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Randomized trial to evaluate azithromycin's effects on serum and upper airway IL-8 levels, and recurrent wheezing in infants with RSV bronchiolitis

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Abstract

Background—Respiratory syncytial virus (RSV) bronchiolitis in infancy is a major risk factor for recurrent wheezing and asthma. As azithromycin attenuated neutrophilic airway inflammation in a murine viral bronchiolitis model, demonstration of similar effects in humans may provide a strategy for the prevention of post-bronchiolitis recurrent wheezing.

Objectives—To investigate whether azithromycin treatment during RSV bronchiolitis reduces serum and nasal lavage IL-8 levels and the occurrence of post-bronchiolitis recurrent wheezing.

Method—A randomized, double-masked, placebo-controlled, proof-of-concept trial in 40 otherwise healthy infants hospitalized with RSV bronchiolitis who were treated with azithromycin or placebo for 14 days. IL-8 levels were measured in nasal lavage and serum on randomization, day 8, and day 15 (nasal lavage only). The occurrence of wheezing episodes was assessed monthly over the ensuing 50 weeks.

Results—Compared to placebo, azithromycin treatment did not reduce serum IL-8 levels at day 8 (p=0.6), but resulted in a greater decline in nasal lavage IL-8 by day 15 (p=0.03). 22% of azithromycin-treated participants experienced at least 3 wheezing episodes compared to 50% of

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participants in the placebo group (p=0.07). Azithromycin treatment resulted in prolonged time to the third wheezing episode (p=0.048), and in fewer days with respiratory symptoms over the subsequent year in comparison to placebo (36.7 vs. 70.1 days; p=0.01).

Conclusion—In this proof-of-concept study, azithromycin treatment during RSV bronchiolitis reduced upper airway IL-8 levels, prolonged the time to a third wheezing episode, and reduced overall respiratory morbidity over the subsequent year.

Keywords

Azithromycin; IL-8; respiratory syncytial virus (RSV); wheezing

Introduction

Respiratory syncytial virus (RSV) is the major cause of bronchiolitis in the first year of life[1–3]. Most children infected with RSV experience mild disease that does not require hospitalization; nevertheless, RSV bronchiolitis is the leading cause of hospitalizations in infants younger than one year of age in the US[4, 5]. Early life RSV bronchiolitis is a major risk factor for subsequent recurrent wheezing and asthma[6–10], and this risk is more profound among infants with severe bronchiolitis requiring hospitalization compared to infants with less severe bronchiolitis involving an outpatient encounter[11, 12]. Among a high risk group of infants hospitalized with RSV bronchiolitis, up to 90% of children experience 2 or more wheezing episodes and almost 50% will be diagnosed with asthma by the age of 6 years[10]. As hospitalized infants have the greatest risk for post-RSV wheezing episodes, they represent an attractive target population in which to explore intervention strategies for the prevention of post-RSV recurrent wheeze. Multiple therapeutic strategies have been explored with a goal of reducing post-RSV wheezing, most with negative results. Numerous trials using treatments typically used for asthma, such as inhaled corticosteroids (ICS)[13–15], systemic corticosteroids[16–18], and montelukast[19, 20] have shown to have limited to no effect on the occurrence of post-bronchiolitis wheezing. These medications have minimal effect on non-eosinophilic airway inflammation[21], the dominant pattern seen during bronchiolitis[22, 23], and this may explain lack of efficacy of these treatments on post-bronchiolitis wheezing.

One potential intervention for the prevention of post-RSV wheezing is a macrolide antibiotic, which provide clinical benefits in inflammatory airway diseases, such as cystic fibrosis and diffuse panbronchiolitis, likely through a combination of anti-inflammatory and antimicrobial activities [24, 25]. Macrolides also have anti-neutrophilic activities *in-vitro*[24], and within the airway in patients with refractory neutrophilic asthma[26]. Moreover, we have previously reported that in a mouse model of viral bronchiolitis, the macrolide azithromycin decreased neutrophil accumulation in the airway, and attenuated neutrophilic inflammation as evidenced by lower levels of bronchial alveolar lavage (BAL) CXCL1 (previously termed KC), the mouse homologue of the major neutrophil chemoattractant, IL-8[27]. Based on these observations, we conducted this proof-of-concept study to evaluate whether these biological effects of azithromycin are evident in infants hospitalized with RSV bronchiolitis. We hypothesized that azithromycin therapy during bronchiolitis would reduce IL-8 levels in serum and upper airway secretions over the following two weeks. A secondary goal of this

study was to generate a preliminary estimate of the effect size for a potential future trial of azithromycin for the prevention of post-RSV recurrent wheezing.

