A randomized control trial of azithromycin for the acute management of wheezy pre-school children

by

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ABSTRACT

Objective: We sought to determine if azithromycin, a macrolide antibiotic with anti-inflammatory(1,2), antibacterial(3) and potential antiviral(4) properties, added to routine therapy of wheezy pre-school children would resolve respiratory symptoms more rapidly, and protect against symptom reoccurrence longer than the addition of placebo.

Study design: We completed a double blind, randomized, placebo-control trial in which pre-school children were randomized to receive five days of azithromycin or placebo in addition to their regular treatment in the emergency department. The primary outcome was time to resolution of symptoms during the following 21 days after enrolment. Secondary outcomes included the number of days children used a short-acting beta-agonist during the 21 days following randomization and the time to reoccurrence during the following six months of follow-up.

Result: A total of the 300 wheezing children were randomized, 222 had sufficient data to be included in primary outcome analysis and 169 were analyzed for secondary outcomes. The treatment groups had similar demographics and clinical parameters at baseline. Median time to resolution of respiratory symptoms was four days for both treatment arms (interquartile range (IQR) 3,6; p=0.28). Median number of days of Short-Acting Beta-Agonist use among those who received azithromycin was four and a half days (IQR 2, 7) and five days (IQR 2, 9; p=0.22) among those who received placebo. Participants who received azithromycin had a 0.91 hazard ratio for time to six-month exacerbation compared to placebo (95% CI 0.61, 1.36, p=0.65). A pre-determined subgroup analysis showed no differences in outcomes for children with their first or repeat episode of wheezing. There was no significant difference in the proportion of participants experiencing an adverse event.

Conclusion: Azithromycin did not reduce the duration of respiratory symptoms nor the time to a respiratory exacerbation in the following six months after treatment among wheezing preschool children presenting to an emergency department. There was no significant effect among either first-time or prior wheezing children.

PREFACE

This thesis is an original work by Patricia Silbernagel, the randomized control trial, of which this thesis is based on, received research ethics approval from the University of Alberta Ethics Board, A double blind, randomized control trial of azithromycin for the acute management of wheezy pre-school children, study ID: Pro00009987, April 2011 (**Appendix 1**).

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I. INTRODUCTION

Preschool wheeze represents significant morbidity for the individual and a significant burden to society. One in three children wheeze prior to their third birthday; almost 50% of children wheeze by six years of age(1,5). Wheeze occurs in approximately eight million pre-school children in the United States. While emergency department visits in Ontario for asthma (all ages) decreased, the emergency department visits for children zero to four years did not change and had the highest asthma admission rates(6). Preschool children who wheeze use 0.15% of the total healthcare budget in the United Kingdom(7). The current management of children who wheeze includes supplemental oxygen, hydration, nutrition, and inhaled short-acting beta agonists(8). While short-acting beta agonists are effective acutely, they do not influence the underlying disease pathogenesis.

Antibiotics are frequently used to treat wheezing children(9,10). New generation macrolide antibiotics (e.g. clarithromycin, azithromycin) demonstrate anti-inflammatory effects in animal and human studies(11). Previous research has suggested a potential benefit for macrolide therapy in both bronchiolitis(12) and asthma(13). This thesis is based on the "Trial for the Treatment of Acute Asthma in Wheezy Pre-school Aged Children" registered at ClinicalTrials.gov number NCT01008761. The purpose of this thesis is to investigate if the treatment of pre-school children presenting an acute episode of wheezing symptoms with five days of azithromycin will reduce the duration of their respiratory symptoms sooner than those children treated with placebo.

A. Epidemiology of wheeze

Wheezing is a respiratory symptom that occurs during exhalation. The wheeze intensity depends on the degree of narrowing of the bronchial segment from which sounds originate(14,15). This continuous

sound last more that 250 msec. and may be audible at the patient's open mouth, by auscultation over the chest or the larynx, or to the unaided ear in some special circumstances. Wheezes can be high or low pitched, consist of single or multiple notes and can occur during inspiration (stridor), expiration or both. The pitch of the wheeze indicates the mass, elasticity and the velocity through an airway almost to the point of closure, sufficient airflow is necessary to produce a sound in addition to the narrowing of the airway(15). The absence of wheezing in a patient with clear symptoms of respiratory distress like asthma, may suggest impending respiratory failure.

One in three children wheeze prior to their third birthday; almost 50% of children wheeze by six years of age(16,17). The prevalence of pre-school wheeze in Canada is 22%, similar to findings in the United States and the United Kingdom(18). The prevalence of wheezing between 1994/1995 and 2000/2001 increased in most Canadian regions for children aged zero to five years old(18). According to Kuehni et al. there was a significant increase of pre-school wheezing prevalence disorders from 1990 to 1998 in the county of Leicestershire, United Kingdom(19). The number of children that reported to had ever wheezed before increased from 16% to 29%, current wheeze increased from 12% to 26%, diagnosis of asthma increased from 11% to 19%, treatment for wheeze increased from 15% to 26%, and admission for wheeze or other chest trouble also increased from 6% to 10%.

B. Healthcare utilization of wheezing children

The number of Ontario emergency department visits for asthma among children aged zero to four years did not change between 1996 and 2005 (14.6 visits/100 asthma individuals in 1996 versus 14.4 visits/100 asthma individuals in 2005) and had the highest number of emergency department visits of any age(20) despite overall Ontario emergency department visits decreasing over the same time period(6). Stevens et. al.(7) determined that their direct and indirect costs were 0.15% of the total United Kingdom healthcare budget in 1998/1999 (£52.75 million) among participants of a randomized controlled trial, following children aged 18 months to five years of age diagnosed with asthma or

wheezing(7). Most of this expenditure was health care costs (76.3%), followed by waged and non-waged employment (18%), and family-borne costs (5.6%). In a prospective study Ungar et. al.(21) concluded that the annual cost of Ontario asthmatic children younger than four years of age who had been prescribed bronchial inhaler medication (bronchodilators or corticosteroids) and had respiratory symptoms (shortness of breath, wheezing, or recurrent wheezing) was statistically significant than older children (5 to 14 years). The annual direct and indirect cost for pre-school children was $1,386. Hospital admissions accounted for 43% of the total costs, followed by medications 31%. Ungar et. al. analysis revealed a higher societal cost for children under four years of age due to wage loss of caregivers(21).

C. Conditions associated with wheeze in pre-school children

The most common conditions associated with wheezing symptoms in pre-school children are asthma(16), bronchiolitis, allergies, gastroesophageal reflux, infections, and obstructive sleep apnea (**Table: 1**)(22–25). Congenital and acquired conditions like cystic fibrosis, anatomical abnormalities, cardiac abnormalities and immune deficiencies can also present with wheezing symptoms(26). Bronchiolitis, a disorder characterized by acute inflammation, increased mucus production, edema, and necrosis of epithelial cells lining small airways, is a common cause of wheezing(27). Bronchiolitis, which usually presents as the first episode of wheezing in pre-school children(27), is the partial or complete inflammation of the small airways (bronchioles), most frequently caused by a virus in children younger than two years of age. Principal viral species causing bronchiolitis are respiratory syncytial virus, human rhinovirus, human metapneumovirus, and influenza. Human rhinovirus (an RNA virus of the *Picornaviridaeis* family) is the most common cause of common cold(28), and can cause upper and lower respiratory infections. The prevalence of preschool-aged children with human rhinovirus increases with age and the presence of human rhinovirus is significantly more common in

children with recurrent wheeze than first-time wheeze(29). Most infants have a respiratory syncytial virus (an RNA virus of the *Paramyxoviridae* family) infection by the three years of age(30) although only one to two percent of children with respiratory syncytial virus are severely ill and need hospitalization(31,32). Martinez et. al.(16) concluded that children with respiratory syncytial virus bronchiolitis were three to four times more likely to wheeze at six years of age but this relationship decreased with age and was almost non-significant by the 13 years of age. Stein et. al.(33) reported that children with respiratory syncytial virus bronchiolitis were more likely to have asthma at 18 years of age (33% vs 7%) when compared to healthy controls. Another study focused on causal effects concluded that premature infants receiving Palivizumab, a monoclonal antibody, for respiratory syncytial virus prophylaxis had 50% reduction in the occurrence of recurrent wheeze(34).

Similar to respiratory syncytial virus, the human metapneumovirus (an RNA virus of the *Paramyxoviridae* family) has been associated with wheeze among children younger than three years of age specially during winter months(35). Unlike respiratory syncytial virus, human metapneumovirus was not significantly associated with need of hospitalization(36). Influenza virus (an RNA virus of the *Orthomyxoviridae* family) is also associated with wheezing symptoms that may cause severe disease like bronchitis and pneumonia(37). More research is needed to clarify if early in life respiratory tract infections are a cause or just an indicator of a disposition to asthma(28).

Table 1. Causes of Wheezing in Children and Infants

Table 1. Causes of Wheezing in Children and Infants
Common causes of wheezing
Allergies Asthma or wheezing Gastroesophageal reflux disease Infections Bronchiolitis Bronchitis Pneumonia Tuberculosis Upper respiratory infection Obstructive sleep apnea
Uncommon causes of wheezing
Bronchopulmonary dysplasia Foreign body aspiration
Rare causes of wheezing
Bronchiolitis obliterans Congenital heart disease Congestive heart failure Cystic fibrosis Immune deficiencies Mediastinal masses Primary ciliary dyskinesia Tracheomalacia Tumor or malignancy Vocal cord dysfunction

Sources: (26,38,39)

1. Environmental exposures that influence wheezing in children

Air pollution, tobacco smoke exposure, breast feeding history, and maternal and child's body mass index are associated with wheezing symptoms. Andersen et. al.(40) concluded that air pollution related to traffic gases (nitrogen dioxide and nitrogen oxide) is associated with wheezing symptoms during the

first three years of life, with the greatest impact in the first year of age, among 205 children participating in the Copenhagen Prospective Study on Asthma birth cohort study. There is also evidence suggesting that the increased body weight of the mother, before and during pregnancy, and the child's increased body mass index are associated with a higher risk to develop wheezing. Leermakers et. al.(41) concluded that maternal pre-gestational obesity and history of atopy or asthma, as well as each standard deviation increase of gestational weight gain were associated with a higher risk of preschool wheezing in a population-based cohort study. Jeong et. al.(42) compared the body mass index at birth and at three years of age and their association with the development of wheezing symptoms in a hospital-based cohort study. Jeong et. al.(42) observed that children's wheezing prevalence significantly increased with each increase in body mass index tertiles (lowest third: 16.0 kg/m2; middle third: 14.8-16.0 kg/m2; highest third: >16.0 kg/m2) at three years of age. The Tucson Children's Respiratory Study reported that infants who were not breastfed for at least one month after birth had higher rates of wheezing during the first four months of life(28).

2. Classification of preschool wheeze

Several studies have attempted to classify pre-school wheezers based on their age of onset of symptoms. The different pre-school wheeze categories may represent different phenotypes associated with different risk factors such as atopic status, family history of atopy, history of age of bronchiolitis. Martinez et. al. using data from the Tucson Children's Respiratory Study(16), identified different wheezing phenotypes based on clinical observations: Never wheezed, transient early wheeze, persistent wheeze and late onset wheeze. The Tucson Children's Respiratory Study(16) is one of the earliest and largest studies to present wheezing classification. The Tucson Children's Respiratory Study followed the course of 1,246 participants from birth into adulthood to investigate the relationship between potential risk factors for acute lower respiratory tract illnesses and their impact on chronic lung diseases such as asthma later in life(43). Early transient wheeze referred to symptoms starting at three years of age and stopping at around six years of age while late-onset wheeze indicated no

symptoms during the first three years of life that developed at six years of age(44). The third phenotype of persistent wheeze indicated symptoms starting from the first three years of life that remain after reaching six years of age.

The Avon Longitudinal Study of Parent and Children (ALSPAC)(45), a United Kingdom birth cohort study, identified six wheezing phenotypes from birth to 81 months of age using longitudinal latent class analysis: never/infrequent (59%), transient early (16%), intermediate (3%), persistent (7%), prolonged early (9%) and late (16%) wheezing. They demonstrated differences in asthma prevalence, lung function levels and atopy between phenotypes. The wheezing phenotypes most strongly associated with atopy and airway responsiveness had later onset (after 18 months of age). The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study (The Netherlands)(46) identified five different wheezing phenotypes comparable to the six previously found in ALSPAC with longitudinal latent class analysis: never/infrequent (75%), transient early (17%), intermediate-onset (3%), late-onset (1.7) and persistent (3.5%). Both cohort studies showed very similar associations of wheezing phenotypes with asthma prevalence, lung function and atopy.

Alternatively, the European Respiratory Society categorized children into having episodic or viral wheeze and multi-trigger wheeze. While episodic or viral wheeze refers to episodes of wheezing that occur in conjunction with clinical evidence indicating a respiratory infection, multi-trigger wheeze refers to episodes of wheezing that occur with or without evidence of an infection(44,47). Episodic or viral wheezing is the most common phenotype of preschool wheezing. It is often caused by upper respiratory tract infections and is usually associated with a good prognosis(48). On the other hand, children with multi-trigger wheeze often have an allergic disposition and persistent symptoms with a higher predisposition of asthma later in life. These phenotypes can vary over time and episodic or viral wheeze may evolve into multi-trigger wheeze or vice versa(49). Episodic wheeze commonly declines over time almost disappearing by the six years of age; it can also continue as episodic wheeze, change into multiple-trigger wheeze or disappear at an older age(50).

3. Relationship between preschool wheeze and asthma

The pathophysiology of wheeze among pre-school and school-age children is not clear with studies showing conflicting results(51,52). Unlike school-age children and adults with asthma, the epithelial reticular basement membrane thickening and the eosinophilic inflammation characteristic of asthma are not present in symptomatic atopic and non-atopic infants with reversible airflow obstruction(53). However, there is contradictory information about these characteristic pathologic features of asthma in preschool children with severe recurrent wheeze. Saglani et. al.(51) confirmed eosinophilic airway inflammation and epithelial reticular basement membrane thickening in children aged one to three years of age. Lezmi et. al.(54) observed a progressive thickening of the epithelial reticular membrane when comparing severe recurrent wheezing children under 36 months, 36 to 59 months and severe asthmatic school-age children. However, without control subjects, Lezmi et. al.(54) could not determine whether the progressive thickening of the epithelial reticular membrane resulted from the underlying pathology or the normal age-related development.

The relationship between pre-school phenotypes of wheeze and asthma as a long-term outcome has a poor predictive value(55,56). The Tucson birth cohort study(16) found that 34% of children wheezed during the first three years of life but 60% of these children had stopped wheezing by the six years of age. Approximately one quarter of school-age children with persistent asthma had wheezed by the six months of age and approximately three quarters by the three years of age(50). Nonetheless, most children that wheeze remit their symptoms, some have persistent symptoms or relapse after a period of remission into adulthood(57). Even though epidemiological studies provide phenotypes to classify wheeze, studies have yet to identify key biological indicators of any predictive value to improve therapeutic approaches(50,58). Response to inhaled corticosteroids, atopy or family history of asthma is of modest clinical value to predict the disappearance or persistence of wheeze overtime.

4. Investigations of preschool wheezing children

The cause of wheezing disorders can often be made by history taking(59). Important previous history elements include whether the wheezing symptoms are new or recurrent, temporal or seasonal variations, co-morbidities and other symptoms. A recent contact with a person with an upper viral infection or pertussis will suggest respiratory infection. A family history of asthma and/or allergies increases the suspicion of asthma. In young infants, congenital causes such as tracheomalacia or congenital heart disease are more likely than in older children(60). A sudden onset of wheeze increases the possibility of a foreign body aspiration.

The physical examination can help augment the findings from the medical history. The physical examination is focused on vital signs, oxygen saturation and observation of respiratory distress symptoms such as, nasal flaring, tracheal tug, intercostal retraction and accessory muscle use. Chest and neck auscultation helps to distinguish the different types of respiratory noises (rattle, stridor, snore, snuffle) from wheezing and define the acoustic characteristics of wheeze(61). The timbre of the wheeze may provide a guide on locating the airways that are obstructed. For example, a polyphonic wheeze (multiple musical notes starting and ending at the same time) are more likely to be produced by dynamic compression of large, central airways. A second example are monophonic wheezes (single or multiple musical notes starting and ending at different times) that are most likely to be produced in small airways, but they can also be produced by pathologies in the extrathoracic large airways(15). Whether wheezing occurs during the inspiratory or expiratory phase of the respiratory cycle does not always help locate whether the wheeze is extra or intrathoracic. Biphasic wheezing (inspiratory and expiratory wheezing) can occur if there is a central, large airway obstruction, asthma or chronic obstructive pulmonary disease. However, some patients with asthma may present with inspiratory wheezing or no wheezing at all(62). Inspiratory wheezing, stridor, may be a sign of upper airway obstruction(63).

Pulmonary function tests and laboratory investigations can help further narrow the differential diagnosis of wheeze. The evaluation, diagnosis and treatment of wheeze is more accurate with

objective measures of lung function tests that most children six years of age and older can undergo. The lung function testing includes the measurement of reversible airway obstruction (pre- and post-bronchodilator spirometry) that is key to diagnose asthma(64). The European and the American Thoracic Societies describe the measurements of flow rates and volumes during a forced expiratory manoeuvre to determine the forced expiratory volume in one second (FEV1) and the forced vital capacity (FVC)(65). The ratio of these measurements FEV1/FVC is a measure of airflow obstruction. The asthma diagnosis is supported when reversible airflow obstruction is present and the appropriate management can be provided(66). Additional tests that may aid in the diagnosis and severity assessment of wheeze include: chest radiography, pulmonary function tests, sweat chloride tests, culture for infection, pH monitoring for gastroesophageal reflux disease (GERD), computerized axial tomography scan (CT), magnetic resonance imaging (MRI), and bronchoscopy (**Table 2**)(38).

