsible for concept and design, data searching, inclusion and exclusion of studies, data analysis, and draft of the manuscript. YZ and AS are responsible for data extraction, assessment of methodological quality, and data analysis. LY is responsible for data searching and assessment of methodological quality. JD is responsible for the assessment of methodological quality and revision of articles. All authors read and approved the final manuscript.

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