# The Azithromycin for Acute Exacerbations of Asthma (AZALEA) Randomized Clinical Trial

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**Conflict of interest disclosures**:
Sebastian Johnston: Institutional funding for a clinical trial, research grant and/or Consultant compensation: AstraZeneca, Boehringer Ingelheim, Centocor, Chiesi, GlaxoSmithKline, Merck, Novartis, Roche/Genentech, Sanofi Pasteur & Synairgen; Shareholding: Synairgen; Licensed Patents: 9; Patents Pending: 1
Christopher Brightling: Grants and consultancy paid to Institution from: GSK, AstraZeneca, Boehringer Ingelheim, Novartis, Chiesi and Roche/Genentech
Rekha Chaudhuri: Grant and Personal Fees for attendance at scientific conferences and advisory board meetings: Novartis Pharmaceuticals, Astra-Zeneca, Teva, GlaxoSmithkline

Dave Singh: Grants and personal fees from: Almirall, AstraZeneca, Boehringher Ingleheim, Chiesi, GlaxoSmithKline, Glenmark, Johnson and Johnson, Merck, NAPP, Novartis, Pfizer, Takeda, Teva, Therevance & Verona. Personal fees from: Genentech & Skyepharma.

Bernard Higgins: Multicentre Study, local PI for studies funded by: Novartis & Roche

Christopher Corrigan: Grant and Personal Fees for attendance at scientific conferences and payments for lectures: Allergy Therapeutics; Grant and Personal Fees for research collaborations and consultancy not connected with the current research: Novartis; Grant for attendance at scientific conferences: Stallergenes, Boehringer Ingelheim & Diagenics; Personal fees for speaking at conferences: Astra Zeneca

All other authors: None

**Main text word count** 3000.

**Key words:** Asthma, Asthma Exacerbation, Antibiotic, Randomized, Controlled Trial

**Trial registration:** ClinicalTrials.gov Identifier: NCT01444469

**Funding:** This project was funded by the Efficacy and Mechanism Evaluation programme of the Medical Research Council and the National Institute of Health Research, UK. **Funders Reference number: 10/60/27**

**The AZALEA Trial team** membership and their affiliations are listed in the **Online Supplement**.

**Disclaimer**: The views expressed in this article are those of the authors and not necessarily those of the National Health Service, the National Institute for Health Research (NIHR), or the Department of Health.

**Abstract**

**Importance:** Guidelines recommend against antibiotic use to treat asthma attacks. A study with telithromycin reported benefit, but adverse reactions limit its use.

**Objective:** To determine whether azithromycin added to standard care of asthma attacks in adults resulted in clinical benefit.

**Design**: The AZithromycin Against pLacebo in Exacerbations of Asthma (AZALEA) randomized, double-blind, placebo-controlled clinical trial ran from September 2011 to April 2014.

**Setting**: UK-based multi-center study in adults requesting emergency care for acute asthma exacerbations.

**Participants**: Adults with a history of asthma for >6 months, recruited within 48 hours of presentation to medical care with an acute deterioration in asthma control requiring a course of oral/systemic corticosteroids.

**Intervention**: Azithromycin 500mg daily or matched placebo for 3 days.

**Main Outcomes**: The primary outcome was diary card symptom score 10 days after randomization, with an hypothesized treatment effect size of -0.3. Secondary outcomes were diary card symptom score, quality of life questionnaires and lung function changes between exacerbation and day 10, and time to 50% reduction in symptom score.

**Results:** Of 4582 patients screened at 31 centers, 199 of a planned 380 were randomized within 48 hours of presentation. The major reason for non-recruitment was receiving antibiotics (2044, 44.6% of screened subjects). Median time from presentation to drug administration was 22 hours. Exacerbation characteristics were well balanced across treatment arms and centers. The primary outcome asthma symptom scores in this likely underpowered study were: mean (SD) 4.14 (1.38) at exacerbation and 2.09 (1.71) at 10 days for azithromycin; 4.18 (1.48) and 2.20 (1.51) for placebo. Using multilevel modeling, there was no significant difference in symptom scores between azithromycin and placebo at day 10 (difference -0.166 [95% confidence interval -0.670 to 0.337]), nor on any day between exacerbation and day 10. No significant between group differences were

observed in quality of life questionnaires or lung function between exacerbation and day 10, or in time to 50% reduction in symptom score.

**Conclusions:** In this randomized population, azithromycin resulted in no statistically or clinically significant benefit. For each patient randomized, >10 failed screening because they had already received antibiotics.

**Trial Registration**: ClinicalTrials.gov Identifier: NCT01444469, https://clinicaltrials.gov/ct2/show/NCT01444469?term=AZALEA&rank=1

Word count: 347 words (including headings)

**List of abbreviations**

| | |
|---|---|
| AZALEA | AZithromycin Against pLacebo for acute Exacerbations of Asthma |
| $FEF_{25-75\%}$ | Forced mid-expiratory flow |
| $FEF_{50\%}$ | Forced expiratory flow at 50% expiration |
| $FEV_1$ | Forced expiratory volume in one second |
| $FEV_1$/FVC | Ratio of forced expiratory volume in one second to forced vital capacity |
| FVC | Forced vital capacity |
| LRTI | Lower respiratory tract infection |
| PCR | Polymerase chain reaction |
| PEF | Peak expiratory flow |
| AQLQ | Asthma quality of life questionnaire |

## Background

Asthma morbidity, mortality and major health care costs result from acute attacks (exacerbations)[1]. The majority of asthma patients report an exacerbation in the last year, with >1/3 children and >1/4 adults requiring consequent urgent medical care[2].

