Title

A Randomised, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy of Oral Azithromycin (500 Mg OD) as a Supplement to Standard Care for Adult Patients with Acute Exacerbations of Asthma (the AZALEA Trial).

Authors

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ABSTRACT

Objective: To evaluate the efficacy of azithromycin during an acute exacerbation of asthma.

Design: Multi-centre, randomised, double-blind, placebo-controlled study.

Setting: Adults presenting to A&E and acute medical units and one primary care centre.

Participants: Adults with a history of asthma presenting within 48 hours (of initial presentation requesting medical care) with an acute deterioration in asthma control (increased wheeze, dyspnoea and/or cough with reduced Peak Expiratory Flow (PEF)) requiring treatment with corticosteroids.

Interventions: Azithromycin (two 250 mg capsules) or two placebo capsules once a day for 3 days.

Primary outcome measure: Diary card summary symptom score, assessed (at) 10 days after randomisation.

Results: 4582 patients were screened at 31 centres, of whom 199 were randomised to treatment. The major reasons for non-recruitment were: already receiving antibiotics (2044, 44.6% of screened subjects), unable to contact (315, 6.9%), declined participation (191, 4.2%), other (660, 14.4%).

Mean age of participants was 39.9 years, gender: 69.8% female, smoking status: never smoked 61.1%, former smoker 22.7% current smoker 16.2%, mean pack years: 3.45, exacerbation severity: Life Threatening Asthma 6.1%, Acute Severe Asthma 59.1%, Moderate Asthma Exacerbation 30.8%, Mild Asthma Exacerbation 4.0%. Median time from presentation to drug administration was 22 hours. Lung function at baseline (exacerbation) was PEF 74.8 %predicted, FEV_1% 64.8% predicted, FEV_1/FVC ratio 69.2%. Baseline characteristics were well balanced across treatment arms and centres.

Mean (SD) scores on the primary outcome asthma symptom score were 4.14 (1.38) at baseline and 2.09 (1.71) at the end of treatment for the azithromycin group and 4.18 (1.48) and 2.20 (1.51) for the placebo group. Using multilevel modelling, there was no statistically significant difference in symptom scores between groups at day 10, similarly no significant between group differences were seen in symptom scores on any other day between baseline and day 10.

No significant between group differences were seen in the acute AQLQ, mini AQLQ, nor in any measure of lung function on any day between baseline and day 10 and there were no differences in time to a 50% reduction in symptom score.

52.7% of patients provided sputum for bacterial culture and/or cell counts, 96.0% provided nasal/throat swabs for virus/atypical pathogen PCR and 92.0% serum for atypical pathogen serology.

Sputum bacterial culture was positive in 6% of subjects, atypical pathogen PCR and/or serology in 4.5% and virus PCR in 18.1%. There were no differences in the primary outcome asthma symptom

score between active and placebo groups in patients with positive sputum bacterial culture, positive atypical bacteria, any bacteria or virus positive tests, although numbers for these analyses were small. **Conclusions:** In the population of patients randomised to treatment in this study, addition of azithromycin to standard medical care resulted in no statistically significant or clinically important benefit. For each subject randomised, more than 10 failed screening because they had already been prescribed antibiotic therapy.

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LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Transaminase
AQLQ	Asthma Quality of Life Questionnaire
AR	Adverse Reaction
AST	Aspartate Transaminase
ATS	American Thoracic Society
BTS	British Thoracic Society
CI	Chief Investigator
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CRGO	Clinical Research Governance Office (at Imperial)
CRN	Clinical Research Network
CTA	Clinical Trials Authorisation
DMEC	Data Monitoring and Ethics Committee
eCRF	Electronic Case Record Form
EME	Efficacy and Mechanism Evaluation
$FEF_{25-75\%}$	Forced Mid-Expiratory Flow Rate
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
GCP	Good Clinical Practice
ICS	Inhaled corticosteroids
ICU	Intensive Care Unit
ICTU	Imperial Clinical Trials Unit
IMP	Investigational Medicinal Product
ITM	Integrated Trial Management
ITT	Intention-to-treat
MHRA	Medicines and Healthcare products Regulatory Authority
NIHR	National Institute for Health Research
OCS	Oral corticosteroids
PC	Predefined Change
PCA	Predefined Change Abnormal

PCR	Polymerase Chain Reaction
PEF	Peak Expiratory Flow
PFT	Pulmonary Function Tests
PI	Principal Investigator
QA	Quality Assurance
QoLQ	Quality of Life Questionnaire
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TSC	Trial Steering Committee

SCIENTIFIC SUMMARY

Background

Asthma is the most prevalent respiratory disease. Major asthma morbidity and mortality result from acute exacerbations: 5-10% of asthmatics have been hospitalised with an exacerbation and more than half of asthma patients reported having an exacerbation in the last year with >1/3 children and >1/4 adults requiring urgent medical care visits as a result.

Respiratory viral infections are the major cause of asthma exacerbations in children (80-85%) and adults (75-80%). However, non-viral respiratory pathogens such as *Mycoplasma pneumoniae* (*M. pneumoniae*) and *Chlamydophila pneumoniae* (*C. pneumoniae*) have also been associated with wheezing episodes and asthma exacerbations in both adults and children.

There is little published evidence that standard bacterial infections are important in the aetiology of asthma exacerbations, however, patients with asthma have increased susceptibility to respiratory bacterial infections, increased carriage of pathogenic respiratory bacteria identified by culture and molecular techniques and impaired interferon responses to bacterial polysaccharides. There is good evidence that bacterial respiratory infections are both more common and more severe in asthma.

A recent study of 361 children reported acute wheezing episodes were associated with both bacterial infection (odds-ratio 2.9, 95% CI 1.9-4.3, $p<0.001$) and with virus infection (odds-ratio 2.8, 95% CI [1.7, 4.4], $p<0.01$). We therefore hypothesised that standard bacterial infections are likely also to be important in the aetiology of asthma exacerbations in adults.

Current asthma guidelines recommend specifically that antibiotic therapy should NOT be administered routinely in asthma exacerbations.

Adults with acute exacerbations of asthma and treated with telithromycin (a ketolide antibiotic closely related to macrolides: both classes being highly active against *M.* and *C. pneumoniae*) as a supplement to standard care, showed a significantly greater reduction in asthma symptoms ($P<0.005$), improvement in lung function ($P=0.001$) and faster recovery ($P=0.03$) when compared to those treated with placebo. This treatment therefore had a clear therapeutic effect; however this study requires confirmation in a second similar study, before revision of guidelines could be considered. Ideally confirmation would be with a further study with telithromycin, however issues with toxicity have limited use of telithromycin to severe life threatening infections.

The macrolide antibiotic azithromycin is an alternative that has been used for many years in the treatment of respiratory disease, but has thus far not been studied in acute exacerbations of asthma. We therefore hypothesised that treatment with azithromycin might be of benefit in treatment of acute asthma exacerbations. The AZALEA study therefore investigated the effectiveness of azithromycin added to standard care for adult patients with acute exacerbations of asthma.

A further mechanistic aim of our study, was to investigate frequencies of standard bacterial, atypical bacterial and viral infections in these exacerbations to determine the relative importance of each of these infections, and to perform subgroup analyses to determine whether any treatment benefit observed is greater in those with evidence of one or more of these infections, with the aim of shedding some light on the possible mechanism(s) of action of azithromycin.

Different patterns of airway inflammation have been identified in asthma exacerbations – these have been classified as neutrophilic, eosinophilic, mixed granulocytic or pauci-granulocytic. However, it is not known whether these different patterns of inflammation are associated with different aetiologies of exacerbation, nor whether they are related to treatment outcome. We therefore, finally aimed to characterise the inflammatory cell profiles in sputum at presentation, to determine whether exacerbation aetiology as well as any possible treatment benefit are related to the types of airway inflammation present.

Objectives

1. Primary Objective

To assess efficacy using diary card summary symptom score, with symptoms including wheezing, breathlessness and coughing at 10 days after randomisation.

2. Secondary Objectives

The secondary objectives of the study were to evaluate:

- The following additional efficacy endpoints:
    - Health status assessed by acute asthma QoLQ (Juniper)
    - Health status assessed by Mini Asthma QoLQ (Juniper)
    - Pulmonary Function tests (FEV_1, FVC, FEV1/FVC ratio, PEF, FEF25-75%, FEF50%)
- Primary and secondary outcomes assessed 5 and 10 days post randomisation to permit better estimation of optimum timing of primary/secondary outcome variables in future similar studies
- Time to 50% reduction in symptom score

Exploratory analyses

- Assessment of efficacy outcomes in relation to initial standard bacteriological, *C. pneumoniae* and/or *M. pneumoniae* and virological status
- Assessment of efficacy outcomes in relation to initial sputum inflammatory cell status

Methods

1. Trial Design

This was a multi-centre, randomised, double-blind, placebo-controlled study. Eligible patients were randomised within 48 hours of initial presentation to medical care with an acute deterioration in asthma control and requiring a course of oral steroids. Patients were randomised to receive either 1) azithromycin or 2) placebo for 3 days, with post-therapy assessments at 5 and 10 days and a follow-up visit at six weeks.

2. Participants

Adult patients with a documented history of asthma for greater than 6 months and presenting within 48 hours (of initial presentation to medical care) with an acute deterioration in asthma control and requiring a course of oral steroids.

Main inclusion criteria

- Adults, either sex, aged 18-55 years or aged 56 to 65 with < 20 pack year smoking history or >65 with <5 pack year smoking history
- Patients with a documented history of asthma for >6 months, **and**
- Patients presenting within 48 hours (of initial presentation to medical care) with an acute deterioration in asthma control (increased wheeze, dyspnoea and/or cough and/or reduced PEF) and requiring a course of oral steroids
- Patients with a PEF or FEV_1 less than 80% of predicted normal or patient's best at presentation, at recruitment, or in the time elapsed between presentation and recruitment

Main exclusion criteria

- Patients with known prolongation of the QT interval, a history of torsades de pointes, congenital long QT syndrome, bradyarrhythmias or uncompensated heart failure, patients on drugs known to prolong the QT interval.
- Smokers aged 56-65 with a ≥20 pack year history, or aged >65 with ≥5 pack year history
- Patients requiring immediate transfer to or referral to the Intensive Care Unit (ICU)
- Patients who took oral or systemic antibiotics within 28 days prior to enrolment
- Patients with known impaired hepatic function (ALT/AST > 2 upper limit of normal)
- Patients with significant lung disease (including Chronic Obstructive Pulmonary Disease (COPD)) other than asthma
- Patients with >20mg oral corticosteroid maintenance therapy
- Patients receiving other medications or who have other disease conditions or infections that could interfere with the evaluation of drug efficacy or safety
- Women breast-feeding or pregnant
- Patients with suspected or known hypersensitivity to, or suspected serious adverse reaction to azithromycin or any of the macrolide or ketolide class of antibiotics, erythromycin or to any excipients thereof
- Patients who have received treatment with any other investigational drug within 1 month prior to study entry, or have such treatment planned for the study period during treatment or follow up phase
- Patients with a concomitant condition making implementation of the protocol or interpretation of the study results difficult
- Patients with mental conditions rendering them unable to understand the nature, scope, and possible consequences of the study.
- Patients unlikely to comply with the protocol.
- No patient was allowed to enrol in this study more than once.

3. Interventions

All patients in the study received, per randomised allocation, treatment with either azithromycin or placebo. The identity of the treatment regimen was blinded by encapsulating active medication in opaque capsules to match the placebo.

Those randomised to azithromycin received 500 mg azithromycin (two 250 mg capsules) once a day for 3 days. Patients randomised to placebo received two placebo capsules once a day for 3 days.

Patients were instructed to take study medication at least 1 hour before or 2 hours after food or antacids.

The time of administration of the study medication was documented on the case report form for patients throughout the study. The first dose was given in the presence of a member of the research team.

4. Outcomes

a. Primary outcome

Diary card summary symptom score, with symptoms including wheezing, breathlessness and coughing assessed (at) 10 days after randomisation

b. Secondary outcomes

i. The following additional efficacy endpoints:

- Health status assessed by acute asthma QoLQ (Juniper)
- Health status assessed by Mini Asthma QoLQ (Juniper)
- Pulmonary Function tests (FEV_1, FVC, FEV_1/FVC ratio, PEF, FEF25-75%, FEF50%)

ii. Primary and secondary outcomes assessed 5 and 10 days post randomisation to permit better estimation of optimum timing of primary/secondary outcome variables in future similar studies (the efficacy of telithromycin was only assessed at 10 days).

iii. Time to 50% reduction in symptom score

Exploratory analyses

- Trends in primary and secondary outcomes over the time course of the exacerbation up to 10 days
- Assessment of efficacy outcomes in relation to initial *C. pneumoniae* and/or *M. pneumoniae* status
- Assessment of efficacy outcomes in relation to initial standard bacteriological status
- Assessment of efficacy outcomes in relation to initial virological status
- Assessment of efficacy outcomes in relation to initial sputum inflammatory cell status

5. Sample size and statistical analysis

The sample size calculations were based on the primary outcome: change from baseline in diary card summary asthma symptom scores at 10 days after randomisation. Our previous study found a mean decrease in symptom score of 1.3 in treatment group, and 1 in the control group, a difference of -0.3 (SD 0.783) between the groups at 10 days.

Using a two-sided t-test at 1% significance level, with 80% power, 161 patients in each group were required to detect the same difference in asthma scores between the groups. A significance level of 1% in the above calculation was chosen to provide greater certainty in assessment of the primary outcome variable, as well as to provide greater power for the subgroup exploratory analyses, as those subgroup analyses that were performed were uninformative in the 280 patient Telicast study.

Taking into account a drop-out rate of 15% in the study, we aimed to recruit 190 patients in each arm of the study.

The clinical efficacy analyses were carried out on an intention-to-treat basis. Outcomes which were recorded at multiple time-points (diary card symptom scores, quality of life questionnaires and pulmonary function tests) were analysed using a three level hierarchical model to take account of the structure in the data.

Results

Recruitment was from 31 sites, the majority (30) of which were secondary care hospitals with one primary care centre. Recruitment lasted 2.5 years, from September 2011 to April 2014. A total of 4582 patients were screened of whom 390 patients met eligibility criteria, 199 were randomised to treatment, 193 (97%) from secondary care hospitals, 6 (3%) from the primary care centre. The major reasons for non-recruitment were: already receiving antibiotics (2044, 44.6% of screened patients), discharged/unable to contact (315, 6.9%), declined participation (191, 4.2%), other (660, 14.4%).

The mean age of study participants was 39.9 years, gender: 69.8% female (n= 139), 30.2% male (n= 60). Underlying asthma severity was classified by treatment before exacerbation: mild intermittent asthma 10.1% (n= 20), regular preventer therapy 28.3% (n=56), initial add-on therapy 29.3% (n= 58), persistent poor control 22.2% (n= 44), continuous or frequent use of oral steroids 10.1% (n= 20). Smoking status included: never smoked 61.1% (n= 121), former smoker 22.7% (n= 45) current smoker 16.2% (n= 32), mean pack. years: 3.45. Exacerbation severity was categorised: near-fatal asthma 0.5% (n=1), life threatening asthma 5.6% (n= 11), acute severe asthma exacerbation 59.1% (n= 117), moderate asthma exacerbation 30.8% (n= 61), mild asthma exacerbation 4.0% (n=8). Median time from presentation to trial drug administration was 22 hours. Lung function at baseline (exacerbation) included PEF 74.8% predicted, FEV_1 64.8% predicted, and FEV_1/FVC ratio 69.2%. Baseline characteristics were well balanced across treatment arms and centres.

Mean (SD) scores on the primary outcome asthma symptom score (from 0 no symptoms to 6 severe symptoms) were 4.14 (1.38) at baseline and 2.09 (1.71) at the end of treatment for the azithromycin group and 4.18 (1.48) at baseline and 2.20 (1.51) at the end of treatment for the placebo group. Using multilevel modelling for the primary outcome, there was no statistically significant difference in symptom scores between groups at day 10 (difference -0.166 [95% CI: -0.670; 0.337]). Similarly no significant between group differences were seen in symptom scores on any other day between baseline and day 10.

No significant between group differences were seen in the acute AQLQ and mini AQLQ nor in any measure of lung function, on any day, between baseline and day 10 and there were no differences in time to a 50% reduction in symptom score.

Only 105 (52.7%) patients provided sputum samples for sputum bacterial culture and/or sputum cell counts, while 191 (96.0%) patients provided nasal/throat swabs for virus/atypical pathogen PCR and 183 (92.0%) patients provided acute (IgM) or acute and convalescent (IgG, IgA) sera for atypical pathogen serology.

Sputum bacterial culture was positive in 6% of subjects (4.1% active, 7.8% placebo), nasal/throat swab and/or sputum atypical pathogen PCR and/or atypical pathogen serology were positive in 4.5%

of patients (5.2% active, 3.9% placebo). Nasal/throat swab and/or sputum virus PCR were positive in 18.1% of patients (16.5% active, 19.6% placebo). There were no differences in the primary outcome asthma symptom score between active and placebo groups in patients with positive sputum bacterial culture, atypical bacteria PCR or serology, (including any bacteria or virus PCR positive tests), though patient numbers for these analyses were low. No subgroup analyses, defined on sputum cell count characteristics, were performed, as numbers per group were too low to be meaningful.

Conclusions

In the population of patients randomised to treatment in this study, addition of azithromycin to standard medical care resulted in no statistically significant, or clinically important benefit. For each patient randomised, approximately 10 were excluded because they had already received antibiotic therapy, despite guideline recommendations that such therapy should not be routinely used. The study may therefore have been underpowered to detect therapeutic benefit in the minority of patients randomised to treatment.

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PLAIN ENGLISH SUMMARY

Acute asthma attacks are common and cause substantial suffering and occasionally death. Current treatments for asthma attacks are not as effective as they should be and new/better treatments are needed. Viral respiratory infections often cause asthma attacks and bacterial respiratory infections have also been associated with some asthma attacks. However, current guidelines recommend antibiotic therapy should NOT routinely be given as the role for bacteria is uncertain. We previously reported that adults experiencing asthma attacks showed a significantly greater reduction in symptoms and faster recovery when given the antibiotic telithromycin compared to placebo ('dummy' treatment). This treatment had clear benefit, however safety concerns have limited use of telithromycin. We therefore investigated whether azithromycin, which is a well tolerated antibiotic similar to telithromycin, might be of benefit in asthma attacks. In addition we looked at 1) how frequently bacteria are detected in asthma attacks and 2) whether those people with a bacterial infection recovered better from an asthma attack. We did not find statistically significant difference between the azithromycin and placebo groups in patient diary scores, nor in any pulmonary function tests. There were no differences between groups in time to recovery. Numbers of bacterial infections were low, and there was no suggestion of treatment benefit in subjects with detectable bacteria.

For every patient randomised to treatment, approximately 10 were excluded as they had already received antibiotic therapy.

In the patients randomised to treatment in this study, azithromycin had no statistically or clinically significant benefit.

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CHAPTER 1: INTRODUCTION

1. BACKGROUND

1.1 Importance of asthma exacerbations

Asthma is the most prevalent respiratory disease, in developed countries it is diagnosed in 5-10% of adults and 10-15% of children, while around 30% of children report wheeze in the last year[1]. The most important asthma morbidity and mortality result from acute exacerbations: 5-10% of asthmatics have been hospitalised with an exacerbation and an estimated/approximately~25,000 Europeans die unnecessarily of asthma each year. Exacerbations also account for ~50% of total expenditure on asthma care[2]. More than half of asthma patients report having an exacerbation in the last year with >1/3 children and >1/4 adults requiring urgent medical care visits as a result[3].

1.2 Aetiology of asthma exacerbations

Viruses and atypical bacteria: Respiratory viral infections are the major cause of asthma exacerbations in children (80-85%)[4, 5] and adults (75-80%)[6-8]. However, non-viral respiratory pathogens such as *Mycoplasma pneumoniae* (*M. pneumoniae*) and *Chlamydophila pneumoniae* (*C. pneumoniae*) have also been associated with wheezing episodes and asthma exacerbations in both adults and children[9-13]. Interestingly, in two of these studies virus detection rates were ~80%[9, 11], while serological positivity for atypical bacterial infection/reactivation can be as high as 40-60%[9, 13] indicating that viral and atypical bacterial infections probably interact in increasing the risk of asthma exacerbations.

Bacterial infections: There is little published evidence that standard bacterial infections are important in the aetiology of asthma exacerbations[14], however, patients with asthma have increased susceptibility to respiratory bacterial infections[15-17], increased carriage of pathogenic respiratory bacteria identified by culture[18] and molecular techniques[19] and impaired interferon responses to bacterial polysaccharides[20]. In addition, viral infection impairs innate responses important in antibacterial immunity[21] and increases bacterial adherence to bronchial epithelial cells[22]. There is therefore good evidence that bacterial respiratory infections are both more common and more severe in asthma, and that viral infection can increase susceptibility to bacterial infection.

A recent study of 361 children with >800 stable and exacerbation airway samples collected during the first 3 years of life and analysed for standard bacteria and respiratory viruses, has confirmed that acute wheezing episodes were associated with both bacterial infection (odds-ratio 2.9, 95% CI 1.9-4.3, p<0.001) and with virus infection (odds-ratio 2.8, 95% CI [1.7, 4.4], p<0.01)[23]. We therefore hypothesise that standard bacterial infections are likely also to be important in the aetiology of asthma exacerbations in adults, and aimed to investigate this in this study.