Table 2. Investigations in Wheezing Children and Infants

Table 2: Investigations in Wheezing Children and Infants	
Causes of wheezing	Suggested testing
Allergies	Skin prick test
Asthma or reactive airway disease	Spirometry, bronchial challenge test with methacholine, cold air, exercise, response to bronchodilator therapy
Gastroesophageal reflux disease	pH monitoring, barium swallow, or endoscopy
Infections: Bronchiolitis Bronchitis Pneumonia Upper respiratory infection	Viral and bacterial swabs, chest radiography
Obstructive sleep apnea	Polysomnography
Bronchopulmonary dysplasia	Chest radiography, bronchoscopy
Foreign body aspiration	Chest radiography, bronchoscopy
Bronchiolitis obliterans	Chest radiography, CT
Congenital vascular abnormalities	Chest radiography, echocardiogram
Congestive heart failure	Chest radiography, echocardiogram
Cystic fibrosis	Sweat chloride test
Mediastinal masses	Chest radiography, CT or MRI
Immune deficiencies	Serum immunoglobulin levels
Primary ciliary dyskinesia	Ciliary brush biopsy, nasal nitric oxide
Tracheomalacia	Chest radiography, laryngoscopy
Tumor or malignancy	Chest radiography, CT or MRI
Vocal cord dysfunction	Flexible bronchoscopy

Source: (38,67)

5. Management of wheezing

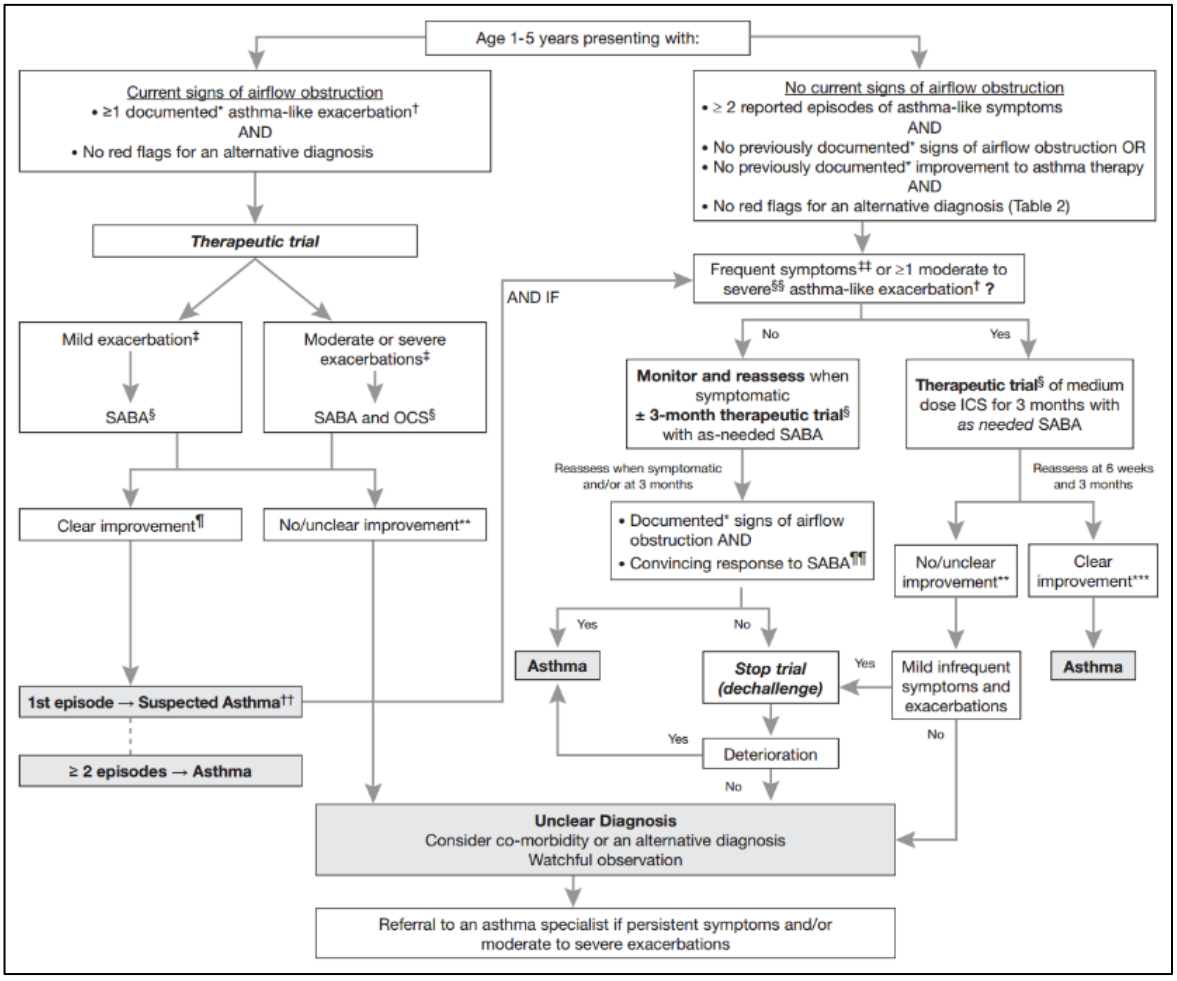
The current acute management of children who wheeze includes supplemental oxygen, hydration, nutrition and inhaled short-acting beta agonists(8). The management of wheezing in pre-school children includes nebulized hypertonic saline that may be administered to hospitalized infants and children. Antibiotics should only be used when signs of coexisting infection are present. Supplemental oxygen may not be administered if the oxyhemoglobin saturation exceeds 90%. Nasogastric or intravenous fluids should be administered to infants that can not maintain hydration orally(27). Inhaled short-acting beta agonists have been the drug of choice for acute symptoms of wheeze. Holmgren et. at., Bentur et. al., and Vangveeravong have demonstrated in randomized controlled trials the efficacy of short-acting beta agonists in infants and preschool children (68–70). Additionally, Nielsen and Bisgaard and Avital et. al. described short-acting beta agonists protective effects against possible triggers such us bacterial endotoxins, air pollution, and viral infections(71,72). Although short-acting beta agonists are effective acutely, they require frequent administration and do not influence the underlying disease pathogenesis.

The management of recurrent pre-school wheeze includes environmental control, fast-acting bronchodilators and inhaled corticosteroids. Inhaled corticosteroids, even though of controversial use in preschool children, have proven effective in preschool recurrent wheezers as well as in older populations(73). Specifically, Guilbert et. al.(74) and Murray et.al.(75) showed that daily use of inhaled corticosteroids reduced asthma-related symptoms and/or exacerbations in pre-school wheezing children through randomized controlled trials.

The Canadian Thoracic Society and the Canadian Paediatric Society have published a position paper with recommendation on diagnosis and treatment for children one to five years of age based on age, wheezing history and response to asthma medications(76). The presence of airflow obstruction and their response to a therapeutic trial of inhaled short-acting beta agonists without a clinical suspicion of an alternative cause must be documented (**Table 1**). An oral corticosteroid is added if the wheezing episode is moderate to severe. In order to prevent the morbidity related to late diagnosis of asthma in

this population(77), asthma is suspected after the first wheezing episode with clear improvement to asthma medication. After two or more asthma-like symptoms or exacerbations with response to asthma therapy asthma can be confirmed(76) (**Figure 1**).

Figure 1. Diagnosis and Management Algorithm for Children One to Five Years of Age



*Documentation by a physician or trained health care practitioner; †Episodes of wheezing with/without difficulty breathing; ‡Severity of an exacerbation documented by clinical assessment of signs of airflow obstruction, preferably with the addition of objective measures such oxygen saturation and respiratory rate, and/or validated score such as the Pediatric Respiratory Assessment Measure (PRAM) score; §See Table 3 for dosing; ¶Based on marked improvement in signs of airflow obstruction before and after therapy or a reduction of ≥3 points on the PRAM score, recognizing the expected time response to therapy; **A conclusive therapeutic trial hinges on adequate dose of asthma medication, adequate inhalation technique, diligent documentation of the signs and/or symptoms, and timely medical reassessment; if these conditions are not met, consider repeating the treatment or therapeutic trial; ††The diagnosis of asthma is based on recurrent (≥2) episodes of asthma-like exacerbations (documented signs) and/or symptoms. In case of a first occurrence of exacerbation with no previous asthma-like symptoms, the diagnostic of asthma is suspected and can be confirmed with re-occurrence of asthma-like symptoms or exacerbations with response to asthma therapy ; ‡‡≥8 days/month with asthma-like symptoms; §§Episodes requiring rescue oral corticosteroids (OCS) or a hospital admission; ¶¶In this age group, the diagnostic accuracy of parental report of a short-term response to as-needed short-acting β2-agonist (SABA) may be unreliable due to misperception and/or spontaneous improvement of another condition. Documentation of airflow obstruction and reversibility when symptomatic, by a physician or trained health care practitioner, is preferred; ***Based on 50% fewer moderate/severe exacerbations, shorter and milder exacerbations, and fewer, milder symptoms between episodes. ICS Inhaled corticosteroid.
Source: Reproduced with permission from the Canadian Thoracic Society (CTS)(76).

Role of antibiotics in wheezing patients

There has been a decline in the recent year in the use of antibiotics partly due to concerns over increasing antibiotic-resistance and changes in the microbiome(78). Standard treatment for wheezing symptoms and asthma do not include antibiotic treatment unless a concomitant respiratory bacterial illness such as pneumonia is suspected. Protracted bacterial bronchitis, continuous wet cough for more than one month, is the most common cause of chronic cough worldwide(79). Up to 90% of children with protracted bacterial bronchitis present with wheezing symptoms(80–82). Protracted bacterial bronchitis is suspected when wheezing children do not improve regardless of asthma medication trial treatment. Among patients with protracted bacterial bronchitis, the wheezing and wet cough will not resolve until proper antibiotic treatment is provided(81,83). There is a high degree of overlap between protracted bacteria bronchitis with other wheezing conditions including asthma, tracheomalacia and bronchomalacia. There is evidence that pre-school children with persistent wheezing but no symptoms of acute pulmonary infection or chronic wet cough have airways colonized by *Haemophilus influenza*, *Streptococcus pneumoniae* or *Moraxella catarrhalis*. The airway colonization suggests that pre-school wheezing children may benefit from antibiotic therapy(84).

D. Macrolide antibiotics

Macrolides are one of the most widely used group of antibiotics(85). Macrolides have broad-spectrum antibacterial activity against aerobic Gram-positive bacteria, certain Gram-negative bacteria, anaerobic bacteria, and intracellular pathogens such as *Mycoplasma* and *Chlamydia*(86). Macrolides also have immune-modulatory and potential antiviral properties beyond the antimicrobial properties(85). Macrolides are used in treatments of respiratory and urogenital tract, skin, and soft tissue infections(87,88). Erythromycin, the prototypical macrolide, has been used in the management of pediatric infections since 1952(89). Erythromycin is the drug of choice in bacterial enteritis,

pertussis, diphtheria and Legionnaire's disease. Erythromycin is also indicated in cases of allergy to penicillin or infections caused by penicillin-resistant organisms(90).

Macrolides' immunomodulatory effects have been studied in-vitro and in animal studies(85). The immunomodulatory effects of macrolides include the inhibition of pro-inflammatory pathways(91,92), modulation of macrophages and monocyte function and phenotype(93,94), anti-neutrophilic inflammation effects(95,96), and a potential inhibition of Th2 immune response(97,98). Also, macrolides reduce airway inflammation by reducing airway mucus secretion(2) and decreasing airway neutrophil accumulation through a reduction in both pro-inflammatory cytokines and adhesion molecule production(99).

Newer generations of macrolides such as azithromycin have better pharmacokinetic properties with fewer adverse events. Azithromycin has a higher concentration in most tissues than serum (10 to 100 times)(100). Azithromycin's half-life in tissue is high with an average of 68 hours which allows for single daily dose administration. Furthermore, due to azithromycin's prolonged tissue half-life, a five-day course is equivalent to a 10-day course of amoxicillin or penicillin V for treatment of acute otitis media and streptococcal pharyngitis(101). The most common use of azithromycin in western countries is upper and lower respiratory tract infections(102).

1. Use of macrolides in wheezing patients

Macrolides have shown to be effective to treat chronic respiratory inflammatory disorders such as panbronchiolitis and cystic fibrosis(95,103). The best example of macrolides effectiveness on wheezing symptoms is its efficacy in diffuse panbronchiolitis(104). The ten-year survival rate of diffuse panbronchiolitis, an idiopathic sinobronchial inflammatory disease seen primarily in Asian populations(105), significantly increased after the introduction of low-dose erythromycin to the regular treatment(106). The five-year survival rate of diffuse panbronchiolitis increased from 12-50% to over 90% since the addition of macrolide therapy(107). Another condition that has benefited from

macrolides antibiotics is cystic fibrosis. Macrolide antibiotics reduce the risk of cystic fibrosis exacerbations, improves lung function and reduces the use of additional antibiotics(108). Moreover, a recent meta-analysis concludes its effectiveness to improve respiratory symptoms in patients with cystic fibrosis(107).

Previous studies have shown the effectiveness of macrolide therapy in both bronchiolitis(12) and asthma(13). Macrolides have been used to prevent severe lower respiratory tract illnesses(108) and reducing inflammation of respiratory airways(109). The early administration of Azithromycin to children (12 to 71 months of age) with previous history of wheezing significantly reduced the progression to severe lower respiratory infection when compared to placebo(108). A small study observed reduction of hospital stay and readmission rates within six months from the administration of Clarithromycin for three weeks to hospitalized infants with respiratory syncytial virus bronchiolitis(12). In a large randomized controlled trial on asthma exacerbations, Johnston et. al.(110) concluded that telithromycin for ten days resolved symptoms faster than placebo. Nonetheless, this improvement could be due to the presence of atypical bacteria. Sixty one percent of patients had positive serology for *M. pneumoniae*, *C. pneumoniae*, or both. Roxithromycin significantly decreased symptoms, eosinophil cationic protein, and eosinophil count in serum and sputum after 8 weeks in patients with aspirin-intolerant asthma(111). In eosinophilic asthmatics, clarithromycin improved symptoms and had a reduction of blood eosinophil and eosinophil cationic protein levels on serum and sputum(112). A randomized control trial of adults with severe, refractory asthma, clarithromycin significantly reduced airway concentrations of interleukin 8 (IL-8), metalloproteinase 9 (MMP-9), neutrophil elastase, neutrophil numbers, and improved quality-of-life score compared with those asthmatics adult on placebo(113). In a large randomized controlled trial on asthma exacerbations, the early administration of azithromycin to preschool children with recurrent severe wheeze prevents the progression of symptoms to a severe lower respiratory tract infections(108). Despite these findings, a recent Cochrane review of macrolides use for chronic asthma, that included twenty-three studies and 1513 individuals, concluded that macrolides given for at least four weeks are not better than placebo(114).

2. Side effects of macrolide antibiotics

The potential harms produced by macrolides include direct adverse events such as gastrointestinal symptoms and higher risk for sudden cardiac death(115–117) and increased antimicrobial resistance(118). The gastrointestinal system is the most commonly affected producing nausea, vomiting and diarrhea(119). Organ systems rarely affected are: heart, liver and central nervous system(119). The Community Paediatrics and Infectious Disease and Immunization Committees of the Canadian Paediatric Society suggest that azithromycin should not be used to treat acute pharyngitis, acute otitis media or community-acquired pneumonia in otherwise healthy children, unless life-threatening beta-lactam allergy to treat acute pharyngitis or pneumonia caused by atypical bacteria(120).

Anaphylaxis / allergy: Macrolides allergy is extremely rare occurring in 0.4% to 3% of treatments(121). Allergic reactions including rash, fever, eosinophilia and joint pain are unusual(90). Transient deafness and allergic reactions are very unusual and mostly occur after fast or high dose administration of erythromycin lactobionate(119).

Cardiac Risk: A recent meta-analysis on macrolides cardiovascular risk, that included eleven studies with data for 6,639,411 individuals, concluded that macrolides are associated with increased risk for sudden cardiac death, ventricular tachyarrhythmias and cardiovascular death but not increased all-cause mortality(122). In 2013 the Food and Drug Administration released a statement warning about the potential for azithromycin-induced QT prolongation, commonly considered a sign of increased cardiac risks(123), and fatal torsade de pointes (polymorphic ventricular tachycardia)(124). The major factors related to the incidence of QT prolongation and torsades de pointes are the preexisting risk factors of QT prolongation (advanced age, female sex, hepatic and renal dysfunction or electrolyte disturbance), the cardiac adverse effects induced by azithromycin, and their coadministration with other drugs that prolong QT interval (antiarrhythmic drugs, antipsychotics, antidepressants or quinolone antibiotics)(125). Compared with other macrolides, azithromycin has minimal cardiovascular toxicity(125).

Gastrointestinal effects: The most common side effect associated with macrolides are gastrointestinal effects occurring in 15% to 20% of patients on erythromycin and 5% or fewer on newer generations of macrolides(119). Erythromycin's most common side effect is epigastric distress that appears to be related to the stimulation of motility due to the agonist effect on motilin receptors in the intestine(126). Newer generation macrolides possess lower incidence of gastrointestinal effects than erythromycin(127). In an Azithromycin safety review of Phase II and III studies, diarrhea/loose stools, abdominal pain, vomiting and nausea had a rate of 7.3% (8.4% was the total rate of adverse events). These adverse events were mild or moderate and the discontinuation of treatment resolved the symptoms(128). The administration of troleandomycin and erythromycin at high dose and for long periods of time has a potential for hepatotoxicity while josamycin, midecamycin, miocamycin, flurithromycin, clarithromycin and roxithromycin has low risk, and spiramycin, rikamycin, dirithromycin and azithromycin is negligible or absent(119).

Bacterial Resistance: The most important determinant of bacterial resistance is suboptimal use of antibacterial medications including using antibiotics to treat nonbacterial infections, overprescribing antibiotics (e.g. using the second drug of choice with a suboptimal spectrum), or inappropriate dosage or length of treatment(129,130). There are an increasing number of pneumococcal strains becoming resistant to macrolides. Furthermore, there is higher resistance of pneumococcal strains to macrolides than there is to penicillin(129,131,132). The prevalence of pneumococcal strains resistant to erythromycin in children with invasive pneumococcal disease decreased in Alberta from 8.8% to 5.8% after the introduction of the Streptococcus pneumoniae seven valent conjugate vaccine (PCV7) (130). In contrast, Quebec has shown that 23% of pneumococcal strains causing invasive infections were resistant to erythromycin(133). Brusselle et. al.(134) reported 87% erythromycin-resistant oropharyngeal streptococci in the azithromycin group and 35% of the subjects in the placebo group after 26 weeks of treatment period in a randomized double-blind placebo-controlled trial of azithromycin for the prevention of exacerbations in severe asthma.

Microbiome: Multiple studies have shown links between microbiota composition and disease severity in chronic airway infections(135,136). Macrolide treatment results in dysbiosis of respiratory

microbiota(137). Studies that have used next-generation sequencing methods have shown gut microbiota disturbances and macrolide resistance as short and long-term effects(138,139). The Bronchiectasis and Low-dose Erythromycin Study (BLESS) randomized controlled trial performed in adult patients (20 to 85 years) with bronchiectasis showed that long-term erythromycin treatment changed the respiratory microbiota(140). Specifically, participants whose baseline airway infection was not dominated by *Pseudomonas Aeruginosa*, erythromycin promoted displacement of *Haemophilus Influenzae* by more macrolide-tolerant pathogens including *Pseudomonas Aeruginosa*. Participants whose baseline airway infection was dominated by *Pseudomonas Aeruginosa*, erythromycin did not produce a significant change in their microbiota after 48 weeks of twice-daily erythromycin.

E. Randomized Controlled Trials

Trials are experimental studies that involve the active manipulation of an intervention in a sample population by the investigator to evaluate causation, prevention, or treatment of a disease(141). Trials are not only important to determine the efficacy of an intervention but also to study the safety and effects produced by the intervention(142). Randomized controlled trials can be tailored to answer a specific research question(143). The first comparative clinical trial was performed by James Lind in 1747(61). Dr. Lind assessed the merits of six existing treatments for scurvy on board the *Salisbury* at sea. The first clinical trial that used a random assignment and had a mask assessment (blind) of patients was performed in 1931(144). Amberson et. al.(144) compared the use of sanocrysin (a gold component) in pulmonary tuberculosis to a control group who received intravenous injections of distilled water(145). The first randomized controlled trial with a systematic enrolment criteria and data collection, similar to what is used in contemporary research(61), was performed by the United Kingdom Medical Research Council in 1946(146). The United Kingdom Medical Research Council studied the use of streptomycin on pulmonary tuberculosis(146).

Randomized controlled trials must always report the purpose, randomization methods, analysis methods, primary and secondary outcomes, size, and characteristics of studied populations. Clinical trials involving pharmaceutical products are categorized into four phases (I to IV)(147). The pharmacokinetics and pharmacodynamics are evaluated in phase I studies. Exploratory and confirmatory therapeutic studies are evaluated during phase II and III randomized control trials. Studies that examine the use of the drug in special populations and uncommon adverse events are evaluated in phase IV studies(148). Studies with non-pharmacological interventions, such as changes of life style or surgical approach, do not fit into those phases(149).

Advantages of Randomized Controlled Trials: Randomized controlled trials are the "gold standard" when evaluating the effects of pharmacological interventions, medical device and/or equipment usage under controlled conditions(145). Randomized controlled trials with appropriate study design, randomization, double blinding, control, and analysis by intention-to-treat provide the strongest empirical evidence of treatments efficacy due to randomized controlled trials ability to make causal inferences(145). The randomization of participants to treatment and control arms ensures that selection bias and confounding factors are evenly distributed among treatment arms(143).

Disadvantages of Randomized Controlled Trials: Randomized controlled trials evaluate the efficacy of an intervention. However, the well-defined structures that characterize randomized control trials make difficult to generalize the results to real-life circumstances(150). The rigorous selection of patients following the inclusion and exclusion criteria make the selected participants differ from the general population(151). Healthier and younger patients are more likely to be included in trials, consequently there is a limited possibility to evaluate complex treatments on patients with polypharmacy, multiple morbidities, or the elderly(152,153). For example, most of the randomized controlled trials in asthma do not include smokers or previous smokers, while most chronic obstructive pulmonary disease trials exclude asthma patients. Therefore, there is no information on

approximately 30% of the population who have poor lung function and unfavourable clinical outcomes(154,155).

Randomized controlled trials may present monitoring bias due to the regular follow-up compared to regular clinical care. The regular follow-up may improve outcome perception and reduce differences between control and treatment arms(156). Furthermore, patients are often followed in specialized centers that have specialists, diagnostic tools and follow international protocols that may not be the standard care in a regular health care setting(157,158). Additional limitations of randomized controlled trials are economical, logistical, and ethical(152). Randomized trials need notable large budgets, especially if long-term effects of an intervention are studied. Consequently, most trials run for two years at maximum(142). Most studies spend approximately 25% of their time recruiting patients. It is estimated that 80% of clinical trials extend their timelines due to slow enrollment(159). The extension of clinical trials can significantly increase operating costs. To prevent patient withdrawals and make studies affordable, clinical trials may have short follow-up periods(160). Therefore, randomized control trials have constrains to measure long-term treatment or chronic diseases(152,156).