Respiratory viral infections are a frequent cause of asthma exacerbations in children[3,4] and adults[5-7]. Atypical bacterial (*Mycoplasma* and *Chlamydophila (C.) pneumoniae*) infection/reactivation is also associated, with serologic positivity rates of 40-60% in some studies[8-12], indicating viral and atypical bacterial infections may interact in increasing asthma exacerbation risk.

People with asthma have increased susceptibility to streptococcal infections[13-15], increased carriage of bacterial pathogens identified by culture[16] and molecular techniques[17] and impaired interferon/$Th_1$ responses to bacterial polysaccharides[18,19]. Viral infection impairs antibacterial innate immune responses[20] and increases bacterial adherence to bronchial epithelium[21]. Thus, bacterial infections are more common and more severe in asthma, viruses increase susceptibility to bacterial infection and acute wheezing episodes in children aged <3 years were associated with both bacterial and virus infection[22].

Asthma exacerbations treated with telithromycin had greater reductions in asthma symptoms, improvement in lung function and faster recovery compared to placebo[12]. However, liver toxicity limits telithromycin to life threatening infections and guidelines recommend antibiotics should NOT be administered routinely in asthma exacerbations[23,24].

The AZALEA study investigated the effectiveness of azithromycin when added to standard care for adult patients with asthma exacerbations, closely following the telithromycin study design, with the aim of providing confirmation or otherwise of those results.

Macrolide antibiotics might benefit asthma exacerbations through antimicrobial activity, anti-inflammatory properties[25] and azithromycin, but not telithromycin, was anti-viral[26] augmenting production of interferons that are deficient in asthma[19,27]. A mechanistic/exploratory aim of AZALEA was to determine whether treatment benefitted patients with these infections.

## Methods

*Study design*

This United Kingdom-based multi-center, double-blind, placebo-controlled study randomized eligible patients to azithromycin 500mg daily or placebo for 3 days on day 1 (Visit 1), with post-therapy assessments/visits on days 5 (Visit 2) and 10 (Visit 3) and for serum sampling at six weeks (Visit 4).

The main inclusion criteria were adults aged 18-55 years with any smoking history, aged 56-65 with <20 pack year smoking history or >65 years with <5 pack year smoking history with a documented history of asthma for >6 months, and recruitment within 48 hours of presentation to medical care with an acute deterioration in asthma control (increased wheeze, dyspnea and/or cough) requiring a course of oral/systemic corticosteroids (based on clinical judgement by attending physicians) and a peak expiratory flow (PEF) or forced expiratory volume in one second ($FEV_1$) less than 80% predicted or patient's best at presentation, at recruitment or in the time elapsed between presentation and recruitment.

The main exclusion criteria were use of oral/systemic antibiotics within 28 days of enrolment, need for intensive care, significant lung disease other than asthma, chronic use of >20mg oral corticosteroid daily, known QT-interval prolongation, history of brady/tachy arrhythmias or uncompensated heart failure and patients on drugs known to prolong the QT interval.

The primary outcome was diary card summary symptom score, with symptoms including wheezing, breathlessness and coughing assessed at 10 days after randomization (as in the telithromycin study)[12]. Secondary outcomes included the acute Asthma Quality of Life Questionnaire (AQLQ), the mini AQLQ, $FEV_1$, forced vital capacity (FVC), $FEV_1$/FVC, forced mid-expiratory flow ($FEF_{25-75\%}$), forced expiratory flow at 50% expiration ($FEF_{50\%}$), PEF and time to 50% reduction in symptom score. Primary and secondary outcomes were assessed over the time course of the exacerbation to 10 days and sub-group analyses were planned in relation to initial standard/atypical bacteriologic and virologic status.

Spontaneous or induced sputum was taken where possible at exacerbation and sent for quantitative bacteria culture. A nasal mucus sample, nasal and throat swabs were taken where possible at exacerbation and these and spontaneous/induced sputum were analyzed by viral and atypical bacterial PCRs and acute and convalescent sera for atypical bacterial serology.

The trial received Research Ethics Committee approval and all patients gave written informed consent. Additional methods are available in the **Online Supplement**.

*Statistical analyses*

The sample size calculations hypothesized a treatment effect size of -0.3 (SD 0.783) based on the primary outcome of the telithromycin study[12] and used a significance level of 1% with 80% power, assuming a drop-out rate of 15%[12]. We proposed to recruit 190 patients to each arm. To run the trial within the project funding one-year timeline, we planned 10 centers, each recruiting ~38 patients. All patients who returned at least one diary card and received study drug were included in the intention-to-treat analyses. As the timing of greatest magnitude of any treatment effect was not known, multilevel modelling was used to calculate the estimated differences in primary and secondary outcomes between treatment arms for each day from randomization to day 10. A Cox model was used to calculate the hazard ratio for time to 50% reduction in symptom score. Details of the statistical model, model selection process and treatment of missing data are in the **Online Supplement**. All analyses were performed using Stata 13. A Statistical Analysis Plan was prepared by the trial statistician prior to unblinding.

## Results

*Recruitment details and clinical characteristics*

Recruitment from 31 sites (30 secondary care hospitals, 1 primary care center) lasted 2.5 years, from September 2011 to April 2014. The recruitment period was longer than planned because of recruitment difficulties arising from the large numbers of patients excluded. A total of 4582 patients were screened of whom 390 patients met eligibility criteria, 199 were randomized, 97 to active treatment, 102 to placebo (**Figure 1**). The major reason for non-recruitment was already receiving antibiotics (2044, 44.6% of screened patients).