1.3 Treatment of asthma exacerbations

When asthma exacerbations occur, treatment options are limited to bronchodilators and (cortico)steroids. Beyond the addition of magnesium in severe exacerbations, treatments have developed very little in the last 50 years. Current therapeutic strategies are of limited efficacy and development of new approaches addressing the aetiological agents causing the exacerbations is urgently needed. Current asthma guidelines recommend specifically that antibiotic therapy should NOT be administered routinely in asthma exacerbations[24].

1.4 New approaches to treatment of asthma exacerbations

If atypical bacteria are causal or contributory factors in asthma exacerbation, then treatment with antibiotics with activity against mycoplasma and chlamydia species would be expected to be beneficial in asthma exacerbations. Adults with acute exacerbations of asthma and treated with telithromycin (a ketolide antibiotic closely related to macrolides: both classes being highly active against *M.* and *C. pneumoniae*) as a supplement to standard care, showed a statistically significantly greater reduction in asthma symptoms ($P<0.005$), improvement in lung function ($P=0.001$) and faster recovery ($P=0.03$) when compared to those treated with placebo[13]. The magnitude of the treatment effect was (also) highly clinically significant, with the improvement in symptoms resulting from telithromycin treatment being approximately 50% greater than with standard therapy (plus placebo). Improvement in lung function was almost 100% greater, and importantly, recovery time to a 50% improvement in clinical symptoms 3 days faster in those receiving active treatment. This treatment therefore had a clear therapeutic effect; however this study requires confirmation in a second similar study, before revision of guidelines could be considered. Ideally confirmation would be with a further study with telithromycin. However, issues with toxicity have limited use of telithromycin to severe life threatening infections.

The macrolide antibiotic azithromycin is a safe and well tolerated alternative that has been used for many years in the treatment of respiratory disease, but has thus far not been studied in acute exacerbations of asthma. We therefore hypothesised that treatment with azithromycin might be of benefit in acute asthma exacerbations. The AZALEA study investigated the effectiveness of azithromycin as a supplement to standard care for adult patients with acute exacerbations of asthma, following as closely as possible the design of the telithromycin study, with the aim of providing confirmation or otherwise of those results.

1.5 Mechanisms of activity of macrolide/ketolide antibiotics in treatment of asthma exacerbations

Macrolide/ketolide antibiotics might have therapeutic effect/benefit in treating asthma exacerbations through treatment of either standard or atypical bacteria or both. In addition, both macrolide and ketolide antibiotics have anti-inflammatory properties that are independent of their antibacterial activity which may be beneficial in reducing airway inflammation, which is known to be important in the pathogenesis of asthma exacerbations[7, 25]. In addition to these three possible mechanisms of action, we also believe antiviral activity is a 4th possible mechanism.

We have previously reported that impaired type I and type III interferon production by virus infected bronchial epithelial cells and macrophages is important in the pathogenesis of asthma exacerbations[20, 26]. We have also recently shown that azithromycin, but not erythromycin or telithromycin, significantly increased rhinovirus induced type I and type III interferon and interferon-stimulated anti-viral protein production in primary bronchial epithelial cells, as well as significantly reducing rhinovirus replication and release in bronchial epithelial cells[27]. Azithromycin has also been shown to reduce illness severity in a mouse model of viral bronchiolitis[28]. Thus azithromycin has potential to have direct anti-viral activity by augmenting production of those interferons we have already shown to be deficient in asthma exacerbations[20, 26], and this activity may make it a better treatment option than telithromycin, which does not appear to have this property[27]. A further mechanistic aim of our study therefore, was to investigate frequencies of standard bacterial, atypical bacterial and viral infections in these exacerbations to determine the relative importance of each of these infections, and of possible co-infections with one or more agents, in the aetiology of acute exacerbations of asthma in adult subjects. We have also performed subgroup analyses to determine whether any treatment benefit observed is greater in those with evidence of one or more of these infections,

with the aim of shedding some light on the possible mechanism(s) of action of azithromycin in this context.

1.6 Concerns regarding antimicrobial resistance

This clinical trial is important as there are significant concerns regarding development of resistance against macrolide antibiotics. Although these concerns are somewhat mitigated by the short course of therapy being studied (relative for example to ongoing clinical trials investigating long term treatment in severe asthma), determining whether azithromycin has efficacy in this context will, if the study were negative, this would help limit inappropriate use of antibiotics (in a recent study of adult asthma exacerbations, 57% of subjects received antibiotics[29]).

If the study were positive, then determining the frequencies of detection of standard bacterial, atypical bacterial and viral infections in these exacerbations, combined with the subgroup analyses assessing efficacy of the intervention in those with evidence of one or more of these infections would help guide use of such therapies in subgroups of asthma exacerbations that may respond better to such therapies, as well as guiding future investigation of efficacy of alternative antibiotics with shorter durations of action or different spectra of antimicrobial/viral/inflammatory activity.

1.7 Choice of and duration of therapy

Although the course of therapy is only 3 days, azithromycin has a multiple-dose, tissue half-life of 68 hours and will therefore persist in the lung at significant concentrations for around 10 days after a 3 day course of therapy[30]. The main aim of this study was to determine whether the telithromycin results could be validated in a study with a similar antibiotic, with a similar mechanism and duration of action. telithromycin was given for 10 days (the standard licensed duration of therapy for other respiratory indications) and the primary outcome variable was assessed at 10 days[13]. Since our aim was to determine whether the telithromycin results can be validated, we felt it was important to use the same primary outcome variable and as similar a duration of action as is possible (given that we cannot use telithromycin due to liver toxicity). This was one reason why we chose to study azithromycin rather than other macrolide antibiotics. Other reasons for choosing azithromycin include its antiviral activity not shared with other macrolides[27], a more favourable drug interaction profile[30] and excellent concentration at sites of infection[30].

1.8 Are patterns of airway inflammation associated with aetiology and treatment outcomes?

Different patterns of airway inflammation have been identified in both stable asthma and during exacerbations – these have been classified as neutrophilic, eosinophilic, mixed granulocytic or pauci-granulocytic. However, it is not known whether these different patterns of inflammation are associated with different aetiologies for the exacerbation, nor whether they are related to treatment outcome. Our final aim was therefore to characterise the inflammatory cell profiles in sputum at presentation, to determine whether exacerbation aetiology as well as any possible treatment benefit were related to the types of airway inflammation present (neutrophilic, eosinophilic, mixed or pauci-granulocytic).

1.9 Need for the AZALEA study

There are no systematic reviews of, and no published reports of clinical trials investigating efficacy of azithromycin in the treatment of (acute) asthma exacerbations. At time of protocol development for this study there were no similar studies registered on Clinicaltrials.gov. The only somewhat similar study is NCT00266851 which planned to enrol 200 adult patients with asthma, either stable persistent or in exacerbation and treat for 3 months, to answer the question: will a 12-week treatment with the antibiotic, azithromycin, result in a statistically significant and clinically meaningful improvement in overall asthma symptoms and other patient-oriented asthma outcomes one year after initiation of treatment of adult primary care patients with asthma. Thus the aims, design, timing of outcome analysis and treatment length are clearly very different from the AZALEA study.

CHAPTER 2: RESEARCH OBJECTIVES

2.1 Primary Objective

To assess efficacy of azithromycin using diary card summary symptom score, with symptoms including wheezing, breathlessness and coughing assessed at 10 days after randomisation.

2.2 Secondary Objectives

- The assess the following additional efficacy endpoints:
    - Health status assessed by acute asthma QoLQ (Juniper)
    - Health status assessed by Mini Asthma QoLQ (Juniper)
    - Pulmonary Function tests (FEV_1, FVC, FEV_1/FVC ratio, PEF, FEF25-75%, FEF50%)
- Primary and secondary outcomes assessed 5 and 10 days post randomisation to permit better estimation of optimum timing of primary/secondary outcome variables in future similar studies
- Time to 50% reduction in symptom score

Exploratory analyses

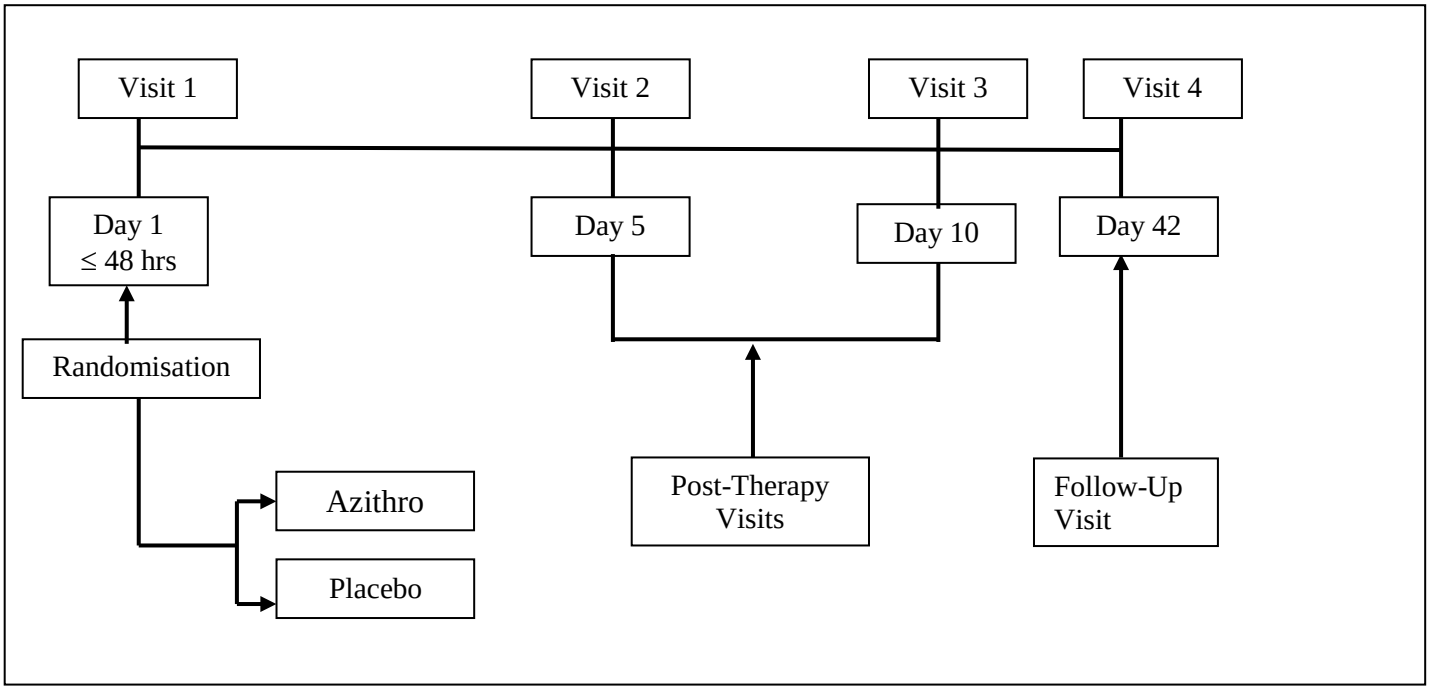
- Trends in primary and secondary outcomes over the time course of the exacerbation up to 10 days
- Assessment of efficacy outcomes in relation to initial *C. pneumoniae* and/or *M. pneumoniae* status
- Assessment of efficacy outcomes in relation to initial standard bacteriological status
- Assessment of efficacy outcomes in relation to initial virological status
- Assessment of efficacy outcomes in relation to initial sputum inflammatory cell status

CHAPTER 3: METHODS

3.1 **Trial design**

This was a multi-centre, randomised, double-blind, placebo-controlled study. Eligible patients were randomised within 48 hours of initial presentation to medical care with an acute deterioration in asthma control and requiring a course of oral steroids. Patients were randomised on a 1:1 basis to receive either 1) azithromycin or 2) placebo. The duration of therapy with study medication (active or placebo) was 3 days, with post-therapy assessments/visits up to 10 days and a follow-up visit at six weeks.

The following diagram summarises the design for the study:



3.2 **PARTICIPANTS**

Adult patients with a documented history of asthma for greater than 6 consecutive months and presenting within 48 hours (of initial presentation to medical care) with an acute deterioration in asthma control and requiring a course of oral steroids.

3.2.1 Inclusion criteria

- Adults, of either sex, aged 18-55 years or aged 56 to 65 with < 20 pack. years smoking history or >65 years with <5 pack. years smoking history
- Patients with a documented history of asthma for >6 consecutive months, **and**
- Patients presenting within 48 hours (of initial presentation to medical care) with an acute deterioration in asthma control (increased wheeze, dyspnoea and/or cough and/or reduced PEF) and requiring a course of oral steroids
- Patients with a PEF or FEV_1 less than 80% of predicted normal or patient's best at presentation, at recruitment or in the time elapsed between presentation and recruitment
- Patients must be able to complete diaries and quality of life questionnaires
- Patients must sign and date an informed consent prior to any study procedures.

3.2.2 Exclusion criteria

- Patients with known prolongation of the QT interval, a history of torsades de pointes, congenital long QT syndrome, bradyarrhythmias or uncompensated heart failure, patients on drugs known to prolong the QT interval and patients with ongoing proarrhythmic conditions such as uncorrected hypokalemia or hypomagnesemia, clinically significant bradycardia, and patients receiving Class IA (quinidine, procainamide) or Class III (dofetilide, amiodarone, sotalol) antiarrhythmic agents.
- Smokers aged 56-65 years with a $\geq$20 pack. year history, or aged >65 years with $\geq$5 pack. year history
- Patients requiring immediate transfer/referral to ICU
- Patients who used oral or systemic antibiotics within 28 days prior to enrolment
- Patients with known impaired hepatic function (ALT/AST > 2 times upper limits of normal)
- Patients with significant lung disease (including COPD) other than asthma
- Patients taking >20mg oral corticosteroid daily as maintenance therapy
- Patients requiring other antibiotic therapy
- Patients who are receiving other medications or who have other disease conditions or infections that could interfere with the evaluation of drug efficacy or safety
- Women who are breast-feeding, or are pregnant, as demonstrated by a urine pregnancy test carried out before exposure to study medication or the start of any study procedure that could pose a risk to the foetus

- Patients with suspected or known hypersensitivity to, or suspected serious adverse reaction to azithromycin or any of the macrolide or ketolide class of antibiotics, erythromycin or to any excipients thereof
- Patients who have received treatment with any other investigational drug within 1 month prior to study entry, or have such treatment planned for the study period during treatment and follow up phase
- Patients with a concomitant condition (including clinically relevant cardiovascular, hepatic, neurological, endocrine, or other major systemic disease) making implementation of the protocol or interpretation of the study results difficult
- Patients with mental conditions rendering them unable to understand the nature, scope, and possible consequences of the study.
- Patients unlikely to comply with the protocol, e.g., uncooperative attitude, inability to return for follow-up visits.
- No patient was allowed to enrol in this study more than once.

3.3 **INTERVENTIONS**

All patients in the study received, per randomised allocation, treatment with either azithromycin or placebo. The identity of the treatment regimen was blinded by encapsulating active medication in opaque capsules to match the placebo.

Those randomised to azithromycin received 500 mg azithromycin (two 250 mg capsules) once a day for 3 days (this is the routine dose given in clinical care). Those patients randomised to the placebo received two placebo capsules once a day for 3 days. The duration of treatment with study medications was 3 days. Patients were instructed to take study medication at least 1 hour before, or 2 hours after, food and if they were taking antacids to take the study drug at least 1 hour before, or 2 hours after, the antacids.

The time of administration of the study medication, and the labeling on the study medication containers was documented on the case report form for patients throughout the study. The first dose was given in the presence of a member of the research team.

3.4 **OUTCOMES**

3.4.1 Primary outcome

Diary card summary symptom score, with symptoms including wheezing, breathlessness and coughing assessed (at) 10 days after randomisation

3.4.2 Secondary outcomes

i. The following additional efficacy endpoints:
- Health status assessed by acute asthma QoLQ (Juniper)
- Health status assessed by Mini Asthma QoLQ (Juniper)
- Pulmonary Function tests (FEV_1, FVC, FEV_1/FVC ratio, PEF, FEF25-75%, FEF50%)

ii. Primary and secondary outcomes assessed 5 and 10 days post randomisation to permit better estimation of optimum timing of primary/secondary outcome variables in future similar studies (the efficacy of telithromycin was only assessed at 10 days).

iii. Time to 50% reduction in symptom score

3.4.3 Exploratory analyses

- Trends in primary and secondary outcomes over the time course of the exacerbation up to 10 days
- Assessment of efficacy outcomes in relation to initial *C. pneumoniae* and/or *M. pneumoniae* status
- Assessment of efficacy outcomes in relation to initial standard bacteriologic status
- Assessment of efficacy outcomes in relation to initial virological status
- Assessment of efficacy outcomes in relation to initial sputum inflammatory cell status

3.5 DATA COLLECTION

3.5.1 Electronic CRF (eCRF)

Data management was through the InForm ITM (Integrated Trial Management) System, a web-based data entry system that builds an Oracle database for each individual clinical trial. Trial data was captured on a bespoke web-based electronic case record form (eCRF) with built-in validation rules to identify data entry errors in real time and a full audit trail of data entry and changes. All persons entering data were trained prior to start up and given personal login details with access to forms restricted according to site and role. The eCRF was designed in accordance with the requirements of the trial protocol and access to the eCRF was password-protected and included controlled level of access.

3.5.2 Timescale of trial evaluations

Daily evaluations:

Visit 1 (day 1) for each patient occurred within 48 hours of initial presentation to medical care with an acute deterioration in asthma control and requiring a course of oral steroids. Patients were then seen by the research team for Visit 2 (day 5 +/- 1 day) and Visit 3 (day 10 +/- 1 day). At visit 1 patients were instructed regarding recording of information in the symptom diary cards and asked to complete the diary each day for 10 days at the end of the day. Symptom diary cards were reviewed at Visit 2 and 3 and recordings entered onto the eCRF.

Follow up evaluation:

This final follow up evaluation took place at Visit 4 (day 42 +/- 2 weeks) to obtain a final serology sample for atypical pathogens and record any AEs.

3.5.3 Schedule of investigations

Table 1: Summary of tests and investigations

Study Procedure	Visit 1 Day 1 Within 48 hrs of initial presentation	Visit 2 Day 5 (+/- 1 day)	Visit 3 Day 10 (+/- 1 day)	Visit 4 Follow up Visit Day 42 (+/- 2 weeks)
Informed consent	X			
Inclusion/Exclusion criteria review	X			
Demographics	X			
Medical/Surgical history	X			
Record previous & concomitant treatments	X	X	X	
Pulmonary function tests ($FEFV_1$, FVC, FEV_1/FVC ratio, $FEF_{25-75\%}$,, $FEF_{50\%}$, peak flow)	X	X	X	
Urine pregnancy test*	X			
Serology for atypical pathogens	X			X
Nose and throat swab and nasal mucus in tissue for PCR	X			
Spontaneous/induced sputum for PCR	X			
Culture of sputum for standard bacteria (quantitative)	X			
Sputum for cell differential and mediators in supernatant	X			
Full Blood Count (FBC)	X			
Dispense diary- Diary training	X			
Diary review		X	X	
Return Diary to investigator		X	X	
Health outcomes assessment - Acute Asthma QoLQ (Juniper)	X	X	X	
Health outcomes assessment - MiniAQLQ (Juniper)	X	X	X	
Randomisation and Dispense study medication	X			
Collect and count unused drug		X		
AE review		X	X	X

KEY:

* if indicated

3.6 CLINICAL INVESTIGATIONS

3.6.1 Pulmonary Function Tests (PFTs)

A spirometer meeting all American Thoracic Society (ATS) recommendations was used for these measurements. PFTs were performed at Visits 1 to 3. PFTs were measured three times in a consistent position (standing or sitting) throughout the study. The best FEV_1, FVC, FEV_1/FVC ratio, $FEF_{25-75\%}$, $FEF_{50\%}$ and peak flow were recorded in the CRF as stated below:

1. Forced expiratory volume in one second (FEV_1) in litres;
2. Forced vital capacity (FVC) in litres;
3. Forced expiratory volume in one second/forced vital capacity (FEV_1/FVC) ratio;
4. Forced Mid-Expiratory Flow Rate ($FEF_{25-75\%}$) in litres/sec;
5. Forced Expiratory Flow Rate at 50% ($FEF_{50\%}$) in litres/sec;
6. Peak expiratory flow (PEF) in litres/min

3.6.2 Patient's Daily Recordings

All patients were supplied with a diary in which to record salbutamol (reliever) use, asthma symptom ratings and number of night-time awakenings due to asthma symptoms. At Visit 1 patients were instructed regarding recording of information in the diary (see below) and asked to complete the diary each day for 10 days at the end of the day (with the nocturnal questions referring to the previous night). They were reminded of the recording instructions at Visits 2 and 3 and to return all of the completed diary cards to the site at Visit 4 All diary cards were retained in the participant files for data entry and monitoring.