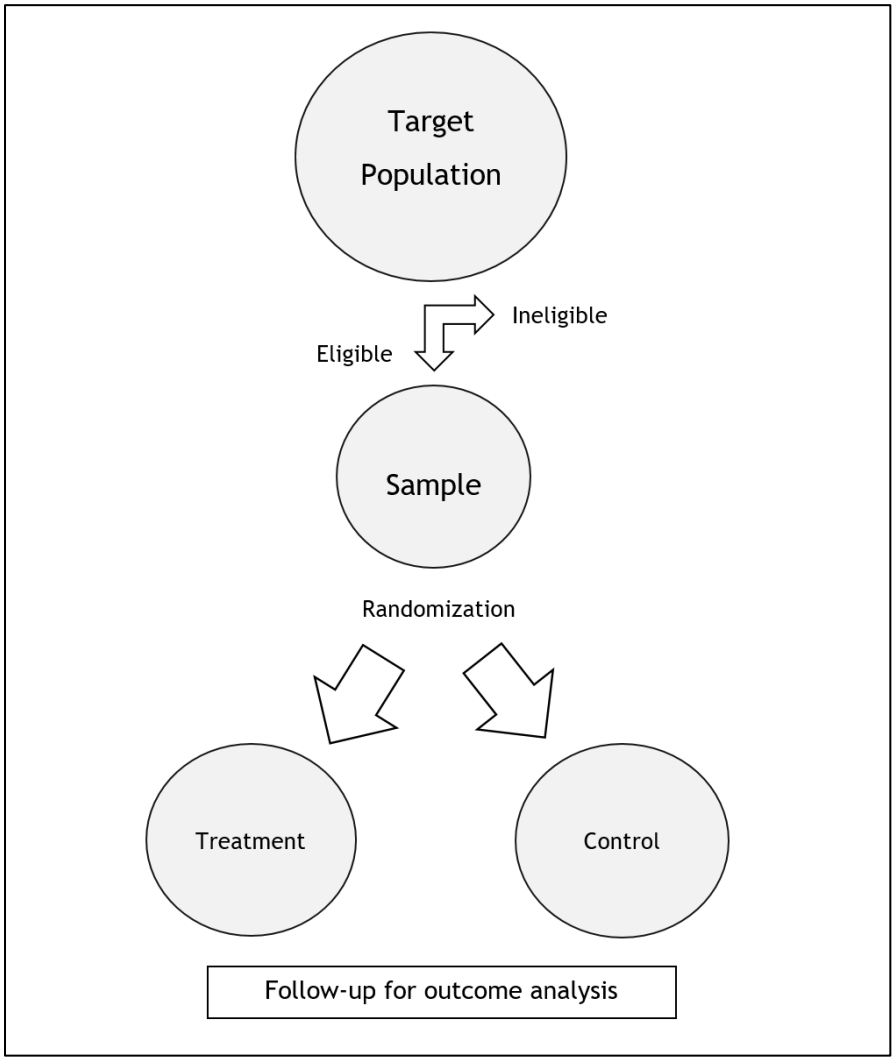
Considerations in designing a randomized controlled trial: The design of a randomized controlled trial includes the study design, the study population, sample size calculations and effect size estimates, enrollment of participants, intervention(s), follow-up visit description and schedule, ascertainment of response variables and the assessment of adverse events(161).

Study design: Three different randomized controlled trials designs can be completed according to treatment administration between groups under standardized conditions(141,162). The “parallel group design” is the least complex. Investigators study two groups nearly identical in size over a defined period of time(163). The “cross-over design”, a more complex trial, is where all treatments are administered one after the other to each group(163,164). Crossover designs switch the order of treatment for all groups at the same time and often have a wash-out period between treatments(141). Limitations of the crossover designs include the period and the carry-over effects. Period effects describe how chronic diseases can have cyclical patterns regardless of treatment. The carry-over effect

is seen when the impact of one treatment continues after reversing the order of treatments(163). The "factorial design" allows investigators to evaluate more than one treatment, e.g. different doses of the same drug or different study drugs(165). The factorial design is used to evaluate safety or efficacy compared to a control group(166).

Study and Sample Population: A sample population is selected from the target population being studied (**Figure 2**). Individuals from the sample population are randomly assigned to the treatment or the control groups. Control patients receive either another treatment (e.g. standard treatment) or a placebo(167). The inclusion and exclusion criteria information define and validate the study population that present the characteristics of interest of the study(147). Study participants that constitute the study sample are selected from the study population according to the inclusion and exclusion criteria of the study(168). The selection of participants is also related to the avoidance of adverse effects on a particular population and the exclusion of participants with comorbidities that might affect the outcome due to early termination or affect an accurate evaluation of the outcome of interest(169). Furthermore, poor adherence to medication should also be avoided since investigators need cooperation to take their assigned intervention and return or complete the follow-up to be able to observe the true effect of the intervention(170). Patients are followed for a pre-specified time after randomization. Results of randomized controlled trials depend on a comparison of findings between the intervention to a control group. The study sample size is calculated according to the working hypothesis ("superiority" or "equivalence"). The results must be calculated in an intention-to-treat analysis that include all the patients who were initially randomized(156).

Figure 2. Design of Randomized Controlled Trials



Sample size calculations and effect size estimates: The outcome measures and the secondary response variables are fundamental for determining the analysis and what conclusions can be drawn from the study. The sample size calculations and statistical power (the ability to identify significant differences between treatment groups) are crucial when planning a randomized controlled trial(171). The number of subjects needed for studies depends on the objective or phase of the study. The sample size of randomized control trials that explore toxicity or if a treatment has a therapeutic effect (Phase

I or II studies) range between 10 to 50 subjects. Studies that compare new treatments to standard therapy or placebo typically enroll between 100 to 1000 subjects. Those randomized controlled trials that examine the long-term effects of a drug after it is widely used typically enroll between hundreds and thousands of subjects(153). Factors that determine the number of participants needed in a study include: the clinical difference between treatment and control groups, the background rate of the characteristic being studied, and the probability of statistical *alpha* (type I) and *beta* (type II) errors(172). Type I errors refer to the probability of a conclusion that there is a difference between treatment when in fact they are equivalent. Type II errors refer to the probability of a conclusion that treatments are not different when in fact they are different(173). Even though type I and II errors can not be completely avoided, the likelihood of them occurring can be reduced by increasing the sample size(174).

Enrolment of participants: Study participants must be presented with all the information related to the study (study information sheet) after potential participants have been screened for inclusion and exclusion criteria. Participants must understand the study material and their participation must be voluntary (informed consent)(147). The informed consent form must be approved by the ethics committee or institutional review board of each one of the participating institutions(141). The informed consent must be provided in simple language and basic elements include the research nature of the study, the purpose, the expected duration and tasks or procedures needed from the participant, likely benefits and possible adverse events, how the confidentially of the information provided will be maintained, and contact numbers in case of questions or in the event of a research-related injury(175).

Randomization: Randomization is defined as the "act of assigning or ordering that is the result of a random process"(176). Participants are randomly allocated to either treatment or placebo group after they have granted consent(141). Randomization ensures that the allocation of known and unknown confounders, variables associated with the exposure and the outcome conditional to the exposure(177), are allocated randomly across each study group(178). Methods of randomizing participants include: simple, blocked, and stratified fixed allocation(171). Simple randomization, the most basic from of randomization, can be achieved by the flipping of an unbiased coin to assign participants to treatment groups or a simple randomization schedule using a random number producing algorithm software(179). Block allocation ensures balance in the number of participants assigned to each treatment groups and balance time of enrolment(180). Stratified randomization ensures that a previously(181) known characteristic or risk factor that might affect the outcome is equally distributed between treatment groups(182). In stratified allocation, participants are designated a specific stratum according to the confounding characteristic before they are assigned a treatment group(141).

Blinding: Masking or blinding ensures that participants and researchers are unaware of treatment allocation. Blinding prevent bias during enrolment, follow-up, and analysis of the study(153). Participants are unaware of the intervention assignment in a single blind design. A double-blind design implies that participants and investigators are unaware of treatment assignment. Investigators are unaware of intervention assignment during data collecting and analysis. In a triple blind design, the Data and Safety Monitoring board is unaware of intervention assignment(147). Blinding is not possible in some trials due to the nature of the disease or the treatment that is being studied. Sham surgery or masking a community intervention may not be possible(141,181).

Data Collection: High quality data collection must be ensured through the study(183). An operation manual with definitions of data collection procedures can be produced and training sessions can be arranged to ensure standardized measurements(184). Data collection should focus on addressing the research questions formulated in the protocol(185). The baseline examination refers to characteristics that study subjects have before the intervention. Baseline characteristics are often utilized to classify study subjects according to the presence or absence of a condition of interest(186) and provide the information necessary to extrapolate the results to other populations(187). The baseline characteristics are important to determine if the randomization process generated comparable treatment and controlled groups(186).

Adverse Event monitoring: The collection and monitoring of adverse events ensures a reliable assessment of harms from the intervention(153). Monitoring safety of participants in a randomized controlled trial is crucial to measure the balance between benefits and harms of an intervention(188). Every adverse event is recorded and compared among treatment arms to determine if the adverse event is caused by the intervention or a result of the disease itself(188). A serious adverse event may threaten life or function and must immediately be reported to regulators(189). Mechanisms to assess for harms during randomized controlled trials include having either a qualified clinician or a Data and Safety Monitoring Board in charge of the monitoring and reporting of the adverse events(153). The board has the advantage of being and independent source of monitoring and assessment to maintain safety and study validity(153).

Analysis: Data analysis procedures are pre-established and present baseline characteristics, primary and secondary outcomes and adverse events(141). The main purpose of the description and comparison of baseline characteristics is to assess generalizability of the study. Baseline differences between treatment and control groups should not be statistically tested as per the Consolidated Standards of Reporting Trials statement(190). Random allocation makes baseline differences between groups are due to chance rather than bias(186). Adjustment for variables that differ significantly at baseline is likely to bias the estimated treatment effect(191). Whether these differences are significant does not have any implications with the validity of the results of the study(192).

Considerations during analysis are the intention-to-treat analysis and how to handle missing data. The intention-to-treat approach reflects a practical clinical scenario reflecting noncompliant patients and patients that may stop taking the medication. The intention-to-treat analysis implies that participants are analyzed as randomized; regardless of intervention assignment or even if they completed or received the intervention(193,194). The exclusion of noncompliant patients from analysis might create important differences between treatment groups' outcomes(195). Patients may not take the study drug or may drop out of the study due to their response to treatment. For example, patients might feel better sooner due to the treatment provided or they may present adverse events(196).

Missing data is a major concern in analyzing randomized controlled trials data(197). Missing data may reduce the power of the analysis or bias the results of the study(198,199). The best way to deal with missing data is to limit the problem during the design and collection stage of the study according to the National Research Council (US) Panel on Handling Missing Data in Clinical Trials(199). Three different scenarios may occur regarding missing data(199,200). 1) Missing data completely at random means that complete cases represent all original cases as when they were randomized during enrolment. 2) Missing at random means that characteristics collected can account for differences in the distribution of missing data between treatment arms. 3) Missing not at random means that collected characteristics do not account for the distribution of missing data among treatment arms. There is no best method to control for missing data at the analysis stage(197). Three different methods to adjust for missing data include: 1) Complete-case analysis, where only complete cases are analyzed. 2) Single imputation methods, where missing values are replaced with plausible ones such as the mean for the observed cases of a particular variable. 3) Methods based on statistical model, where assumptions about the distribution of outcomes and predictors are taken into a model(199–201).

Termination of trial: The termination of a study is pre-planned and scheduled(202) based on the approximate time to recruit the sample size and follow-up needed to complete the study. A study can be terminated earlier if the intervention is deemed beneficial, harmful or futile(203,204). Other important considerations for termination includes unmasking of the intervention and dissemination of results. The unmasking can occur at the same time for all participants (common closeout) or it could occur at different times (anniversary closeout) per completion of follow-up through a closeout visit, phone call or letter. All data collection must be completed before unmasking. The dissemination of results is also important and it can be done through scientific journals to the scientific community, and though leaflets in hospital or waiting rooms or the institution's websites. The dissemination of results to the general public is also an excellent way to engage the community and promote research participation(205,206).

II. METHODS

A. OBJECTIVES

We examined the use of azithromycin for the treatment of pre-school children who presented with an acute wheezing episode. We sought to determine whether treatment with azithromycin for five days would resolve their acute symptoms sooner (up to Day 21) and would allow these children to remain symptom free for a longer period of time (up to Day 189) compared to those children treated with placebo.

B. HYPOTHESES

To assess the effectiveness of azithromycin among pre-school wheezing children we proposed the following hypotheses:

- Primary hypothesis: Treatment of pre-school children with an acute episode of wheezing symptoms with five days of azithromycin would reduce the duration of their respiratory symptoms sooner than those treated with placebo.
- Secondary Hypothesis: Treatment of pre-school children with acute wheezing symptoms with five days of azithromycin would allow these children to remain free of subsequent wheezy episodes longer than those treated with placebo. Secondary outcomes will include:
    - Number of days a short-acting beta agonist was used
    - Time to disease exacerbation: Unscheduled visit to a nurse/physician for respiratory problems or the use of oral corticosteroids for acute respiratory symptoms (cough, wheeze, or respiratory distress) that occurred after the child's initial symptoms resolved.

C. STUDY DESIGN

We completed a prospective, double-blinded, placebo-control randomized controlled trial in pre-school wheezing children from 12 to 60 months of age. Patients were recruited from January 2011 to May 2014 from the emergency departments of the Alberta Children's Hospital and Stollery Children's Hospital. The study was approved by the Health Research Ethics Boards of the University of Alberta and the University of Calgary. Informed consent was obtained from the parents or legal caretakers by a trained research assistant or the study coordinator. This study was not sponsored by any pharmaceutical company. Trial Registry: www.clinicaltrials.gov; Identifier: NCT01008761.

1. Patients

Subjects included in the study were children 12 to 60 months of age who presented to one of the participating emergency departments, with wheeze on auscultation, and whose parents consented to their enrollment in the study. Subjects excluded from the study were those who had taken antibiotics during the past 30 days, those whom physicians in the emergency department intended to prescribe antibiotics, those with known hypersensitivity to macrolides, those with significant comorbidities, those who were already enrolled in another study, those who did not communicate in English, those without access to a telephone, and those who were unable to follow up during the 21 days after enrollment (**Table 3**).

Table 3. Inclusion and Exclusion Criteria

Table 3. Inclusion and Exclusion Criteria
Inclusion criteria
12-60 months of age
Presented to one of the participating emergency departments
Wheezing on physical exam (noted by physician or nurse)
Exclusion criteria
Use of antibiotics during the 30 days previous to the study
Contraindication for use of macrolides
Significant co-morbidities
Current enrolment in another study, or enrolment within four weeks previous to the study
Language barrier
No access to a telephone
Not available to complete follow-up

2. Stratification and Intervention

We classified children according to their history of wheeze as first-time wheezers or previous wheezers during enrolment. Children were defined as first-time wheezers if they:

1. Never had symptoms of wheeze prior to this episode, or
2. Current symptoms of wheeze occurred for less than one month with no resolution of symptoms for more than one week during the one-month period.

Patients were randomized to receive either azithromycin or placebo. The placebo was produced by the Drug Development and Innovation Centre at the University of Alberta. Subjects were given 10 mg/kg/day for day one, then five mg/kg for four days. Each bottle contained sufficient drug to adequately dose children who weigh up to 30 kg (which is above the 95%ile weight for 60-month-old children). The research assistant expelled excess study drug/placebo from each syringe, so that the remaining volume of study drug/placebo equalled the appropriate dose based on the child's weight.

The dose was administered through an oral syringe by a registered nurse in the emergency department. If the child vomited up the first dose within 20 minutes of administration, a second dose was given. A syringe was marked with the calculated dose level for days two to five, labelled "study drug," and provided to the parents upon discharge from the emergency department. If patients were prescribed a metered-dose salbutamol inhaler (Airomir™, 100 µg per puff) by the attending physician, a dose counter (Doser™, Meditracker) and a spacer with mask (OptiChamber Advantage®, Philips Respironics) were provided to follow-up and count salbutamol usage. Children who were not prescribed salbutamol on discharge were still eligible for the study.

3. Randomization

The allocation sequence was generated by the Drug Development and Innovation Centre at the University of Alberta using random-number generating software (2 x 2 variable block ranging between four and eight per block), with stratification by study site and first versus previous episode of wheeze. The Drug Development and Innovation Centre produced the blinded study packages, completed the block randomization, and numbered the bottles. The allocation sequence was concealed from research assistants, investigators and participants. Participants were enrolled by the research assistant in the emergency department with assignment to intervention based on the randomization sequence and wheezing history. The master code was available to both sites if unmasking was deemed necessary.

4. Blinding

All investigators, research assistants, and participants were masked to allocation of treatment until analysis was completed. Participants, study personnel, study investigators and data analysts were blinded to allocation group.

5. Data Collection

Data collection started during enrolment and continued until day 189 of follow-up. Data collection involved the administration of several questionnaires: one questionnaire to gather enrolment data in the emergency department, one questionnaire to gather discharge data, and several follow-up questionnaires administered on days one, three, five, 14, and 21, when an in-clinic visit was performed. The follow-up survey continued to be administered two weeks after the in-clinic visit and then every six weeks, concluding on day 189 of follow-up. The monitoring of adverse events was done during the days the study drug was administered and 30 days after the last dose. Study data were collected and stored using REDCap (Research Electronic Data Capture) at each center(207).

Table 4. Study Design

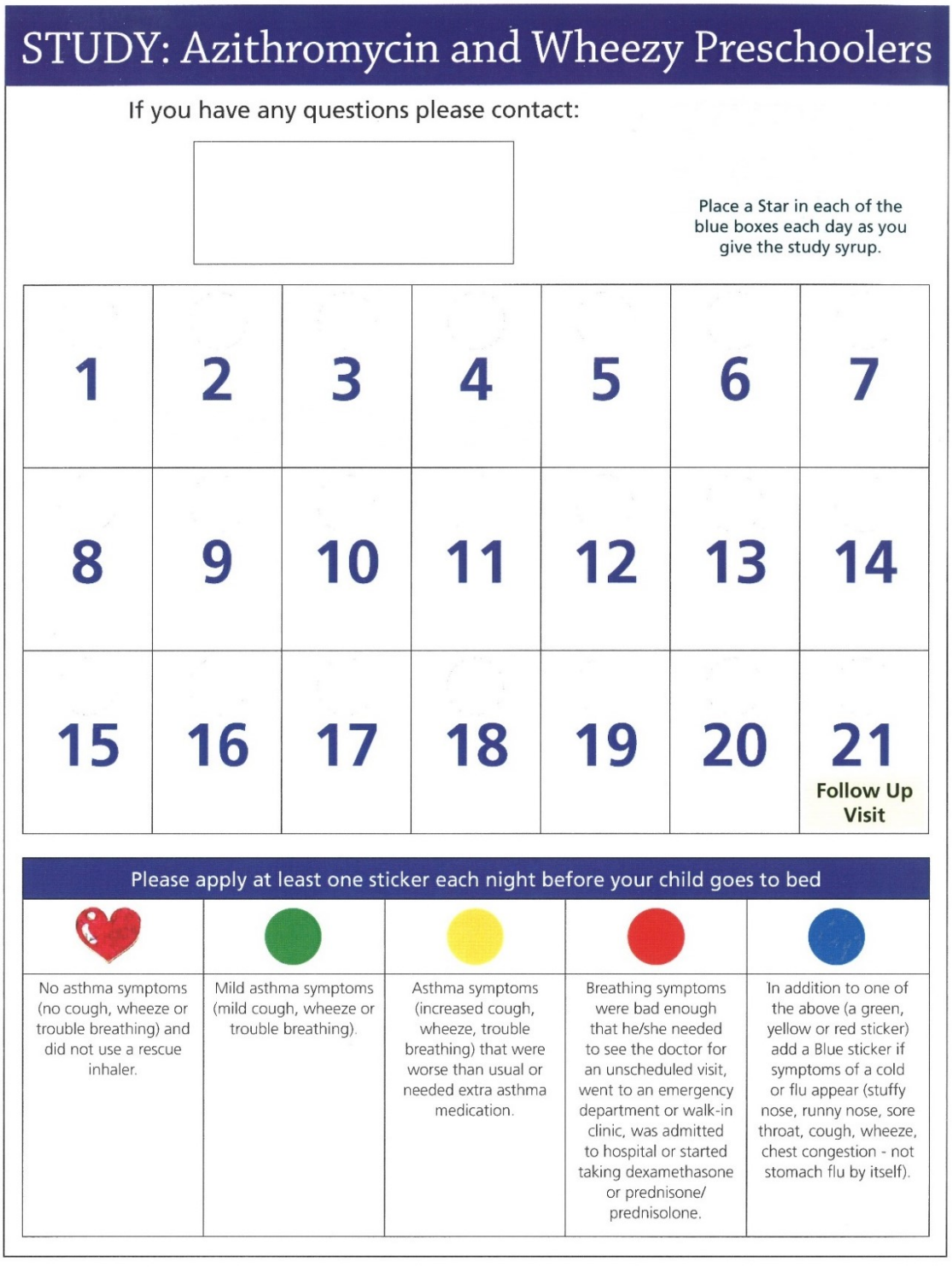
Table 4: Study Design						
Data Collection Instrument	Enrolment and Randomization	ED Discharge Vitals	Follow-up Day1	Follow-up Day3	Follow-up Day5	Follow-up Day14
Emergency Enrolment	x					
Emergency Discharge		X				
Follow-up			x	x	x	x
In-Clinic Visit						
• Daily Diary						
• Clinical Assessment						
• Baseline Questionnaire						
• Skin Prick Test						
Monitoring Adverse Events	x	X	x	x	x	x

Table 4: Study Design (continuation)						
Data Collection Instrument	In-Clinic Visit Day21	Follow-up Day 35	Follow-up Day63	Follow-up Day105	Follow-up Day 147	Follow-up Day189
Emergency Enrolment						
Emergency Discharge						
Follow-up	x	X	x	x	x	x
In-Clinic Visit						
• Daily Diary	x					
• Clinical Assessment	x					
• Baseline Questionnaire	x					
• Skin Prick Test	x					
Monitoring Adverse Events	x	X				

Enrolment in the emergency department. During enrolment, information about the symptoms of the current respiratory episode, the description of vital signs, symptoms at triage, and a detailed description of the administration of the study drug were collected. The clinical information was obtained directly from the parents or legal guardian of the child and included: coughing, wheezing, runny nose and difficulty breathing. The emergency department vital signs at admission were recorded from the clinical chart. The information related to the administration of the drug included the calculation of the first dose provided in the emergency department and the doses for days two to five. Calculations were taken from a standardized chart according to weight, reviewed by a registered nurse and recorded in the emergency enrolment questionnaire (**Appendix 2**). Parents were also provided with a copy of the informed consent form, a *daily diary* (**Figure 3**) with stickers to record asthma symptoms, and a *base line questionnaire* (**Appendix 3**) to be returned during the in-clinic visit on day 21.