Clinical characteristics of randomized patients are summarized in **Table 1.** Study participants' mean age was 39.9 years, gender 69.8% female, 30.2% male. Underlying asthma severity, smoking status, exacerbation severity and median time from presentation to trial drug administration are in **Table 1**. Pulmonary function at baseline (exacerbation, Visit 1) are in **Table 2** and include PEF 74.8% predicted, $FEV_1$ 64.8% predicted, and $FEV_1$/FVC 69.2% (all means). Baseline characteristics were well balanced across treatment arms and centers.

Of the 199 patients randomized, all attended visit 1 (randomization), 21 (11%) missed Visit 2, 28 (14%) missed Visit 3 and 39 (20%) missed Visit 4, 80% of patients attended all follow-up visits. Missing visits/data were balanced between the treatment arms. Day 1 was defined as the day of administration of study drug.

*Primary outcome analysis*

Mean (SD) asthma symptom scores (from 0=no symptoms to 6=severe symptoms) were 4.14 (1.38) at baseline (exacerbation) and 2.09 (1.71) at day 10 for azithromycin and 4.18 (1.48) and 2.20 (1.51) respectively for placebo. Using multilevel modeling, there was no statistically significant difference in symptom scores between groups at day 10 (difference -0.166 [95% CI: -0.670; 0.337], **Figure 2** and **Online Supplement eTable 3**).

*Secondary outcome analyses*

Multilevel modeling revealed no significant between group differences in symptom scores on any day between baseline and day 10 .(**Figure 2** and **Online Supplement eTable 3**).

No significant between group differences were seen in acute AQLQ, mini AQLQ (**Figure 3a** and **3b** and **Online Supplement eTables 7-10**) nor in any measure of lung function (**Online Supplement eTables 11 and 12**) on any day from baseline to day 10 and there was no difference in time to 50% reduction in symptom score (Hazard Ratio 1.03 [95% CI: 0.71; 1.49]) (**Figure 3c**).

*Pathogen detection results*

105 (52.7%) patients provided sputum for bacterial culture, 191 (96.0%) nasal/throat mucus/swabs for virus/atypical bacterial PCR and 158 (79.4%) acute (IgM) and acute and convalescent (IgG, IgA) sera for atypical bacterial serology.

A bacterial/atypical bacterial test positive occurred in 10.6% of patients (9.3% active, 11.8% placebo). Nasal/throat swab/mucus and/or sputum virus PCRs were positive in 18.1% of patients (16.5% active, 19.6% placebo).

*Subgroup analyses*

There were no differences in the primary outcome asthma symptom score between treatment groups in patients with positive sputum bacterial culture, atypical bacterial PCR/serology or virus PCR tests (including any bacteria/virus positive test) (**Online Supplement eTables 13-15** and **Online Supplement eFigures 6-8**), though patient numbers for these analyses were low.

*Safety*

Adverse events were infrequent (**Online Supplement eTables 16-22**), with more gastrointestinal adverse events in the azithromycin group compared to placebo (35 vs 24 events respectively **Online Supplement eTable 16**). There was an increased frequency of cardiac adverse events (4 vs 2 respectively) in the azithromycin group compared to placebo and a reduced frequency of respiratory, thoracic and mediastinal (63/64 respiratory) adverse events (27 vs 37 respectively) **Online Supplement eTables 16 and 20**), suggesting antibiotic therapy possibly reduced respiratory adverse events in this population.

## Discussion

In the patients with asthma exacerbations randomized to treatment/placebo in this study, addition of azithromycin to standard medical care resulted in no statistically or clinically significant therapeutic benefit. The findings were consistently negative across three different symptom and quality of life scores, including one previously reporting statistically and clinically significant benefit with telithromycin[12]. The findings were also negative for all measures of lung function, including $FEV_1$ which was significantly improved in the previous study[12] and for time to a 50% reduction in asthma symptoms, which was significantly improved in the previous study[12].

Recruitment proved extremely challenging; initially there were 10 centers each aiming to recruit 38 subjects over one winter season, to recruit the planned 380 patients. Our power calculation deliberately mandated large patient numbers to provide statistically robust data to settle the important clinical question regarding antibiotic efficacy in this setting (for comparison the telithromycin study randomized 270 patients)[12]. We also desired larger patient numbers to enhance subgroup analyses aimed at potentially important mechanistic questions. Once recruitment obstacles became clear with such widespread antibiotic usage, a total of 31centers were enrolled, inclusion criteria were relaxed to change eligibility criteria from <24 to <48 hours from time of presentation, to include older subjects with low smoking histories and recruitment was extended to 2 years and 7 months. However, despite all these efforts only 199 subjects were recruited by medication-expiry and funding-end dates and the study was terminated despite not reaching its recruitment target. The study was therefore underpowered and a difference of 0.3 in mean symptom score between treatment arms at 10 days cannot be excluded.