I. Daytime symptom diary scale questions

1 How often did you experience asthma symptoms today?

0	1	2	3	4	5	6
None of the time						All of the time

2 How much did your asthma symptoms bother you today?

0	1	2	3	4	5	6

Not at all bothered — Severely bothered

3 How much activity could you do today?

0 1 2 3 4 5 6

More than usual activity — Less than usual activity

4 How often did your asthma affect your activities today?

0 1 2 3 4 5 6

None of the time — All of the time

II. Nocturnal diary scale question

1 Did you wake up with asthma symptoms? (This can be awakening in the middle of the night or on awakening in the morning?)

□ No □ Once □ More than once □ Awake "all night"

III. Number of inhalations of salbutamol will be recorded in the diary. Each patient should be reminded that Salbutamol should be used only as needed for symptoms, not on a regular basis or prophylactically.

IV. Study medication will be recorded in the diary. Any concomitant medication use will be recorded in the diary.

V. Adverse Events - patients will record all unusual health related events in the diary regardless of relationship to medication.

3.6.3 Clinical sample collection

Respiratory samples

- A nasal mucus sample and nasal and throat swab was taken where possible at Visit 1 for PCR for viruses and atypical bacteria. Nasal mucus samples were taken using a clean soft tissue subsequently placed into a freezer plastic bag, stored at -80^{0}C and transferred at intervals to Imperial College for analysis. Sites were supplied with Flocked swabs for nasal and throat sample collection. Swabs were taken and then placed into a bijoux with PBS or normal saline, frozen at -80^{0}C and sent to Imperial College at intervals for analysis.

- At Visit 1 in patients with a productive cough, deep expectorated sputum was collected after rinsing the mouth with sterile water. Deep cough specimen was collected into a sterile Petri dish and patients instructed not to expectorate saliva or postnasal discharge into the container.
- In patients unable to produce an adequate sample of spontaneous sputum, sputum was induced according to published protocols using isotonic saline [7, 31] if the visit took place at the recruiting site.
- Sputum supernatants and cytospin slides were sent in batches by sites to Imperial College to be processed for Polymerase Chain Reaction (PCR), standard bacteriology and cytospin and supernatant production. Anyone responsible for sputum processing at sites received specific training from the University of Leicester which was documented by either a training certificate or written confirmation that previous training in the area was sufficient and additional training was not required.
- If sputum was not obtained at visit 1 because of nonproductive cough or for any other reason, this was documented on the case report form. If there was no attempt to collect sputum this was reported as a protocol deviation.

Serology

Acute (Visit 1) and convalescent (Follow up visit day 42) serum samples were obtained, and analysed in Imperial College laboratories for atypical pathogens. At Visit One (Day 1) and Visit Four (Day 42) 10mls of blood was collected, processed at sites to obtain serum and transferred immediately to a -80°C freezer. At intervals these stored aliquots were sent to Imperial College for analysis. In addition, at Visit One (Day 1) an additional 3mls of blood was collected from patients for standard hospital analysis for a Full Blood Count (FBC).

3.6.4 Health outcomes data

Health outcomes were measured to determineoverall assessment of symptom resolution during the first ten days based on global subject diary assessment.

Health Status was assessed at visits 1 to 3 using – Acute AsthmaQoLQ (Juniper)
– Mini Asthma AQoLQ (Juniper)

Each site was provided with:

- Acute Asthmas Quality of Life Questionnaire (AQLQ)
- Mini AQLQ
- Background information, administration and analysis on AQLQ

- AQLQ coloured cards

All staff at sites who were delegated responsibility to administer the AQLQs were asked to familiarize themselves with the contents of the above before administering any questionnaires.

Questionnaires were all Interviewer administered and not Self-Administered. The Acute AQLQ contains a response sheet with columns for 'Responses 1st, 2nd and 3rd' which were used to record the responses at Visit 1, 2 and 3 respectively. A new mini AQLQ was printed for each visit when the mini AQLQ was administered and patient responses recorded directly onto the AQLQ. All patient responses/completed AQLQs were kept in the participant files for Source Data Verification (SDV) and also entered into the InForm eCRF database.

Sites were asked, if possible, for the AQLQ to be the first questionnaire completed during a clinic visit to precede any discussion with a health professional as this may have influenced how the patient completed the questionnaire.

3.7 PHARMACOVIGLILANCE DEFINITIONS AND PROCEDURES

3.7.1 Definitions

3.7.1.1 Adverse Event (AE):

An Adverse Event was defined as any untoward medical occurrence in a patient (or clinical trial subject) administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

The adverse event may have been:

- A new illness
- Worsening of a sign or symptom of the condition under treatment, or of a concomitant illness
- An effect of the study medication, including comparator
- A combination of two or more of these factors.

If the event met the criteria of serious, then the event was reported as a serious adverse event (see below).

3.7.1.2 Adverse Reaction (AR)

All AEs judged by either the reporting investigator or the Sponsor as having reasonable causal relationship to a medicinal product were reported as adverse reactions.

3.7.1.3 Unexpected Adverse Reaction

An AR, the nature or severity of which was not consistent with the summary of product characteristics (SmPC) for azithromycin was reported as an Unexpected Adverse Reaction. Side effects documented in the SmPC which occurred in a more severe form than anticipated were also considered to be unexpected.

3.7.1.4 Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)

Any untoward medical occurrence or effect that at any dose:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity** – *there is a substantial disruption of a person's ability to carry out normal life functions*
- **Is a congenital abnormality or birth defect**

Medical judgement was exercised in deciding whether an AE/AR was serious in other situations. Important AE/ARs that were not immediately life-threatening or did not result in death or hospitalisation but may have jeopardised the subject or may have required intervention to prevent one of the other outcomes listed in the definition above, were also considered serious.

Hospitalisation of the patient as a direct result of the asthma exacerbation was not recorded as an SAE as this was part of the patients' routine clinical care and not related to their participation in the trial.

3.7.1.5 Suspected Unexpected Serious Adverse Reaction (SUSAR)

Any suspected adverse reaction related to an IMP that was both unexpected and serious.

3.7.2 Causality

The assignment of the causality of adverse events and reactions was made by the investigator responsible for the care of the participant using the definitions in the table below. If any doubt about the causality existed the local investigator would inform the Chief Investigator.

Relationship	Description
Unrelated	There is no evidence of any causal relationship
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

3.7.3 Period of observation

For the purposes of this study, the period of observation extended from the time the subject gave informed consent until 7 days after the last dose of study medication.

3.7.4 Reporting Procedures

All adverse events that occurred after the subject had signed the informed consent were documented on the pages provided in the case report form. The trial eCRF included dedicated forms for reporting SAEs.

3.7.4.1 Non serious AR/AEs

All such events, whether expected or not, were recorded in the relevant case report form. These were reported to the MHRA and REC on the annual safety report form on the anniversary of the date a favourable opinion for the study was given.

3.7.4.2 Serious AR/AEs/SUSARs

Fatal or life threatening SAEs and SUSARs were reported to the Chief Investigator (who reported to the Sponsor) on the day that the local site became aware of the event. The SAE form included nature of event, date of onset, severity, corrective therapies given, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). Additional information was sent to the CI and Sponsor within 5 days if the reaction had not resolved at the time of reporting.

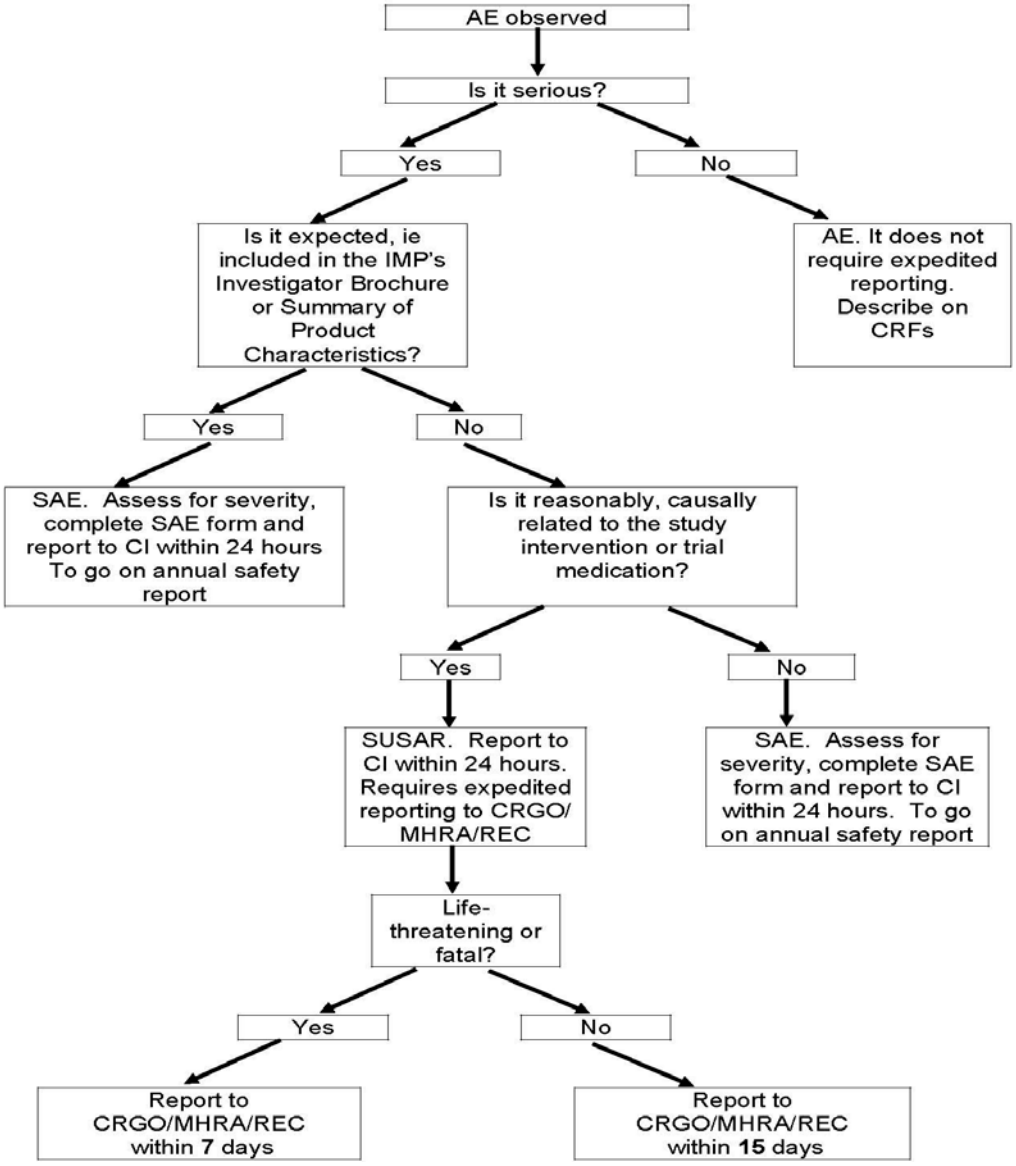
SAEs

Investigators were advised to report SAEs via the eCRF within 24 hours of becoming aware of the event and to include an assessment of expectedness and causality in the SAE report. Each SAE report was reviewed by the Clinical Trials Unit and Chief Investigator. A flowchart is given below to show the reporting procedures (Figure 1).

SUSARs (Suspected Unexpected Serious Adverse Reaction)

If an AE was considered serious, unexpected and related to the IMP (possible, probable or definitely related) this would have met the definition of Suspected Unexpected Serious Adverse Reaction requiring expedited reporting to the MHRA, REC and Sponsor. There were no SUSARs for the AZALEA trial.

Figure 1 Reporting procedure for Adverse Events



3.7.5 Annual Safety reports

Annual Safety reports were provided to the REC and MHRA, in accordance with clinical trial regulations, on the anniversary of the Clinical Trial Authorisation each year. A total of three annual safety reports were submitted over the course of the trial.

3.8 Statistical considerations

3.8.1 Sample size

The sample size calculations were based on the primary outcome: change from baseline in diary card

summary asthma symptom scores at 10 days after randomisation. Our previous study[13] found a mean decrease in symptom score of 1.3 in the treatment group, and 1 in the control group, resulting in the difference of -0.3 (SD 0.783) between the groups at 10 days.

Using a two-sided t-test at 1% significance level, with 80% power, 161 patients in each group were needed to be detect the same difference in asthma scores between the groups. The significance level of 1% in the above calculation was chosen to provide greater certainty in assessment of the primary outcome variable, as well as to provide greater power for the subgroup exploratory analyses, as those subgroup analyses that were performed were uninformative in the 280 patient Telicast study[13].

Taking into account the drop-out rate of 15% in the study [13], we proposed to recruit 190 patients in each arm of the study. To be able to run the trial within the project timelines, we initially intended to involve 10 centres.

3.8.2 Randomisation

Randomisation was web-based via access to a secure Imperial College server performed using the InForm ITM (Integrated Trial Management) System, a web based data entry system that builds an Oracle database for each individual clinical trial. Patient allocation was stratified by centre performed in random length blocks. Either the Research Nurse or Principal Investigator at each site enrolled and then randomised each patient into the study using the InForm database.
The randomisation lists were generated by an Imperial Clinical Trials Unit (ICTU) statistician. Details such as the block size were kept confidential and held separately by ICTU.

3.8.3 Blinding

This was a double blind trial so all participants and care providers and therefore those assessing outcomes were blinded to study treatment.

The identity of the study medications was blinded, packaged and supplied to the investigator by Sharp Clinical Services with code break envelopes. Over-encapsulated azithromycin capsules and placebo capsules were placed into child-resistant tamper-evident containers and a randomised label applied to each container.

Emergency identification of study medication/unblinding

If it was medically imperative to know what study medication the subject was receiving, the investigator or authorised person would be able to contact the on call pharmacist who could open the relevant code break envelope that corresponded to the randomisation label on the patients study drug container, exposing the blinded information. Clear instructions were provided to sites to ensure that no unnecessary or unintentional un-blinding occurred and clear guidelines on when a code break envelope should be opened were given. Any code break must have been documented in the code break log and the Trial Manager notified in writing as soon as possible.

There was no requirement for unblinding during the AZALEA Trial therefore no patients were unblinded before the statistical analysis took place.

3.8.4 Statistical methods

The modelling process of patient diary scores was based on the methods outlined in the Statistical Analysis Plan (SAP). All patients who returned at least one diary card (and received study drug) were included in the analysis but only those diary cards which were collected in the investigated 10 days timeframe were included. Clinical efficacy analyses were carried out on an intention-to-treat basis. Multilevel modelling was used to calculate the estimated differences in diary scores for each day between the treatment arms. As outlined in the SAP, different models were compared, their goodness of fit was assessed by residual plots. The models differed in their change over time term, which was the interaction between time and treatment. Linear, quadratic, square root relationships and the use of splines were investigated. Details of the statistical model, model selection process and the statistical details of the covariates and fixed and random effects of the model can be found in Appendix 2. Similar models were used to assess the day 10 differences in change in Acute AQLQ and Mini AQLQ scores and pulmonary functions between the two treatment arms.

All analyses were performed using Stata 13 [38].

3.8.5 Missing data

Before starting data analysis, the level and pattern of the missing data in the baseline variables and outcomes was analysed by forming appropriate tables. Additionally, the likely causes of any missingness were investigated. This information was used to determine whether the level and type of missing data had the potential to introduce bias into the analysis or to substantially reduce the precision of estimates related to treatment effects.

Missing data in the patient diary took one of several forms: no patient diary returned for any day (patient missingness), all data missing for one or more days (day missingness) and data missing for some but not all the individual questions for a particular day (item missingness). Of these, the level of item missingness was expected to be minimal. If any item missingness occurred in diary scores, the scores for the missing questions were interpolated from the previous and subsequent day scores.
If any item missingness occurred in AQLQ scores the summary score for that day was treated as missing.
Missing data for the pulmonary function tests were expected to be due to the spirometer not recording some measures. As this was unrelated to the patient outcome, it was reasonable to assume that this missingness was uninformative and that multi-level models fitted to all observed data would provide unbiased parameter estimates.

3.8.6 Statistical Analysis Plan

A SAP was prepared by the trial investigators and trial statistician and reviewed and agreed by the TSC and DMEC prior to the end of the recruitment period.

3.9 Trial organisation

3.9.1 Trial Management

The UKCRC registered Imperial Clinical Trials Unit (ICTU) was responsible for trial management, quality assurance, trial statistics and development and maintenance of the trial database. A dedicated Trial Manager and Clinical Trials Monitor were appointed through ICTU to oversee the day to day management and monitoring of the project from set up to close.

3.9.2 Trial Sponsor

The Sponsor of the trial was Imperial College London. The Sponsor's role is clearly set out in the European Clinical Trials Directive and NHS Research Governance documents. Imperial College London signed a clinical trial agreement with each of the participating centres prior to the start of the recruitment at each centre.

3.9.3 Ethical considerations

The trial was conducted in accordance with the Declaration of Helsinki (http://www.wma.net/) on research involving human subjects. The study protocol, patient information sheet and consent form were submitted to the Research Ethics Committee (REC) prior to the start of the study and a favourable opinion was obtained on the 15th June 2011.

3.9.4 Consent

Patients were given the patient information sheet, given sufficient time to consider participation and discussed the trial with the research staff prior to consent and enrolment. Full written informed consent was taken using the ethically approved consent form.

3.9.5 Research governance

The trial was carried out in accordance with the NHS Research Governance Framework and local NHS permission was granted by the Research and Development departments at each participating site prior to recruitment commencing.

3.9.6 Regulatory requirements

As a randomised trial of an IMP, AZALEA was conducted in accordance with the European Clinical Trials Directive and the Medicines for Human Use (Clinical Trials) Regulations 2004 as well as ICH GCP guidelines. The trial received clinical trials authorisation (CTA) from the Medicines and Healthcare Regulatory Agency (MHRA) on the 21st July 2011 and was registered in the European Community with a EudraCT number: 2011-001093-26.

3.9.7 Trial registration

The trial was registered on the clinicaltrials.gov clinical trial database with the following reference: NCT01444469 .

3.9.8 NIHR CRN portfolio

The AZALEA trial was adopted on the NIHR Clinical Research Network (CRN) portfolio with the UKCRN ID number 11358. Accrual data were uploaded onto the NIHR CRN database on a monthly basis.

3.9.9 Summary of protocol amendments

The following amendments were made to the trial protocol following approval of the first version of the document by the REC and (MHRA):

- Version 2: In addition to minor typographical clarifications to the wording of the protocol the following changes were made: addition of a throat swab (in case sufficient sample was not obtained from the nasal mucus and nasal swab); refinement of inclusion criteria to include FEV_1 as well as PEF as a measurement of lung function; refinement of exclusion criteria to clarify the type of antibiotic use that would be excluded and inclusion of a statement that hospitalisation as a direct result of the asthma exacerbation was not an SAE
- Version 3: Refinement of inclusion criteria to include patients aged over 65 years with less than 5 pack years smoking history
- Version 4: Refinement of the eligibility criteria to include patients presenting within 48 hours (of initial presentation to medical care) with an acute deterioration of asthma control (instead of 24 hours as in the previous protocol version), recruitment extension to April 2014 and minor administrative changes
- Version 5: Protocol amendment to introduce participant reimbursements for completing study visits and returning all symptom diaries
- Version 6: Addition of an extra exclusion criterion to reflect guidelines released from the FDA on the use of azithromycin

3.9.10 Trial Committees

3.9.10.1 Trial Steering Committee

A Trial Steering Committee (TSC) was established to oversee the conduct of the study. The TSC met seven times over the course of the trial; on 06/01/2012, 05/07/2012, 18/01/2013, 04/04/2013, 31/10/2013, 10/04/2014 and 24/07/2015. Copies of the minutes from each meeting were sent to the funder, the Efficacy and Mechanism Evaluation program (EME) of the National Institute of Health Research (NIHR). The TSC approved the trial protocol prior to the start of the study and received regular recruitment reports throughout the duration of the trial.

The TSC membership is listed below:

Independent members

Professor Wisia Wedzicha – Chair

Professor Peter Calverley - Independent Member

Professor Ratko Djukanovic – Independent Member

Ms Leanne Metcalf, Asthma UK – Patient representative, Independent Member

Professor Mike Thomas – Independent Member

Non-members in attendance

Professor Deborah Ashby – Senior Statistician

Professor Chris Brightling – Principal Investigator, Leicester

Mrs Mary Cross – Operations Manager, Imperial Clinical Trials Unit

Professor Sebastian Johnston – Chief Investigator

Ms Laura Robison – Trial Manager (until February 2013)

Dr Zahid Sattar – Trial Manager (until April 2015)

Dr Jane Warwick – Senior Statistician (until June 2014)

Dr Alexina Mason – Junior Statistician (until Jan 2015)

Dr Ernie Wong – Research Fellow, Imperial College

3.9.10.2 Data Monitoring and Ethics Committee

An independent Data Monitoring and Ethics Committee (DMEC) was established to review SAE reports and any ongoing safety issues. The DMEC meetings took place on 31/05/2012, 29/11/2012, 02/12/2013 and 24/07/2015.

The first DMEC meeting to agree the charter outlining operational details and responsibilities took place early in the trial, on 31/05/2012. The DMEC provided feedback reports for each meeting to the Chair of the TSC and this was reviewed at subsequent TSC meetings as applicable.