Methods

Participants

Potential participants for the APW-RSV study were initially identified by as a result of having a positive NP RSV swab result in the St Louis Children's Hospital (SLCH) virology laboratory during two consecutive winter RSV seasons (2011–2012 and 2012–2013). This initial screening was performed irrespective of site of care (in-patient, emergency department, or outpatient), clinical indication for obtaining the nasal swab, or patient age. Based upon this initial screen, all infants 1–18 months of age with positive NP swabs for RSV and bronchiolitis requiring inpatient care at SLCH were further screened (Figure 1) by a study coordinator to determine eligibility. Infants were eligible to enroll in the Azithromycin to Prevent Wheezing following RSV Bronchiolitis (APW-RSV) study if they were 1–18 months of age, otherwise healthy, were hospitalized with a first episode of lower respiratory tract symptoms, and had a nasopharyngeal swab (DFA or multiplex kit) result confirming infection with RSV. Additional eligibility criteria were duration of respiratory symptoms from onset to admission less than 5 days and randomization within 7 days of the onset of respiratory symptoms. Exclusion criteria included: history of previous wheeze, any previous treatment with corticosteroid (systemic or inhaled), treatment with bronchodilators prior to the current RSV bronchiolitis episode, use of anti–gastroesophageal reflux medication, treatment with any antibiotics within past 2 weeks (4 weeks for macrolide antibiotics), prematurity (gestational age < 36 weeks), or any chronic disease (lung, cardiac, renal, or hepatic disease).

During the hospitalization, the infants were treated according to a predefined care-path, the St Louis Children's Hospital Bronchiolitis Pathway, a set of orders based on the American Academy of Pediatrics guidelines for treatment of bronchiolitis [28]. The St Louis Children's Hospital Bronchiolitis Pathway is an evidence based pathway that focuses on supportive treatments such as: nasal suctioning, supplemental oxygen, and IV fluids for infants who are not able to feed adequately. A trial of inhaled albuterol is permitted, but the treatment should be discontinued if albuterol does not provide clinical benefit. The Pathway strongly discourages use of systemic corticosteroids and antibiotics. All decisions regarding medical treatment during the hospitalization, other than those related to study participation, were made by the child's primary attending physician. The study protocol was approved by the Washington University Institutional Review Board. Participants' parents provided written informed consent, and a data and safety monitoring board monitored the study.

Study design and treatment

APW-RSV was a randomized, double-masked, placebo-controlled, proof-of-concept trial (Figure 2). All participants, their families, investigators, and study staff were blinded to study medication allocations. Each eligible participant was randomly assigned to receive either placebo or azithromycin in random blocks based on a computer-generated randomization scheme. Study treatments were either azithromycin oral suspension (Teva

Pharmaceuticals, Sellersville, PA) 10 mg/kg once daily for 7 days, followed by 5 mg/kg once daily for additional 7 days; or an oral placebo suspension that was matched in taste and appearance to the azithromycin oral suspension. Adherence to study medication was calculated based on measurements of medication bottle weight obtained before and after the treatment phase.

Outcome measures: general overview

The APW-RSV trial included co-primary outcomes including biological and clinical outcomes. The primary biological outcome of this trial evaluated the effect of azithromycin treatment on serum and nasal lavage IL-8 levels, while the effect of the treatment on recurrent wheezing was the primary clinical outcome of the study.

IL-8 measurements

Serum and nasal lavage samples for IL-8 measurements were obtained on randomization and 8 days later. An additional nasal lavage sample was obtained on day 15 (Figure 2). The change in serum IL-8 levels from randomization to day 8 was the primary biological outcome, and the changes in nasal lavage IL-8 levels from randomization to day 8 and to day 15 were prespecified as secondary outcomes.

Nasal lavage samples were obtained by the study coordinator: each of the child's nostrils was flushed with 3 ml of 0.9% saline, and then the nasal fluid and upper airway secretions were aspirated by a syringe bulb.

Serum and nasal lavage IL-8 levels were measured using the BD (BD Biosciences, San Diego, CA) human cytometric bead array (CBA), per manufacturer's instructions[29, 30]. Serum IL-8 was measured by the enhanced sensitivity IL-8 Flex Set (lower detection limit of 69.9 fg/m), while the nasal lavage IL-8 was measured by the IL-8 Flex Set (lower detection limit of 1.2 pg/mL). Data acquisition occurred on an LSR II BD flow cytometer using DIVA software. Results were analyzed using FCAP Array Software v3.0 (BD Biosciences, San Jose, CA).

Clinical outcomes assessment

The primary clinical outcome was the proportion of participants who experienced 2 or more additional wheezing episodes, assessed by monthly telephone calls over the 50 weeks following the treatment period, and at a final clinic visit 1 year after randomization (Figure 2). The proportion of children who experienced 3 or more wheezing episodes was specified as secondary outcome. We defined the occurrence of a wheezing episode each time a parent/guardian answered yes to either "Has your child had wheezing with colds?" or "Has your child had wheezing without colds?" as previously utilized[10]. Additional secondary pre-specified outcomes assessed over the 50 weeks following the treatment period included: the time to 2nd and 3rd wheezing episode; the proportion of children with physician diagnosis of asthma; the number of days with respiratory symptoms; the proportion of children who were prescribed ICS; number of days with use of rescue albuterol; the number of courses of oral corticosteroids (OCS); the number of emergency department (ED) visits for respiratory symptoms; and the number of adverse and severe adverse reactions. We also prospectively

monitored for potential gastrointestinal side effects of the study medication by contacting the participant families 3 times a week during the treatment period.