The different outcomes of the present and previous study[12], which employed closely related therapies in very similar study designs, requires interpretation/explanation. The antibiotics studied are different, albeit related. Both drugs were used at their standard recommended doses and durations of therapy. The shorter duration of treatment with azithromycin (3 days vs 10 days with telithromycin) is unlikely to explain the difference in outcome, as azithromycin has a very long tissue half-life and

is likely to have remained at therapeutic doses in the lung for around 10 days[28]. Azithromycin, but not telithromycin has anti-viral activity[26], so this is an unlikely explanation. In terms of anti-bacterial activity against relevant respiratory bacteria, telithromycin is reportedly more active than azithromycin against *S. pneumoniae*, but has similar activity against both *M. catarrhalis* and *H. influenzae*[29-31]. Since the present study only detected 3 *S. pneumoniae,* 1 *M. catarrhalis* and no *H. influenzae* infections in the active treatment arm, differences in activity against these organisms seem unlikely to explain the differing outcomes. In terms of anti-inflammatory activities, both drugs reportedly have similar activities when compared[25].

A remarkable finding of this study was the number of patients (2044) excluded as they were already receiving antibiotic therapy for their asthma exacerbation, despite treatment guidelines recommending such therapy should not be routinely given[23,24]. For each patient randomized, more than 10 were excluded for this reason. This important finding has obvious and worrying implications regarding antibiotic stewardship[32], in addition, such high antibiotic usage may also have directly influenced study outcome as it is possible that patients who might potentially have benefitted from antibiotic therapy for their asthma exacerbation (through having sputum production, sputum purulence, fever), were excluded from the study through already having received them. The population remaining to be randomized could theoretically have been selected against for antibiotic responsiveness, through having no clinical indication that antibiotic therapy might be of benefit. This is possible as patients being screened had often been seen by their primary care practitioner, by emergency room medical staff and by a member of the on call respiratory/medical team, so in many instances three independent doctors/teams had assessed them, including their suitability for antibiotics. It is likely therefore that those not prescribed antibiotics were negatively selected against, for suitability for antibiotics. This interpretation is supported by the very low bacterial/atypical bacterial positivity rate found in this study: only 9.3% of azithromycin treated subjects.

It is also possible that the population randomized were in other ways not representative of the larger population screened, as over 2000 other patients were excluded from the study for other reasons

297 (**Figure 1**). The telithromycin study did not report numbers of patients screened[12], so it is not
298 possible to determine to what extent these caveats may also have applied to that study.
299 A further difference is that all patients randomized to this study were required to be prescribed
300 oral/systemic corticosteroid treatment, while in the telithromycin study only 34.1% of patients
301 randomized to active treatment required corticosteroid therapy[12]. Requirement for corticosteroid
302 treatment in this study was designed to reduce the number of milder exacerbations studied. However,
303 if our study included largely non-bacterially infected subjects, this could have resulted in us studying
304 possible anti-inflammatory effects of azithromycin, in the face of the powerful anti-inflammatory
305 effects of corticosteroids, with predictably negative results.
306 The clinical characteristics of the patients in our study compared to those in the telithromycin study
307 were similar in terms of mean age (39.9 years in our study, vs 39.5 in the telithromycin study),
308 gender (30.2% male vs 32%), smoking status (mean of 3.44 pack years vs 2.15), exacerbation
309 symptom score severity (4.16 vs 2.9) and lung function at exacerbation (PEF 74.8% predicted vs
310 55.2%, $FEV_1$ 64.8% predicted vs 67.2%, $FEV_1$/FVC 69.2% vs 72%)[12]. Differences in clinical
311 characteristics do not seem a likely explanation for the difference in outcome of the two studies.
312 The studies differed strikingly in one regard: 61% of telithromycin-treated but only 5.2% of
313 azithromycin-treated patients had a positive test for current atypical bacterial infection[12]. Both
314 studies used similar sampling and detection methods, though the laboratories performing the analyses
315 differed (GR Micro, London UK for telithromycin, Prof Johnston's laboratory for this study). PCR
316 detection rates were very low in both studies (3 positive in the telithromycin study and 0 positive in
317 this study). In contrast, serological positives differed markedly: the telithromycin study positives
318 were almost all *C. pneumoniae* IgM positives, while in our study only one sample was IgM positive
319 for this organism. Both studies used the same assay (Medac *C. pneumoniae* IgM sandwich ELISA,
320 Medac, Hamburg, Germany) so the discrepancy between the results of this assay is difficult to
321 explain. This major difference in frequency of *C. pneumoniae* IgM positivity may have contributed
322 to the difference in clinical outcomes between the two studies.

Sputum culture for standard bacteria was not performed in the telithromycin study[12]. In the present study 105 (52.8%) subjects provided sputum for bacterial culture and positivity was observed in 6.0% (4.1% active, 7.8% placebo), These results, together with the negative outcomes in relation to therapy, suggest that the role of standard bacterial infection in the population studied was unlikely to be important.

Interpretation of the outcome of this study must be considered in the light of prior knowledge that non-infectious agents can also trigger exacerbations, and of other randomized placebo controlled studies investigating the effects of similar therapies in acute wheezing episodes. In addition to the telithromycin study reporting positive outcomes in asthma exacerbations in adults[12], azithromycin treatment during bronchiolitis in infancy was reported to reduce nasal lavage IL-8, the occurrence of post-bronchiolitic wheezing[33] and the duration of acute episodes of asthma-like symptoms in 1-3 year old children[34]. Furthermore, in 1-6 year old children with histories of recurrent severe lower respiratory tract infections (LRTIs), azithromycin early during an apparent RTI reduced the likelihood of severe LRTI[35]. Finally low-dose azithromycin prophylaxis for 6 months in subjects with exacerbation-prone severe asthma did not reduce the primary outcome (rate of severe exacerbations and LRTIs requiring treatment with antibiotics) however in a predefined subgroup analysis according to inflammatory phenotype, azithromycin benefitted subjects with non-eosinophilic severe asthma[36]. We therefore carried out a similar post hoc analysis, but found no evidence of benefit in this subgroup (**Online Supplement**). Thus further study of azithromycin in acute exacerbations of asthma in adults and children in settings of low antibiotic usage and stratifying on blood/sputum cell counts seems justified.