The DMEC membership is listed below:

Independent members

Professor Jonathan Grigg – Chair

Dr Stephen Bremner – Independent Statistician

3.9.11 Data management

Pre-defined data ranges were included in the eCRF which raised automated queries if data outside of the expected range were entered. In addition to the automated queries, the trial data were reviewed on a regular basis by the Trial Monitor to look for discrepancies and errors. In addition to the regular checks performed by the Trial Monitor, the Trial Statistician also performed a series of checks on snapshots of data to look for inconsistencies.

3.9.12 Risk assessment and Monitoring Plan

A risk assessment was performed by the ICTU Quality Assurance (QA) Manager prior to the start of the trial. The result of the risk assessment indicated that the study was medium risk and that 50% of trial data, 100% consent forms and 100% SAEs should be source verified. A monitoring plan was prepared in accordance with the risk assessment to specify the frequency of monitoring visits and amount of source data verification required.

The requirements of a medium risk trial for monitoring are:

At least 2 monitoring visit to be performed or 1-3 per annum/SDV of 50% of subjects for eligibility, existence, drug delivery (to patients), end points, AEs/ SDV of 100% of consent forms and SAEs/Verify research approvals, drug accountability, regulatory documents and archiving.

3.9.13 Monitoring visits

A site initiation visit was performed at all participating centres. Interim monitoring visits were carried out depending on the recruitment rate, and closeout visits were carried out at all centres following the final follow-up visit for the last patient recruited. The monitoring visits were conducted mainly by the Trial Monitor.

3.9.14 Investigational Medicinal Product Manufacturer

Over-encapsulation of azithromycin capsules and production of matching placebo was undertaken by Sharp Clinical Services, an MHRA licensed manufacturing unit with expertise in manufacturing and over-encapsulating IMP.

3.9.15 Patient and Public Involvement

Patient representatives were consulted during preparation of the patient information sheets. The TSC membership included a patient representative from Asthma UK who was invited to attend all TSC meetings and included in all relevant correspondence.
In addition, the Trial Manager attended the National Heart and Lung Institute at the Royal Brompton Hospital on several occasions to meet the respiratory consumer group. At the group meetings an update on study progress was given and any relevant issues including the following were discussed:

1) Patient leaflet and poster – the group were asked for their comments and feedback on the language and appropriateness of these as a tool to help introduce the study to patients.

2) Ongoing issues affecting recruitment such as whether the group felt patients would be more likely to participate in the study if they were approached by a study doctor rather than nurse (in looking at why consenting to the study was low) and whether introducing patient payments for visits would increase the number of visits attended (in looking at why the level of missing visits was high).

Useful feedback was received from the group and incorporated into study documents and procedures where relevant as the study progressed.

3.9.16 Data Archiving

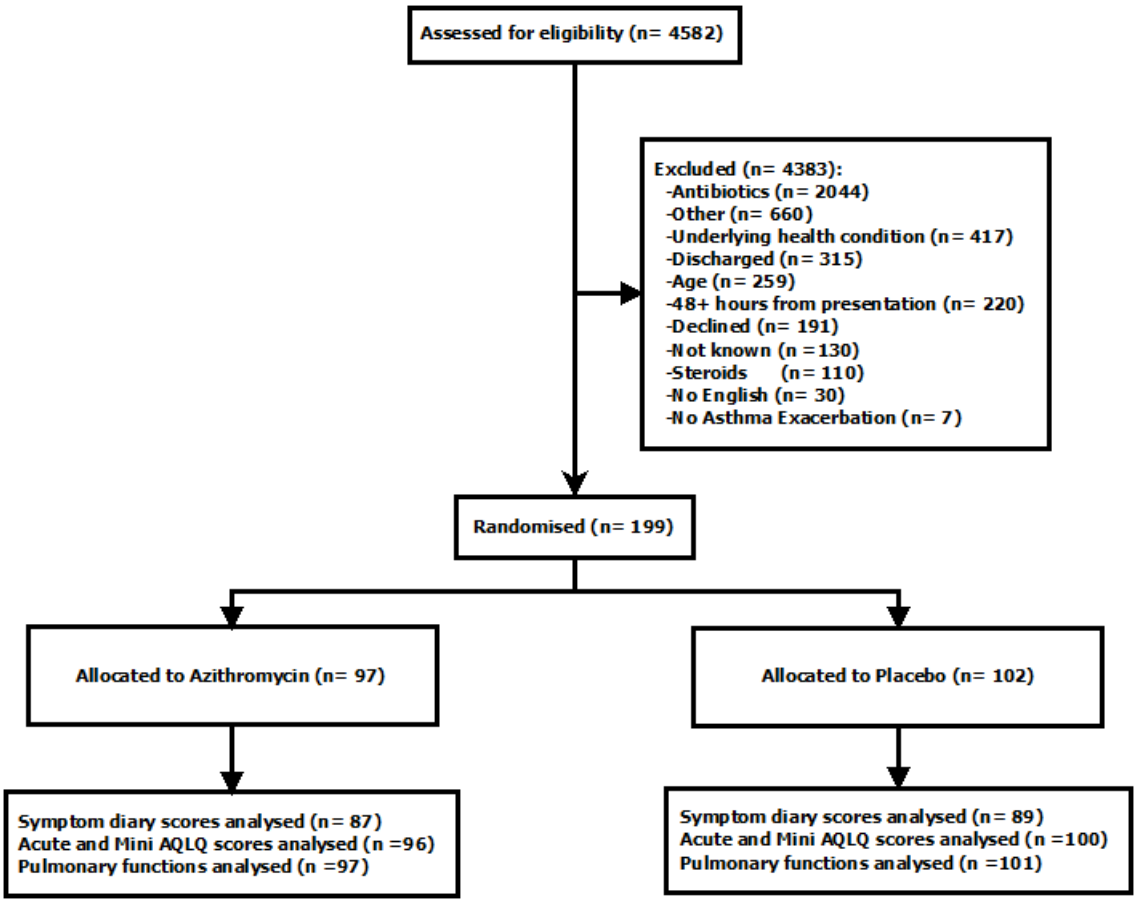
This trial complies with the Imperial College records management policy which includes the retention schedule for data by type; transferring records to the College archive facility (records include both paper and electronic records), there is a specific section relating to Clinical Trials Transfer of records; retrieval of records and access records are also included in the policy. Data is kept for a minimum of 10 years to comply with Imperial College policy. All available trial data can be obtained from the corresponding author.

CHAPTER 4: RESULTS

4.1 Participant flow

The flow of patients is summarised in Figure 2 including number of patients screened, randomised and completing the trial.

Figure 2. CONSORT diagram of the AZALEA trial



4.1.1 Screening

4582 patients were screened at the participating hospitals/centres over the duration of the trial. Of the 390 patients meeting the eligibility criteria, 199 were randomised to the trial as 191 declined to participate. Table 2 summarises the number of screened and eligible patients recruited to the trial and Table 3 the reasons for non- recruitment.

Table 2: Summary of screening data for all trial sites

Centre[1]	Date Opened	Patients Screened[2]	Recruited
Queen Alexandra, Portsmouth	05/12/2011	298	56
Birmingham Heartlands	10/01/2012	520	29
Western & Royal Infirmary, Glasgow	16/12/2011	1333	28
University Hospital of North Tees	16/10/2012	199	10
Nottingham City & QMC	02/04/2012	172	10
Glenfield Leicester	02/02/2012	182	10
St Mary's, London	17/10/2011	414	11
New Cross, Wolverhampton	25/06/2013	25	8
East Surrey Hospital	27/03/2013	84	7
Blackpool Victoria	14/12/2012	78	6
Rowden GP Surgery, Chippenham	11/03/2013	11	6
Ipswich Hospital	24/04/2013	108	3
St James's University Hospital, Leeds	16/01/2013	100	3
Worcestershire Acute Hospitals NHS Trust	27/03/2013	43	2
Countess of Chester	07/02/2013	39	2
Norfolk and Norwich University Hospital	11/01/2013	43	3
Gloucestershire Royal Hospital	20/01/2014	45	1
Princess Royal Hospital, Telford	09/12/2013	32	1
Musgrove Park Hospital, Taunton	25/03/2013	85	1
Royal Berkshire Hospital	07/02/2013	136	1
Freeman, Newcastle	02/04/2012	32	1
Guy's and St Thomas' NHS Foundation Trust	18/04/2012	253	0
Charing Cross Hospital, Imperial College Healthcare NHS Trust	22/11/2011	131	0
Derriford Hospital, Plymouth	21/03/2013	96	0
University Hospital of South Manchester NHS Foundation Trust	12/06/2012	56	0
Hammersmith Hospital, Imperial College Healthcare NHS Trust	17/01/2012	31	0
Sherwood Forest Hospitals NHS Foundation Trust	01/11/2012	22	
Great Western Hospitals NHS Trust, Swindon	25/03/2013	-	0
Barnsley Hospital	21/10/2013	-	0
James Cook Hospital, South Tees	12/12/2013	14	0
Leighton Hospital, Crewe	04/12/2013	-	0
Total		4582	199

[1] Ordered by number of patients recruited

[2] Patients presenting with an acute exacerbation of asthma and considered for AZALEA (includes those recruited)

Table 3: Reasons for exclusion

Reason	Number of patients	Comments
No English	**30**	
>48 hours from presentation	**220**	includes >24 hours from presentation
Antibiotics	**2044**	
No asthma exacerbation	**7**	includes no asthma exacerbation and no exacerbation
Underlying health condition	**417**	includes COPD and co-morbidities
Declined	**191**	
Other	**660**	
Steroids	**110**	patients not requiring steroids are listed as 'steroids' or 'no steroids' in comments
Age	**259**	
Discharged	**315**	includes 'unable to contact'
Not known	**130**	Reason and time of screening was not recorded
Total	**4383**	

4.1.2 Recruitment and retention

Recruitment lasted for 2.5 years, from September 2011 to April 2014. The actual recruitment period was longer than the original target of 1 year. The delays in starting the trial and continuing issues with slower recruitment were associated with the following:

a) Delays in opening sites to recruitment

b) In the initial year of recruitment we encountered an unusually mild winter with lower than anticipated numbers of asthma exacerbations

c) One of the greatest factors restricting recruitment was patients being prescribed antibiotics by doctors (A&E and GP), despite the current British Thoracic Society (BTS) guidelines stating antibiotics should not be routinely given to treat asthma exacerbations.

d) Research teams at some recruiting sites were based at a different hospital to the A&E department; consequently the research nurse and Investigator were not always available to travel to the other site to recruit patients.

4.1.3 Recruitment rate

The target recruitment rate for the study was 3 to 4 patients per month per centre, based on the original ten centres recruiting and a target recruitment figure of 380.

Accrual of patients during the whole study period is presented in Figure 3. Monthly and cumulative accrual of patients is shown in Figure 4.

Figure 3: Accrual of patients into the AZALEA trial

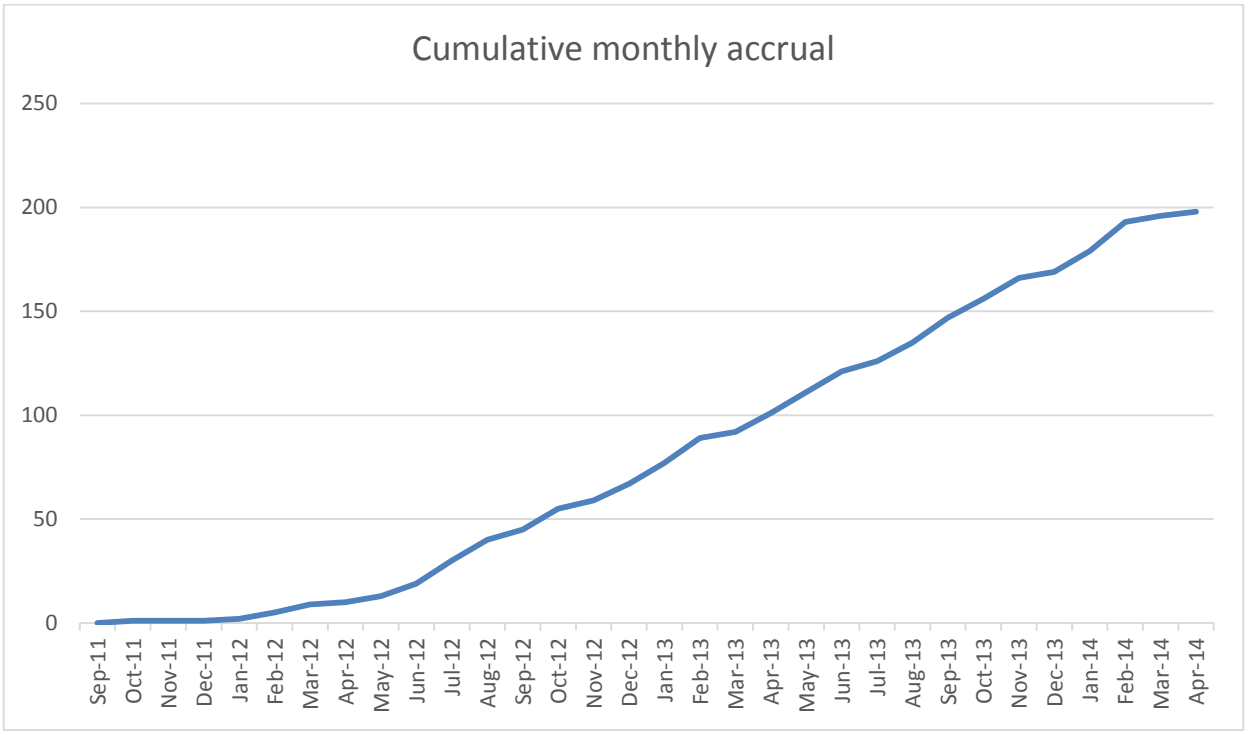
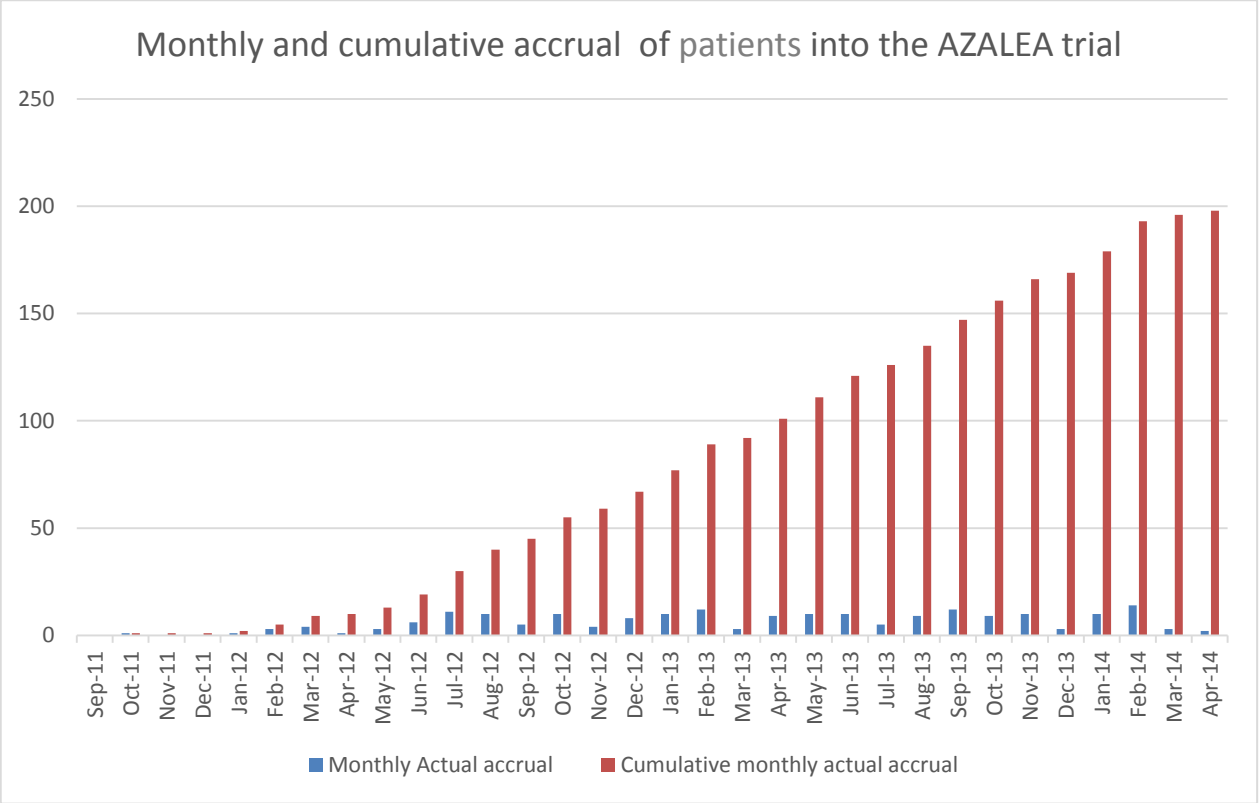


Figure 4: Monthly and cumulative accrual of patients into the AZALEA trial



4.2 Baseline data

4.2.1 Clinical characteristics

Baseline characteristics of all randomised patients were summarised by treatment group using appropriate median and interquartile range for continuous, number and percentage for categorical variables in Table 4. In order to check for any differences in baseline characteristics between centres, the same variables for centres that recruited at least 10 patients were compared in Table 5. Baseline characteristics were well balanced across treatment arms and centres.

Table 4: Baseline characteristics of patients by treatment group

Factor	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)		
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 or frequent use of 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)

Table 5: Baseline characteristics of patients by centre (N≥10)

	Centre					
Factor	**BIR**	**GLA**	**LEI**	**POR**	**SMH**	**UNT**
N	29	28	10	56	11	10
Age (years), median (IQR)	38.2 (28.3, 43.4)	32.6 (27.45, 44.8)	50.35 (26.7, 62.6)	37.15 (26.25, 49.5)	42.3 (23.2, 58.2)	41.75 (39.1, 58.7)
Gender						
F	22 (76%)	18 (64%)	4 (40%)	39 (70%)	8 (73%)	8 (80%)
M	7 (24%)	10 (36%)	6 (60%)	17 (30%)	3 (27%)	2 (20%)
Asthma Severity						
step 1: mild intermittent asthma	0 (0%)	5 (18%)	1 (10%)	5 (9%)	1 (9%)	0 (0%)
step 2: regular preventer therapy	8 (28%)	3 (11%)	3 (30%)	12 (21%)	7 (64%)	5 (50%)
step 3: initial add-on therapy	15 (52%)	8 (29%)	3 (30%)	13 (23%)	1 (9%)	4 (40%)
step 4: persistent poor control	4 (14%)	9 (32%)	2 (20%)	17 (30%)	2 (18%)	1 (10%)
step 5: continuous or frequent use of oral steroids	2 (7%)	3 (11%)	1 (10%)	9 (16%)	0 (0%)	0 (0%)
Smoking status						
never smoked	18 (62%)	19 (68%)	7 (70%)	29 (52%)	9 (82%)	5 (50%)
former smoker	5 (17%)	5 (18%)	2 (20%)	16 (29%)	1 (9%)	5 (50%)
current smoker	6 (21%)	4 (14%)	1 (10%)	11 (20%)	1 (9%)	0 (0%)
Pack years, median (IQR)	5 (2, 10)	2 (2, 8)	8 (1, 15)	7 (2, 15)	2 (1, 3)	10 (10, 20)
Asthma Exacerbation						
Mild Asthma Exacerbation	0 (0%)	3 (11%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)
Moderate Asthma Exacerbation	5 (17%)	12 (43%)	3 (30%)	12 (21%)	2 (18%)	5 (50%)
Acute Severe Asthma	24 (83%)	13 (46%)	5 (50%)	38 (68%)	8 (73%)	5 (50%)
Life Threatening Asthma	0 (0%)	0 (0%)	1 (10%)	5 (9%)	1 (9%)	0 (0%)
Near-Fatal Asthma	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)
Time from presentation to study drug, median (IQR)	20 (9, 26)	23 (18, 36)	11.5 (6, 19)	23 (17, 30)	19 (16, 21)	24 (24, 27)

BIR = Birmingham Heartlands;
GLA = Western & Royal Infirmary, Glasgow;
LEI = Glenfield Leicester;
POR = Queen Alexandra, Portsmouth;
SMH = St Mary's Hospital, Imperial College Healthcare NHS Trust
UNT = University Hospital of North Tees

4.2.2 Biological samples

Sputum bacteriology results by treatment arm are shown in Table 6 and 7. Sputum virology results are shown in Table 8 and nasal virology in Table 9. Joint result of sputum and nasal swab virology are shown in Table 10.