Sample size determination and statistical analysis

The APW-RSV study was designed to have a total sample size of 40 participants to detect 25% reduction in serum IL-8 levels with azithromycin treatment compared to placebo, assuming an overall α level of 0.05, using a 2 sided test, and a 20% withdrawal rate. The follow-up period of this pilot study was designed in order to measure the impact of the intervention on the occurrence of recurrent wheezing, using the intention to treat approach.

A logarithmic transformation was used on both the serum and nasal lavage IL-8. The transformed IL-8 levels in the nasal lavage samples collected at three time points were compared between the two groups using mixed-model repeated-measures analysis of covariance with the baseline IL-8 as the covariate. Statistical contrasts were used within the mixed-model analysis to compare the placebo and treatment groups with respect to changes in IL-8 concentration from baseline to day eight and from baseline to day fifteen. Analysis of covariance was used for serum IL-8 concentration comparisons as only two time points are available.

The chi-squared test of independence was used to evaluate the relationship between the treatment arm and categorical variables such as: having at least two episodes of wheezing; having at least three episodes of wheezing; asthma diagnosis. Time to second and third wheezing episode was assessed using Kaplan-Meier survival analysis.

All tests were 2-sided and based on a significance criterion of $p \leq 0.05$. All analyses were performed using SAS version 9.3 (SAS Institute Inc). No interim data analysis was performed.

Results

Enrolment, study completion, and adherence to study medications

Forty patients were randomized into the trial, and 38 completed a full year of follow up (Figure 1): one patient did not complete any of the study visits, and one completed 11 out of the 12 months of follow up. Adherence to study medication, assessed by medication bottle weight, was 89% in the azithromycin group and 82% in the placebo group.

Baseline characteristics

The mean (SD) age of the study participants at randomization was 3.8 (2.9) months, 59% were males, and 64% were Caucasian. The mean (SD) duration of hospitalization was 63.4 (53.2) hours. Baseline characteristics did not differ between the two groups (Table 1). Post-randomization acute RSV bronchiolitis course of the study participants is presented in table E1 (See table E1 in this article's Online Repository, available at jacionline.org).

Serum and nasal lavage IL-8 levels

Compared to placebo, azithromycin treatment did not result in a reduction of serum IL-8 levels by day 8 (p=0.62; Figure 3). Median (IQR) serum IL-8 levels in the azithromycin group vs. the placebo group were 14,676 (7,931 – 19,270) fg/mL vs. 12,795 (6,265 – 17,763) fg/mL at randomization, and 6,971 (4,115 – 10,389) fg/mL vs. 5,050 (3,343 – 7,459) fg/ml at day 8, respectively.

In contrast, azithromycin treatment, compared to placebo, resulted in a significant reduction of nasal lavage IL-8 levels measured between randomization and day 15 (p=0.026, Figure 4), but not at day 8 (p=0.11), or when assessed including all 3 times points (p=0.076, Figure 4). Median (IQR) nasal lavage IL-8 levels in the azithromycin group vs. the placebo group were 6,055 (5,168–12,959) pg/ml vs. 4,381 (2,493–9,027) pg/ml at randomization, 2,217 (1,229–8,256) pg/ml vs. 4,395 (2,423–7,511) pg/ml at day 8, and 865 (357–2,212) pg/ml vs. 2,318 (948–5,476) pg/ml at day 15, respectively.

Recurrent wheezing and asthma

The proportion of participants who experienced 2 or more wheezing episodes over the 50 weeks following the initial episode of RSV bronchiolitis did not differ between the azithromycin and placebo groups (39% vs. 50%; p=0.5, respectively). Azithromycin treatment resulted in a numerically, but not statistically significant, smaller proportion of participants who experienced 3 or more subsequent wheezing episodes (22% vs. 50%; p=0.07). A Kaplan-Meier survival analysis revealed a significant prolongation of the time until a third episode of wheezing among participants who received azithromycin compared to those who received placebo (p=0.048; Figure 5). The proportion of participants with a physician diagnosis of asthma did not differ between the azithromycin and placebo groups (11% vs. 25%; p=0.5, respectively).

Other secondary clinical outcomes

Participants treated with azithromycin experienced significantly fewer days with respiratory symptoms (cough, wheeze, or shortness of breath) over the ensuing 50 weeks (36.7 (28.0) vs. 70.1 (43.1) days; p=0.01). Six participants in the placebo group and one participant in the azithromycin group were prescribed ICS over the follow-up period (p=0.09). Days with use of rescue albuterol (p=0.26), the number of courses OCS (p=0.6), and the number of ED visits for respiratory symptoms (p=0.11) did not differ between the treatment groups.