The daily diary (**Figure 3**) was used to collect respiratory symptoms daily. The daily diary was provided during enrolment and returned in the day 21 in-clinic visit. Parents described their child's respiratory symptoms using different coloured stickers during the 21 days after enrolment. Five different stickers were used: one heart and four different colour dots. The heart sticker was used when the participant did not present any asthma symptoms nor used a rescue medication inhaler during that calendar day. Three of the four colour stickers were used to describe the severity of the asthma symptoms: green for mild, yellow for worse than usual and red for severe asthma symptoms that required an unscheduled medical visit or the use of oral corticosteroids. The last blue sticker was used in addition to the four previously described stickers. The blue sticker was added if symptoms of a cold or flu appeared in addition to the asthma symptoms. The daily diary was based on a calendar previously used to assess respiratory symptoms in a randomized controlled trial(208).

Figure 3. Daily Diary



STUDY: Azithromycin and Wheezy Preschoolers

If you have any questions please contact:

Place a Star in each of the blue boxes each day as you give the study syrup.

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21 Follow Up Visit

Please apply at least one sticker each night before your child goes to bed

No asthma symptoms (no cough, wheeze or trouble breathing) and did not use a rescue inhaler.	Mild asthma symptoms (mild cough, wheeze or trouble breathing).	Asthma symptoms (increased cough, wheeze, trouble breathing) that were worse than usual or needed extra asthma medication.	Breathing symptoms were bad enough that he/she needed to see the doctor for an unscheduled visit, went to an emergency department or walk-in clinic, was admitted to hospital or started taking dexamethasone or prednisone/ prednisolone.	In addition to one of the above (a green, yellow or red sticker) add a Blue sticker if symptoms of a cold or flu appear (stuffy nose, runny nose, sore throat, cough, wheeze, chest congestion - not stomach flu by itself).

The *base line questionnaire* (**Appendix 3**), provided during enrolment and returned on the day 21 in-clinic visit, included questions about past medical history, previous episodes of wheezing, shortness of breath, coughing, phlegm and asthma, as well as previous or usual asthma medication use. The baseline questionnaire also included information on the biological parents' allergy and asthma symptoms, and environmental characteristics such us smoke exposure, type of dwelling and pets at home.

Discharge from the emergency department: After discharge, the research assistant collected information related to the vital signs at discharge, the medication provided by the attendant physician in the emergency department, the medicine prescribed to be used at home, the final diagnosis, and whether patients were discharged from the emergency department or admitted to hospital (**Appendix 4**).

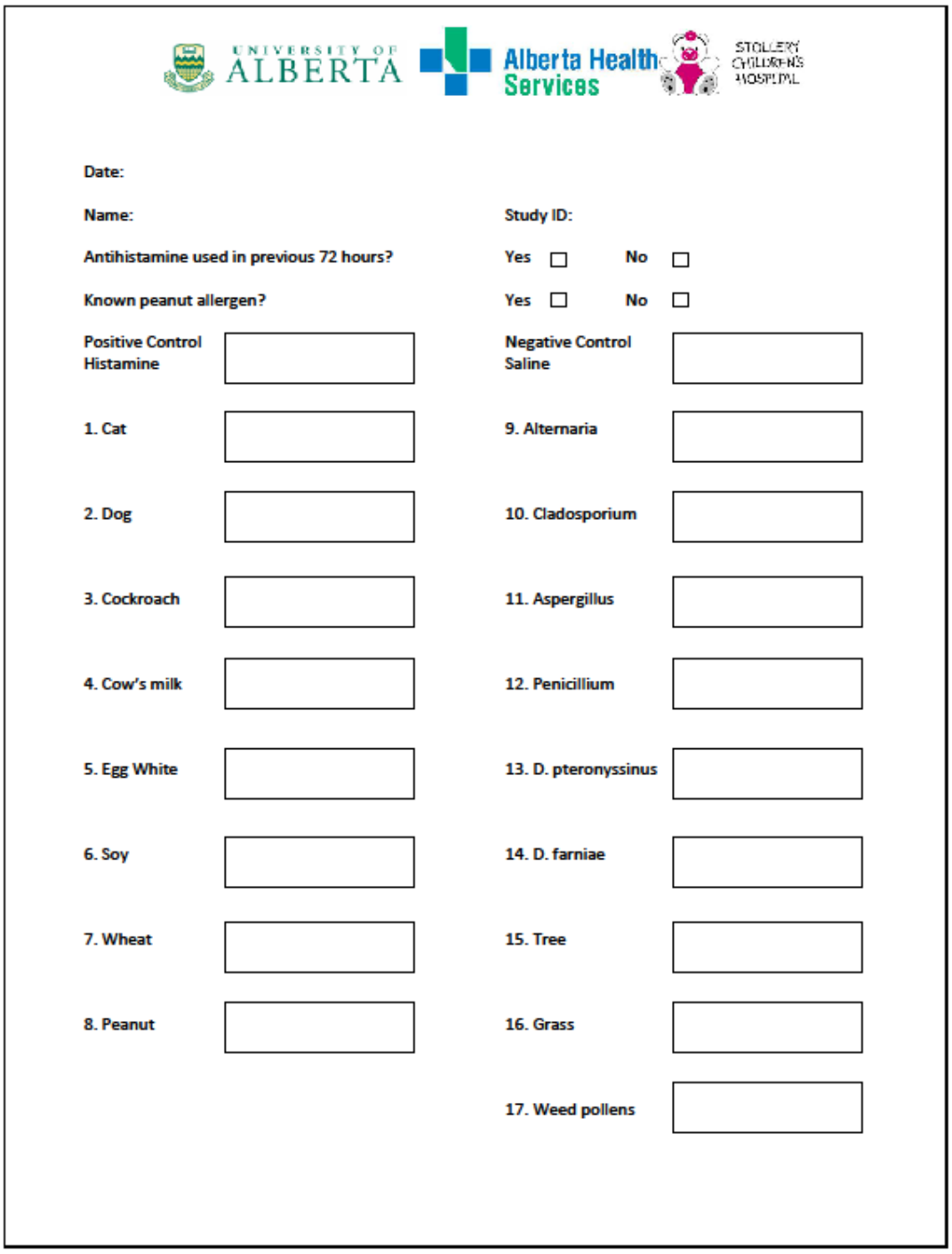
Telephone Follow-Up and In-Person Follow-Up (Day 21). Parents were contacted on days one, three, five, and 14 by phone or e-mail to complete the follow-up questionnaire and to determine whether any adverse event had occurred (**Appendix 5**). The same questionnaire was also administered during the in-clinic visit on day 21. Parents were asked about respiratory symptoms (wheezing, shortness of breath and cough) and if they taken their child to a doctor due to respiratory symptoms. Parents were also reminded to provide the study drug to their children (up to the 5th day) and to complete the *daily diary* (respiratory symptoms and medication). The in-clinic visit was scheduled during the first follow-up on day 1, and parents were called with a reminder a week before the scheduled day.

In-Clinic Visit (Day 21). An in-clinic visit was schedule on day 21 to perform a skin prick test and a clinical examination. The daily diary that indicated the asthma symptoms for the 21 days after enrolment and the baseline questionnaire regarding past medical history (family and environmental information) was collected during the in-clinic visit. A regular follow-up questionnaire was also administered (**Appendix 6**).

The *skin prick test* (SPT) was performed (**Figure 4**) to assess the child's atopic status during the in-clinic visit. Highly standardized ALK allergens, including tree, grass, weed pollen, cat, dog, D. pteronyssinus, D. farinae, cockroach, Alternaria, Cladosporium, Aspergillus, Penicillium, cow's milk,

egg white, soy, wheat and peanut were tested. The same batch of each allergen was used for all subjects. The wheal and flare was outlined in pen, at 10 minutes for histamine and at 15 minutes for allergens, and was transferred to paper (hard copy) using adhesive tape. The maximum weal and flare diameters and their mid-point perpendicular responses were measured.

Figure 4. Skin Prick Test



UNIVERSITY OF ALBERTA

Alberta Health Services

STOLLERY CHILDREN'S HOSPITAL

Date:

Name: Study ID:

Antihistamine used in previous 72 hours? Yes ☐ No ☐

Known peanut allergen? Yes ☐ No ☐

Positive Control Histamine		Negative Control Saline	
1. Cat		9. Alternaria	
2. Dog		10. Cladosporium	
3. Cockroach		11. Aspergillus	
4. Cow's milk		12. Penicillium	
5. Egg White		13. D. pteronyssinus	
6. Soy		14. D. farniae	
7. Wheat		15. Tree	
8. Peanut		16. Grass	
		17. Weed pollens	

The *clinical examination* (**Appendix 6**) was performed to assess respiratory symptoms: nasal flaring, tracheal tug, intercostal indrawing, stridor, prolong expiration, crackles, wheeze, any signs of inflammation and atopic dermatitis. This examination was based on the child clinical assessment used by the Canadian Healthy Infant Longitudinal Development (CHILD) study(209).

Follow-Up After Day 21. A follow-up questionnaire was administered on days 35, 63, 105, 147 and 189 (**Appendix 5**). Specifically, parents were asked if their children had been taken to a physician's office, urgent care clinic, or emergency department for treatment of worsening respiratory symptoms, and if oral corticosteroids were prescribed.

Monitoring for Adverse Events. Patients were actively monitored for the occurrence of adverse events on days one, three, five, 14, 21 and 35. Adverse events included symptoms related and unrelated to the study syrup. The following symptoms were included in the follow-up questionnaire (**Appendix 5**): abdominal pain, nausea, vomiting, headache, rash, diarrhea/watery stools, jaundice, hives, irregular heart rate, faint or any other symptom believed to be related to the study syrup. We collected adverse event information to inform the principal investigator if necessary, and to follow up on the resolution of symptoms. Any symptoms consistent with adverse effects to azithromycin, such as diarrhea or loose stools, nausea, abdominal pain and vomiting (common side effects), symptoms of allergic reaction (hives), changes in mood or behaviour, or arrhythmia/tachycardia, resulted in a review of the case by the study investigators. The investigators could decide whether to break the code and/or terminate the study based on the particulars of the case. If upon review there were concerns about the patient, the code was broken and the randomization drug discontinued. In the eventuality that a significant number of adverse events had emerged, a safety monitoring committee consisting of a pediatric pulmonologist, allergist, and general pediatrician would have been assembled. The committee had the authority to consider terminating the study if there were profound adverse effects that reflected concerns for all children.

6. Primary and Secondary Outcomes

The *primary outcome measure* was the time taken for children to resolve their acute respiratory symptoms: wheeze, cough, respiratory distress, and shortness of breath. This outcome was measured with the daily diary (**Figure 3**). The resolution of symptoms was defined as three consecutive days of their child having either no (heart sticker) or usual (green sticker) respiratory symptoms, as recorded in the daily diary.

The *secondary outcomes* included short-acting beta agonist use and the time to disease exacerbation. Only children prescribed a short-acting beta agonist at discharge from the emergency department were included in the secondary outcome analysis as established prior to data collection and analysis. The number of days the child used salbutamol during the 21 days following study enrolment was assessed by dose counter (Doser™, Meditracker) among study participants prescribed salbutamol (Airomir™, 100 µg per puff) in the emergency department. The time to disease exacerbation was assessed during each follow-up (**Appendix 5**) administered at days 35, 63, 105, 147 and 189. Disease exacerbation was defined as an unscheduled visit to a physician/nurse practitioner or treatment with an oral corticosteroid for acute symptoms of cough, wheeze, or respiratory distress (based on parental perception) that occurred after the child's initial symptoms resolved, during the following six months of follow-up.

7. Power Calculation

We based our power calculations on a previous study focused on the effects of telithromycin on acute asthma in adults(110) that demonstrated a 50% reduction in asthma symptoms among those using telithromycin (16% asthma symptoms in the placebo group versus 8% in the telithromycin group). We used these data to estimate the magnitude of effect for our sample size calculation (a 50% decrease in the number of days to symptom resolution). We determined that we needed a total enrolment of 440 subjects, divided into first-time wheezers (n=110) and previous wheezers (n=330). Using one-way

ANOVA, a total of 440 enrolled subjects would have provided 80% power to detect a 1.18 day difference in the two treatment groups at the .05 level. This calculation assumed a conservative standard deviation of 6 days in symptoms duration and a 10% loss to follow-up, and sufficient power to detect a 1.36 day difference between azithromycin and placebo. We considered the reduction of symptoms by one day or more to be meaningful because of its socio-economic implications.

8. Statistical Methods

The baseline characteristics in the two groups were compared overall in terms of means, standard deviation, and range. Median, inter-quartile range, percentages and 95% confidence intervals were calculated where appropriate. All randomly assigned participants were included in the adverse events analysis.

a) Primary Outcome Analysis

The time to resolution of symptoms was compared between treatment groups using Mann-Whitney U test. A pre-specified subgroup analyses between treatment groups by type of wheeze (first time vs. previous) was also performed. The data are presented as medians and interquartile range (IQR) accordingly. All analyses were carried out in Stata version 13.

b) Secondary Outcome Analysis

The number of days children used a short-acting beta agonist inhaler from drug and placebo groups were compared using Mann-Whitney U test. A pre-specified subgroup analyses between treatment groups by type of wheeze (first time vs. previous) was also performed. The data are presented as medians and interquartile range (IQR) accordingly.

Survival rates were expressed as the percentage free of disease exacerbation for 189 days calculated using the Kaplan-Meier method. Kaplan-Meier curves comparing treatment arms are shown up to 189

days follow-up. Cox proportional hazards was used to calculate the hazard ratios and 95% confidence intervals between treatment groups. All analyses were carried out in Stata version 13.

c) Ancillary Analysis

The time to resolution of symptoms (primary outcome) was compared between treatment groups by atopy status (atopic vs. non-atopic) and other characteristics using Mann-Whitney U test. The data were presented as medians and interquartile range (IQR) accordingly.

Survival rates (secondary outcome) comparing azithromycin and placebo by atopy status (atopic vs. non-atopic) were expressed as the percentage free of disease exacerbation for 189 days calculated using the Kaplan-Meier method. Kaplan-Meier curves comparing azithromycin and placebo by atopy status (atopic vs. non-atopic) are shown up to 189 days follow-up. Cox proportional hazards was used to calculate the hazard ratios and 95% confidence intervals between treatment groups by sub-group analysis.

The difference of short-acting beta agonist puffs used per day during the first 21 days of follow-up between treatment groups was examined using generalized estimating equations (GEE). Only children prescribed a short-acting beta agonist inhaler at discharge from the emergency department was included in this analysis. All analyses were carried out in Stata version 13.

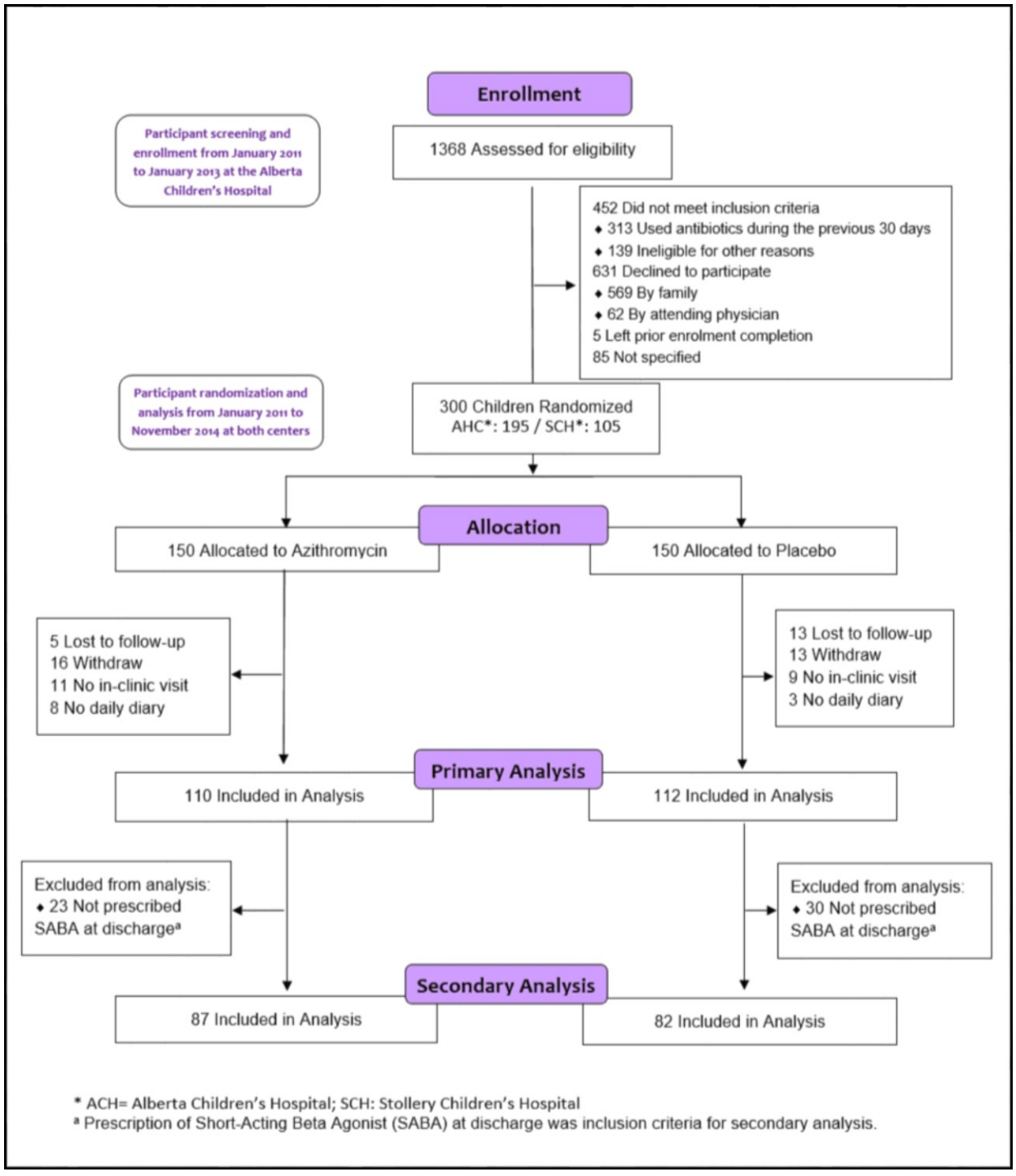
III. RESULTS

A. Study Participants

A total of 1368 children were assessed for eligibility at The Alberta Children's Hospital from January 2011 to January 2013. Thirty-three percent of children (453/1368) did not meet inclusion criteria with the majority of ineligible children (69%; 313/453) excluded due to use of antibiotics 30 days prior to screening. Of the 915 eligible children, 62% (569/915) of families declined to participate in the study, 62 children (7%) were deemed ineligible for the study by the emergency department attending physician, and 5 children (0.5%) left the emergency department before enrolment completion (**Figure 5**). The number of eligible children at the Stollery Children's Hospital was not available.