Table 6: Sputum bacteriology results

Factor	Active	Placebo
N	97	102
Sputum samples collected*	52 (53.6%)	53 (52.0%)
Streptococcus pneumoniae		
Negative	21 (21.6%)	21 (20.6%)
Positive	3 (3.1%)	3 (2.9%)
Result Not Available	73 (75.2%)	78 (76.5%)
Haemophilus influenzae		
Negative	24 (24.7%)	20 (19.6%)
Positive	0 (0.0%)	4 (3.9%)
Result Not Available	73 (75.2%)	78 (76.5%)
Moraxella catarrhalis		
Negative	23 (23.7%)	22 (21.6%)
Positive	1 (1.0%)	1 (1.0%)
Result Not Available	73 (75.2%)	79 (77.5%)
Any bacteria positive including Sputum, Nasal and Serology results	9 (9.3%)	12 (11.8%)

*not all of the collected samples were sufficient quantity/quality for processing

Table 7: Atypical bacteriology results

Factor	Active	Placebo
N	97	102
Sputum:		
C.pneumoniae-negative and M.pneumoniae-negative	40 (41.2%)	36 (35.3%)
Not available	57 (58.8%)	66 (64.7%)
Nasal:		
C.pneumoniae-negative and M.pneumoniae-negative	95 (97.9%)	96 (94.1%)
Not available	2 (2.1%)	6 (5.9%)
Serology:		
C.pneumoniae-positive and M.pneumoniae-negative	1 (1.0%)	1 (1.0%)
C.pneumoniae-negative and M.pneumoniae-positive	4 (4.1%)	3 (2.9%)
C.pneumoniae-negative and M.pneumoniae-negative	79 (81.4%)	77 (75.5%)
Not available	13 (13.4%)	21 (20.6%)
C. pneumoniae and M. pneumonia including Sputum, Nasal and Serology results		
C.pneumoniae-positive and M.pneumoniae-negative	1 (1.0%)	1 (1.0%)
C.pneumoniae-negative and M.pneumoniae-positive	4 (4.1%)	3 (2.9%)
C.pneumoniae-negative and M.pneumoniae-negative	79 (81.4%)	77 (75.5%)
Not available	13 (13.4%)	21 (20.6%)

Table 8: Sputum virology

Factor	Active	Placebo
N	37	34
Rhinovirus		
Negative	31 (84%)	26 (76%)
Positive	6 (16%)	8 (24%)
Other Picornaviruses		
Negative	35 (95%)	31 (91%)
Positive	2 (5%)	3 (9%)
Adenoviruses		
Negative	37 (100%)	34 (100%)
Bocavirus		
Negative	37 (100%)	34 (100%)
Respiratory Syncytial Virus		
Negative	34 (92%)	30 (88%)
Positive	3 (8%)	4 (12%)
Influenza AH1/AH3/B		
Negative	37 (100%)	34 (100%)
Parainfluenza viruses 1-3		
Negative	37 (100%)	34 (100%)
HMPV		
Negative	37 (100%)	34 (100%)
Coronaviruses 229E and/or OC43		
Negative	37 (100%)	34 (100%)
Any virus		
Negative	27 (73%)	20 (71%)
Positive	10 (27%)	14 (29%)

Table 9: Nasal virology results

Factor	Active	Placebo
N	95	96
Rhinovirus		
Negative	88 (92.6%)	87 (90.6%)
Positive	7 (7.4%	8 (8.3%)
Result Not Available	0	1 (1.1%)
Other Picornaviruses		
Negative	95 (100%)	95 (98.9%)
Positive	0	0
Result Not Available	0	1 (1.1%)
Adenoviruses		
Negative	95 (100%)	95 (98.9%)
Positive	0	0
Result Not Available	0	1 (1.1%)
Bocavirus		
Negative	95 (100%)	95 (98.9%)
Positive	0	0
Result Not Available	0	1 (1.1%)
Respiratory Syncytial Virus		
Negative	95 (100%)	95 (98.9%)
Positive	0	0
Result Not Available	0	1 (1.1%)
Influenza AH1/AH3/B		
Negative	95 (100%)	95 (98.9%)
Positive	0	0
Result Not Available	0	1 (1.1%)
Parainfluenza viruses 1-3		
Negative	95 (100%)	95 (98.9%)
Positive	0	0
Result Not Available	0	1 (1.1%)
HMPV		
Negative	95 (100%)	95 (98.9%)
Positive	0	0
Result Not Available	0	1 (1.1%)
Coronaviruses 229E and/or OC43		

Negative	93 (97.9%)	93 (96.9%)
Positive	2 (2.1%)	2 (2%)
Result Not Available	0	1 (1.1%)
Any virus		
Negative	86 (90.5%)	85 (88.5%)
Positive	9 (9.5%)	10 (10.4%)
Result Not Available	0	1 (1.1%)

Table 10: Sputum and Nasal virology results

Factor	Active	Placebo
N	95	95
Rhinovirus		
Negative	85 (89%)	82 (86%)
Positive	10 (11%)	13 (14%)
Other Picornaviruses		
Negative	93 (98%)	92 (97%)
Positive	2 (2%)	3 (3%)
Adenoviruses		
Negative	95 (100%)	95 (100%)
Bocavirus		
Negative	95 (100%)	95 (100%)
Respiratory Syncytial Virus		
Negative	92 (97%)	91 (96%)
Positive	3 (3%)	4 (4%)
Influenza AH1/AH3/B		
Negative	95 (100%)	95 (100%)
Parainfluenza viruses 1-3		
Negative	95 (100%)	95 (100%)
HMPV		
Negative	95 (100%)	95 (100%)
Coronaviruses 229E and/or OC43		
Negative	93 (98%)	93 (98%)
Positive	2 (2%)	2 (2%)
Any viral test		
Negative	79 (83%)	75 (79%)
Positive	16 (17%)	20 (21%)

4.2.3 Pulmonary function tests

Pulmonary function tests results at baseline (visit 1) are shown in Table 11 by treatment arm and in Table 12 by centres with ≥10 patients recruited.

Table 11: Baseline pulmonary functions by treatment arm

	Active							
Pulmonary function	**N**	**Mean**	**SD**	**P25**	**Median**	**P75**	**Min**	**Max**
FEV1(litres)	95	1.9	0.7	1.4	1.8	2.5	0.6	4.1
FEV1 %predicted (%)	93	63.2	21.8	48	63	79	16	113
FVC(litres)	96	2.8	1.0	2.0	2.7	3.5	0.9	5.3
FEV1/FVC ratio	94	69.7	13.3	62.0	70.0	79.0	35.0	93.0
FEF25-75%(litres/sec)	80	1.6	0.9	0.9	1.4	2.1	0.3	3.9
FEF50%(litres/sec)	76	1.9	1.1	1.1	1.7	2.6	0.3	4.5
PEF(litres/min)	95	288	108	211	283	361	5.0	526
PEF %predicted (%)	94	76.6	108.6	47.0	67.5	79.0	15.0	1094
	Placebo							
Pulmonary function	**N**	**Mean**	**SD**	**P25**	**Median**	**P75**	**Min**	**Max**
FEV1(litres)	96	2.1	0.8	1.5	2.0	2.6	0.6	4.5
FEV1 %predicted (%)	96	66.3	21.0	52.5	64.0	84.0	23.0	107
FVC(litres)	96	3.1	1.0	2.4	3.0	3.6	1.3	6.9
FEV1/FVC ratio	96	68.8	13.7	58.0	69.0	79.5	40.0	96.0
FEF25-75%(litres/sec)	87	1.7	1.1	0.9	1.4	2.4	0.2	5.6
FEF50%(litres/sec)	84	2.0	1.3	1.1	1.7	2.8	0.2	6.1
PEF(litres/min)	97	320	102	247	335	389	4.0	628
PEF %predicted (%)	96	72.9	21.4	56.5	74.0	90.0	26.0	126

P25 = 25% percentile

P75 = 75% percentile

Table 12: Baseline pulmonary functions by centres (N≥10), median (IQR)

	Centre, median (IQR)					
Factor	**BIR**	**GLA**	**LEI**	**POR**	**SMH**	**UNT**
N	29	28	10	56	11	10
FEV1(litres)	2.0 (1.5, 2.5)	2.1 (1.8, 2.9)	2.0 (1.4, 2.6)	1.9 (1.4, 2.8)	1.6 (1.3, 2.3)	1.8 (1.2, 2.2)
FEV1 %predicted (%)	65 (47, 78)	73 (57, 93)	66 (48, 82)	60 (50, 79)	62 (38, 77)	56 (49, 68)
FVC(litres)	2.8 (2.3, 3.6)	3.2 (2.6, 3.9)	2.9 (2.5, 3.2)	2.9 (2.1, 3.6)	2.7 (1.9, 3.7)	2.4 (2.0, 3.0)
FEV1/FVC ratio	68 (60, 81)	74 (64, 85)	69 (65, 79)	71 (62, 84)	67 (50, 74)	68 (60, 73)
FEF25-75%(litres/sec)	1.3 (1.0, 2.2)	2.0 (1.3, 2.5)	1.5 (0.9, 2.3)	1.6 (0.9, 2.4)	0.9 (0.5, 2.0)	1.1 (0.7, 1.7)
FEF50%(litres/sec)	1.5 (1.2, 2.1)	2.2 (1.7, 2.9)	1.9 (1.1, 3.0)	2.1 (1.1, 2.8)	1.3 (0.7, 2.4)	1.2 (0.8, 2.1)
PEF(litres/min)	284 (211, 343)	369 (260, 427)	270 (193, 397)	329 (264, 399)	322 (244, 372)	262 (162, 351)
PEF %predicted (%)	63 (46, 83)	78 (67, 93)	65 (43, 82)	75 (56, 88)	78 (45, 98)	62 (41, 75)

BIR = Birmingham Heartlands;
GLA = Western & Royal Infirmary, Glasgow;
LEI = Glenfield Leicester;
POR = Queen Alexandra, Portsmouth;
SMH = St Mary's Hospital, Imperial College Healthcare NHS Trust
UNT = University Hospital of North Tees

4.3 Data completeness

Each patient should have four visits: a randomisation visit (visit 1) and three follow-up visits (visits 2-4). The timing of these visits and the associated data collection schedule are shown in Table 13.

Table 13: Data collection schedule

	Visit 1 Day 1	Visit 2 Day 5 (±1 day*)	Visit 3 Day 10 (±1 day)*	Visit 4 Day 42 (±15 days)
Demographics	X			
Pulmonary function tests	X	X	X	
Other biological samples	X			X
Return Diary to investigator		X	X	
Acute Asthma QLQ (Juniper)	X	X	X	
MiniAQLQ (Juniper)	X	X	X	
Adverse Event review		X	X	X

* can be varied by ±2 days in exceptional circumstances

The number of patients missing each visit is shown in Figure 5. Of the 199 patients randomised, all attended visit 1 which coincided with randomisation, but 21 (11%) missed visit 2, 28 (14%) missed visit 3 and 39 (20%) missed visit 4.

Figure 5: Number of missed visits (from 199 subjects)

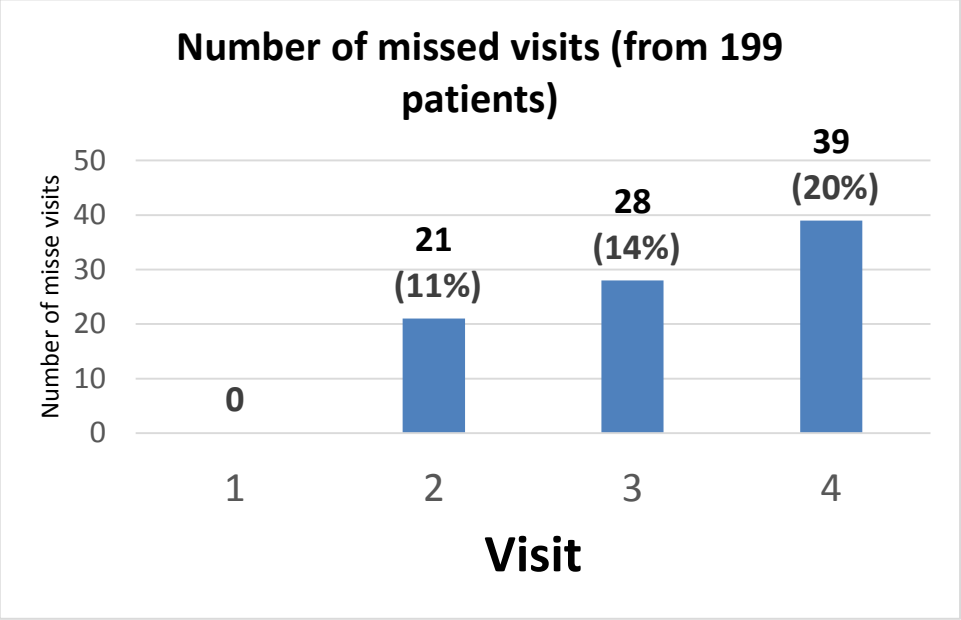


Table 14 shows the pattern of the missed visits. 80% of the patients attended all the follow-up visits.

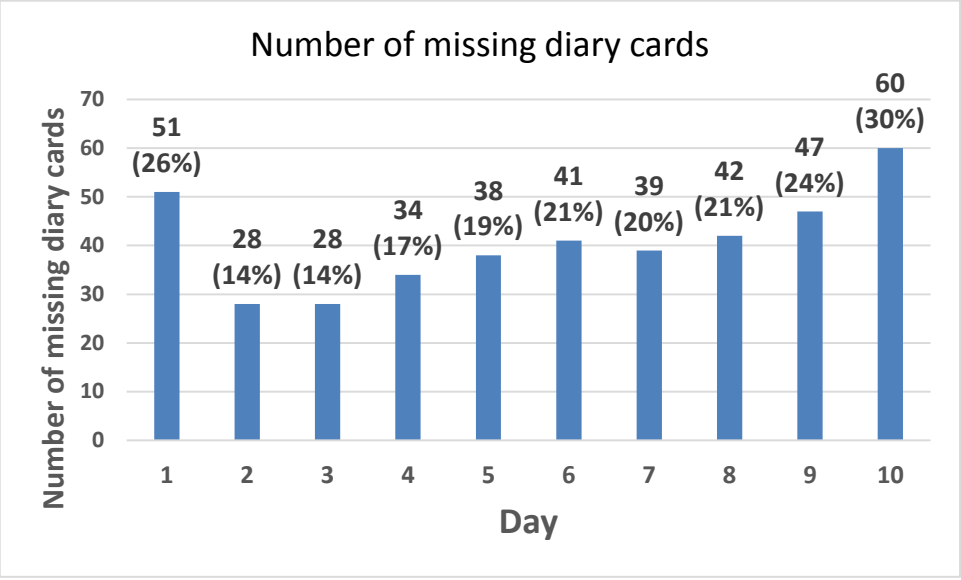
Table 14: Pattern of missed visits

Visits missing	No.	%
None	159	80
Visit 2 only	1	0.5
Visit 2 and 4	1	0.5
Visit 2,3 and 4	19	9.5
Visit 3 and 4	9	4.5
Visit 4 only	10	5
Total	**199**	**100**

4.3.1 Missing diary cards

Figure 6 shows the extent of the missingness for the diary cards. Day 1 was defined as the day of administration of the study drug (See Chapter 3). The highest level of missingness was observed on Day 10 (30%), although the second highest was observed on Day 1 (26%).

Figure 6: Number and percentage of missing diary cards by day (from 199 patients)



A breakdown of diary card missingness for centres with at least 10 recruited patients is shown in Table 15. A relatively high missingness was observed in the largest centre (Queen Alexandra, Portsmouth, POR), where 41% of the diary cards were missing on the last two days.

Table 15: Percentage of missing diary cards by centre that recruited ≥10 patients

Site*	Recruited	Day (%)									
		1	2	3	4	5	6	7	8	9	10
POR	**56**	39%	23%	21%	29%	29%	36%	32%	34%	41%	41%
BIR	**29**	28%	14%	14%	14%	14%	17%	14%	14%	14%	21%
GLA	**28**	21%	7%	7%	14%	18%	18%	18%	18%	18%	21%
SMH	**11**	27%	36%	36%	36%	36%	36%	36%	36%	36%	36%
LEI	**10**	10%	0%	0%	0%	0%	0%	0%	10%	10%	20%
UNT	**10**	20%	10%	10%	10%	20%	10%	10%	10%	20%	20%

*BIR = Birmingham Heartlands;
GLA = Western & Royal Infirmary, Glasgow;
LEI = Glenfield Leicester;
POR = Queen Alexandra, Portsmouth;
SMH = St Mary's Hospital, Imperial College Healthcare NHS Trust
UNT = University Hospital of North Tees

Table 16 shows the most common patterns of diary card missingness. Regardless of whether Day 1 was defined as the day of randomisation or day of administration of the study drug, 10% of the patients did not complete their diary card on the first day. Apart from that the missingness of the diary score records can be considered as standard drop out.

Table 16: Patterns of diary card missingness

Frequency	Percentage	Day									
		1	2	3	4	5	6	7	8	9	10
111	63%	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
17	10%	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓
13	7%	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗
4	2%	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗
4	2%	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓
3	2%	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗
2	1%	✗	✓	✓	✓	✗	✗	✗	✗	✗	✗
2	1%	✓	✓	✓	✗	✗	✗	✗	✗	✗	✗
2	1%	✓	✓	✓	✓	✗	✗	✗	✗	✗	✗
18	10%	Other patterns									
TOTAL											
176	100%										

4.3.2 Missing asthma questionnaires

Asthma questionnaires were missing if the patient did not attend visits 2 or 3. In addition there were 18 missing overall acute asthma scores (3 at visit 1, 7 at visit 2 and 8 at visit 3) and 18 missing

overall mini-asthma scores (3 at visit 1, 7 at visit 2 and 8 at visit 3) because one or more items were missing. The missingness was balanced between the treatment arms.

4.3.3 Missing pulmonary function tests

Results from the pulmonary function tests were missing for unattended visits, and in addition some test results were missing for other patients. There were complete results for 160, 150 and 142 patients at visits 1, 2 and 3 respectively (out of a possible 199, 171 and 163 patients who attended visits 1, 2 and 3 respectively). Most of the missing results are for $FEF_{25-75\%}$ and $FEF_{50\%}$, which were missing in 5 cases out of the 6 recruited patients at Rowden Surgery and in 5 cases out of the 8 recruited patients at New Cross Hospital, Royal Wolverhampton.

4.4 Primary outcome analysis

4.4.1 Exploratory analysis of the primary outcome

As a check for outliers and imbalances, a series of longitudinal plots (one for each centre) of diary score for each patient, differentiating between treatment arm were produced (see Appendix 2). Figure shows the mean diary scores and their standard errors for each treatment arm against day. Boxplots of diary scores by treatment arm for each day were also produced to show the distribution of the observed scores graphically in Figure 8. Table 17 shows the observed mean diary scores and standard deviations for each treatment arm by day and the number of observations. Additionally, a table of summary statistics of the diary scores by day and treatment arm was produced, including the number of observations, mean, standard deviation, median, lower and upper quartiles (Table 18).