Adverse events

During the 50 weeks of follow-up, 2 participants in the azithromycin group were re-hospitalized for wheezing episodes (3 and 11 months after enrolment) and one patient in the placebo group was hospitalized 3 times for wheezing (all within the first 3 months of the study). Gastrointestinal adverse events (diarrhea, vomiting, or abdominal pain) during the active treatment phase were recorded in 7 children treated with azithromycin and in 8 children treated with placebo, and none were severe enough to warrant discontinuation of study medication.

Discussion

This proof-of-concept pilot trial in infants hospitalized with RSV bronchiolitis revealed that azithromycin treatment for 2 weeks, when added to routine bronchiolitis care, resulted in a reduction in a marker of neutrophilic airway inflammation, nasal lavage IL-8 levels, but not in serum IL-8 over the treatment period. In addition, the azithromycin-treated participants experienced significant delays in the time until a third episode of wheezing following the acute bronchiolitis, and significantly fewer days with respiratory symptoms over the ensuing year.

It has been estimated that up to 13% of childhood asthma cases could be prevented by primary prevention of RSV bronchiolitis[31]. This concept is supported by two recent trials demonstrating reductions in early life wheezing among late-preterm children who received the anti-RSV monoclonal antibody palivizumab[32, 33]. Although palivizumab is an effective modality for the prevention of RSV infection and subsequent wheezing, its use has limitations: it is expensive, requires monthly injections during the RSV season, has not been studied in full term infants, and is unlikely to be feasible for routine use in large populations. Therefore, there is a need to identify other pharmacological interventions that could be used in children hospitalized with RSV bronchiolitis that may prevent subsequent recurrent wheezing. Conventional asthma controller medications have shown to have limited to no efficacy for the prevention of post-RSV recurrent wheezing[13–20], possibly related to their limited effect on neutrophilic airway inflammation that predominate the inflammatory process during viral bronchiolitis[22, 23, 34]. Therefore, a medication with anti-neutrophilic properties has the mechanistic rationale to serve as an intervention for the prevention of post-RSV recurrent wheezing.

IL-8 is a potent neutrophil chemoattractant and activator and is produced by epithelial and immune cells as a response to RSV infection and replication[34–36]. Increased upper airway IL-8 levels were reported to be associated with markers of acute bronchiolitis severity[23, 37, 38] and IL-8 dependent neutrophil recruitment to the lung. Subsequent neutrophil degranulation can result in additional epithelial cell damage in addition to that caused by the virus[34]. Therefore, an intervention to reduce airway IL-8 might attenuate ongoing neutrophil-driven damage to the epithelium, and subsequently prevent the respiratory sequelae of recurrent wheezing caused by RSV. Macrolide treatment in patients with refractory neutrophilic asthma resulted in a reductions of BAL IL-8 and the neutrophil mediators neutrophil elastase and matrix metalloproteinase[26]. Moreover, in our previous study of a mouse model of viral bronchiolitis, we demonstrated that azithromycin attenuated airway neutrophilic inflammation and reduced BAL concentration of CXCL1 (the mouse homologue of IL-8)[27], prompting consideration of this as a wheezing prevention modality in children.

We chose IL-8 as the main cytokine of interest based on its major role in the pathogenesis of neutrophilic inflammation, our previous findings in the mouse model, and its associations with clinical parameters of acute bronchiolitis severity [23, 27, 37, 38]. We chose serum, and not nasal lavage, IL-8 levels as our primary biological outcome, as the only previous human study that had investigated the effect of macrolide treatment on cytokines levels during RSV

bronchiolitis, investigated this effect in serum but not in airway fluid[39]. However, we found that azithromycin had no significant effect on serum IL-8 levels suggesting that, at least in RSV bronchiolitis, the potential anti-inflammatory effects of azithromycin may be predominantly directed toward the airway rather than through systemic anti-inflammatory effects. Since no previous human data were available on the kinetics of airway IL-8 levels during RSV bronchiolitis, and serum was not drawn on day 15 to minimize blood draws in infants, we defined the primary biological outcome to be serum IL-8 measured at day 8 after randomization.

To the best of our knowledge, the only previous trial for the prevention of post-RSV wheezing utilizing a macrolide was performed by Tahan et. al.[39]. In this relatively small randomized control trial, 3 weeks of treatment with the macrolide clarithromycin among 21 children hospitalized for RSV bronchiolitis was initially reported to be associated with a fewer readmissions for wheezing during the subsequent 6 months. However, subsequent communications refuted these findings[40,41]. Tahan et. al.[39] also reported that clarithromycin treatment resulted in reduced serum cytokines (IL-8, IL-4 and eotaxin), but the serum IL-8 finding was not confirmed in our study, potentially due to differences in timing of sampling (1 vs. 3 weeks), choice of macrolide, method of outcome ascertainment, or other unknown reasons.