A total of 300 wheezing children were enrolled in the study between January 2011 and May 2014. The Alberta Children's Hospital enrolled 195 children and the Stollery Children's Hospital enrolled 105 children. Children were randomized into two treatment arms: 150 children into azithromycin and 150 children into placebo. Recruitment was extended for 2 years but eventually the study was terminated due to exhaustion of funds. For the primary outcome analysis, 222(74%) eligible families that returned the daily diary were included in the analysis. Of the 78 children excluded from primary analysis: 31 families (40%) did not return a complete daily diary (**Figure 5**), 29 families (37%) withdrew consent to participate in the study and 18 (23%) were lost to follow-up. Participants not included in the analysis had similar characteristics compared to those included in the analysis. Participants were mostly males (over 70% for both groups; **Table 5**) with a mean age at recruitment of 31.5 months (SD: 16.0) for those not included and 32.6 months for those included in the analysis (SD: 13.9). Similarly, over 70% of those included as well as of those not included in the analysis had previous wheezing history (**Table 5**). For the secondary outcome analysis, 169 eligible children were included in the analysis. As per protocol 53 children that were not prescribed a short-acting beta agonist at discharged from the emergency department were excluded. Over 93% of participants (279/300) provided adverse event information.

Figure 5. Study Design



1. Demographic Characteristics

Demographic characteristics were similar between treatment arms. Participants were mostly males: 70% males in the azithromycin group and 74% males in the placebo group. The mean age in months in the azithromycin arm was 34.8 (SD: 13.6) and 30.5 (SD: 13.9) in the placebo group (**Table 5**).

Demographic characteristics were also similar between first-time and previous wheezers by treatment arm sub-groups. Participants were mostly males: among first-time wheezers 74% males in the azithromycin group and 70% males in the placebo group; among previous wheezers 68% males in the azithromycin group and 76% males in the placebo group. The mean age in months among first-time wheezers in the azithromycin group was 31.0 (SD: 12.7) and 26.5 (SD: 13.1) in the placebo group; among previous wheezers the mean age in months was 36.2 (13.8) in the azithromycin group and 32.2 (13.9) in the placebo group (**Appendix Table 8a**).

Table 5. Demographic Characteristics - All Randomized Participants

Table 5. Demographic Characteristics – All Randomized Participants						
Participants/Characteristics	Not included (N=78)	Not included Azithromycin (N=40)	Not included Placebo (N=38)	Included (N=222)	Included Azithromycin (N=110)	Included Placebo (N=112)
Male (n (%))	55(70.5)	30(75.0)	25(65.8)	159(72.0)	77(70.0)	83(74.1)
Age in months – mean (SD)	31.5/75 (16.0)	31.2/37 (17.6)	31.8/38 (14.5)	32.6 (13.9)	34.8 (13.6)	30.5 (13.9)
Inhalers used prior ED (n(%))	42(53.9)	22(55.0)	20(52.6)	141(63.5)	73(66.4)	68(61.7)
One inhaler	18(23.1)	10(25.0)	8(21.1)	60(27.0)	32(29.1)	28(25.0)
Two inhalers	20(25.6)	11(27.5)	9(23.7)	73(32.9)	37(33.6)	36(32.1)
Three inhalers	3(3.9)	1(2.5)	2(5.3)	8(3.6)	4(3.6)	4(3.6)
Type of inhaler(n(%))						
Short-acting beta agonist	37(47.4)	20(50.0)	17(44.7)	135(60.8)	69(62.7)	66(58.9)
Inhaled corticosteroid	24(30.8)	12(30.0)	12(31.6)	80(36.0)	39(35.5)	41(36.6)
Previous Wheezing History	58(74.4)	29(72.5)	29(76.3)	158(71.2)	79(71.8)	78(70.5)
Symptoms at triage (n(%))						
Cough	73(92.3)	39(97.5)	33(86.8)	217(97.7)	109 (99.1)	108(96.4)
Runny nose	54(69.2)	30(75.0)	24(63.2)	187(84.2)	90(81.8)	97(86.6)
Wheeze	72(92.3)	38(95.0)	34(89.5)	221(99.6)	110(100.0)	111(99.1)
Difficulty breathing	67(85.9)	36(90.0)	31(81.6)	210(94.6)	106(96.4)	104(92.9)
Labored breathing	62(79.5)	30(75.0)	32(84.2)	198(89.2)	100(90.9)	98(87.5)
Accessory muscle use	52(66.7)	28(70.0)	24(63.2)	183(82.4)	92(83.6)	91(81.3)
Moderate retractions	13(16.7)	5(12.5)	8(22.1)	54(24.3)	27(24.6)	27(24.1)
Moderate severity of Wheezing	28(35.9)	14(35.0)	14(36.8)	90(40.5)	51(46.4)	39(34.8)

Data given as number or mean, percentage (%) or standard deviation (SD) as appropriate.

Previous use of inhalers and respiratory symptoms at triage were similar between treatment arms. Over 60% of participants (n=141) used at least one inhaler prior to the enrolment visit at one of the participating emergency departments. The most commonly used inhaler was a short-acting beta agonist with 63% participants in the azithromycin group and 59% participant in the placebo group. The use of an inhaled corticosteroid was also common with 36% participants in the azithromycin group and 37% participant in the placebo group (**Table 6**). Wheezing was the most common symptom at triage presented in 100% of participants in the azithromycin group and 99% participant in the placebo group. The presence of cough, runny nose, difficulty breathing, and wheezing severity are also similar between treatment arm sub-groups (**Table 6**).

Previous use of inhalers was different between first-time and previous wheezers. First-time wheezers had lower inhaler use prior visiting the emergency department, approximately 20% of first-time wheezers versus 80% of previous wheezers. Cough, runny nose, wheezing, difficulty breathing, and wheezing severity were similar between first-time and previous wheezers and treatment arm sub-group (**Appendix Table 8a**).

2. Baseline Questionnaire Characteristics

A total of 213(96%) baseline questionnaires were completed and included in the analysis. The azithromycin group with 110 participants retuned 104(95%) questionnaires and the placebo group with 112 participants retuned 109(97%) questionnaires. Previous medical history characteristics were similar between treatment arms and subgroups. A total of 43% participant in the azithromycin group and 41% participants in the placebo group had a history of allergies or eczema. Approximately 60% of participants had a family history of atopy: 63% participant in the azithromycin group and 59% participants in the placebo group. Around 25% reported a significant illness in the past and approximately 5% had ever required endotracheal intubation in both treatment arms. Approximately

20% of participants reported having a smoker at home and 7% reported regular exposure to smoke outside the household. Approximately 45% of participants attended day care (**Table 6**).

The distribution of eczema, family atopy, smokers in household, and attendance to day care are similarly balanced between first-time and previous wheezers by treatment arm sub-groups (**Appendix Table 8b**). However, previous significant illness was higher among previous wheezers and a fraction of them required endotracheal intubation: 0% of first-time wheezers and approximately 7% of previous wheezers (**Appendix Table 8b**).

Table 6. Previous Medical History

Table 6: Previous Medical History		
Participants/Previous Medical History	**Azithromycin (N=110)**	**Placebo (N=112)**
Diagnosis of allergies/eczema	47 (42.7)	46 (41.1)
Family history of atopy	69 (62.7)	66 (58.9)
Previous significant illness	33 (30.0)	20 (17.9)
Required endotracheal intubation	6 (5.5)	5 (4.5)
Smokers in household	26 (23.6)	20 (17.9)
Regular exposure to smoke outside household	8 (7.3)	8 (7.1)
Attend day care	49 (44.6)	53 (47.3)

Data given as number and percentage (%).

Wheeze and shortness of breath history were similarly balanced between treatment arms. Because the baseline questionnaire was returned in the 21 day in-clinic visit subjects might have included the enrolment episode in the description of symptoms (**Table 7**). Approximately 80% of participants reported wheezing during the last year. Less than half of participants reported to have woken up with tightness of chest during the last year. Over 70% of participants reported shortness of breath and

approximately 40% of them had woken up at night by an attack of shortness of breath during the last year. Less than 5% reported continuous shortness of breath.

Wheeze and shortness of breath was predominant among previous wheezers: approximately 55% of first-time wheezers versus over 70% of previous wheezers reported wheezing during the last year. A total of 26% first-time wheezers in the azithromycin group and 30% in the placebo group, and 39% of previous wheezers in the azithromycin group and 44% in the placebo group had woken up at night by an attack of shortness of breath during the previous twelve months. Repeated frequency of shortness of breath was predominant among previous wheezers: approximately 6% of first-time wheezers versus 40% of previous wheezers (**Appendix Table 8c**).

Table 7. Wheeze and Shortness of Breath History

Table 7: Wheeze and Shortness of Breath History		
	Azithromycin (N=110)	**Placebo (N=112)**
Wheeze and chest tightness		
Ever wheeze before*	97 (88.2)	98 (87.5)
Wheeze without a cold	52 (47.3)	43 (38.4)
Breathless when wheezing ever	66 (60.0)	69 (61.6)
Wheeze during the last year	89 (80.9)	89 (79.5)
Woken up with tightness of chest during last 12 months	43 (39.1)	55 (49.1)
Shortness of breath (SOB)		
Shortness of breath ever	77 (70.0)	83 (74.1)
SOB during the day at rest	34 (30.9)	31 (27.7)
SOB during the day at rest during the last 12 months	33 (30.0)	29 (25.9)
SOB during moderate physical activity during the last 12 months	27 (24.6)	21 (18.8)
SOB following strenuous activity during the last 12 months	41 (37.3)	36 (32.1)
Woken up at night by an attack of SOB during the last 12 months	39 (35.5)	45 (40.2)
Frequency of shortness of breath		
Rarely	40 (36.4)	50 (44.6)
Repeatedly	34 (30.9)	29 (25.9)
Continuously	3 (2.7)	4 (3.6)

Data given as number and percentage (%).
*Has your child ever had wheezing noise coming from his/her chest? "Wheezing" means a whistling sound, however high or low pitched and however faint. (Baseline Questionnaire provided during enrolment and handed over during 21 Day visit).

Asthma history characteristics were similarly balanced between treatment arms. A total of 46% participant in the azithromycin group and 44% participants in the placebo group noted an asthma episode at least once (**Table 8**). Over 80% of participants reported an emergency department visit due to asthma or wheezing and approximately 30% were hospitalized.

Table 8. Asthma History

Table 8: Asthma History		
	Azithromycin (N=110)	**Placebo (N=112)**
Ever asthma	52 (47.3)	52 (46.4)
Ever diagnosed asthma by MD	45 (40.9)	39 (34.8)
Ever had an asthma attack	50 (45.5)	49 (43.8)
Ever had any treatment for asthma or wheezing	74 (67.3)	84 (75.0)
Ever had any treatment for asthma or wheezing in the last 12 months	73 (66.4)	79 (70.5)
ED visit for asthma or wheezing	91 (82.7)	97 (86.6)
Hospitalized for asthma or wheezing	36 (32.7)	27 (24.1)

Data given as number and percentage (%).

Cough, phlegm and other symptoms history were similarly balanced between treatment arms. A total of 48% participant in the azithromycin group and 53% participants in the placebo group had coughed at night without a cold at least once (**Table 9**). Approximately 8% of participants reported usual phlegm on getting up during the last year. Over 40% of participants in both treatment arms reported eczema and over 35% reported urticaria (**Table 9**).

Cough, phlegm and other symptoms were more frequent among previous wheezers. Among first-time wheezers: 32% in the azithromycin group and 52% in the placebo group had cough at night without a cold at least once. Among previous wheezers 54% in the azithromycin group and 53% in the placebo group had cough at night without a cold at least once (**Appendix Table 8d**).

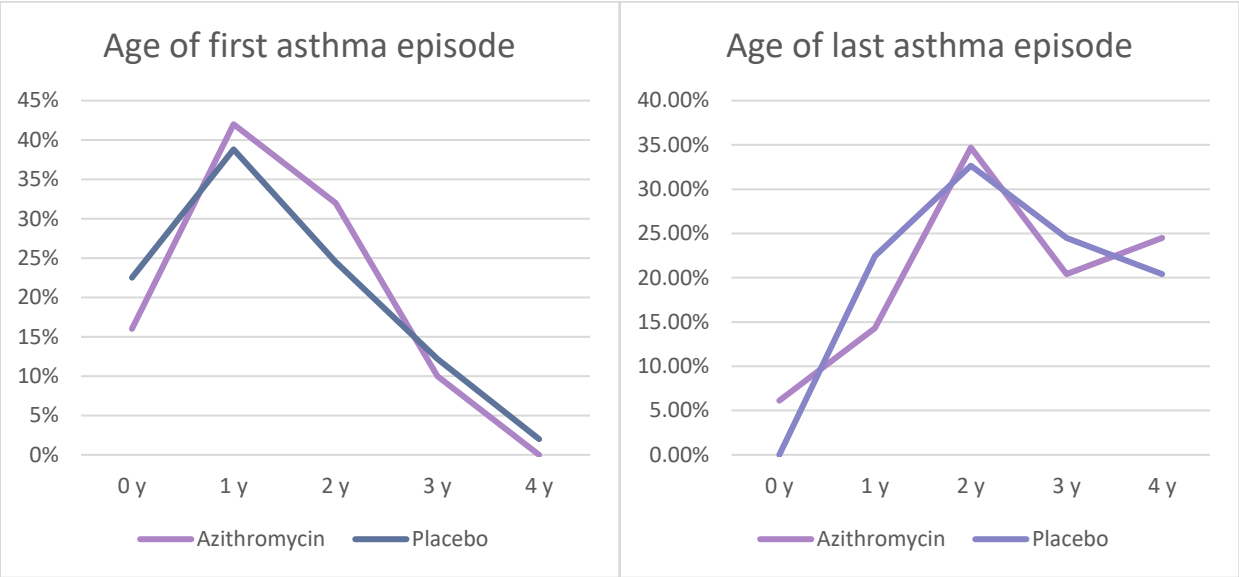
Table 9. Cough, Phlegm and Other Symptoms History

Table 9: Cough, Phlegm and Other Symptoms History		
	Azithromycin (N=110)	**Placebo (N=112)**
Ever coughed at night without a cold	53 (48.2)	59 (52.7)
Woken up coughing without a cold (last 12 months)	43 (39.1)	47 (42.0)
Usually cough on getting up (last 12 months)	29 (26.4)	28 (25.0)
Coughing on getting up most mornings for at least three months in a row each year	11 (10.0)	7 (6.3)
Usually phlegm on getting up (last 12 months)	9 (8.2)	8 (7.1)
Phlegm on getting up most mornings for at least three months in a row each year	6 (5.5)	2 (1.8)
Hay fever symptoms ever	30 (27.3)	38 (34.8)
Hay fever symptoms during the last 12 months	23 (20.9)	37 (33.0)
Eczema ever	53 (48.2)	49 (43.8)
Eczema during the last 12 months	42 (38.2)	43 (38.4)
Urticaria ever	38 (34.6)	43 (38.4)
Urticaria during the last 12 months	27 (24.6)	38 (33.9)
Other allergies ever	28 (25.5)	32 (28.6)
Other allergies during the last 12 months	19 (17.3)	17 (15.2)

Data given as number and percentage (%).

The age of first and last asthma attack reported by parents were similar between treatment arms. Most participants had their first asthma attack when they were one year old (**Figure 6**) and the last when they were two years old (**Figure 6**).

Figure 6. Age of First and Last Asthma Episode



Biological parent's atopy history was similar between treatment arms and sub-groups. Hay fever was the most frequent condition in both parents: 32% of fathers in the azithromycin group and 35% of fathers in the placebo group and 27% of mothers in the azithromycin group and 43% of mothers in the placebo group (**Table 10**). Biological parent's atopy history was also similar between first-time and previous wheezers by treatment arm sub-groups (**Appendix Table 8e**).

Table 10. Biological Parents' History

Table 10: Biological Parents' History		
Family atopy history	**Azithromycin (N=110)**	**Placebo (N=112)**
Participants/Father's history		
Previous history of asthma	29 (26.4)	28 (25.0)
Ever had hay fever symptoms	35 (31.8)	39 (34.8)
Ever had eczema	22 (20.0)	24 (21.4)
Ever had other allergies	37 (33.6)	36 (32.1)
Participants/Mother's history		
Previous history of asthma	30 (27.3)	33 (29.5)
Ever had hay fever symptoms	29 (26.4)	48 (42.9)
Ever had eczema	30 (27.3)	37 (33.0)
Ever had other allergies	51 (46.4)	51 (45.5)

Data given as number and percentage (%).

Environmental factors were similar between treatment arms and sub-groups. The most common housing type was house or townhouse with a total of 77% participant in the azithromycin group and 80% participants in the placebo group (**Table 11**). Approximately half of the participant had a pet at home. The most common pet at home was dog: 35% of participants in the azithromycin group and 31% of participants in the placebo group (**Table 11**). Environmental factors were also similar between first-time and previous wheezers by treatment arm sub-groups (**Appendix Table 8f**).

Table 11. Environmental Factors

Table 11: Environmental Factors		
Participants/House type	**Azithromycin (N=110)**	**Placebo (N=112)**
Apartment building	13 (11.8)	9 (8.0)
House or townhouse	85 (77.3)	90 (80.4)
Duplex	5 (4.6)	8 (7.1)
Farm with animals	1 (0.9)	2 (1.8)
Pets at home	58 (52.7)	53 (47.3)
Dogs	38 (34.6)	35 (31.3)
Cats	23 (20.9)	19 (17.0)
Others	12 (10.9)	17 (15.2)

Data given as number and percentage (%).

Medication usage characteristics were similar between treatment arms. The most commonly rescue medication used was Ventolin with a total of 86% participant in the azithromycin group and 88% participants in the placebo group. The most commonly controller medication used was the inhaled corticosteroid Qvar with a total of 27% participant in the azithromycin group and 29% participants in the placebo group. Leukotriene receptor antagonists were used by less than 6% of participants in both treatment arms. Anticholinergics were used by less than 2% of participants in both treatment arms (**Table 12**). Previous wheezers presented higher use of rescue medication before activity compared to first-time wheezers (**Appendix Table 8g**).

Table 12. Rescue and Controller Medication

Table 12: Rescue and Controller Medication		
	Azithromycin (N=110)	**Placebo (N=112)**
Rescue medication		
Short-acting beta2-agonist		
Ventolin	95(86.4)	98(87.5)
Airomir	1(0.9)	0(0.0)
Salbutamol Nebule	0(0.0)	3(2.7)
Use of rescue medication before activity	11(10.0)	15(13.4)
Controller medication		
Inhaled Corticosteroids		
Flovent	28(25.5)	28(25.0)
Pulmicort	1(0.9)	1(0.9)
Becloforte	1(0.9)	0(0.0)
Qvar	30(27.3)	32(28.6)
Advair	2(1.8)	0(0.0)
Alvesco	9(8.2)	10(8.9)
Anticholinergic		
Atrovent	2(1.8)	3(2.7)
Leukotriene receptor antagonist (LTRA)		
Singulair	6(5.5)	5(4.5)

Data given as number and percentage (%).

Emergency department and discharge medication usage was similar between treatment arms and sub-groups. The medication most commonly used in the emergency department was a short-acting beta agonist, used by 96% of participants in both treatment arms. Ipratropium bromide (Atrovent) and steroids were also used by over 80% of participants in the emergency department. At discharge 79% of participants in the azithromycin group and 73% of participants in the placebo group were prescribed a

short-acting beta agonist. Over 50% of participants were prescribed oral corticosteroids and inhaled corticosteroids in both treatment arms (**Table 13**).

Among first-time wheezers: 97% in the azithromycin group and 94% in the placebo group were provided a short-acting beta agonist in the emergency department. Among previous wheezers 96% in both treatment arms were provided a short-acting beta agonist in the emergency department. Over 70% of first-time and previous wheezers in both treatment arm sub-groups were prescribed a short-acting beta agonist at discharge from the emergency department (**Appendix Table 8h**).