Figure 7: Observed symptom diary scores by day (with standard error bars)

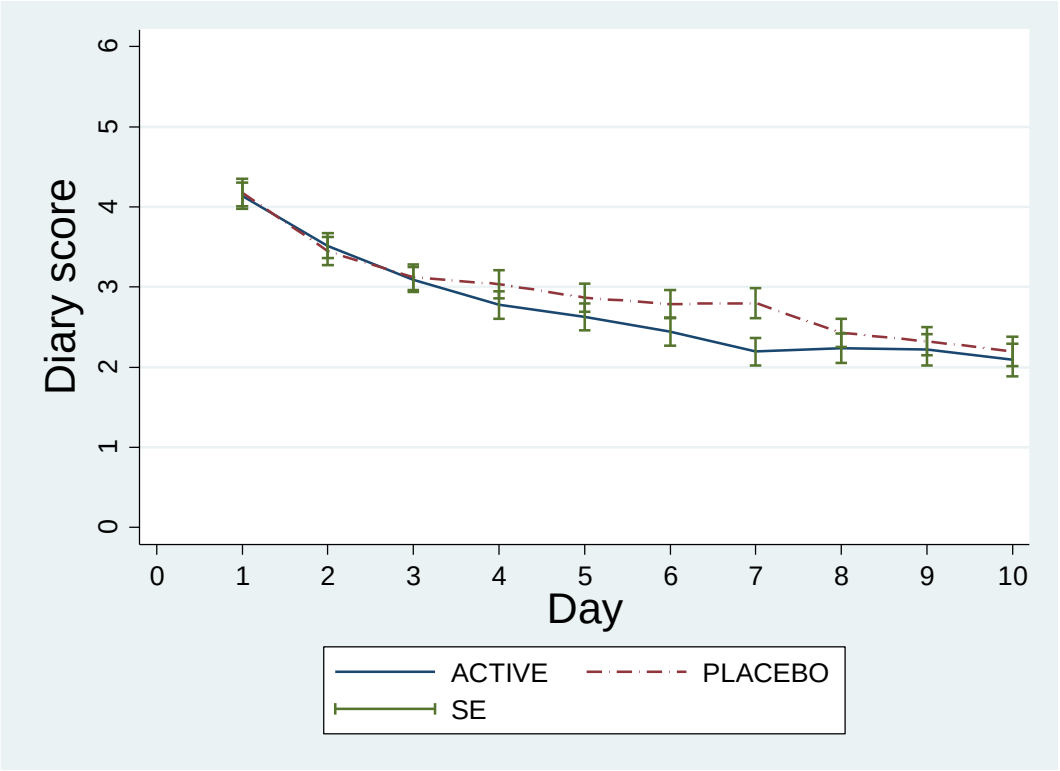


Figure 8: Boxplots of observed symptom diary scores

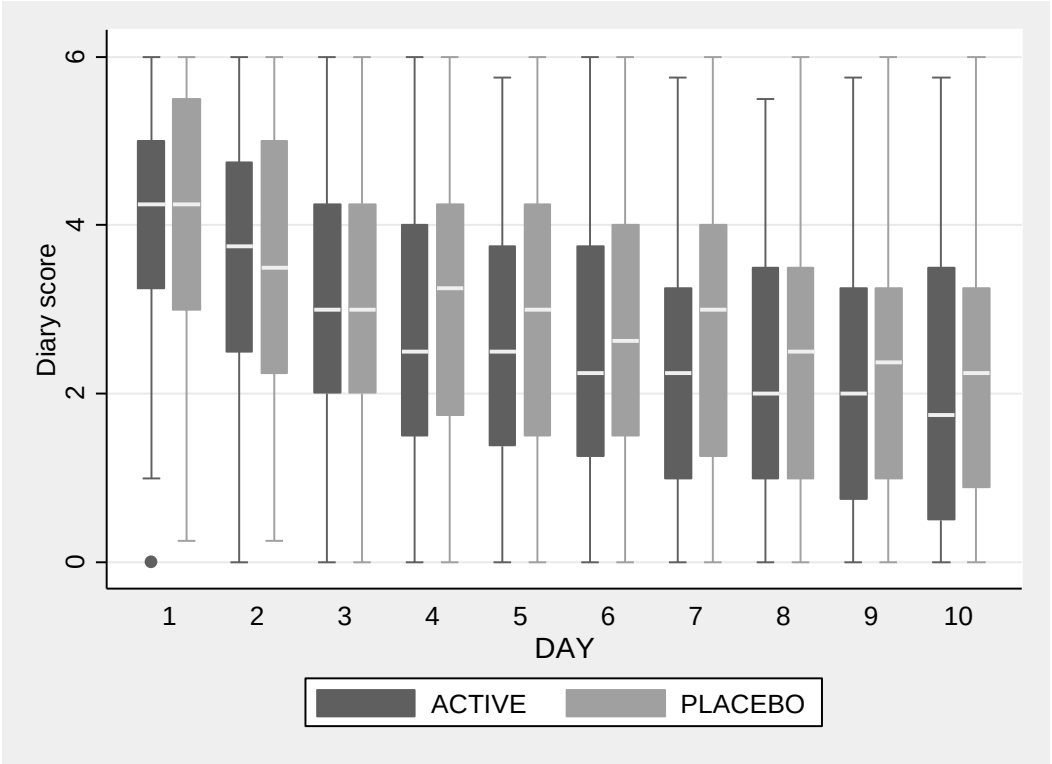


Table 17: Observed mean symptom scores for each day by treatment group and their standard deviation

	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10
Placebo (SD)	4.18 (1.48)	3.45 (1.62)	3.12 (1.47)	3.04 (1.57)	2.87 (1.58)	2.79 (1.56)	2.80 (1.69)	2.43 (1.53)	2.32 (1.55)	2.20 (1.51)
N	77	86	85	81	81	80	79	77	74	68
Active (SD)	4.14 (1.38)	3.51 (1.42)	3.09 (1.45)	2.78 (1.58)	2.63 (1.51)	2.44 (1.54)	2.19 (1.53)	2.24 (1.61)	2.22 (1.71)	2.09 (1.71)
N	71	85	86	84	80	78	81	80	78	71

Table 18: Detailed statistics of observed diary scores

	Placebo				Active		
	N	**Diary score, mean (SD)**	**Diary score, median (IQR)**		**N**	**Diary score, mean (SD)**	**Diary score, median (IQR)**
day 1	77	4.18 (1.48)	4.25 (3.00, 5.50)	**day 1**	71	4.14 (1.38)	4.25 (3.25, 5.00)
day 2	86	3.45 (1.62)	3.50 (2.25, 5.00)	**day 2**	85	3.51 (1.42)	3.75 (2.50, 4.75)
day 3	85	3.12 (1.47)	3.00 (2.00, 4.25)	**day 3**	86	3.09 (1.45)	3.00 (2.00, 4.25)
day 4	81	3.04 (1.57)	3.25 (1.75, 4.25)	**day 4**	84	2.78 (1.58)	2.50 (1.50, 4.00)
day 5	81	2.87 (1.58)	3.00 (1.50, 4.25)	**day 5**	80	2.63 (1.51)	2.50 (1.38, 3.75)
day 6	80	2.79 (1.56)	2.63 (1.50, 4.00)	**day 6**	78	2.44 (1.54)	2.25 (1.25, 3.75)
day 7	79	2.80 (1.69)	3.00 (1.25, 4.00)	**day 7**	81	2.19 (1.53)	2.25 (1.00, 3.25)
day 8	77	2.43 (1.53)	2.50 (1.00, 3.50)	**day 8**	80	2.24 (1.61)	2.00 (1.00, 3.50)
day 9	74	2.32 (1.55)	2.38 (1.00, 3.25)	**day 9**	78	2.22 (1.71)	2.00 (0.75, 3.25)
day 10	68	2.20 (1.51)	2.25 (0.88, 3.25)	**day 10**	71	2.09 (1.71)	1.75 (0.50, 3.50)

4.4.2 Results

A linear change was assumed in the model for the diary score over time with different slopes for the two treatment arms. Additionally, equal mean scores were assumed at baseline for the two groups as any inequality could only have occurred by chance, due to randomisation. In order to reduce bias caused by the observed difference at baseline, the main effect of the interaction term was not included in the model as an independent covariate. Sensitivity analysis with the inclusion of this

covariate was conducted. The estimated mean diary score at baseline (day 1) in the whole study population was 3.66 (95% CI: 3.41; 3.90). In addition to the decrease observed in the placebo group, the decrease of the diary score in the azithromycin group was slightly greater. On average the difference in change compared to the placebo group was -0.018 per day (95% CI: -0.074 , 0.037). The estimated differences with their 95% confidence intervals for each day can be found in Table 19. The mean "natural" background daily decrease (decrease in placebo group) in diary score was -0.18 (95% CI for the first day alone: -0.22; 0.14). On day 10, the difference between the two groups was not statistically significant. The estimated mean diary score was lower in the azithromycin group by -0.166 (95% CI: -0.670; 0.337). On Day 5 the difference was -0.074 (95% CI: -0.298; 0.150) between the two groups.

Table 19: Estimated difference in change of diary scores from baseline and 95% confidence intervals for azithromycin compared to the placebo

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Difference in Change from baseline	0	-0.018	-0.037	-0.055	-0.074	-0.092	-0.111	-0.129	-0.148	**-0.166**
95% Confidence Interval	- -	-0.074 0.037	-0.149 0.075	-0.223 0.112	-0.298 0.150	-0.372 0.187	-0.446 0.224	-0.521 0.262	-0.595 0.299	**-0.670** **0.337**

4.5 Secondary outcome analysis

For all secondary outcomes, an exploratory analysis and assessment of missing data was completed prior to the main analysis. This was analogous to that outlined for the primary outcome in Chapter 4.4. Multilevel models, similar to those specified for the primary outcome, were used to analyse the acute asthma and mini-asthma questionnaires and also for the pulmonary function tests. Details of the models used can be found in Appendix 2. Missingness was assessed in Chapter 4.3.2 for the acute asthma and mini-asthma questionnaires and for the pulmonary function tests in Chapter 4.3.3.

4.5.1 Acute asthma and mini asthma questionnaires analysis

Figure 9 shows the mean Acute AQLQ scores and the standard errors for each treatment arm against visit. Boxplots of Acute AQLQ by treatment arm for each visit are shown in Figure 10.

Table 20 shows the observed mean and standard deviation of Acute AQLQ scores for each treatment arm by visit and the number of observations.

Figure 9: Observed mean acute AQLQ scores and Standard errors by visits for each treatment arm

Figure 10: Boxplots of observed acute AQLQ scores

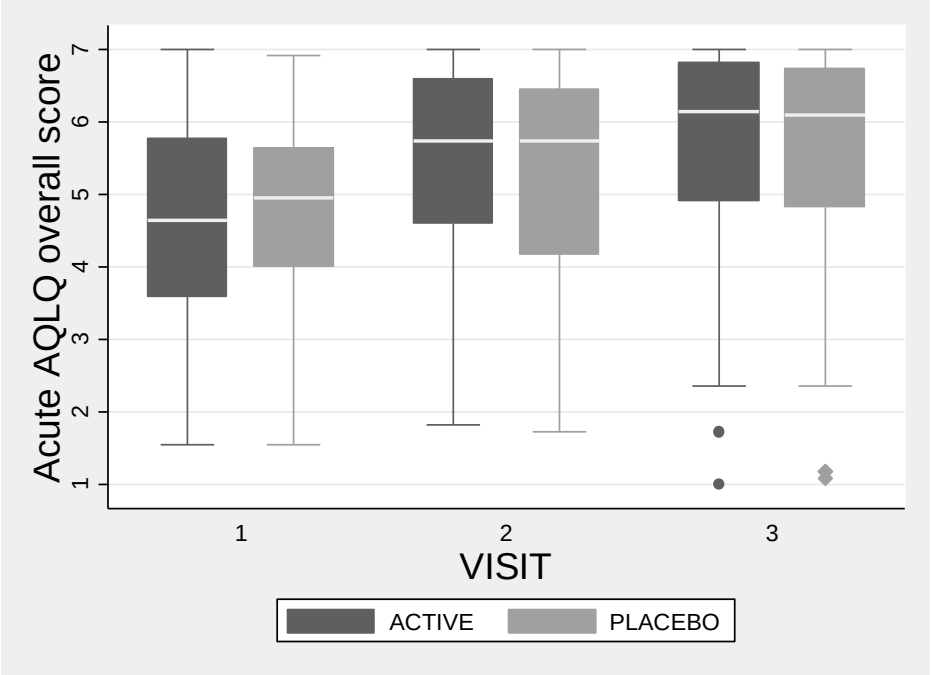


Table 20: Detailed statistics of observed acute AQLQ scores

Placebo						
		Acute AQLQ				
Visit	**N**	**Mean**	**Sd**	**Median**	**P25**	**P75**
1	100	4.8	1.3	5.0	4.0	5.6
2	87	5.3	1.4	5.7	4.2	6.4
3	83	5.6	1.5	6.1	4.8	6.7
Active						
		Acute AQLQ				
Visit	**N**	**Mean**	**Sd**	**Median**	**P25**	**P75**
1	96	4.6	1.4	4.6	3.6	5.8
2	84	5.4	1.3	5.7	4.6	6.6
3	80	5.6	1.5	6.1	4.9	6.8

As for the primary outcome, multilevel modeling was carried out assuming equal mean scores at baseline and linear change for the acute AQLQ and mini-AQLQ scores over time with different slopes for the two treatment arms. Differences in the change of acute AQLQ scores for each visit with the 95% confidence intervals can be found in Table 21. At visit 3 (day 10) there was no statistically significant difference between the two groups. According to the model, at visit 3, there was 0.130 (95% CI: -0.276; 0.539) greater acute AQLQ score estimated in the azithromycin group than the placebo group. Details of the model can be found in Appendix 2.

Table 21: Estimated difference in acute AQLQ score by visits

Acute AQLQ score	**Visit 1 (Day 1)**	**Visit 2 (Day 5)**	**Visit 3 (Day 10)**
Difference in change compared to Placebo group	0	0.065	0.130
95% Confidence Interval	-	-0.138; 0.269	-0.276; 0.539

The same analyses were conducted for Mini-AQLQ scores as for acute AQLQ scores. Figure 11 shows the mean Mini-AQLQ scores and the standard errors for each treatment arm against visit. Boxplots of Mini-AQLQ, by treatment arm, for each visit, are shown in Figure 12 . Table 22 shows the observed mean and standard deviation of Mini-AQLQ scores for each treatment arm by visit.

Figure 11: Observed mini AQLQ scores by visits

Figure 12: Boxplots of observed mini AQLQ scores

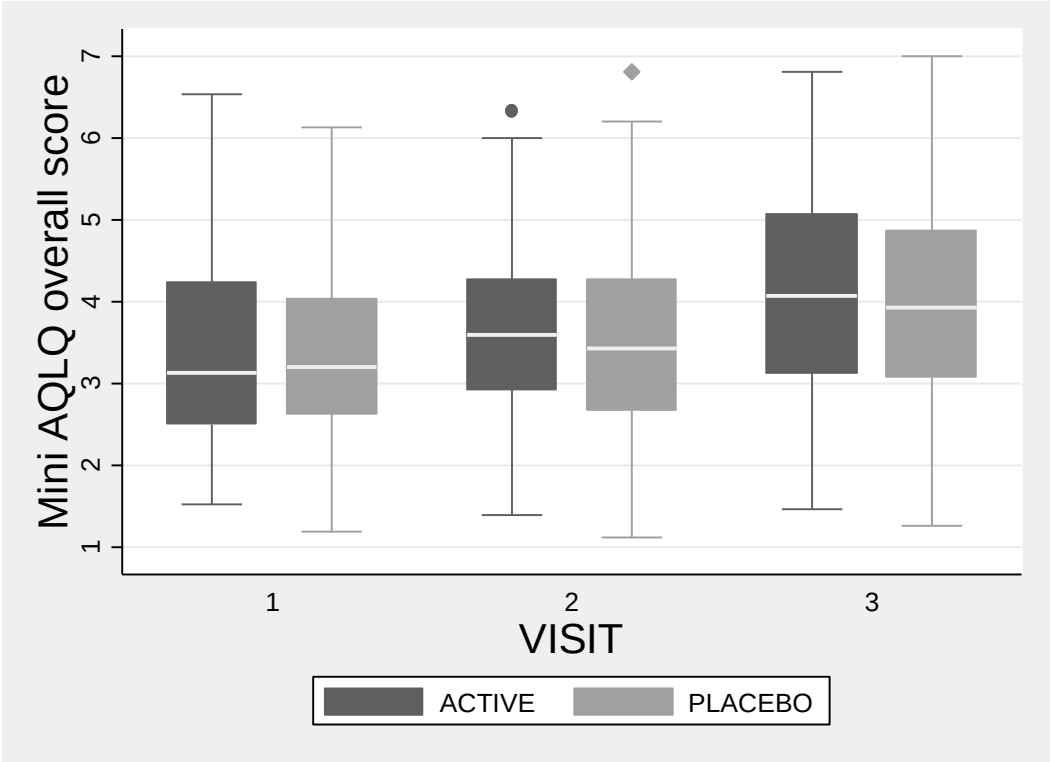


Table 22: Detailed statistics of observed mini AQLQ scores

Placebo					
	Mini AQLQ				
Visit	**Mean**	**Sd**	**Median**	**P25**	**P75**
1	3.4	1.1	3.2	2.6	4.0
2	3.6	1.2	3.4	2.7	4.3
3	4.1	1.3	3.9	3.1	4.9
Azithromycin					
	Mini AQLQ				
Visit	**Mean**	**Sd**	**Median**	**P25**	**P75**
1	3.4	1.2	3.1	2.5	4.2
2	3.6	1.1	3.6	2.9	4.3
3	4.1	1.3	4.1	3.1	5.1

Differences in the change of Mini AQLQ scores for each visit with 95% confidence intervals are shown in Table 23. At visit 3 (day 10) there was no statistically significant difference between the two groups. According to the model, at visit 3 there was -0.042 (95% CI: -0.409; 0.325) lower Mini AQLQ score estimated in the azithromycin group than the placebo group. Details of the model can be found in Appendix 2.

Table 23: Estimated difference in mini AQLQ score azithromycin compared to placebo by visits

Mini AQLQ	Visit 1 (Day 1)	Visit 2 (Day 5)	Visit 3 (Day 10)
Difference in change compared to Placebo group	0	-0.020	-0.042
95% Confidence interval	-	-0.204; 0.163	-0.409, 0.;325

4.5.2 Pulmonary function test analysis

For the pulmonary function tests similar exploratory analyses and multilevel modelling was conducted as for AQLQ scores. Details of the models used can be found in Appendix 2. Table 24 shows the observed pulmonary function test values (mean and standard error) for each visit by treatment arm.

Table 25 shows the estimated differences in change for azithromycin compared to placebo group with 95% confidence intervals by visit for each pulmonary function test.

Table 24: Observed mean (SD) pulmonary function test results by visit and treatment arm

Active Group				Placebo group		
Visit 1 Day	**Visit 2 Day**	**Visit 3 Day**		**Visit 1 Day**	**Visit 2 Day**	**Visit 3 Day**
97	85	80	N*	101	90	83
1.94 (0.74)	2.23 (0.77)	2.30 (0.83)	**FEV1(litres), mean (SD)**	2.11 (0.79)	2.34 (0.83)	2.38 (0.91)
2.80 (1.03)	3.13 (1.00)	3.25 (1.08)	**FVC(litres), mean (SD)**	3.09 (1.05)	3.40 (1.10)	3.38 (1.09)
69.66 (13.33)	71.71 (12.02)	71.00 (12.38)	**FEV1/FVC ratio, mean (SD)**	68.83 (13.71)	69.28 (12.24)	70.02 (12.71)
1.59 (0.89)	1.85 (0.94)	1.77 (0.92)	**FEF25-75%(litres/sec), mean (SD)**	1.74 (1.14)	1.83 (1.08)	1.94 (1.20)
1.92 (1.06)	2.12 (1.05)	2.19 (1.08)	**FEF50%(litres/sec), mean (SD)**	2.04 (1.26)	2.15 (1.24)	2.32 (1.35)
288.0 (107.5)	345.0 (109.0)	363.3 (108.4)	**PEF(litres/min), mean (SD)**	320.2 (102.6)	349.5 (110.1)	356.8 (118.1)

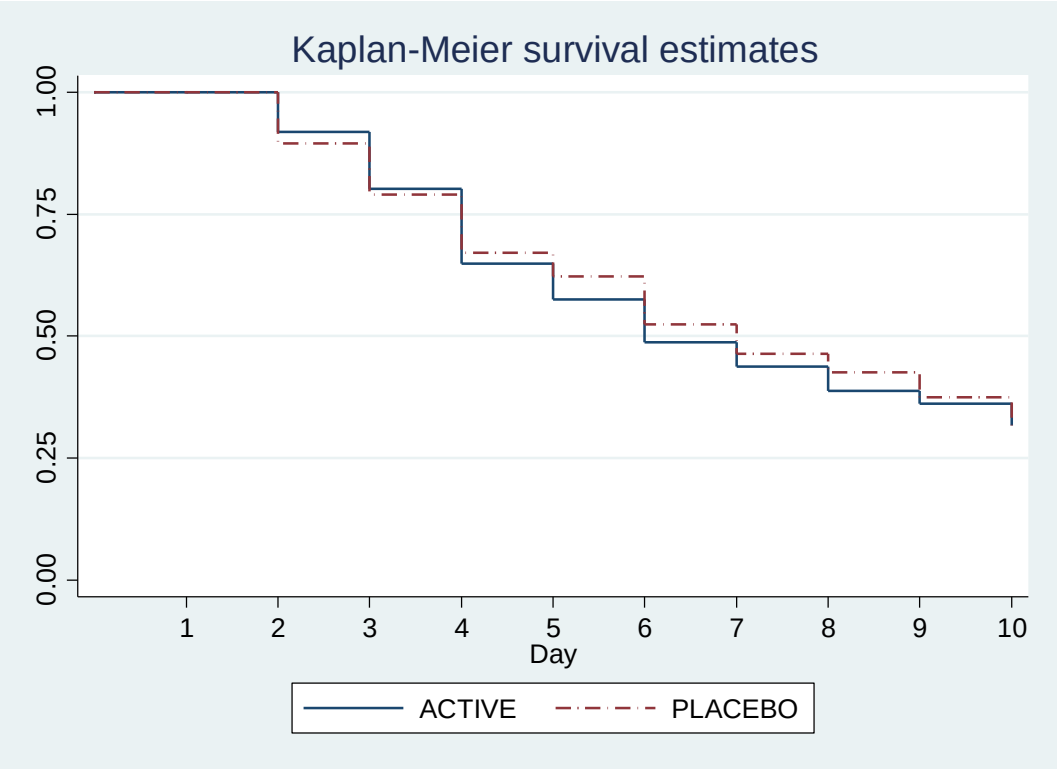
Table 25: Estimates of pulmonary function and 95% CI in brackets

	Difference in change compared to Placebo at visit 3 (Day 10)	**Difference in change compared to Placebo at visit 2 (Day 5)**	**Per visit change in Placebo**	**Baseline average***
FEV1(litres)	0.050 (-0.132; 0.231)	0.024 (-0.067; 0.116)	0.164 (0.099; 0.228)	2.011 (1.875; 2.146)
FVC(litres)	0.038 (-0.166; 0.243)	0.019 (-0.083; 0.122)	0.200 (0.127; 0.272)	2.959 (2.809; 3.110)
FEV1/FVC ratio	1.379 (-1.559; 4.316)	0.689 (-0.779; 2.158)	0.365 (-0.732; 1.463)	69.5 (67.7; 71.4)
FEF25-75%(litres/sec)	0.036 (-0.192; 0.265)	0.018 (-0.096; 0.132)	0.116 (0.035; 0.197)	1.631 (1.470; 1.792)
FEF50%(litres/sec)	0.045 (-0.234; 0.324)	0.022 (-0.117; 0.162)	0.161 (0.062; 0.260)	1.931 (1.750; 2.112)
PEF(litres/min)	18.03 (-8.56; 44.62)	9.016 (-4.278; 22.31)	24.66 (15.01; 34.31)	296.3 (272.0; 321.6)

4.5.3 Time to 50% reduction in symptom score analysis

Kaplan-Meier curves of time to 50% reduction in symptom score for each treatment arm (truncated at 10 days) are shown in Figure 13.