The unique pharmacokinetics and pharmacodynamics properties of azithromycin might explain the differential effect observed between serum and upper airway IL-8 levels. In general, azithromycin accumulates in the intracellular tissues: it has sustained lung tissue penetration and extensive phagocytic accumulation that results in human alveolar macrophages and BAL concentrations that are up to 100 fold higher than the corresponding serum concentration[42, 43]. Moreover, azithromycin concentrations in PMN cells were reported to be more than 2000 fold higher than the corresponding plasma levels[44]. The intracellular accumulation property of azithromycin results in a very long half life in the airway, as azithromycin persists in measurable quantity in human airway macrophages for 21 days after the last dose of a 5 days course (i.e., 26 days)[43]. The optimal dose and duration of azithromycin treatment needed to provide a potential anti-inflammatory effect are not established. Previous studies in animal models of viral bronchiolitis revealed immunologic sequelae up to 21 days after infection[45]. As azithromycin has a very long half-life in the lung tissue[46], 14 days of treatment with azithromycin should result in at least 23 days with effective anti-microbial concentrations in the lung tissue[46], and in at least 35 days of measurable quantity in airway macrophages[43], which will cover this period of immunologic events. In addition, we aimed to minimize the likelihood of potential drug adverse reaction; thus, we adopted this dosing regimen which was also reported to have an excellent safety profile in very-low birth-weight pre-term infants[47].

Upper airway colonization with capsular polysaccharide bacteria was reported to be a predictor of subsequent recurrent wheezing and asthma diagnosis at the age of 5 years[48]. In addition, the co-detection of upper airway rhinovirus with polysaccharide bacteria in children was associated with an increased risk of experiencing asthma exacerbations[49]. Therefore, we cannot exclude that some of the beneficial effects of azithromycin detected in our study are mediated by its effects on airway bacterial colonization. Alternatively, direct

anti-viral activity of the macrolide may be a potential mechanism for the beneficial effects of azithromycin detected in our study. Results from in-vivo models of viral infections, which utilized human respiratory epithelial cells, showed that azithromycin treatment inhibited rhinovirus replication and release[50], and that clarithromycin treatment reduced RSV[51] and influenza[52] supernatant titers.

A major strength of our study is the use of a homogenous study population, as we enrolled only otherwise healthy full-term infants experiencing acute RSV bronchiolitis. However, this rigorous approach somewhat limits the generalizability and the clinical applicability of the study results. Given the proof-of-concept nature of this trial, minimization of population heterogeneity was imperative. Future trials of this approach should consider inclusion of a more heterogeneous population of infants.

Our research question was investigated among a group of patients experiencing the most severe disease since all required hospitalization, and these children experience the greatest morbidity in terms of subsequent wheezing and asthma[11, 12, 53]. High levels of medication adherence and retention of participants during follow-up further strengthen our findings. Finally, we evaluated the potential effects of azithromycin on both a biologic endpoint (IL-8 as a marker for neutrophilic inflammation) and on a clinical endpoint (recurrent wheezing).

Our study has some limitations. IL-8 was measured in nasal lavage samples as a surrogate for lower airway fluids. However, measurement of cytokines in upper airway samples is informative, as nasal IL-8 has been previously shown to correlate with clinical outcomes of acute bronchiolitis severity[23, 37, 38]. This sampling approach avoids the risks and ethical considerations associated with performing lower airway sampling via bronchoscopy in children for research purposes. While azithromycin treatment was associated with a significant reduction in nasal lavage IL-8 levels at day 15, this effect did not reach statistical significance level at all time points suggesting that confirmation in a larger study is required. The potential effect of azithromycin in the prevention of recurrent wheezing was evident using the outcome measure of 3 or more episodes of wheezing, and not with 2 or more episodes of wheezing. The lack of effect of treatment on the outcome of 2 or more episodes of wheezing is not unexpected, as we previously reported that 70% infants hospitalized with RSV bronchiolitis experienced at least 2 wheezing episodes in the year following the acute bronchiolitis[10], while the occurrence of 3 or more episodes of wheezing had a better correlation with the development of asthma. Therefore, we suggest that the outcome of 3 or more episodes of wheezing is a more sensitive and clinically important outcome to evaluate the effect of azithromycin treatment in future post-RSV wheezing prevention trials. The relatively small sample size is another limitation, as the main goal of this pilot study was to prove the biologic plausibility of the intervention and not to perform a definitive evaluation of the utility of this intervention for recurrent wheezing prevention. Therefore, although the overall trend toward improved clinical outcomes is encouraging, we cannot firmly conclude that azithromycin treatment during RSV bronchiolitis reduces the occurrence of recurrent wheezing. These potential beneficial effects need to be evaluated in a larger and a more definitive clinical trial with longer duration of follow-up.