In conclusion, in the patients randomized to treatment/placebo in this study, addition of azithromycin to standard medical care resulted in no statistically significant, or clinically important benefit. For each patient randomized, more than 10 were excluded because they had already received antibiotics.

**Acknowledgement**

We would like to thank the patients who took part in the trial, Josephine Marange, Research Nurse, Birmingham Heartlands Hospital, UK for assistance with patient recruitment, Elena Kulinskaya, Senior Statistician, Imperial College, London, for help with the power calculations and the independent members of the Trial Steering Committee and Data Monitoring and Ethics Committee (membership listed in the **Online Supplement**). This study was funded by the Efficacy and Mechanisms Evaluation programme of the Medical Research Council (MRC), in partnership with the National Institute for Health Research (NIHR) (Funders Reference number: 10/60/27). The trial was supported by the NIHR Comprehensive Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London. SLJ is an NIHR Senior Investigator and was supported by European Research Council FP7 Advanced Grant 233015, a Chair from Asthma UK (CH11SJ) and MRC Centre grant G1000758.

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all raw data and the corresponding author had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

## References


1. Weiss KB, Sullivan SD. The health economics of asthma and rhinitis. I. Assessing the economic impact. *J Allergy Clin Immunol.* Jan 2001;107(1):3-8.
2. Rabe KF, Vermeire PA, Soriano JB, Maier WC. Clinical management of asthma in 1999: the Asthma Insights and Reality in Europe (AIRE) study. *Eur Respir J.* Nov 2000;16(5):802-807.
3. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *Bmj.* May 13 1995;310(6989):1225-1229.
4. Chauhan AJ, Inskip HM, Linaker CH, et al. Personal exposure to nitrogen dioxide (NO2) and the severity of virus-induced asthma in children. *Lancet.* Jun 7 2003;361(9373):1939-1944.
5. Johnston SL, Pattemore PK, Sanderson G, et al. The relationship between upper respiratory infections and hospital admissions for asthma: a time-trend analysis. *Am J Respir Crit Care Med.* Sep 1996;154(3 Pt 1):654-660.
6. Wark PA, Johnston SL, Moric I, Simpson JL, Hensley MJ, Gibson PG. Neutrophil degranulation and cell lysis is associated with clinical severity in virus-induced asthma. *Eur Respir J.* Jan 2002;19(1):68-75.
7. Grissell TV, Powell H, Shafren DR, et al. Interleukin-10 gene expression in acute virus-induced asthma. *Am J Respir Crit Care Med.* Aug 15 2005;172(4):433-439.
8. Wark PA, Johnston SL, Simpson JL, Hensley MJ, Gibson PG. Chlamydia pneumoniae immunoglobulin A reactivation and airway inflammation in acute asthma. *Eur Respir J.* Oct 2002;20(4):834-840.
9. Esposito S, Blasi F, Arosio C, et al. Importance of acute Mycoplasma pneumoniae and Chlamydia pneumoniae infections in children with wheezing. *Eur Respir J.* Dec 2000;16(6):1142-1146.
10. Cunningham AF, Johnston SL, Julious SA, Lampe FC, Ward ME. Chronic Chlamydia pneumoniae infection and asthma exacerbations in children. *Eur Respir J.* Feb 1998;11(2):345-349.
11. Johnston SL, Martin RJ. Chlamydophila pneumoniae and Mycoplasma pneumoniae: a role in asthma pathogenesis? *Am J Respir Crit Care Med.* Nov 1 2005;172(9):1078-1089.
12. Johnston SL, Blasi F, Black PN, Martin RJ, Farrell DJ, Nieman RB. The effect of telithromycin in acute exacerbations of asthma. *N Engl J Med.* Apr 13 2006;354(15):1589-1600.
13. Talbot TR, Hartert TV, Mitchel E, et al. Asthma as a risk factor for invasive pneumococcal disease. *N Engl J Med.* May 19 2005;352(20):2082-2090.
14. Klemets P, Lyytikainen O, Ruutu P, et al. Risk of invasive pneumococcal infections among working age adults with asthma. *Thorax.* Aug 2010;65(8):698-702.
15. Pilishvili T, Zell ER, Farley MM, et al. Risk factors for invasive pneumococcal disease in children in the era of conjugate vaccine use. *Pediatrics.* Jul 2010;126(1):e9-17.
16. Jounio U, Juvonen R, Bloigu A, et al. Pneumococcal carriage is more common in asthmatic than in non-asthmatic young men. *Clin Respir J.* Oct 2010;4(4):222-229.
17. Hilty M, Burke C, Pedro H, et al. Disordered microbial communities in asthmatic airways. *PLoS One.* 2010;5(1):e8578.
18. Message SD, Laza-Stanca V, Mallia P, et al. Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production. *Proc Natl Acad Sci U S A.* Sep 9 2008;105(36):13562-13567.
19. Contoli M, Message SD, Laza-Stanca V, et al. Role of deficient type III interferon-lambda production in asthma exacerbations. *Nat Med.* Sep 2006;12(9):1023-1026.
20. Oliver BG, Lim S, Wark P, et al. Rhinovirus exposure impairs immune responses to bacterial products in human alveolar macrophages. *Thorax.* Jun 2008;63(6):519-525.
21. Avadhanula V, Rodriguez CA, Devincenzo JP, et al. Respiratory viruses augment the adhesion of bacterial pathogens to respiratory epithelium in a viral species- and cell type-dependent manner. *J Virol.* Feb 2006;80(4):1629-1636.
22. Bisgaard H, Hermansen MN, Bonnelykke K, et al. Association of bacteria and viruses with wheezy episodes in young children: prospective birth cohort study. *Bmj.* 2010;341:c4978.
23. British Guideline on the Management of Asthma. *Thorax.* May 2008;63 Suppl 4:iv1-121.
24. Global Strategy for Asthma Management and Prevention (2015 update). http://ginasthma.org/wp-content/uploads/2016/01/GINA_Report_2015_Aug11-1.pdf.
25. Kobayashi Y, Wada H, Rossios C, et al. A novel macrolide solithromycin exerts superior anti-inflammatory effect via NF-kappaB inhibition. *J Pharmacol Exp Ther.* Apr 2013;345(1):76-84.