Table 13. Emergency Department and Discharge Medication

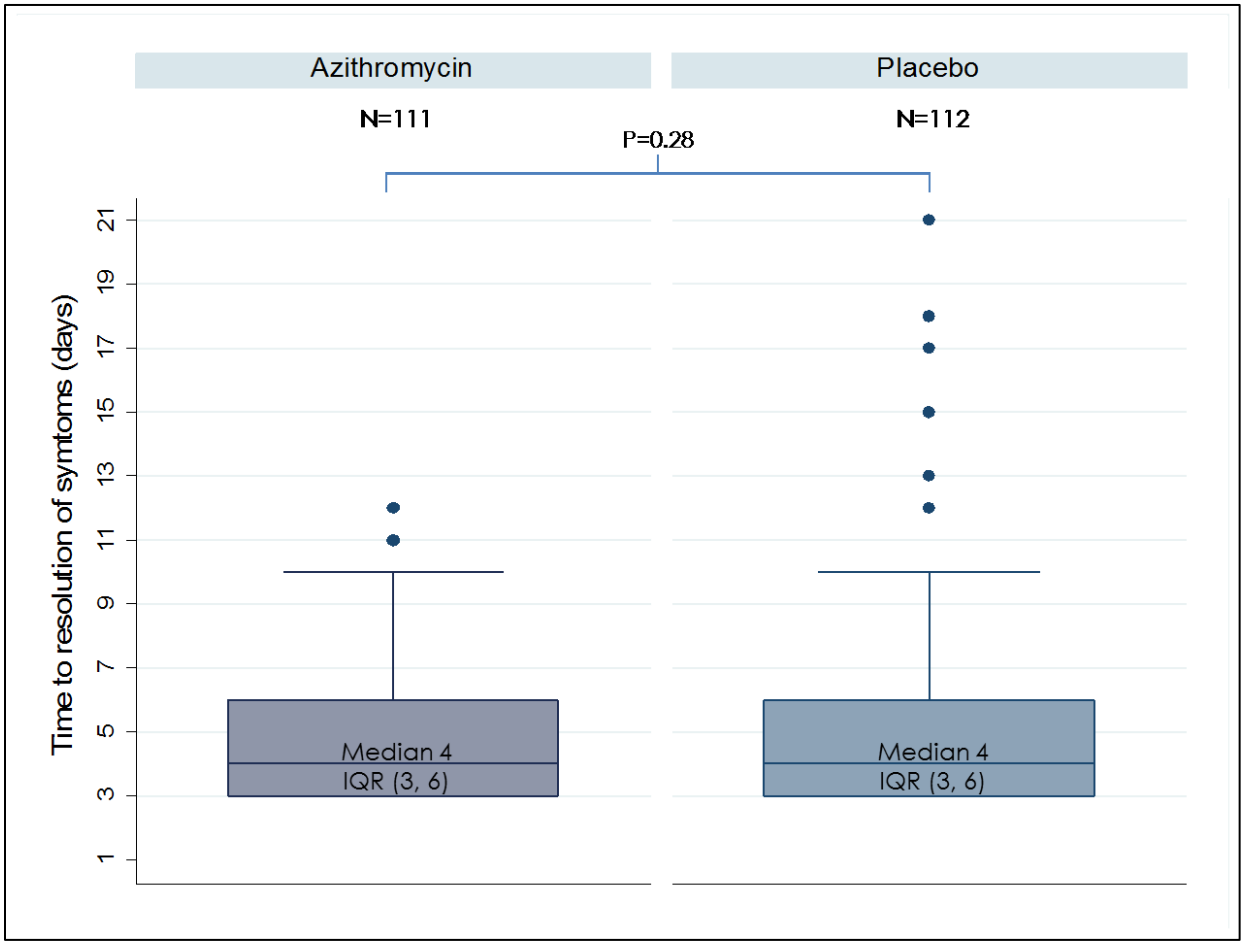
Table 13: Emergency Department and Discharge Medication		
	Azithromycin (N=110)	**Placebo (N=112)**
Emergency department medication		
Salbutamol	106(96.4)	107(95.5)
Atrovent	92(83.6)	82(73.2)
Other bronchodilators	8(7.3)	8(7.2)
Steroids	94(85.5)	90(80.2)
Epinephrine	0(0.0)	3(2.7)
Discharge medication		
Short-acting beta agonists	87(79.1)	82(73.2)
Oral corticosteroids	65(59.1)	70(62.5)
Inhaled corticosteroids	63(57.3)	57(50.9)

Data given as number and percentage (%).

B. Time to symptom resolution (primary outcome)

The time to resolution of symptoms was not normally distributed among treatment groups (Shapiro-Wilk's p<0.005) (**Appendix Figure 1**). The median time to resolution of symptoms among both treatment arms was four days (IQR 3, 6) and was not statistically significant between treatment arms (p=0.28) (**Figure 7, Table14**).

Figure 7. Primary outcome: Time to resolution of symptoms



1. First time wheezers versus previous wheezers

There was not a significant difference in time to resolution of symptoms by prior wheezing status (**Table14**). Among first-time wheezers the median time to resolution of symptoms was four days (IQR 3,6) for azithromycin and four days (IQR 3,5; p=0.40) for placebo. Among previous wheezers the median time to resolution of symptoms was four days (IQR 3,6) for azithromycin and four days (IQR 3,7; p=0.49) for placebo.

Table 14. Median time to resolution of symptoms by treatment

	Median time (days) (IQR)		
	Azithromycin	**Placebo**	**p-value**
Total sample (n=222)	4 (3 to 6)	4 (3 to 6)	0.28
First time wheeze (n=64)	4 (3 to 6)	4 (3 to 5)	0.40
Prior wheeze (n=158)	4 (3 to 7)	4 (3 to 6)	0.49
Non-atopic (n=70)	5 (3 to 7)	4.5 (3 to 7)	0.94
Atopic (n=138)	4 (3 to 6)	4 (3 to 5)	0.05

2. Post Hoc Analysis: Non-atopic versus atopic

There was not significant difference in time to resolution of symptoms among non-atopics but there was a significant difference among atopic children (**Table 14**). Among non-atopic participants the median time to resolution of symptoms was five days (IQR 3,7) for azithromycin and four and a half days (IQR 3,7; p=0.94) for placebo. Among atopic participants the median time to resolution of symptoms was four days (IQR 3,6) for azithromycin and four days (IQR 3,5; p=0.05) for placebo. Even

though there is a borderline significant difference among atopic participants who received placebo, the median time to resolution of symptoms is similar for azithromycin and placebo groups. There is only a slight difference in the interquartile range.

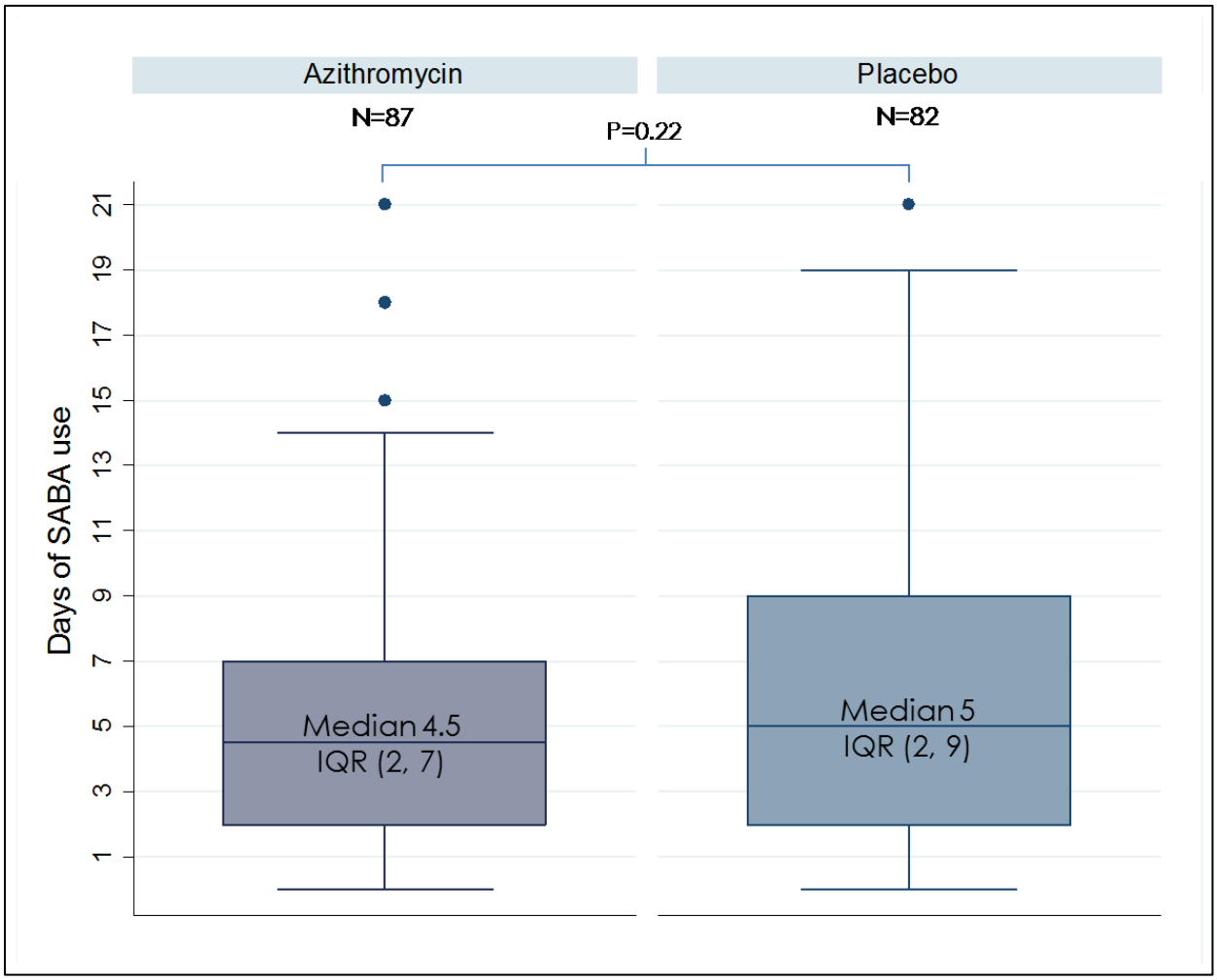
3. Post Hoc Analysis: Average change in duration of symptoms (days) in azithromycin versus placebo by subgroup

There was not significant difference in time to resolution of symptoms by subgroup. Specifically, there were no significant differences between treatment arms by gender, location (Alberta Children's Hospital in Calgary versus Stollery Children's Hospital in Edmonton), family history of atopy, or other environmental characteristics such as smoke exposure. Among males the median time to resolution of symptoms was four days (IQR 3,6; p=0.30) for both treatment arms. Among females the median time to resolution of symptoms was four days (IQR 3,7) for azithromycin and four days (IQR 3,6; p=0.71) for placebo. Among children attending the Alberta Children's Hospital as well as children attending the Stollery Children's Hospital the median time to resolution of symptoms was four days (IQR 3,6; p=0.52) for both treatment arms. Among children with no previous history of family atopy the median time to resolution of symptoms was four days (IQR 3,5) for azithromycin and four days (IQR 3,6; p=0.59) for placebo. Among children with previous history of family atopy the median time to resolution of symptoms was five days (IQR 4,6) for azithromycin and four days (IQR 3,6; p=0.08) for placebo. Among children without smokers in household the median time to resolution of symptoms was four days (IQR 3,6; p=0.53) for both treatment arms. Among children with smokers in household the median time to resolution of symptoms was four days (IQR 3.8,6) for azithromycin and four days (IQR 3,5.8; p=0.15) for placebo. Among children that do not attend daycare the median time to resolution of symptoms was four days (IQR 3,5) for azithromycin and four days (IQR 3,6; p=0.97) for placebo. Among children that attend daycare the median time to resolution of symptoms was five days (IQR 3,7) for azithromycin and four days (IQR 3,5; p=0.06) for placebo.

C. Short-acting beta agonist use (secondary outcome)

The number of days a short-acting beta agonist was used was not normally distributed among treatment groups (Shapiro-Wilk's p<0.005). The median number of days that a short-acting beta agonist was used among those who received azithromycin was four and a half days (IQR 2,7) and five days (IQR 2,9) among those who received placebo (**Figure 8**), this difference between drug and placebo arms was not statistically significant (p=0.22).

Figure 8. Secondary outcome: Short-acting beta agonist use



1. First time wheezers versus previous wheezers

There was no significant difference in the number of days a short-acting beta agonist was used by prior wheezing status. Among first-time wheezers the median number of days children used a short-acting beta-agonist was four days (IQR 2,6) for those who received azithromycin and three days (IQR 0.3, 6.5; p=0.42) for those who received placebo. Among previous wheezers the median number of days children used a short-acting beta agonist was five days (IQR 1.3, 7.0) for those who received azithromycin and six days (IQR 3,10; p=0.10) for those who received placebo.

2. Post Hoc Analysis: Non-atopic versus atopic

There was no significant difference in the number of days a short-acting beta agonist was used by prior atopy status. Among non-atopic children the median number of days children used a short-acting beta agonist was four days (IQR 3.0, 7.5) for those who received azithromycin and seven days (IQR 3, 10; p=0.13) for those who received placebo. Among atopic children the median number of days children used a short-acting beta agonist was five days (IQR 1.5, 7.0) for those who received azithromycin and four and a half days (IQR 2.0, 8.8; p=0.83) for those who received placebo.

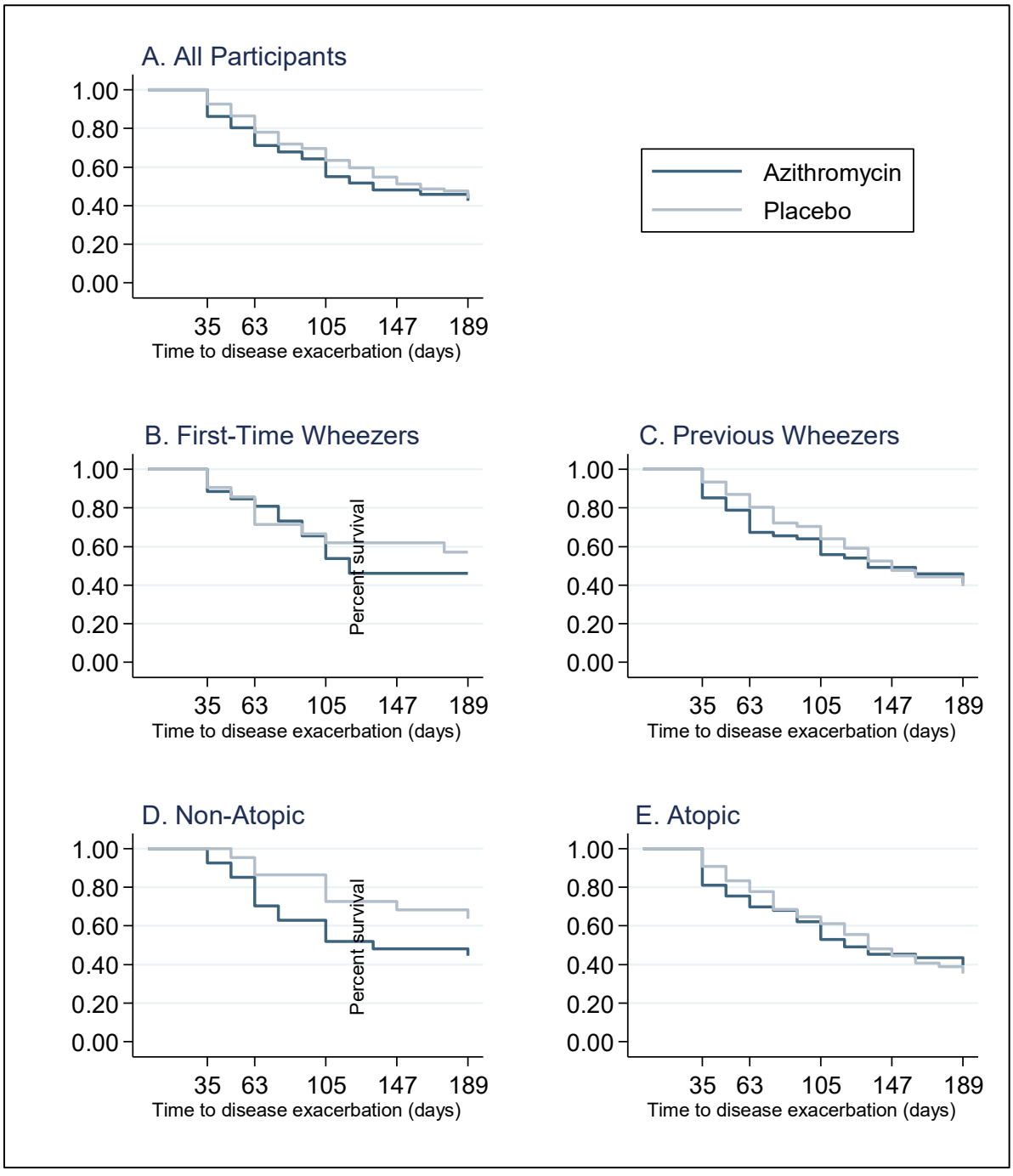
3. Post Hoc Analysis: Number of short-acting beta agonist puffs used per day

The mean number of puff used per day among children in the azithromycin group was 1.75 (SD: 4.5) and 1.82 (SD: 3.5) in the placebo group. There was no significant clinical or statistical difference in the number of short-acting beta agonist puffs used per day between treatment arms. Children randomized to placebo used 0.07 more puffs per day than children randomized to azithromycin (95% CI: -0.5, 0.6; p=0.82).

D. Time to disease exacerbation (secondary outcome)

A total of 169 participants were included in the time to disease exacerbation analysis. Nighty six participants (57%) presented with a disease exacerbation at six-month follow-up (unscheduled visit to a physician/nurse practitioner or treatment with an oral corticosteroid for acute respiratory symptoms). The 189 day exacerbation free rate was not significantly different between treatment groups (hazard ratio 0.91; 95% CI: 0.61 to 1.36; p=0.650) (**Figure 9**). A sensitivity test did not find significant difference using the log rank test (p=0.64).

Figure 9. Secondary Outcome: Kaplan-Meier Curves Showing Time to Respiratory Disease Exacerbation



1. First time wheezers versus previous wheezers

A total of 23/47 first-time wheezers (49%) presented a disease exacerbation at six-month follow-up. The 189 day exacerbation free rate was not significantly different between treatment groups (hazard ratio 0.78; 95% CI: 0.34 to 1.80; p=0.56) (**Figure 9**). A sensitivity analysis did not find significant difference using the log rank test (p=0.55).

A total of 73/122 previous wheezers (60%) presented a disease exacerbation at six-month follow-up. The 189 day exacerbation free rate was not significantly different between treatment groups (hazard ratio 0.96; 95% CI: 0.61 to 1.52; p=0.86) (**Figure 9**). A sensitivity analysis did not find significant difference using the log rank test (p= 0.86).

2. Post Hoc Analysis: Non-atopic versus atopic

A total of 23/49 non-atopic children (47%) presented a disease exacerbation at six-month follow-up. The 189 day exacerbation free rate was not significantly different between treatment groups (hazard ratio 0.53; 95% CI: 0.23 to 1.26; p=0.15) (**Figure 9**). A sensitivity analysis did not find significant difference using the log rank test (p= 0.13).

A total of 67/107 atopic (63%) presented a disease exacerbation at six-month follow-up. The 189 Day exacerbation free rate was not significantly different between treatment groups (hazard ratio 1.02; 95% CI: 0.63 to 1.65; p=0.93) (**Figure 9**). A sensitivity analysis did not find significant difference using the log rank test (p= 0.93).

E. Adverse Events

All randomly assigned participants were included in the adverse event analysis. The proportion of participants experiencing an adverse event was higher in the placebo group than in the azithromycin group: 97 (69.8%) and 80 (57.1%), respectively (**Table 15**). There were no serious or life threatening adverse events. Two subjects discontinued study medication, one with vomiting and diarrhea and one with periorbital erythema. We disclosed the allocation assignment information to the parents of the patient with the vomiting and diarrhea. The allocation disclosure was provided to parents with help of an external party to avoid unblinding study personnel. Subsequently, this subject withdrew the study. The participant with erythema discontinued the study syrup after the first dose and continued regular follow-up in the study. Both participants were assigned to azithromycin group.