In summary, the results of this proof of concept trial revealed that 2 weeks of treatment with azithromycin during RSV bronchiolitis resulted in a reduction of upper airway IL-8 levels over the treatment period. Furthermore, azithromycin treatment resulted in a delay in the time to 3 or more wheezing episodes and in fewer days with respiratory symptoms over the following year. The observations from this pilot trial in children confirm the anti-IL-8 effects of azithromycin within the airway that we have previously detected in the mouse model, and provide biologic and clinical rationale to conduct a more definitive post-bronchiolitis wheezing prevention trial. The results of our current study should be viewed as hypothesis generating pending confirmation, and should not be extended to routine clinical care in an effort to prevent post-bronchiolitis wheezing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

APW-RSV	Azithromycin to Prevent Wheezing following RSV Bronchiolitis
BAL	Bronchoalveolar lavage
ICS	Inhaled corticosteroids
RSV	Respiratory syncytial virus

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Clinical Implications

The results of this proof-of-concept clinical trial suggest that the addition of azithromycin treatment during RSV bronchiolitis attenuates indicators of neutrophilic airway inflammation and might prevent post-RSV recurrent wheezing.

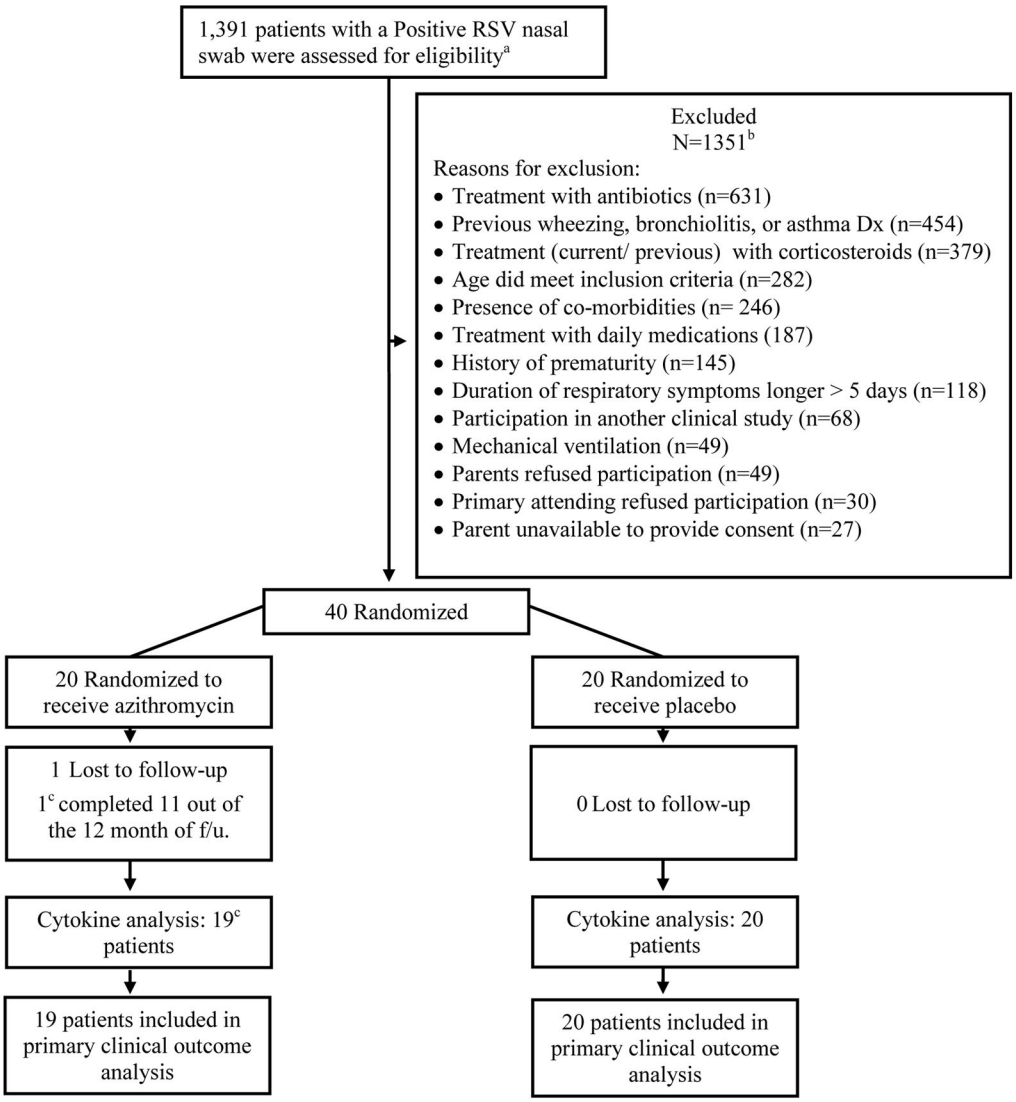

Figure 1.

Participants flow of the APW-RSV trial

APW-RSV: Azithromycin to Prevent Wheezing following RSV Bronchiolitis.

[a]Patients were screened for eligibility if they were hospital in St. Louis children's Hospital during the RSV season with lower respiratory tract symptoms, and had a positive nasopharyngeal swab result confirming infection with RSV.

[b]Some patients were excluded based on more than one exclusion criteria. Details for those screened but not eligible were not collected.

[C]One of the patients who was lost to follow-up had 11 (out of 12) months of follow-up, and contributed all biological samples for cytokine measurements.