26. Gielen V, Johnston SL, Edwards MR. Azithromycin induces anti-viral responses in bronchial epithelial cells. *Eur Respir J.* Sep 2010;36(3):646-654.
27. Wark PA, Johnston SL, Bucchieri F, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med.* Mar 21 2005;201(6):937-947.
28. Zeitlinger M, Wagner CC, Heinisch B. Ketolides--the modern relatives of macrolides : the pharmacokinetic perspective. *Clin Pharmacokinet.* 2009;48(1):23-38.
29. Walsh F, Carnegy F, Willcock J, Amyes S. Comparative in vitro activity of telithromycin against macrolide-resistant and -susceptible Streptococcus pneumoniae, Moraxella catarrhalis and Haemophilus influenzae. *J Antimicrob Chemother.* May 2004;53(5):793-796.
30. Kosowska K, Credito K, Pankuch GA, et al. Activities of two novel macrolides, GW 773546 and GW 708408, compared with those of telithromycin, erythromycin, azithromycin, and clarithromycin against Haemophilus influenzae. *Antimicrob Agents Chemother.* Nov 2004;48(11):4113-4119.
31. De Vecchi E, Nicola L, Larosa M, Drago L. In vitro activity of telithromycin against Haemophilus influenzae at epithelial lining fluid concentrations. *BMC microbiology.* 2008;8:23.
32. Leung E, Weil DE, Raviglione M, Nakatani H. The WHO policy package to combat antimicrobial resistance. *Bull World Health Organ.* May 1 2011;89(5):390-392.
33. Beigelman A, Isaacson-Schmid M, Sajol G, et al. Randomized trial to evaluate azithromycin's effects on serum and upper airway IL-8 levels and recurrent wheezing in infants with respiratory syncytial virus bronchiolitis. *J Allergy Clin Immunol.* May 2015;135(5):1171-1178 e1171.
34. Stokholm J, Chawes BL, Vissing NH, et al. Azithromycin for episodes with asthma-like symptoms in young children aged 1-3 years: a randomised, double-blind, placebo-controlled trial. *The Lancet. Respiratory medicine.* Jan 2016;4(1):19-26.
35. Bacharier LB, Guilbert TW, Mauger DT, et al. Early Administration of Azithromycin and Prevention of Severe Lower Respiratory Tract Illnesses in Preschool Children With a History of Such Illnesses: A Randomized Clinical Trial. *Jama.* Nov 17 2015;314(19):2034-2044.
36. Brusselle GG, Vanderstichele C, Jordens P, et al. Azithromycin for prevention of exacerbations in severe asthma (AZISAST): a multicentre randomised double-blind placebo-controlled trial. *Thorax.* Apr 2013;68(4):322-329.
37. British guideline on the management of asthma. *Thorax.* Nov 2014;69 Suppl 1:1-192.

## Figure legends

**Figure 1. CONSORT diagram of the AZALEA trial.**

**Figure 2: Primary outcome symptom diary scores from randomization to day 10.**

Data are mean with standard error (SE) bars.

**Figure 3: Secondary outcome acute and mini AQLQ scores from randomization to day 10 and time to 50% reduction in symptom diary score.**

(a**)** Acute AQLQ and (b) mini AQLQ mean scores and standard error (SE) bars by visits for each treatment arm and (c) Kaplan-Meier curves of time to a 50% reduction in symptom diary score for each treatment arm (truncated at 10 days).