Table 15. Adverse Events

Table 15: Adverse Events		
	Azithromycin (n=150)	**Placebo (n=150)**
Any adverse event	80/140(57.1)	97/139(69.8)
Abdominal pain/discomfort	23/140(16.4)	34/139(24.5)
Nausea/vomit	22/140(15.7)	29/139(20.9)
Headache	8/140(5.7)	14/139(10.1)
Rash	26/140(18.6)	26/139(18.7)
Hematochezia	0/140(0.0)	3/139(2.2)
Diarrhea/loose or watery stools	42/140(30.0)	44/139(31.7)
Jaundice	3/140(2.1)	0/139(0.0)
Hives	10/140(7.1)	16/139(11.5)
Irregular heart rate	10/140(7.1)	4/139(2.9)

Data given as number and percentage (%).

IV. DISCUSSION

In this multicenter double-blind randomized control trial, we concluded that azithromycin for five days did not reduce the duration of respiratory symptoms in pre-school age children presenting to the emergency department with wheeze. Azithromycin also did not reduce the use of short-acting beta agonists after discharge from the emergency department during the short-term (21 days) follow-up, nor decreased the time to disease exacerbation during the long-term (189 days) follow-up. There were more adverse events in the placebo group than in the azithromycin group. None of the adverse events were serious or life-threatening.

Our findings are consistent with a Cochrane review published in 2014 that concluded that there is not enough evidence to support the use of antibiotics for bronchiolitis. The Cochrane review included seven studies in the analysis, six of them used a macrolide, and three of them used azithromycin. Most of the studies did not find significant differences between the use of antibiotics and placebo in bronchiolitis. Only one double-blind randomized controlled trial, that used IV clarithromycin for three weeks on hospitalized first-time wheezers (with negative skin test and no parental asthma history) with respiratory syncytial virus bronchiolitis, observed a reduction of hospital stay, need for oxygen supplementation and short-acting beta agonists(12). Nevertheless, the latter study has a risk of sampling bias due to a small sample size (21 subjects). A recent study published in 2015 also concluded that three once-weekly doses of azithromycin did not reduced hospital stay, need for oxygen supplementation, 21-day follow-up symptoms, or rehospitalization rate at six-months of follow-up(210).

Unlike previous studies on asthmatic children that reported reduction of bronchial hyperresponsiveness and neutrophils in sputum when treated with azithromycin(211), our results suggest that azithromycin is not useful to treat wheezing symptoms among atopic children. However, this difference was negligible: the median time to resolution of symptoms was four days for both treatment arms. There was only one day difference in the interquartile range interval: azithromycin (IQR 3,6) versus placebo

(IQR 3,5; p=0.05). The length of the intervention may determine the impact of macrolides among atopic children. Beigelman et. al.(95) did not observe a reduction of serum IL-8 levels at day eight but did observe decreased IL-8 levels in nasal lavage fluid by day 15 among hospitalized infants (one to 18 months) with respiratory syncytial virus that received azithromycin for 14 days. Clarithromycin's effectiveness to modulate IL-8 levels and neutrophil accumulation on patients with refractory non-eosinophilic asthma was described after eight weeks of therapy(113). Likewise, the reduction of asthma symptoms in adult acute asthma exacerbations was observed after 10 days of telithromycin therapy(110). In the future, researchers may examine the effects of long-term therapy with macrolides on respiratory tract illness in preschool children.

Research to identify subpopulations that may benefit from antibiotics may be justified(109). Two recently published studies, Bacharier et. al.(108) and Stokholm et. al.(212) reported that early administration of azithromycin to recurrent wheezers can prevent severe lower respiratory tract illnesses and reduce the duration of wheezing episode. Bacharier et. al. included children aged 12 through 71 months, with at least three wheezing episodes during the last twelve months from the AsthmaNet network. Stokholm et. al. included children aged one to three years, with at least five episodes during the list six months from the Copenhagen Prospective Studies on Asthma in Childhood 2010 cohort. Both studies included children with multiple wheezing episodes, at a high risk for asthma. This study included preschool children presenting to the emergency department with and without a wheezing history. Specifically, previous wheezers where those who had wheezed at least once before the enrolment visit. The Bacharier study provided the parents with personal guidelines to administer the intervention at home earlier in the course of illness. Parents were instructed to provide the study medication next time the child presented symptoms that parents described as usual symptoms before the development of a severe lower respiratory tract infection. Our study intervention was provided during the emergency department visit. Bacharier et. al.(108), Stokholm et. al.(212) nor this study observed an improvement in the long-term outcomes for disease exacerbation/time to next episode in those assigned to azithromycin when compared to placebo.

There is evidence that supports the benefit of macrolides for wheezing symptoms in adult chronic asthma. A randomized controlled trial that evaluated the use of telithromycin for ten days in acute asthma exacerbations in adults (18 to 55 years of age), showed a significant reduction of asthma symptoms during the ten days of treatment when compared with placebo(110). An ancillary analysis showed that patients in the telithromycin group had 50% reduction in symptoms when compared to placebo (five versus eight days). A recent in vitro and in vivo study, on airway epithelium effect of azithromycin in adults with moderate-severe asthma, reported that azithromycin improved normal bronchial epithelia differentiation, maintenance and response to damage in vitro. However, the in vivo biopsies did not confirm their in vitro results, probably due to a small sample size (10 participants) with co-morbidities and polypharmacy(213). Even though Slater et. al.(213) did not find that azithromycin protected against the initial challenge with *P. aeruginosa* to mimic respiratory tract infection and damage in vitro, he observed speed-up barrier integrity recovery post-exacerbation during the acute phase response. The transepithelial electrical resistance (measure of epithelial recovery) increased between two to three hours after the challenge with *P. aeruginosa* and was lost twenty-four hours after the challenge.

Other studies also show some evidence of the benefits of azithromycin in specific subpopulations. A study that included sixteen asthmatic children treated with either azithromycin or placebo for eight weeks, observed reduction of bronchial hyperresponsiveness and neutrophils in sputum in the azithromycin group(211). Simpson et. al. in a study of non-eosinophilic refractory asthma (non-allergic classified by sputum analysis)(113) concluded that clarithromycin for eight weeks reduced IL-8 levels, neutrophil accumulation and improved quality of life (wheezing). Amayashu et. al.(112) reported that clarithromycin for eight weeks among adults with eosinophilic (allergic) asthma decreased blood and sputum eosinophilic cationic protein as well as blood and sputum eosinophil count. Shoji et. al.(111) also described antibronchial inflammatory effect associated with eosinophilic infiltration after patients with aspirin-intolerant asthma received roxithromycin for eight weeks when compared with placebo. Specifically, Shoji et. al.(111) reported decrease on symptoms, serum eosinophil and eosinophilic cationic protein as well as sputum eosinophils and eosinophilic cationic protein. These studies warrant

future investigation on macrolide use in specific subpopulations such us aspirin-intolerant and eosinophilic asthma.

The antimicrobial properties of azithromycin may attenuate the severity of respiratory symptoms (wheezing) in rhinoviral infections with bacterial co-infection on children. A recent study evaluated the effect of pathogenic bacteria during rhinovirus infection in children (four to 12 years of age) with and without asthma. Kloepfer et. al.(214) reported an increased detection of specific bacterial pathogens during rhinovirus infection in children with and without asthma diagnosis. The percentage of pathogens observed in asthmatic and non-asthmatic children was similar. However, bacterial pathogens are more prevalent during rhinovirus infection and are associated with increased respiratory and asthma symptoms. Specifically, *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* presence increased during rhinoviral infections. In the absence of rhinoviral infection, *S. pneumoniae* and *M. catarrhalis* showed increased risk for respiratory symptoms but *H. influenzae* was not associated with respiratory symptoms regardless of rhinovirus infection status. Further studies on respiratory syncytial virus and bacterial infections interactions and their effect on respiratory illnesses may lead to new therapeutic approaches.

This study has several strengths compared with previous published randomized controlled trials. The primary outcome focused on detecting the days to resolution of symptoms which has a direct impact on the patients' life quality and socioeconomic impact instead of a laboratory measure such as oxygen saturation or serum/blood eosinophils that may not translate directly into children's daily life. The enrolment in the emergency department allowed for the inclusion of children in an acute care setting regardless of the severity or progression of the disease. Children were included regardless of whether they were discharged from the emergency department or had to be hospitalized after enrolment. This study assessed atopy status through a skin prick test during the 21-day in clinic visit. The 189 days of follow-up allowed us to examine the short-term (21 days) and the long-term effects of azithromycin in wheezing children. The stratification of the study of first-time wheezers and previous wheezers enable to establish if these sub-populations responded differently to azithromycin. Finally, the inclusion of

common adverse events in every follow-up allowed for an active and clear description of all adverse events.

We evaluated for potential biases such as contamination and co-intervention(215). Participants were assessed for the potential co-administration of antibiotics during enrolment and throughout the study. Specifically, participant's emergency department charts were reviewed for treatment with antibiotics during the enrolment visit. Additionally, participant's parents/guardians were asked if any additional medication was provided to their children at each follow-up if. We did not observe any contamination among treatment arms during enrolment and up to twenty-one days after the initiation of the study drug. Similarly, we assessed for co-intervention bias where children would be treated with additional medications for their respiratory symptoms. All medications provided to participants during the enrolment and after discharged were assessed through the emergency medical charts and though each follow-up. No differences were observed between treatment arms in the co-administration of short-acting beta agonist, inhaled corticosteroids and other bronchodilators (**Table 13**).

A limitation of this study was our inability to enroll our targeted sample size despite extending the trial completion date by almost three years beyond our anticipated completion date. We experienced low enrolment rates for multiple reasons: 1) A large proportion of children had already been on antibiotics in the 30 days prior to presentation emphasizing the importance of this study (**Figure 5**) 2) A number of physicians and almost half of families refused to enrol their patients in the study because they were opposed to treating with azithromycin without a clear bacteriological focus. Additionally, due to financial constraints we could not quantify the number of eligible children with wheezing symptoms that visited the emergency department in the Stollery Children's Hospital. Another limitation of this study was the potential for misreporting of respiratory symptoms on the daily diary used by parents to measure respiratory symptoms (primary outcome)(208). An additional limitation of this study was that we could not quantify the rate of macrolide resistance after the administration of azithromycin compared to placebo.

This study collected nasal swabs before the administration of the intervention (enrollment) and 21 days after the first dose, however, samples could not be analyzed due to lack of economic resources. Future

studies could evaluate antibiotic resistance with the nasal swabs collected before and after the administration of azithromycin or placebo. The antibiotic resistance analysis of the Bacharier study reported a slightly increase in the number of subjects with azithromycin resistant organisms on patients treated with azithromycin. Nonetheless, only one site with a total of 81 participants were included in the antimicrobial resistance analysis of the Bacharier study(108). This study has the advantage of having collected nasal swabs samples from all participants, therefore, has a higher probability to find significant differences between groups. A recent published Cochrane review of macrolides for chronic asthma recommends that future research should focus on the measurement and report of resistance as an outcome(114).

A novel non-antibiotic macrolide 12-membered erythromycin A derivative: EM900(216), has potent anti-inflammatory and immunomodulatory effects similar of those of clarithromycin and erythromycin without the macrolide antibacterial activity. Tojima et. al.(217) reported that EM900 low-dose long-term therapy exhibited anti-inflammatory and immunomodulatory effects useful for the treatment of chronic airway inflammation. The development of a macrolide with anti-inflammatory effect and without antimicrobial activity should not promote antibacterial resistance. Further in-vivo and in-vitro studies are necessary to elucidate other parallel mechanisms of action and safety of EM900. The anti-inflammatory and immunomodulatory activity of non-antibiotic macrolides in the management of airway inflammation warrant further studies among wheezing children.

V. CONCLUSION

In this multi-center double-blind randomized control trial, azithromycin for five days in children with wheezing symptoms did not reduce the duration of respiratory symptoms, short-acting beta agonist medication usage after discharge, or time to a respiratory exacerbation among preschool children. We did not find any benefit among first-time wheezers or previous wheezers. Based on this analysis, we would not recommend azithromycin for the treatment of wheeze in preschool wheezing children presenting to the emergency department. Further studies to explore the benefit of azithromycin on severe recurrent wheeze are justified. Recollection of samples to evaluate antimicrobial resistance should be included as a secondary outcome in future studies.

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APPENDICES

Appendix 1: Ethics Approval

Health Research Ethics Board

308 Campus Tower
University of Alberta, Edmonton, AB T6G 1K8
p. 780.492.9724 (Biomedical Panel)
p. 780.492.0302 (Health Panel)
p. 780.492.0459
p. 780.492.0839
f. 780.492.9429

ETHICS APPROVAL FORM - FULL BOARD

Date:	July 6, 2010	
Principal Investigator:	Piushkumar Mandhane	
Study ID:	Pro00009987	
Study Title:	A double blind, randomized control trial of azithromycin for the acute management of wheezy pre-school children	
Approval Expiry Date:	July 5, 2011	
Date of Informed Consent:	Approval Date 06/07/2010	Approved Document Revised information sheet
Funding/Sponsor:	Alberta Lung Association	

Thank you for responding to all issues raised at your presentation of this study at the May 28th, 2010 HREB Biomedical Panel Committee meeting. The protocol involved in this project has been found to be acceptable within the limitations of human experimentation. There are no outstanding ethical issues and the study is approved. In addition to the protocol, the Information Sheet, dated May 31, 2010, along with the Skin Prick Testing Information Sheet, and all other documentation uploaded to Section 7.1 of the application form, have been approved for use. We acknowledge that you are in the process of finalizing your Health Canada submission and that ethical approval is required by Health Canada before they will release your No Objection Letter. As such, this approval is contingent upon the HREB receiving a copy of the Health Canada approval, and no patient recruitment and/or enrollment can be commenced until this has been submitted.

The Research Ethics Board assessed all matters required by section 50(1)(a) of the Health Information Act. Subject consent for access to identifiable health information is required for the research described in the ethics application, and appropriate procedures for such consent have been approved by the HREB Panel. In order to comply with the Health Information Act, a copy of the approval form is being sent to the Office of the Information and Privacy Commissioner.

The ethics approval is valid for one year. This ethics approval will expire on July 5, 2011 . A renewal report must be submitted next year prior to the expiry of this approval if your study still requires ethics approval. If you do not renew on or before the renewal expiry date, you will have to re-submit an ethics application.

Approval by the Health Research Ethics Board does not encompass authorization to access the patients, staff or resources of Alberta Health Services or other local health care institutions for the purposes of research. Enquiries regarding AHS administrative approval, and operational approval for areas impacted by research, should be directed to the AHS Research Administration office, #1800 College Plaza, phone 407-6041.

Sincerely,

S.K.M. Kimber, MD, FRCPC
Chair, Health Research Ethics Board - Biomedical Panel

Note: This correspondence includes an electronic signature (validation and approval via an online system).

UNIVERSITY OF ALBERTA Alberta Health Covenant

Appendix 2: Emergency Enrolment Questionnaire

Emergency Enrolment	
Symptoms	
With this current illness, has your child experienced symptoms of Cough?	
	No
	Yes
	Patient withdrew consent
Cough started	
	Greater than 2 weeks ago
	Less than 2 weeks ago
With this current illness, has your child experienced symptoms of Runny Nose?	
	No
	Yes
	Patient withdrew consent
Date runny nose started	
With this current illness, has your child experienced symptoms of Wheeze?	
	No
	Yes
	Patient withdrew consent
Wheeze Started	Greater than 2 weeks ago
	Less than 2 weeks ago
Date wheeze started	
With this current illness, has your child experienced symptoms of Difficulty Breathing?	
	No
	Yes
	Patient withdrew consent
Date difficulty breathing started	
Since your child became ill, prior to coming to the emergency department, has he/she received any medication through an inhaler, puffer, or nebulizer to help open up their lungs and help them breathe?	
	No
	Yes
	Patient withdrew consent
ED Vital Signs - Admission	
Was temperature taken on presentation?	
	No
	Yes
Temperature at the time of triage or if not available, at the time closest to the triage time.	

Able to obtain Blood Pressure?	
	No
	Yes
Respiratory Rate on presentation	
Heart Rate on presentation	
Oxygen saturations on room air on presentation	
Labored Breathing on presentation	
	No
	Yes
Accessory Muscle use on presentation	
	No
	Yes
Retractions	
	None
	Mild
	Moderate
	Severe
	Not Reported
Wheeze on presentation	
	No
	Yes
Wheezing Severity	
	None
	Mild
	Moderate
	Severe
	Not Reported
Air Entry	
	Normal
	Decreased to the bases
	Widespread decrease
	Absent Air entry
	Not Reported
PRAM score on presentation	
CTAS score on presentation	
Study Drug Administration	
Child Weight	
Initial Dose in mg	
Initial Dose weight(kg) X 10mg	
Initial Dose Calculation (16.7*10)	

Initial Dose in ml
[Initial Dose in mg]=
Initial Dose (ml) Calculated ([day1_dose_mg]/50
Study Drug Dose Day 2-5 in mg
Day 2-5 Dose weight(kg) X 5mg
Day 2-5 Dose Calculation (16.7*5)
Study Drug Dose Day 2-5 in ml (40mg/ml)
[Day 2-5 Dose in mg]=
Day 2-5 Dose (ml) Calculated ([day2_dose_mg]/50)
Drug Administration
Date first dose of Azithromycin was given
Time first dose of Azithromycin was given
Dose (mg) of Azithromycin given on day 1
Dose (ml) of Azithromycin given on day 1
The dose as marked on the syringe has been checked by or given by the RN looking after the patient. No Yes Patient withdrew consent
Dose check by whom? - First name
Dose check by whom? - Last name
Dose check by whom? Any notes
Dose administered by whom? - First name
Dose administered by whom? - Last name
Dose administered by whom? Any notes
Oral study drug vomited? No Yes

Appendix 3: Baseline Questionnaire

Baseline Questionnaire
History
Has your child ever been diagnosed with environmental allergies or eczema? No Yes
Has either parent or any siblings ever been diagnosed with environmental allergies, asthma or eczema? No Yes
Has your child had any other significant illness in the past? No Yes
Please specify
Has your child ever required a tube to be placed down his/her throat in order to help them breathe? No Yes
Please specify
Are there any smokers in the household? No Yes
Has your child been regularly exposed to second hand cigarette smoke outside the home? No Yes
Does the child attend daycare? No Yes
Wheeze and Chest Tightness
Has your child ever had wheezing noise coming from his/her chest? "Wheezing" means a whistling sound, however high or low pitched and however faint. No Yes Unknown
Has your child, ever had this wheezing noise when he/she did not have a cold? No Yes Unknown
Has your child ever been at all breathless when the wheezing noise was present?

No Yes Unknown
Has your child had wheezing or whistling in your chest at any time in the last 12 months? No Yes Unknown
Has your child woken up with a feeling of tightness in his/her chest at any time in the last 12 months? No Yes Unknown
Shortness of Breath
Has your child ever have trouble with your shortness of breath or breathing? No Yes Unknown
How often has he/she had this trouble? Rarely: less than once a month Repeatedly: but it always gets completely better between episodes Continuously: so that his/her breathing is never quite right
Has your child ever had an attack of shortness of breath that came on during the day when they were at rest? No Yes Unknown
Has your child had an attack of shortness of breath that came on during the day when they were at rest, at any time in the last 12 months? No Yes Unknown
Is your child troubled by shortness of breath when hurrying on the same level or walking up a slight hill in the last 12 months? No Yes Unknown
Has your child had an attack of shortness of breath that came on following strenuous activity at any time in the last 12 months? No

Yes Unknown
Has your child been woken at night by an attack of shortness of breath at any time in the last 12 months? No Yes Unknown
Cough and Phlegm from the chest
Has your child ever coughed in the bed at night when he/she does not have a cold? (More than the occasional night) No Yes Unknown
Has your child woken up coughing when he/she did not have a cold in the last 12 months? No Yes Unknown
Does your child usually cough on getting up or first thing in the morning in the last 12 months? No Yes Unknown
Does your child cough on getting up or first thing in the morning, on most mornings for at least 3 months in a row each year? No Yes Unknown
Does your child usually bring up phlegm from his/her chest on getting up or first thing in the morning in the last 12 months? No Yes Unknown
Does your child bring up phlegm from his/her chest on getting up or first thing in the morning, on most mornings for at least 3 months in a row each year? No Yes Unknown
Asthma
Has your child ever had asthma? No Yes Unknown

Has your child ever been diagnosed with asthma by a doctor? No Yes Unknown
Has your child ever had an asthma attack? No Yes Unknown
How old was your child when he/she had his/her first asthma attack?
How old was your child when he/she had his/her last attack
Which months of the year does your child usually have attacks of asthma (check all that applies)? January/February March/April May/June July/August September/October November/December
Has your child ever had any treatment for asthma or wheezing? No Yes Unknown
Has your child had any treatment for asthma or wheezing in the last 12 months? No Yes Unknown
Has your child ever needed treatment at an emergency department for asthma or wheezing? No Yes Unknown
How many times has your child ever needed treatment at an emergency department for asthma or wheezing in his/her lifetime?
How many times has your child ever needed treatment at an emergency department for asthma or wheezing in the last 12 months
Has your child ever needed to be admitted to hospital for treatment for asthma or wheezing? : i.e. hospital is not just an emergency room visit No Yes Unknown
How many times has your child ever need to be admitted to the hospital for treatment for asthma or wheezing in his/her lifetime?