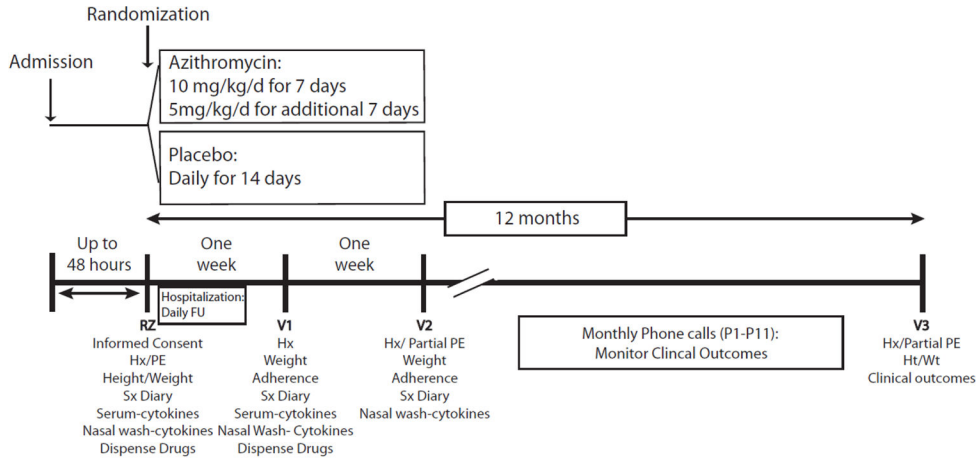

Figure 2.

APW-RSV Study design

Hx/PE: History/Physical exam; Sx: Symptom

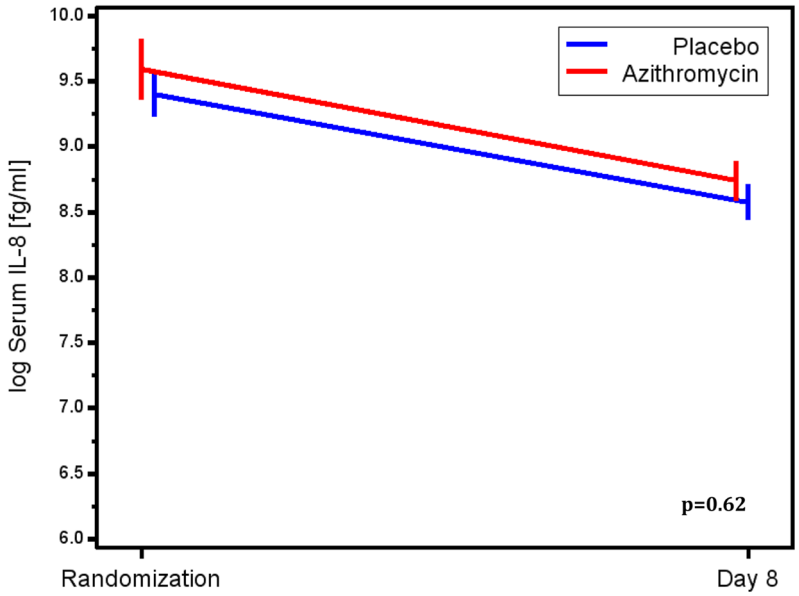

Figure 3.

Mean serum IL-8 levels (on a logarithmic scale) between randomization to day 8. p=0.62 for the change in serum IL-8 concentration measured between randomization and day 8 compared between the treatment groups