| **Table 1: Baseline characteristics of patients by treatment group** | **Active** | **Placebo** |
|---|---|---|
| **N** | 97 | 102 |
| **Age (years), median (IQR)** | 39.1 (28.9, 49.5) | 36.15 (25.4, 49.3) |
| **Gender** | | |
| **Female** | 64 (66.0%) | 75 (73.5%) |
| **Male** | 33 (34.0%) | 27 (26.5%) |
| **Asthma Severity (N = 198)**[37] | | |
| **step 1: mild intermittent asthma** | 7 (7.2%) | 13 (12.9%) |
| **step 2: regular preventer therapy** | 30 (30.9%) | 26 (25.7%) |
| **step 3: initial add-on therapy** | 31 (32.0%) | 27 (26.7%) |
| **step 4: persistent poor control** | 22 (22.7%) | 22 (21.8%) |
| **step 5: continuous/frequent oral steroids** | 7 (7.2%) | 13 (12.9%) |
| **Smoking status** | | |
| **never smoked** | 60 (61.9%) | 61 (60.4%) |
| **former smoker** | 26 (26.8%) | 19 (18.8%) |
| **current smoker** | 11 (11.3%) | 21 (20.8%) |
| **Pack years, median (IQR) (min/max) (N=75)* (current/former smokers)** | 5 (1, 15) (0/127) | 5 (2, 12) (0/22) |
| **Asthma Exacerbation (N = 198)** | | |
| **Mild Asthma Exacerbation** | 5 (5.2%) | 3 (3.0%) |
| **Moderate Asthma Exacerbation** | 26 (26.8%) | 35 (34.7%) |
| **Acute Severe Asthma** | 61 (62.9%) | 56 (55.4%) |
| **Life Threatening Asthma** | 4 (4.1%) | 7 (6.9%) |
| **Near-Fatal Asthma** | 1 (1.0%) | 0 (0.0%) |
| **Time from presentation to study drug, median (IQR) (N = 192)** | 21 (12, 29) | 22 (14, 28) |

**Table2: Baseline (exacerbation) pulmonary function by treatment arm**

| | **Active** | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| **Pulmonary function** | **N** | **Mean** | **SD** | **P25** | **Median** | **P75** |
| **$FEV_1$(liters)** | 95 | 1.9 | 0.7 | 1.4 | 1.8 | 2.5 |
| **$FEV_1$ %predicted (%)** | 93 | 63.2 | 21.8 | 48 | 63 | 79 |
| **FVC(liters)** | 96 | 2.8 | 1.0 | 2.0 | 2.7 | 3.5 |
| **$FEV_1$/FVC ratio** | 94 | 69.7 | 13.3 | 62.0 | 70.0 | 79.0 |
| **$FEF_{25-75\%}$(liters/sec)** | 80 | 1.6 | 0.9 | 0.9 | 1.4 | 2.1 |
| **$FEF_{50\%}$(liters/sec)** | 76 | 1.9 | 1.1 | 1.1 | 1.7 | 2.6 |
| **PEF(liters/min)** | 95 | 288 | 108 | 211 | 283 | 361 |
| **PEF %predicted (%)** | 94 | 76.6 | 108.6 | 47.0 | 67.5 | 79.0 |
| | **Placebo** | | | | | |
| **Pulmonary function** | **N** | **Mean** | **SD** | **P25** | **Median** | **P75** |
| **$FEV_1$(liters)** | 96 | 2.1 | 0.8 | 1.5 | 2.0 | 2.6 |
| **$FEV_1$ %predicted (%)** | 96 | 66.3 | 21.0 | 52.5 | 64.0 | 84.0 |
| **FVC(liters)** | 96 | 3.1 | 1.0 | 2.4 | 3.0 | 3.6 |
| **$FEV_1$/FVC ratio** | 96 | 68.8 | 13.7 | 58.0 | 69.0 | 79.5 |
| **$FEF_{25-75\%}$(liters/sec)** | 87 | 1.7 | 1.1 | 0.9 | 1.4 | 2.4 |
| **$FEF_{50\%}$(liters/sec)** | 84 | 2.0 | 1.3 | 1.1 | 1.7 | 2.8 |
| **PEF(liters/min)** | 97 | 320 | 102 | 247 | 335 | 389 |
| **PEF %predicted (%)** | 96 | 72.9 | 21.4 | 56.5 | 74.0 | 90.0 |