Figure 13: Kaplan-Meier curves of time to 50% reduction in symptom score for each treatment arm (truncated at 10 days).



4.6 Sub-studies

The same model as outlined for the primary outcome was used for subgroup analyses which including the following:

- Bacteria culture positive or negative in sputum: Table 26
- Viral tests positive or negative in nasal swab, throat swab or sputum: Table 27
- Atypical bacteria positive or negative in nasal swab, throat swab, sputum or serological testing: Table 28

Table 26: Estimated Day 10 difference in change of diary scores from baseline with 95% confidence intervalswith azithromycin compared to the placebo group in Bacteria positive or negative subgroup

Group	Whole study population (N=176)	*Bact. Sputum missing* (N=93)*	Bact. Sputum Positive (N= 12)	Bact. Sputum Negative (N= 71)
Day 10 difference in change	-0.166	*-0.114*	1.178	-0.410
95% CI	-0.670; 0.337	*-0.821; 0.594*	-0.497; 2.853	-1.183; 0.364

Table 27: Estimated Day 10 difference in change of diary scores from baseline with 95% confidence intervals for azithromycin compared to placebo in viral test positive or negative subgroups

Group	Whole study population (N=176)	Viral testing positive (N=31)	Viral testing negative (N= 138)
Day 10 difference in change	-0.166	-0.100	*-0.106*
95% CI	-0.670; 0.337	-1.170; 0.969	*-0.683; 0.472*

Table 28: Estimated Day 10 difference in change of diary scores from baseline with 95% confidence intervals for azithromycin compared to the placebo group in atypical and any bacteria positive or negative subgroups

Group	Whole study population (N=176)	*Atypical* bact. Positive (N=8†)*	*Atypical* bact. Negative (N=157)*	Any Bact. Test positive (N=20)
Day 10 difference in change	-0.166	*1.391*	0.044	0.198
95% CI	-0.670; 0.337	*-1.214; 3.996*	-0.465; 0.554	-1.546; 1.942

* *C. pneumoniae* or *M. pneumoniae*

†There were 9 patients with positive atypical bacteriology test results, but one of them had no diary score records

Figure 14: Observed mean diary scores and standard errors of the any bacterial test positive subgroup (N=20) by treatment arm

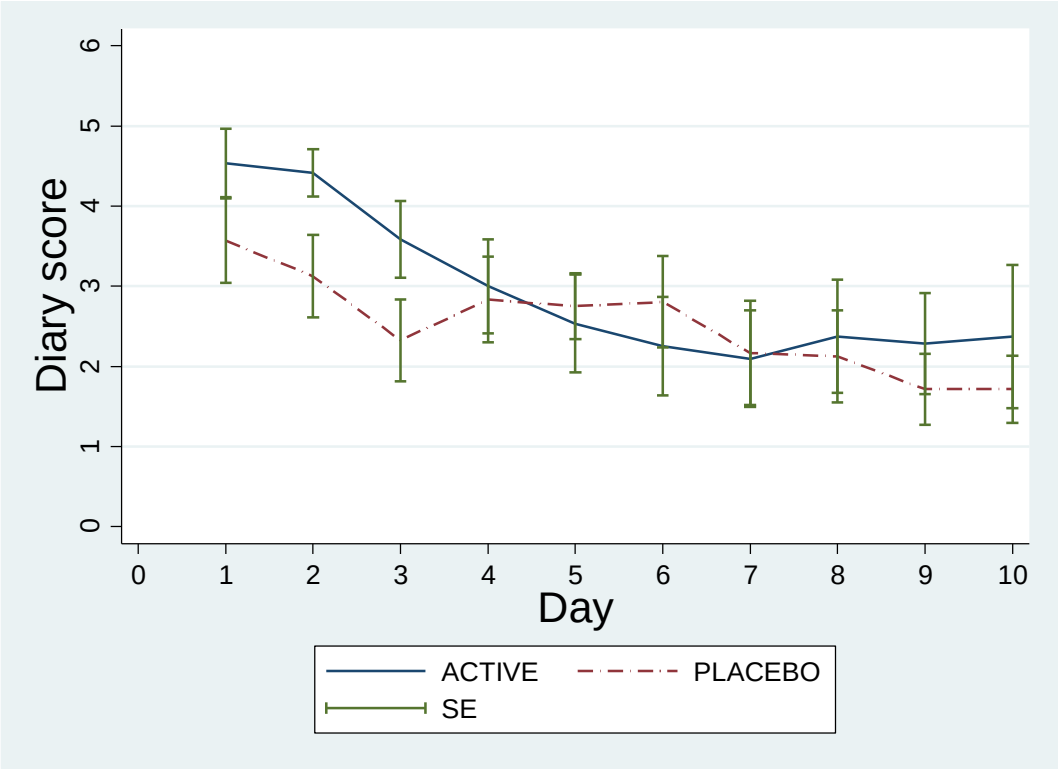


Figure 15: Observed mean diary scores and standard errors of the atypical bacterial test positive subgroup (N=8) by treatment arm

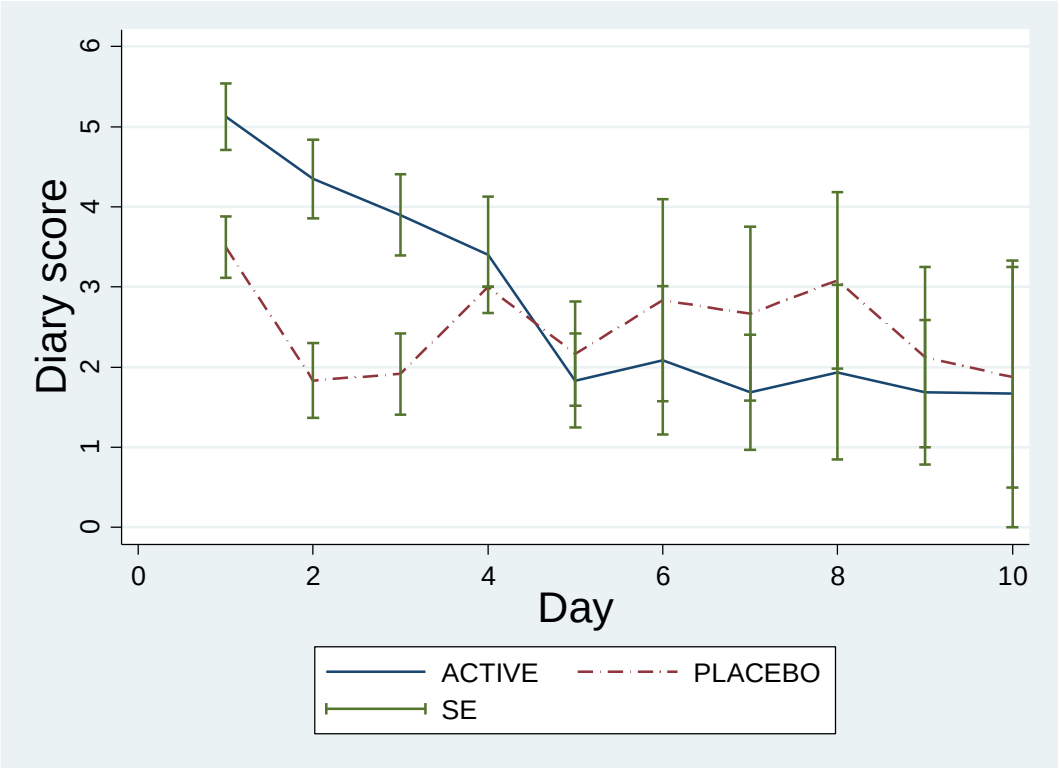
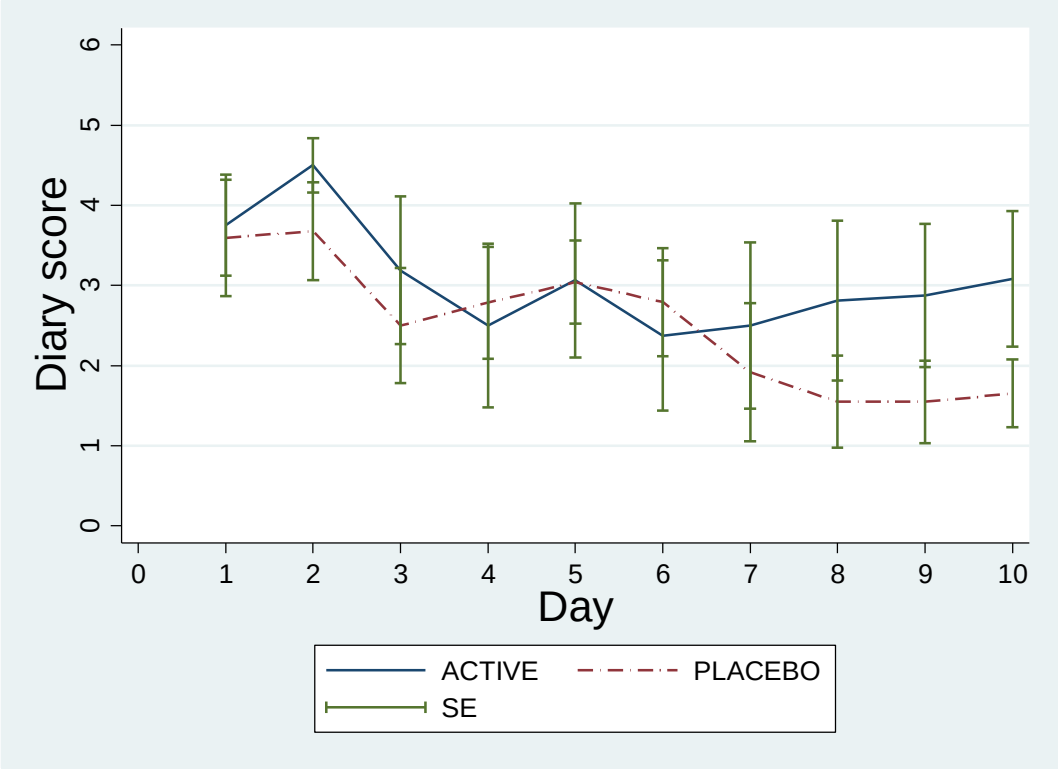


Figure 16: Observed mean diary scores and standard errors of the Bacteria culture positive in sputum subgroup (N=12) by treatment arm



4.7 Protocol deviations

Protocol deviations as recorded in the protocol deviation form in InForm are summarised by site and category in Table 29.

Table 29: Number of protocol deviations by centre⋆ and category†	Centre															
Category	A23	A24	A26	A30	BIR	BVH	GLA	LEI	NNU	NOC	NOT	POR	SMH	STJ	UNT	Total
DNA visit	0	0	0	0	8	2	6	1	0	0	0	4	1	0	0	22
Incomplete AQLQ	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Incomplete mini AQLQ	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Incomplete set of symptom diaries	0	0	0	0	1	0	7	2	0	0	1	16	0	0	1	28
Nasal Mucus not collected	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
No Haematology	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	3
Prohibited medication	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Spirometry	0	0	1	0	0	0	0	0	0	1	0	3	0	0	1	6
Sputum sample	1	0	0	0	4	0	0	0	0	0	0	0	0	0	0	5
Study drug compliance	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2
Visit outside protocol schedule	0	1	2	0	0	2	0	4	0	2	0	0	1	1	4	17
Vital signs not performed	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Withdrawn/ineligible	3	1	0	2	0	0	1	0	0	1	0	1	0	0	1	10
Total	4	2	4	3	14	4	18	7	2	4	1	25	2	1	7	98

a patient is only counted once in each category, but may have protocol deviations in > 1 category

⋆ A23 = East Surrey Hospital A24 = Countess of Chester; A26 = Worcester Acute Hospital;
A30 = Ipswich Hospital, NHS Trust; BIR = Birmingham Heartlands;
BVH = Blackpool Victoria Hospital; GLA = Western & Royal Infirmary, Glasgow; LEI = Glenfield Leicester; NNU = Norfolk and Norwich University Hospital; NOC = Nottingham City Hospital
NOT = Queen's Medical Centre, Nottingham; POR = Queen Alexandra, Portsmouth; SMH = St Mary's Hospital, Imperial College Healthcare NHS Trust
STJ = St James's University Hospital; UNT = University Hospital of North Tees

† as assigned by trial manager, monitor or operations manager, based on description

DNA visit = patient did not attend one or more visits
Incomplete mini QLQ = quality of life questionnaire not completed
Incomplete set of symptom diaries = patient did not return one or more symptom diaries
Nasal Mucus not collected = nasal mucus not performed
No Haematology = Haematology not performed or incomplete set of results

Spirometry = spirometry not performed or incomplete set of results
Sputum sample = no attempt to collect sputum sample (induced or spontaneous) Study drug compliance = patient was partially or non-compliant to taking study drug Visit outside protocol schedule = visit conducted outside protocol time frame Withdrawn/ineligible = withdrawn from study and/or ineligible

4.8 Safety data analysis

Protocol reporting of adverse events were from the time the patient gave informed consent until seven days after the last dose of study medication. Using the information recorded on the adverse event eCRF, each adverse event was categorised using MedDRA coding System Organ Class (SOC) terms by a designee of the Chief Investigator. The number of adverse events and patients affected in each category by treatment arm can be found in Table 30 and Table 31.

Table 30: Number of adverse events by SOC category and treatment arm

	Arm		
Adverse Event Category*	Active	Placebo	Total
	No.	No.	No.
Cardiac disorders	4	2	6
Eye disorders	2	1	3
Gastrointestinal disorders	35	24	59
General disorders	18	25	43
Infections and infestations	0	1	1
Musculoskeletal and connective tissue disorders	4	6	10
Nervous system disorders	15	14	29
Psychiatric disorders	1	2	3
Reproductive system and breast disorders	0	1	1
Respiratory, thoracic and mediastinal disorders	27	37	64
Skin and subcutaneous disorders	0	1	1
Total	106	114	220

*as advised by Chief Investigator or designee, based on description

Table 31: Number of patients affected by SOC category (a patient is only shown once in each category)

	Arm		
Adverse Event Category*	**Active**	**Placebo**	**Total**
	No.	**No.**	**No.**
Cardiac disorders	4	2	6
Eye disorders	2	1	3
Gastrointestinal disorders	25	20	45
General disorders	16	19	35
Infections and infestations	0	1	1
Musculoskeletal and connective tissue disorders	3	4	7
Nervous system disorders	14	13	27
Psychiatric disorders	1	2	3
Reproductive system and breast disorders	0	1	1
Respiratory, thoracic and mediastinal disorders	20	28	48
Skin and subcutaneous disorders	0	1	1
Total† (number of patients affected)	**85(51)**	**92 (52)**	**177 (103)**

*as advised by Chief Investigator or designee, based on description

†a patient may have more than one adverse event in any category

Table 32 shows the number of adverse events by category and relationship to study medication. The relationship is missing for four adverse events, and these are shown as "Unknown". No adverse events were definitely related to the study medication.

Table 32: Number of Adverse Events by SOC category and Relationship to Study Medication

	Relationship to study Medication					
Adverse Event Category*	**Not related**	**Unlikely**	**Possible**	**Probable**	**Unknown**	**Total**
	No.	**No.**	**No.**	**No.**	**No.**	**No.**
Cardiac disorders	3	2	1	0	0	6
Eye disorders	1	2	0	0	0	3
Gastrointestinal disorders	9	5	36	7	2	59
General disorders	20	11	11	0	1	43
Infections and infestations	1	0	0	0	0	1
Musculoskeletal and connective tissue disorders	6	3	1	0	0	10
Nervous system disorders	8	13	8	0	0	29
Psychiatric disorders	0	3	0	0	0	3
Reproductive system and breast disorders	1	0	0	0	0	1
Respiratory, thoracic and mediastinal disorders	49	14	0	0	1	64
Skin and subcutaneous disorders	0	0	1	0	0	1
Total	**98**	**53**	**58**	**7**	**4**	**220**

*as advised by Chief Investigator or designee, based on description

Multiple adverse events were reported for some patients, with 51 patients (just less than half of those with adverse events) reporting more than one. Ten adverse events were reported for one subject. Table 33 provides further detail about the distribution of the 220 adverse events between the 103 patients who reported adverse events.

Table 33: Number of Adverse Events Reported for Individual patients

	Treatment Arm		
Number of Adverse Events	**Active**	**Placebo**	**Total**
	No.	**No.**	**No.**
1	24	28	52
2	12	9	21
3	7	6	13
4	4	4	8
5	3	2	5
6	1	1	2
8	0	1	1
10	0	1	1
Total	**51**	**52**	**103**

Details of the adverse events classified as cardiac disorders are given in Table 34. None of these were classified as a serious adverse event. Serious Adverse Events are presented in Table 35 and Table 36.

Table 34: Listing of adverse events classified as Cardiac Disorders

Age (years)	Subject	Arm	Description	Site*	Relation	Severity	Outcome	Action †	Duration
26	NOC072	PLACEBO	chest pain	NOC	Not related	Moderate	Recovered	None	Intermittent
36	NOC077	ACTIVE	chest pain	NOC	Not related	Mild	Not yet recovered	None	Continuous
22	POR086	ACTIVE	palpitations	POR	Unlikely	Mild	Recovered	None	Intermittent
38	POR120	ACTIVE	chest pain and pain under left arm pit	POR	Unlikely	Mild	Recovered	None	Single Episode
55	POR195	ACTIVE	chest pain	POR	Not related	Mild	Recovered	None	Single Episode
42	SMH058	PLACEBO	feeling of tachycardia	SMH	Possible	Mild	Recovered	None	Single Episode

*NOC = Nottingham City Hospital; POR = Portsmouth Hospitals NHS Trust; SMH = St Mary's Hospital, Imperial College Healthcare NHS Trust

†Action taken concerning study medication

Table 35: Serious Adverse Events

Subject	Age (years)	Arm	Classification	Action taken	Event Description	Site	Relation to study drug	Severity
GLA052	18	PLACEBO	Serious	Hospitalisation required	Pt became wheezy and short of breath, 13/10/12, presented to accident and emergency on 14/10/2012 and was admitted overnight. Diagnosis exacerbation of asthma.	GLA	Unlikely	Moderate
NNU152	22	PLACEBO	Serious	Hospitalisation required	Exacerbation of underlying Asthma. Admitted to Hospital at 9am on 7/Oct/2013 with extreme symptoms of breathlessness.	NNU	Not related	Severe
A32197	47	PLACEBO	Serious	Hospitalisation required	Acute exacerbation of asthma	A32	Not related	Moderate
A29207	49.	ACTIVE	Serious	Hospitalisation required	shortness of breath and wheeze- non- infective exacerbation of asthma	A29	Not related	Moderate

Table 36: Serious Adverse Events continued

Subject	Frequency	Comments	Ongoing	Outcome	Category
GLA052	Single Episode		No	Recovered	Respiratory, thoracic and mediastinal disorders
NNU152	Unknown	Continuation of patients existing underlying condition. Classed as AE	No	Recovered	Respiratory, thoracic and mediastinal disorders
A32197	Single Episode	Admitted to hospital in Chester with asthma exacerbation for 3 nights.	No	Recovered	Respiratory, thoracic and mediastinal disorders
A29207	Single Episode	Patient was admitted with shortness of breath and kept in overnight	Yes	Not yet recovered	Respiratory, thoracic and mediastinal disorders

4.9 Compliance with study drug schedule

Any unused drug was collected during the end-of-therapy visit (visit 2, day 5), and study medication compliance assessed by counting unused capsules. Using this information, further details are provided in Table 37 on the protocol deviations associated with study drug compliance, including a table of level of compliance and additional comments by treatment arm.

Table 37: Summary of unused study medication and associated protocol deviations (visit 2)

PatientNumber	Quantity of study treatment returned	Participant	Treatment arm	Protocol deviation	Comment
A26119	3	partially compliant	Active	"acute AQLQ missing data at visit 2"	Has only 4 diary cards
NOC050	4	partially compliant	Placebo		"patient withdrawn from study after first dose" Recorded as a form comment NOT PD
POR051	4	compliant	Placebo		"patient withdrawn from study by investigator compliant with study medication up to withdrawal. PATIENT WAS NOT UNCOMPLIANT" Recorded as form comment NOT PD
POR041	2	compliant	Active	-	-

CHAPTER 5: DISCUSSION

This study has found that in the population of patients with acute severe asthma randomised to treatment, 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 scoring tools, including one which was used in a previous study reporting statistically and clinically significant therapeutic benefit with the ketolide antibiotic, telithromycin[13]. The findings were also consistently negative for all measures of lung function, including FEV_1, which was significantly improved in the previous study with telithromycin[13]. Furthermore, time to a 50% reduction in asthma symptoms, which in a post hoc analysis was also significantly improved in the previous study with telithromycin, was not improved with azithromycin[13].

The different outcomes of the present study and the previous Telicast study [13], which employed closely related therapies in very similar designs, requires some interpretation.

Clearly the two antibiotics studied are different, albeit related. The different outcomes could be explained by differences in their biological properties, including anti-bacterial, anti-inflammatory and anti-viral activities, and possibly other properties eg pharmacokinetics[32]. We have reported that azithromycin, but not telithromycin has anti-viral activity[27], 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 in vitro activity to azithromycin against both *M. catarrhalis* and *H. influenzae* [33-35]. 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 provide an explanation for the differing outcomes. In terms of anti-inflammatory activities, both drugs reportedly share such properties, and similar activities when compared[36].