How many days has your child ever need to be hospitalized for treatment for asthma or wheezing in his/her lifetime?
How many times has your child need to be admitted to the hospital for treatment for asthma or wheezing in the last 12 months?
How many days has your child need to be hospitalized for treatment for asthma or wheezing in the last 12 months?
In the last 12 months, how many days has your child's asthma been severe enough to prevent him/her from regular daily activities
Other Symptoms
Has your child ever had hay fever symptoms such as eye/nose rhinitis, rhinoconjunctivitis No Yes Unknown
Has your child had hay fever symptoms such as eye/nose rhinitis, rhinoconjunctivitis in the last 12 months No Yes Unknown
Has your child ever had eczema No Yes Unknown
Has your child had eczema in the last 12 months No Yes Unknown
Has your child ever had urticaria (hives) No Yes Unknown
Has your child had uriticaria (hives) in the last 12 months No Yes Unknown
Has your child ever had other allergies No Yes Unknown
Please specify:
Has your child had other allergies in the last 12 months

No Yes Unknown
Please specify:
Father's History (biological father)
Has your child's father ever had asthma No Yes Unknown
Has your child's father ever had hay fever symptoms such as eye/nose rhinitis, rhinoconjunctivitis No Yes Unknown
Has your child's father ever had eczema No Yes Unknown
Has your child's father ever had other allergies No Yes Unknown
Please specify:
Mother's History (biological mother)
Has your child's mother ever had asthma No Yes Unknown
Has your child's mother ever had hay fever symptoms such as eye/nose rhinitis, rhinoconjunctivitis No Yes Unknown
Has your child's mother ever had eczema No Yes Unknown
Has your child's mother ever had other allergies No Yes Unknown

Please specify:
Environment
Where do you live? Apartment building (any multiple family dwelling) House or Townhouse (single dwelling) Duplex (house) Farm with animals
Are there any pets in your home? No Yes Unknown
Dogs in the home No Yes
Number of dogs in the home
Cats in the home No Yes
Number of cats in the home
Other pets in the home No Yes
Number of other pets in the home
Type of other pets in the home
Medications
What medication is used as rescue medication? (i.e. to relieve symptoms when they occur) Ventolin Airomir Bricanyl Berotec Combivent Oxeze Salbutamol Nebule Symbicort
Do you give rescue medication before activity to prevent symptoms? No Yes
What medication is used as controller medication? (i.e. to help keep asthma under control or to prevent asthma) Flovent Pulmicort

	Becloforte
	Qvar
	Atrovent
	Combivent
	Spiriva
	Advair
	Symbicort
	Oxeze
	Serovent
	Intal
	Tilade
	Ketotifen
	Alvesco
Is your child taking Singulair?	
	No
	Yes

Appendix 4: Emergency Discharge Questionnaire

Emergency Discharge	
ED Vital Signs - Discharge	
Discharge date	
Discharge time	
Temperature on discharge	
Blood Pressure on discharge	
Respiratory Rate on discharge	
Heart Rate on discharge	
Oxygen saturations on room air on discharge	
Labored Breathing on discharge	
	No
	Yes
Accessory Muscle use on discharge	
	No
	Yes
Wheeze on discharge	
	No
	Yes
Air Entry	
	Normal
	Decreased to the bases
	Widespread decreased
	Absent air entry
	Not reported
PRAM score on discharge	
ED Co-Interventions	
Was epinephrine administered in the ED	
	No
	Yes
How many doses of epinephrine was given	
Was salbutamol (ventolin) administered in the ED	
	No
	Yes
How many doses of salbutamol (ventolin) was given	
Was Atrovent (ipatromium Bromide) administered in the ED	
	No
	Yes

How many doses of Atrovent (ipatromium Bromide) was given
Were any other bronchodilators administered in the ED No Yes
How many doses of the OTHER bronchodilator were given
Were antibiotics administered No Yes
Were steroids administered No Yes
Were any other medications (not previously listed) given No Yes
Please give details
Medications on Discharge from the Emergency Department
Other than the study medication, was the patient discharged home from the ED on any medications? No Yes
Oral Corticosteroids (prednisone, prednisolone, dexamethasone) No Yes
Oral Steroid Dose
Oral Steroid Units
Oral Steroid Frequency
Aerosolized beta-2 agonists (salbutamol/terbutaline) No Yes
Aerosolized beta-2 agonists Dose
Aerosolized beta-2 agonists Units
Aerosolized beta-2 agonists Frequency
Inhaled corticosteroids (qvar, pulmicort, flovent, alvesco) No Yes
Inhaled corticosteroids (qvar, pulmicort, flovent, alvesco) Dose
Inhaled corticosteroids (qvar, pulmicort, flovent, alvesco) Units
Inhaled corticosteroids (qvar, pulmicort, flovent, alvesco) Frequency
Other Medication No Yes

Name of the other medication
Other medication Dose
Other medication Units
Other medication Frequency
Discharge Diagnosis and Disposition
Discharge Diagnosis
Disposition

Appendix 5: Follow-up Questionnaire

Follow-up
Follow-up Questions
Did your child receive the study syrup today? No Yes
How would you describe your Child's breathing problem today compared to the day you were seen at the hospital? Worse Same Improved Resolved
Is your child's activity impaired because of this illness? No Yes
Is your child's sleep impaired because of this illness? No Yes
Does your child have noisy breathing? (wheezing or whistling sound) No Yes
Is your child experiencing breathlessness? (unable to talk in full sentences without taking a breath and or having difficulty drinking his/her bottle or eating) No Yes
Is your child experiencing shortness of breath? (with play or activity does your child seem to have increased difficulty breathing than normal) No Yes
Does your child have persistent troublesome coughing? No Yes
Since we last spoke have you given your child the Study inhaler (Ventolin/Salbutamol)? No Yes Not applicable
Number of different days that the study inhaler was needed?
Average number of puffs needed per day
Is your infant taking any other medications today?

None oral steroids inhaled steroids antibiotics other
Since the last time we spoke to you, have you taken your Child to see a doctor? No Yes
Since we last spoke, has your child missed day care or preschool? No Yes
Since we last spoke, have you or any other caregivers in the home missed work for wages? No Yes
Patient Symptoms
Since we last spoke, has your child been experiencing any abdominal pain or discomfort? No Yes
Since we last spoke, has your child complained of feeling nauseated and or vomited? No Yes
Since we last spoke, has your child complained of a headache? No Yes
Since we last spoke, has your child developed a rash? No Yes
Since we last spoke, have you noticed any blood in your child's bowel movements? No Yes
Since we last spoke has your child had diarrhea/loose or watery stools? No Yes
Since we last spoke have you noticed any yellowish color to your child's skin or the whites of his/her eyes? No Yes
Since we last spoke have you noticed any hives on your child? No Yes
Since we last spoke, has your child fainted

No Yes
Since we last spoke, has your child experienced an irregular heart rate? No Yes
Is there anything you have noticed about your child that you think might be related to taking the Study syrup? No Yes
Can you please describe what you have noticed?

Appendix 6: Clinical Assessment

Clinical Assessment	
Child's weight	
Child's length/height	
Chest	
Nasal flaring	No/Yes
Tracheal Tug	No/Yes
Intercostal indrawing	No/Yes
Stridor	No/Yes
Prolong expiration	No/Yes
Crackles	No/Yes
Wheeze	No/Yes
Skin: Inflammation	
Face	No/Yes
Earfold	No/Yes
Scalp	No/Yes
Arm	No/Yes
Wrist	No/Yes
Buttocks	No/Yes
Legs	No/Yes
Feet	No/Yes
Diagnosis of atopic dermatitis	
An itchy skin condition (or parental report of scratching or rubbing on a child)?	No Yes
History of involvement of the skin creases of elbows, behind knees, front of ankles or around neck?	No Yes
History of general dry skin in the last year?	No Yes
Visible flexural eczema or eczema involving the cheeks/forehead and outer limb	No Yes
Atopic Dermatitis is defined as the child having an itchy skin condition (or parental report of scratching or rubbing in a child) & 1 or more of the above (creases, skin, flexural surfaces). Does the child meet a criterion for diagnosis of atopic dermatitis?	No Yes

Degree of Severity	
	Mild - Single site or no more than 2 sites, minor symptoms (little itching/rubbing), minor crusting and papules, not excoriated or oozing, not needing frequent medical attention **Moderate** - Neither mild nor severe **Severe** - Multiple sites, with extensive crusting or papules or excoriations or oozing or lichenification, sleep loss, needing frequent medical attention

Appendix 7: Adverse Events

Adverse Events
If any of the "patient symptoms" questions are positive (i.e. yes), please complete an adverse event form.
Where is the case from? Calgary Edmonton
Description of the adverse event:
Have you called the study co-ordinator or site principal investigator? No Yes
What date did the adverse event start?
Is it during immediate post-administration period (between 30 and 45 min) No Yes
Is there a reasonable possibility that the AE may have been caused by the investigational product? No Yes
When did adverse event stop?
Is it a medically attended visit? No Yes
What is the severity of AE? Mild Moderate Severe
What is the outcome? Recovered/resolved Recovering/resolving Not recovered/not resolved Recovered with sequelae/resolved with sequelae
Advice Given

Appendix 8: First-Time Wheezers vs. Previous Wheezers Tables

Appendix Table 8a: Demographic Characteristics

Participants/Enrolment Characteristics	First Time Wheezers		Previous Wheezers	
	Azithromycin (N=31)	Placebo (N=33)	Azithromycin (N=79)	Placebo (N=79)
Male (n (%))	23(74.2)	23(69.7)	54(68.4)	60(76.0)
Age in months – mean (SD)	31.0(12.7)	26.5(13.1)	36.2(13.8)	32.2(13.9)
Inhalers used prior ED (n (%))	7(22.6)	6(18.2)	66(83.5)	62(78.5)
one inhaler	3(9.7)	4(12.1)	29(36.7)	24(30.4)
two inhalers	3(9.7)	2(6.1)	34(43.0)	34(43.0)
three inhalers	1(3.2)	0(0.0)	3(3.8)	4(5.1)
Symptoms at triage				
Cough (n (%))	31(100.0)	32(97.0)	78(98.7)	76(96.2)
Runny nose (n (%))	25(80.7)	28(84.9)	65(82.3)	69(87.3)
Wheeze (n (%))	31(100.0)	32(97.0)	79(100.0)	79(100.0)
Difficulty breathing (n (%))	30(96.8)	32(97.0)	76(96.2)	72(91.1)
Labored breathing (n (%))	29(93.6)	31(93.9)	71(89.9)	67(84.8)
Accessory muscle use (n (%))	25(80.7)	28(84.9)	67(84.8)	63(79.8)
Moderate retractions (n (%))	6(19.4)	10(30.3)	21(26.6)	17(21.5)
Moderate severity of wheezing (n (%))	9(29.0)	11(33.3)	42(53.2)	28(35.4)
Type of inhaler				
Short-acting beta agonist	7(22.6)	6(18.2)	62(78.5)	60(76.0)
Inhaled corticosteroid	3(9.7)	2(6.1)	36(45.6)	39(49.4)

Data given as number and percentage (%).

Appendix Table 8b: Previous Medical History

Participants/Previous Medical History	First Time Wheezers		Previous Wheezers	
	Azithromycin (N=31)	Placebo (N=33)	Azithromycin (N=79)	Placebo (N=79)
Allergies/eczema	11(35.5)	15(45.5)	36(45.6)	31(39.2)
Family history of atopy	18(58.1)	19(57.6)	51(64.6)	47(59.5)
Previous significant illness	9(29.0)	2(6.1)	24(30.4)	18(22.8)
Required tube placement	0(0.0)	0(0.0)	6(7.6)	5(6.3)
Smokers in household	7(22.6)	4(12.1)	19(24.1)	16(20.3)
Regular exposure to smoke outside household	2(6.5)	1(3.0)	6(7.6)	7(8.9)
Attend day care	12(38.7)	15(45.5)	37(46.8)	38(48.1)

Data given as number and percentage (%).

Appendix Table 8c: Wheeze and Shortness of Breath History

	First Time Wheezers		Previous Wheezers	
	Azithromycin (N=31)	Placebo (N=33)	Azithromycin (N=79)	Placebo (N=79)
Wheeze before ever*	25(80.7)	25(75.8)	72(91.1)	73(92.4)
Wheeze without a cold	11(35.5)	8(24.2)	41(51.9)	35(44.3)
Breathless when wheezing ever	17(54.8)	19(57.6)	49(62.0)	50(63.3)
Wheeze during the last year	20(64.5)	22(66.7)	69(87.3)	67(84.8)
Woken up w/tightness of chest during last 12 months	5(16.1)	17(51.5)	38(48.1)	38(48.1)
Shortness of breath ever	15(48.4)	22(66.7)	62(78.5)	61(77.2)
SOB during the day at rest	8(25.8)	6(18.2)	26(32.9)	25(31.7)
SOB during the day at rest during the last 12 months	8(25.8)	5(15.2)	25(31.7)	24(30.4)
SOB during moderate physical activity during the last 12 months	6(19.4)	3(9.1)	21(26.6)	18(22.8)
SOB following strenuous activity during the last 12 months	10(32.3)	6(18.2)	31(39.2)	30(38.0)
Woken up at night by an attack of SOB during the last 12 months	8(25.8)	10(30.3)	31(39.2)	35(44.3)
Participants with SOB/Frequency of SOB				
Rarely	12(38.7)	19(57.6)	28(35.4)	31(39.2)
Repeatedly	2(6.5)	2(6.1)	32(40.5)	27(34.2)
Continuously	1(3.2)	1(3.0)	2(2.5)	3(3.8)

Data given as number and percentage (%).* Has your child ever had wheezing noise coming from his/her chest? “Wheezing” means a whistling sound, however high or low pitched and however faint. (Baseline Questionnaire provided during enrolment and handed over during day 21 visit).

Appendix Table 8d: Cough and Phlegm History

Participants/Cough and phlegm history	First Time Wheezers		Previous Wheezers	
	Azithromycin (N=31)	Placebo (N=33)	Azithromycin (N=79)	Placebo (N=79)
Ever coughed at night without a cold	10(32.3)	17(51.5)	43(54.4)	42(53.2)
Woken up coughing without a cold (last 12 months)	6(19.4)	13(39.4)	37(46.8)	34(43.0)
Usually cough on getting up (last 12 months)	3(9.7)	8(24.2)	26(32.9)	20(25.3)
Coughing on getting up most mornings for at least 3 months in a row each year	1(3.2)	1(3.0)	10(12.7)	6(7.6)
Usually phlegm on getting up (last 12 months)	2(6.5)	2(6.1)	7(8.9)	6(7.6)
Phlegm on getting up most mornings for at least 3 months in a row each year	0(0.0)	1(3.0)	6(7.6)	1(1.3)
Hay fever symptoms ever	10(32.3)	13(39.4)	20(25.3)	26(32.9)
Hay fever symptoms during the last 12 months	9(29.0)	12(36.4)	14(17.7)	25(31.7)
Eczema ever	12(38.7)	18(54.6)	41(51.9)	31(39.2)
Eczema during the last 12 months	10(32.3)	16(48.5)	32(40.5)	27(34.2)
Urticaria ever	12(38.7)	16(48.5)	26(32.9)	27(34.2)
Urticaria during the last 12 months	9(29.0)	16(48.5)	18(22.8)	22(27.9)
Other allergies ever	6(19.4)	7(21.2)	22(27.9)	25(31.7)
Other allergies during the last 12 months	2(6.5)	1(3.0)	17(21.5)	16(20.3)

Data given as number and percentage (%).

Appendix Table 8e: Biological Parents' History

	First Time Wheezers		Previous Wheezers	
	Azithromycin (N=31)	Placebo (N=33)	Azithromycin (N=79)	Placebo (N=79)
Father's history				
Previous history of asthma	3(9.7)	11(33.3)	26(32.9)	17(21.5)
Ever had hay fever symptoms	12(38.7)	14(42.4)	23(29.1)	25(31.7)
Ever had eczema	6(19.4)	9(27.3)	16(20.3)	15(19.0)
Ever had other allergies	8(25.8)	10(30.3)	29(36.7)	26(32.9)
Mother's history				
Previous history of asthma	7(22.6)	5(15.2)	23(29.1)	28(35.4)
Ever had hay fever symptoms	6(19.4)	14(42.4)	23(29.1)	34(43.0)
Ever had eczema	7(22.6)	13(39.4)	23(29.1)	24(30.4)
Ever had other allergies	15(48.4)	14(42.4)	36(45.6)	37(46.8)

Data given as number and percentage (%).

Appendix Table 8f: Environmental Factors

	First Time Wheezers		Previous Wheezers	
	Azithromycin (N=31)	Placebo (N=33)	Azithromycin (N=79)	Placebo (N=79)
House type				
Apartment building	3(9.7)	3(9.1)	10(12.7)	6(7.6)
House or townhouse	24(77.4)	27(81.8)	61(77.2)	63(79.8)
Duplex	2(6.5)	3(9.1)	3(3.8)	5(6.3)
Farm with animals	0(0.0)	0(0.0)	1(1.3)	2(2.5)
Pets at home	17(54.8)	19(57.6)	41(51.9)	34(43.0)
Dogs	13(41.9)	12(36.4)	25(31.7)	23(29.1)
Cats	6(19.4)	7(21.2)	17(21.5)	12(15.2)
Others	4(12.9)	5(15.2)	8(10.1)	12(15.2)

Data given as number and percentage (%).

Appendix Table 8g: Medication

	First Time Wheezers		Previous Wheezers	
	Azithromycin (N=31)	Placebo (N=33)	Azithromycin (N=79)	Placebo (N=79)
Rescue medication				
Short-acting beta2-agonist (SABA)				
Ventolin	21(67.7)	27(81.8)	74(93.7)	71(89.9)
Airomir	1(3.2)	0(0.0)	0(0.0)	0(0.0)
Salbutamol Nebule	0(0.0)	0(0.0)	0(0.0)	3(3.8)
Use of rescue medication before activity	0(0.0)	2(6.1)	11(13.9)	13(16.5)
Controller medication				
Inhaled Corticosteroids				
Flovent	3(9.7)	8(24.2)	25(31.7)	20(25.3)
Pulmicort	0(0.0)	0(0.0)	1(1.3)	1(1.3)
Becloforte	0(0.0)	0(0.0)	1(1.3)	0(0.0)
Qvar	10(32.3)	4(12.1)	20(25.3)	28(35.4)
Advair	0(0.0)	0(0.0)	2(2.5)	0(0.0)
Alvesco	3(9.7)	1(3.0)	6(7.6)	9(11.4)
Anticholinergic				
Atrovent	0(0.0)	3(9.1)	2(2.5)	0(0.0)
Leukotriene receptor antagonist (LTRA)				
Singulair	1(3.2)	1(3.0)	5(6.3)	4(5.1)

Data given as number and percentage (%).

Appendix Table 8h: Emergency Department and Discharge Medication

	First Time Wheezers		Previous Wheezers	
	Azithromycin (N=31)	Placebo (N=33)	Azithromycin (N=79)	Placebo (N=79)
Emergency department medication				
Salbutamol	30(96.8)	31(93.9)	76(96.2)	76(96.2)
Atrovent	28(90.3)	22(66.7)	64(81.0)	60(76.0)
Other bronchodilators	2(6.5)	3(9.1)	6(7.6)	5(6.3)
Steroids	26(83.9)	23(69.7)	68(86.1)	67(84.8)
Epinephrine	0(0.0)	2(6.1)	0(0.0)	1(1.3)
Discharge medication				
Short-acting beta agonists	26(83.9)	21(63.6)	61(77.2)	61(77.2)
Oral corticosteroids	20(64.5)	17(51.5)	45(57.0)	53(67.1)
Inhaled corticosteroids	16(51.6)	11(33.3)	47(59.5)	46(58.2)

Data given as number and percentage (%).

Appendix Figure 1: Distribution of Time to Resolution of Symptoms Among Treatment Groups