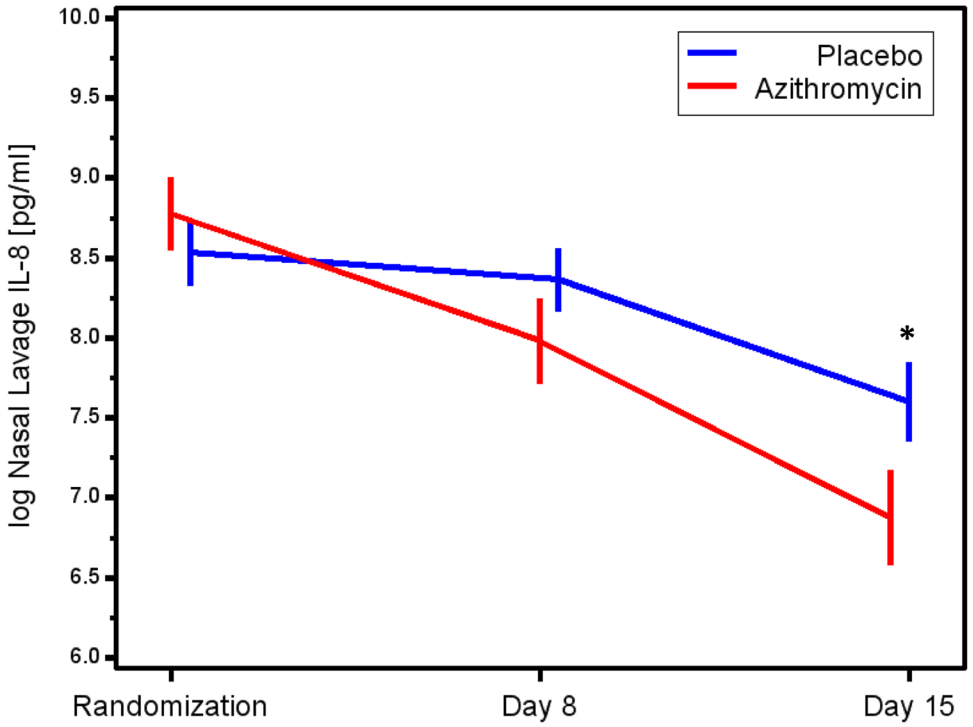

Figure 4.

Mean nasal lavage IL-8 levels (on a logarithmic scale) over the treatment period.

* p=0.03 for the change in nasal lavage IL-8 concentration measured between randomization and day 15 compared between the treatment groups.

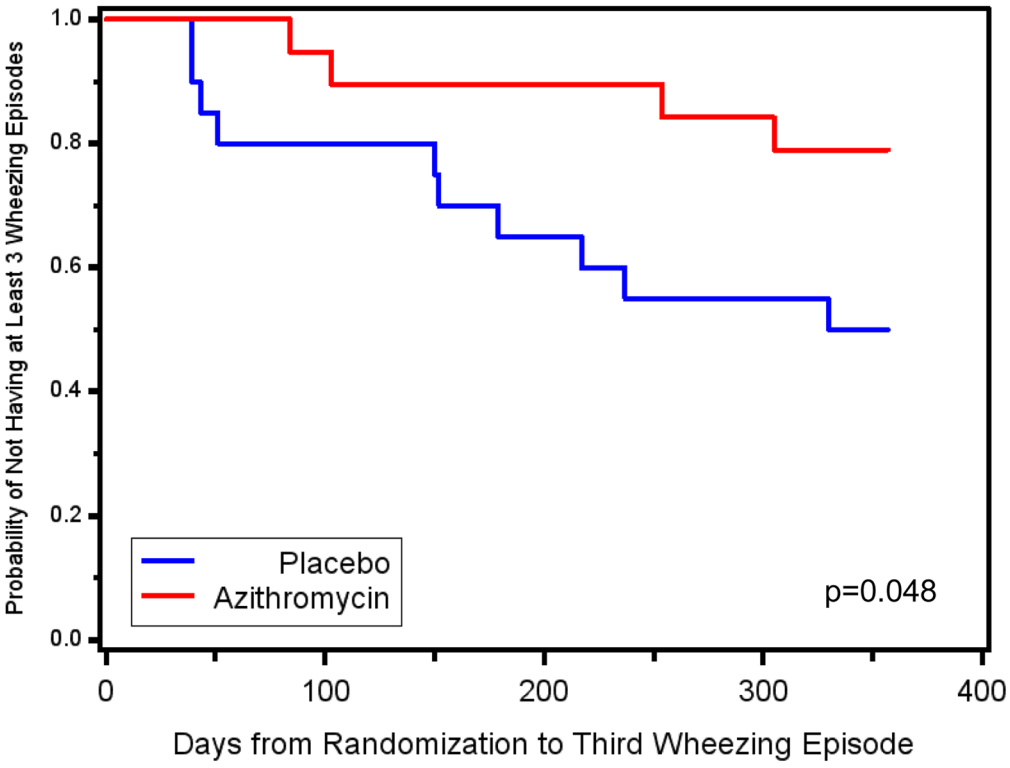

Figure 5.
Probability of not having at least three episodes of wheezing

Table 1

Baseline characteristics[a] of the APW-RSV study participants

	All participants	Azithromycin (n=19)	Placebo (n=20)
Age at enrollment (months)[b]	3.8 (2.9)	3.7 (3.7)	3.9 (2.0)
Gender (male)	59.0%	47.4%	70.0%
Race (Caucasian)	64.1%	63.2%	65.0%
Birth weight (kg) [b]	3.4 (0.5)	3.3 (0.6)	3.4 (0.4)
Length of pregnancy (wks) [a]	38.8 (1.4)	38.9 (1.3)	38.7 (1.4)
Maternal smoking during pregnancy	7.7%	10.5%	5.0%
History of breast feeding	28.2%	31.6%	25.0%
Tobacco smoke exposure	35.9%	42.1%	30%
Pet exposure	61.5%	63.1%	60.0%
History of eczema	17.9%	21.1%	15.0%
Parental history of asthma	41.0%	42.1%	40.0%
Duration of hospitalization (hours) [b]	63.4 (53.2)	58.0 (41.4)	68.5 (63.2)
Lowest O2 saturation on room air[b]	90.8 (4.3)	91.4 (4.9)	90.3 (3.6)

Data are expressed as proportion of children in each group except as noted.

[a] $p>0.05$ for the baseline characteristics comparisons between the treatment groups.

[b] Data represents the mean (SD).