SD = standard deviation, P25 = $25^{th}$ percentile, P75 = $75^{th}$ percentile

**Figure 1. CONSORT diagram of the AZALEA trial**

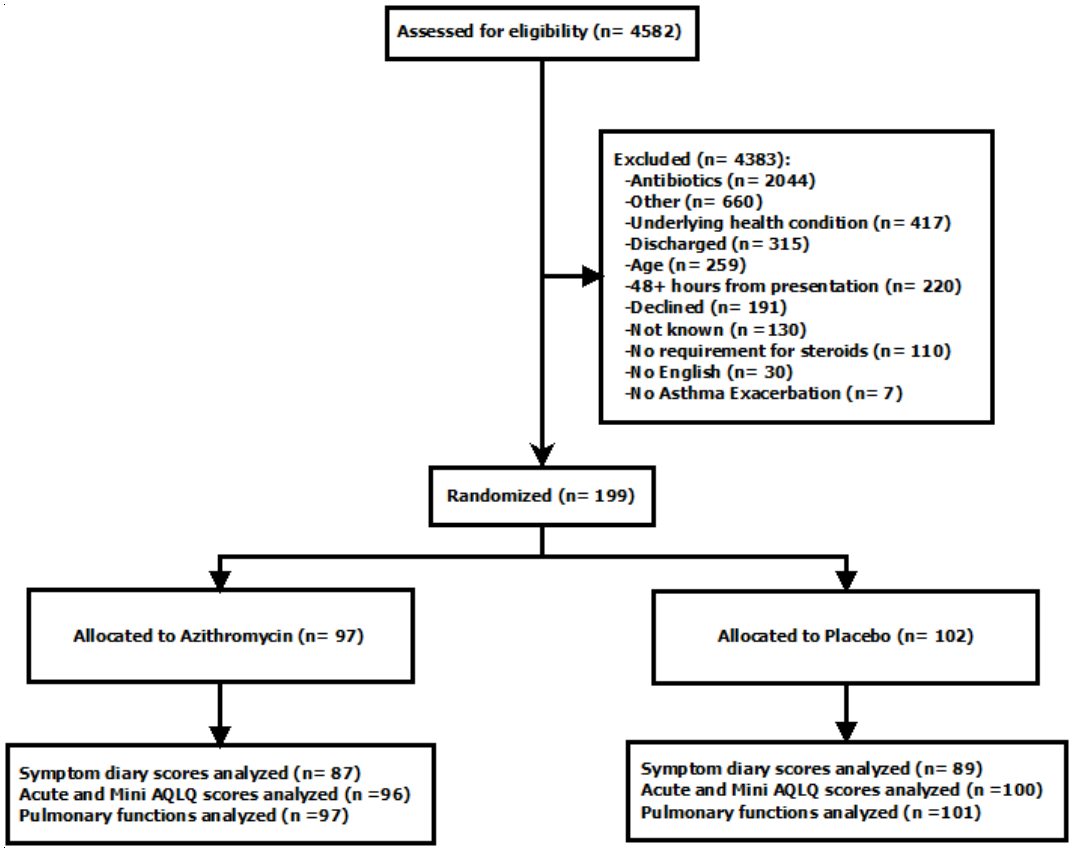


**Figure 2: Primary outcome symptom diary scores from randomization to day 10.**

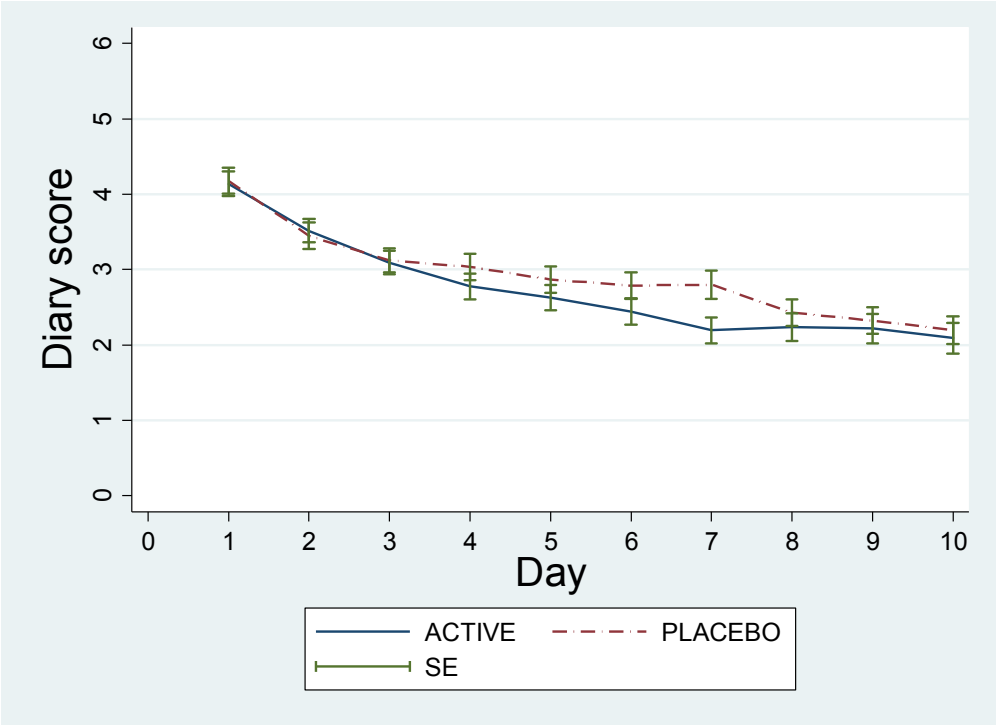
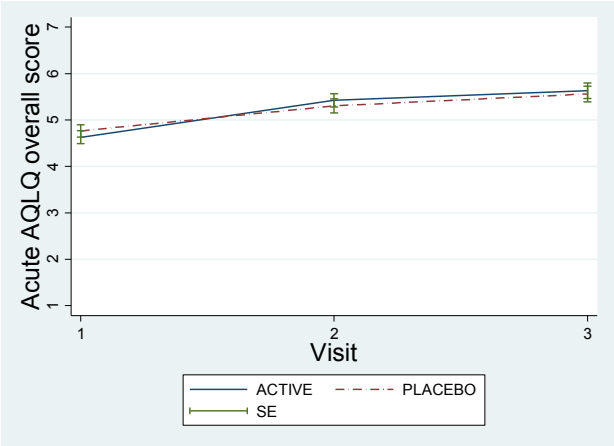
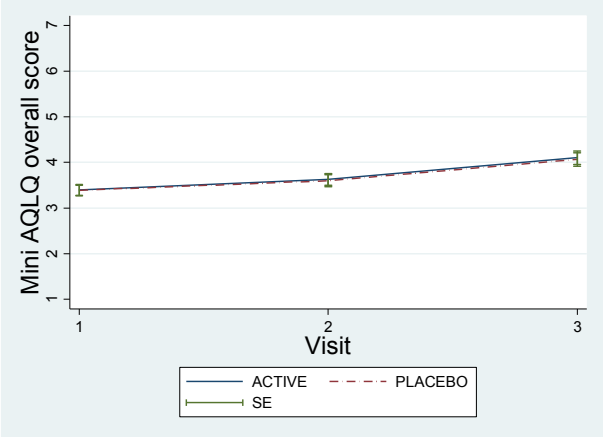


**Figure 3: Secondary outcome acute and mini AQLQ scores from randomization to day 10 and time to 50% reduction in symptom diary score.**

**(a)**




**(b)**




**(c)**