A remarkable finding of this study was the number of patients (2044) that had to be excluded as they were already receiving antibiotic therapy for their asthma exacerbation, despite such therapy not being routinely recommended in treatment guidelines. Indeed, for each patient randomised, approximately 10 had to be excluded because they had already received antibiotic therapy for their asthma exacerbation, despite guideline recommendations that such therapy should not be routinely used. It is therefore possible that patients who might potentially have benefitted from antibiotic therapy for their asthma exacerbation (through

having sputum production, sputum purulence, fever etc), were excluded from the study through already having received them. The population remaining to be randomised could theoretically have been selected against for antibiotic responsiveness, through having no clinical indication that antibiotic therapy might be of benefit – ie no sputum production, no sputum purulence, no fever. This is possible as in the experience of many investigators in this trial, patients being screened had often been seen by their primary care practitioner in the days preceding their emergency room attendance, they had also been seen by emergency room medical staff and many subsequently also by a member of the on call respiratory/medical team, so that in many, three independent doctors/teams had assessed them for treatment including suitability for antibiotics. It is likely therefore that those not prescribed were strongly negatively selected against for suitability for antibiotics.

It is also possible that the population randomised were in other ways not representative of the larger population screened, as 10 fold more (over 2000 other patients) were excluded from the study for other reasons (Figure 2). The telithromycin study did not report numbers of patients screened[13], so it is not possible to determine to what extent these caveats may also have applied to that study.

Recruitment proved extremely challenging; initially there were 10 centres each aiming to recruit 38 subjects over one winter season, to meet the power calculation aim of recruiting 380 patients (note that the telithromycin study successfully randomised 270 patients)[13]. Our power calculation deliberately aimed at a larger number to provide statistically robust data to settle this important clinical question regarding efficacy. We also required larger numbers of patients to enable subgroup analyses aimed at potentially important mechanistic questions. Once recruitment obstacles became clear with such widespread antibiotic usage, more centres were enrolled to an eventual total of 31. Inclusion criteria were relaxed to include changing the eligibility criteria from <24 to <48 hours from time of presentation, to include older subjects, with low smoking histories (to exclude COPD), and recruitment time was extended to further winter seasons totalling 2 years and 7 months. However, despite all these efforts only 199 subjects were recruited by the medication expiry date, and in the absence of any further funding, the study had to be terminated, despite not reaching its recruitment target. It is therefore quite possible that the study was underpowered to detect therapeutic benefit.

A possible trend in favour of active treatment for the primary outcome, symptom scoring diary, was noted as both the mean and the median symptom scores tended to favour active

treatment over several days (Figures 9 and 10). If true, this might favour type II error. However, the possibility that the study reached negative conclusions due to being underpowered is less likely given spread of the data around these possible trends, and the consistent nature of all of the other outcomes including the other symptoms scoring tools, all measures of lung function and time to 50% recovery in symptoms.

A further difference between our study and the telithromycin study was that we required all patients randomised to require oral or systemic steroid treatment, while in the telithromycin study only 34.1% of patients randomised to active treatment, required steroid therapy/treatment[13]. This requirement for steroid treatment in our study was designed to strengthen the conclusions, in reducing the number of milder exacerbations. However, if the population randomised to the present study included largely non-bacterially infected subjects, as argued above, this could have resulted in us studying possible anti-inflammatory effects of our drug, over a very short period in the face of the powerful anti-inflammatory effects of steroids, with predictably negative results. This difference in design could be one explanation for the difference in outcome of the two studies.

The clinical characteristics of the patients in our study compared to those in the telithromycin study were quite similar in terms of mean age (39.9 years in our study, vs 39.5 in the telithromycin study), gender (30.2% male vs 32% male), smoking status (mean of 3.44 vs 2.15 pack years), exacerbation symptom score severity at baseline (exacerbation) (4.16 vs 2.9), and lung function at baseline (exacerbation) (PEF %predicted 74.8% vs 55.2%, FEV_1% predicted 64.8% vs 67.2%, FEV_1/FVC ratio 69.2% vs 72%)[13]. Overall, differences in clinical characteristics does not seem a likely explanation for the difference in outcome of the two studies.

However, the two studies differed strikingly in one important aspect; 61% of telithromycin treated patients had a positive test for *C. pneumoniae* and/or *M. pneumoniae[13]*, while in the present study only 4.5% tested positive for one or both of these two organisms. Both studies used similar methods of sampling, including sputum (where collected), nasopharyngeal swabs for PCR, acute serology for IgM, and acute and convalescent serology for IgA and IgG. The serological testing methodologies were very similar, though different PCR assays were used, and the laboratories performing the analyses differed (G. R. Micro, London UK for telithromycin, Prof Johnston's laboratory for this study). The different detection methods may not have yielded directly comparable results. Of note, in both studies, PCR detection rates were very low (3 positive for *C. pneumoniae* and/or *M. pneumoniae* in the telithromycin

study and 0 positives in this study). In contrast, serological positives were very high in the telithromycin study, but low in this study. The telithromycin positives were mostly IgM positives for *C. pneumoniae*, while in our study IgM positivity for this organism was low with only a single positive sample. Both studies used the same assay (Medac *C. pneumoniae* IgM sandwich ELISA, Medac, Hamburg, Germany) so the discrepancy between the outcomes of this assay is difficult to explain. It might however, contribute to the difference in clinical outcomes between the two studies.

Sputum culture for standard bacteria was not performed in the telithromycin study, and no sputum sample cultured *C. pneumoniae* and/or *M. pneumoniae*. In the present study 105 (52.8%) subjects provided sputum samples for standard bacterial culture and bacterial culture positivity was observed in 6.0% of subjects (4.1% active, 7.8% placebo), These results, together with the negative outcomes in relation to therapy, suggest that the role of bacterial infection in the population studied was unlikely to be important.

Adverse events were infrequent, with a slight preponderance of gastrointestinal adverse events in the azithromycin group (35 events) compared to the placebo group (24 events) as is common in antibiotic studies. Interestingly, there was a similar reduction in respiratory, thoracic and mediastinal adverse events in the azithromycin group (27 events) compared to the placebo group (37 events). A total of 63 of these 64 events, proved to be respiratory (the other was backache), suggesting that antibiotic therapy possibly reduced respiratory adverse event in this population.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

In the population of patients with acute severe asthma randomised to treatment in this study, addition of azithromycin to standard medical care resulted in no statistically or clinically significant therapeutic benefit. For each patient randomised, approximately 10 were excluded because they had already received antibiotic therapy despite guideline recommendations that such therapy should not be routinely used. The study may therefore, have been underpowered to detect therapeutic benefit in the selected, minority of patients randomised to treatment.

In the face of such widespread use of antibiotics in naturally occurring asthma exacerbations, without convincing evidence that they are beneficial, it is difficult to come up with coherent recommendations in practice, as a randomised placebo controlled study of an antibiotic added to therapy in those already receiving an antibiotic, appears meaningless, while performing an adequately powered study in representative numbers of patients not receiving antibiotic therapy has proved extremely challenging.

Further scientific studies, including in human experimentally induced asthma exacerbation studies, should be performed to determine whether there is evidence that bacteria do or do not contribute to the pathogenesis of asthma exacerbations. Such studies should include standard culture techniques, as well as modern molecular techniques. If bacteria do appear to contribute to asthma exacerbation pathogenesis in this human experimentally induced asthma exacerbation setting, a randomised placebo controlled study in this controlled experimental setting would be the logical next step.

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- Barnsley Hospital NHS Foundation Trust – Barnsley Hospital
- Blackpool Teaching Hospitals NHS Foundation Trust – Blackpool Victoria Hospital
- Countess of Chester Hospital NHS Foundation Trust – Countess of Chester Hospital
- Gloucestershire Hospitals NHS Trust - Gloucestershire Royal Hospital
- Great Western Hospitals NHS Trust, Swindon
- Guy's and St Thomas' NHS Foundation Trust
- Heart of England NHS Foundation Trust – Birmingham Heartlands
- Imperial College Healthcare NHS Trust – St Mary's Hospital, Charing Cross Hospital and Hammersmith Hospital
- Ipswich Hospital NHS Trust – Ipswich Hospital
- Leeds Teaching Hospitals NHS Trust – St James' University Hospital
- Mid Cheshire Hospitals NHS Foundation Trust - Leighton Hospital, Crewe
- Newcastle upon Tyne Hospitals NHS Foundation Trust – Freeman Hospital
- NHS Greater Glasgow and Clyde - Western & Royal Infirmary
- NHS Wiltshire – Rowden GP Surgery, Chippenham
- Nottingham University Hospitals NHS Trust - Nottingham City & QMC
- Norfolk & Norwich University Hospitals NHS Foundation Trust - Norfolk and Norwich University Hospital
- North Tees and Hartlepool NHS Foundation Trust – University Hospital of North Tees
- Plymouth Hospitals NHS Trust – Derriford Hospital, Plymouth
- Portsmouth Hospitals NHS Trust - Queen Alexandra Hospital
- Royal Berkshire NHS Foundation Trust – Royal Berkshire Hospital
- Royal Wolverhampton Hospitals NHS Trust – New Cross Hospital
- Sherwood Forest Hospitals NHS Foundation Trust
- Shrewsbury and Telford Hospital NHS Trust - Princess Royal Hospital, Telford

- South Tees Hospitals NHS Foundation Trust – James Cook Hospital
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- Taunton and Somerset NHS Foundation Trust – Musgrove Park Hospital, Taunton
- University Hospitals of Leicester NHS Trust – Glenfield Hospital
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Patients participating in the trial

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REFERENCES

1. Asher, M.I., et al., *Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys.* Lancet, 2006. **368**(9537): p. 733-43.
2. Weiss, K.B. and S.D. Sullivan, *The health economics of asthma and rhinitis. I. Assessing the economic impact.* J Allergy Clin Immunol, 2001. **107**(1): p. 3-8.
3. Rabe, K.F., et al., *Clinical management of asthma in 1999: the Asthma Insights and Reality in Europe (AIRE) study.* Eur Respir J, 2000. **16**(5): p. 802-7.
4. Johnston, S.L., et al., *Community study of role of viral infections in exacerbations of asthma in 9-11 year old children.* Bmj, 1995. **310**(6989): p. 1225-9.
5. Chauhan, A.J., et al., *Personal exposure to nitrogen dioxide (NO2) and the severity of virus-induced asthma in children.* Lancet, 2003. **361**(9373): p. 1939-44.
6. Johnston, S.L., et al., *The relationship between upper respiratory infections and hospital admissions for asthma: a time-trend analysis.* Am J Respir Crit Care Med, 1996. **154**(3 Pt 1): p. 654-60.
7. Wark, P.A., et al., *Neutrophil degranulation and cell lysis is associated with clinical severity in virus-induced asthma.* Eur Respir J, 2002. **19**(1): p. 68-75.
8. Grissell, T.V., et al., *Interleukin-10 gene expression in acute virus-induced asthma.* Am J Respir Crit Care Med, 2005. **172**(4): p. 433-9.
9. Wark, P.A., et al., *Chlamydia pneumoniae immunoglobulin A reactivation and airway inflammation in acute asthma.* Eur Respir J, 2002. **20**(4): p. 834-40.
10. Esposito, S., et al., *Importance of acute Mycoplasma pneumoniae and Chlamydia pneumoniae infections in children with wheezing.* Eur Respir J, 2000. **16**(6): p. 1142-6.
11. Cunningham, A.F., et al., *Chronic Chlamydia pneumoniae infection and asthma exacerbations in children.* Eur Respir J, 1998. **11**(2): p. 345-9.
12. Johnston, S.L. and R.J. Martin, *Chlamydophila pneumoniae and Mycoplasma pneumoniae: a role in asthma pathogenesis?* Am J Respir Crit Care Med, 2005. **172**(9): p. 1078-89.
13. Johnston, S.L., et al., *The effect of telithromycin in acute exacerbations of asthma.* N Engl J Med, 2006. **354**(15): p. 1589-600.
14. Pattemore, P.K., S.L. Johnston, and P.G. Bardin, *Viruses as precipitants of asthma symptoms. I. Epidemiology.* Clin Exp Allergy, 1992. **22**(3): p. 325-36.
15. Talbot, T.R., et al., *Asthma as a risk factor for invasive pneumococcal disease.* N Engl J Med, 2005. **352**(20): p. 2082-90.
16. Klemets, P., et al., *Risk of invasive pneumococcal infections among working age adults with asthma.* Thorax, 2010. **65**(8): p. 698-702.
17. Pilishvili, T., et al., *Risk factors for invasive pneumococcal disease in children in the era of conjugate vaccine use.* Pediatrics. **126**(1): p. e9-17.
18. Jounio, U., et al., *Pneumococcal carriage is more common in asthmatic than in non-asthmatic young men.* Clin Respir J. **4**(4): p. 222-9.
19. Hilty, M., et al., *Disordered microbial communities in asthmatic airways.* PLoS One, 2010. **5**(1): p. e8578.
20. Contoli, M., et al., *Role of deficient type III interferon-lambda production in asthma exacerbations.* Nat Med, 2006. **12**(9): p. 1023-6.
21. Oliver, B.G., et al., *Rhinovirus exposure impairs immune responses to bacterial products in human alveolar macrophages.* Thorax, 2008. **63**(6): p. 519-25.
22. Avadhanula, V., et al., *Respiratory viruses augment the adhesion of bacterial pathogens to respiratory epithelium in a viral species- and cell type-dependent manner.* J Virol, 2006. **80**(4): p. 1629-36.
23. Bisgaard, H., et al., *Association of bacteria and viruses with wheezy episodes in young children: prospective birth cohort study.* Bmj, 2010. **341**: p. c4978.
24. *British Guideline on the Management of Asthma.* Thorax, 2008. **63 Suppl 4**: p. iv1-121.
25. Message, S.D., 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, 2008. **105**(36): p. 13562-7.

26. Wark, P.A., et al., *Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus.* J Exp Med, 2005. **201**(6): p. 937-47.
27. Gielen, V., S.L. Johnston, and M.R. Edwards, *Azithromycin induces anti-viral responses in bronchial epithelial cells.* Eur Respir J, 2010. **36**(3): p. 646-54.
28. Beigelman, A., et al., *Azithromycin attenuates airway inflammation in a mouse model of viral bronchiolitis.* Respir Res. **11**: p. 90.
29. Bafadhel, M., et al., *Procalcitonin and C reactive protein in hospitalised adult patients with community acquired pneumonia, exacerbation of asthma and chronic obstructive pulmonary disease.* Chest, 2010.
30. Amsden, G.W., *Erythromycin, clarithromycin, and azithromycin: are the differences real?* Clin Ther, 1996. **18**(1): p. 56-72; discussion 55.
31. Wark, P.A., et al., *Safety of sputum induction with isotonic saline in adults with acute severe asthma.* Clin Exp Allergy, 2001. **31**(11): p. 1745-53.
32. Zeitlinger, M., C.C. Wagner, and B. Heinisch, *Ketolides--the modern relatives of macrolides : the pharmacokinetic perspective.* Clin Pharmacokinet, 2009. **48**(1): p. 23-38.
33. Walsh, F., et al., *Comparative in vitro activity of telithromycin against macrolide-resistant and -susceptible Streptococcus pneumoniae, Moraxella catarrhalis and Haemophilus influenzae.* J Antimicrob Chemother, 2004. **53**(5): p. 793-6.
34. Kosowska, K., 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, 2004. **48**(11): p. 4113-9.
35. De Vecchi, E., et al., *In vitro activity of telithromycin against Haemophilus influenzae at epithelial lining fluid concentrations.* BMC Microbiol, 2008. **8**: p. 23.
36. Kobayashi, Y., et al., *A novel macrolide solithromycin exerts superior anti-inflammatory effect via NF-kappaB inhibition.* J Pharmacol Exp Ther, 2013. **345**(1): p. 76-84.

APPENDIX 1. PATIENT INFORMATION SHEET AND CONSENT FORM

The use of the antibiotic Azithromycin in treatment of patients following acute asthma attacks.

AZALEA

PARTICIPANT INFORMATION SHEET

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish and ask us if there is anything that is not clear or if you would like more information.

Part 1 tells you the purpose of this study and what will happen to you if you take part.
Part 2 gives you more detailed information about the conduct of the study

PART 1

What is the study about?

Acute attacks (exacerbations) of asthma are common and cause a great deal of suffering in asthmatic patients. Current treatments for asthma attacks are not completely effective and new and better treatments are needed. Viruses often cause asthma attacks and bacterial lung infections have also been associated with asthma attacks. However, the role for bacteria is uncertain. Current asthma guidelines for doctors treating asthma exacerbations do not recommend the routine use of antibiotics. We would like to investigate whether or not azithromycin, which is a safe and well tolerated antibiotic (an antibacterial) that has been used for many years in the treatment of respiratory disease, might be of benefit in asthma attacks. As there is some evidence that azithromycin has anti-viral properties this may add to its benefits (antibiotics don't usually affect viruses). By looking at the effect of azithromycin on asthma attacks this will help us to show whether or not azithromycin should be recommended during an acute asthma attack in addition to the usual care that is provided to these patients as it may help them recover quicker from the exacerbation. We will also be able to look at why azithromycin may be effective - if it is having an anti-bacterial and/or anti-viral effect.

- **Why have I been invited?**

You have been invited to take part in this research study because you have presented with an exacerbation of your asthma which is the condition we are looking at. We are planning to enrol 380 participants into this study who present to medical care with an asthma exacerbation across different AZALEA research sites across the UK.

- **Do I have to take part?**

It is up to you to decide to join the study. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason and this would not affect the standard of care you receive.

- **What will happen to me if I agree to take part?**

- In addition to your current visit we will ask you to attend an additional 3 visits during which we will take some samples from you (see below for details) and ask you to complete some questionnaires. These visits will be at 5 and 10 days from your current visit and then a final follow up visit in 6 weeks time; the total time you will be in the research will be 6 weeks.
  -
- The study will involve a brief interview and medical examination to find out if you are suitable for the study. A member of the research team will review your medical records and ask some questions about you and any medicines you might be taking. These data will be entered into a central study database and be given a code so that you cannot be identified from the information in the database. Only the study team will have access to identifying information about you and this will be kept confidential as described below.
  -
- A summary of the research procedures at each of the study visits is shown in the table below. The research procedures that will occur as part of your current visit should take no longer than 2 hours and all subsequent visits will take around one hour.
-

Study Procedure	Visit 1 Day 1 (current visit)	Visit 2 Day 5	Visit 3 Day 10	Visit 4 Follow up Visit 6 weeks time
Informed Consent	X			
Review of medical notes and brief medical examination	X			
Breathing tests	X	X	X	
Blood sample	X			X
Nose swab, throat swab and collection of nasal mucus	X			
Sputum (spit) sample	X			
Complete symptom diary	X	X	X	
Complete 2 asthma questionnaires	X	X	X	
Dispense study drug or placebo (‘dummy’ treatment)	X			

-
- **Study procedures**

Informed Consent

At your current visit you will be seen by the Study Doctor/Nurse who will discuss the details of the study and answer any questions you may have. If you wish to take part, the Study Doctor or Nurse will then ask you to sign a consent form and you will be given a signed copy of the form to keep.

Study Visits

We will be in contact with you by telephone to co-ordinate the visits and follow up with you.

Review of medical notes/brief medical examination

The Study Doctor or Nurse will ask you about any current or previous medical conditions and any medications which you take. There is some information we will need to take from your medical notes to help with our research and we will keep this confidential.

Breathing tests

You will be asked to perform a few breathing tests that involve blowing into a tube (spirometry). You will be familiar with these from routine appointments to assess your asthma.

Blood sample

We will collect 30mls of blood (2 tablespoons worth) today and 30mls from you in 6 weeks time.

Nose and throat swab and collection of nasal secretion

A sample of nasal mucus will be taken using a nose swab and a throat swab will be taken. You will also be asked to blow into a tissue to collect a sample of nasal mucus.

Sputum (spit) sample

You will be asked to produce a deep cough into a sterile dish. If there is not enough spit we may also induce a sputum sample from you which will involve breathing in saline (a salty solution) through a nebuliser (a device used to breath in a salty mist/spray through a mouthpiece) until there is enough sputum available. This should take no longer than 20 minutes and we will check your breathing throughout with a spirometer (described above). You are free to ask to stop the procedure at any time or it may be stopped by the study doctor or nurse if they feel this is necessary.

Symptom diaries and asthma questionnaires

You will be given a symptom diary to complete at your current visit and be asked to complete it at home each day for the next 10 days. It should take you less than 5 minutes to complete. You will also be given two asthma questionnaires to complete at your current visit and in 5 and 10 days time when you return for visits. These should take up to 20 minutes in total for you to complete.

Study drug/placebo

- Sometimes we don‘t know which way of treating patients is best. To find out, we need to compare different treatments. We put people into groups and give each group a different

treatment. The results are compared to see if one is better. To try to make sure the groups are the same to start with, each patient is put into a group by chance (randomly). In this study 50% of participants will receive the study drug (azithromycin) and 50% will receive a placebo ('dummy' treatment). As the study is a 'double-blind trial' this means that neither you nor your doctor will know in which treatment group you are (although, if your doctor needs to find out he/she can do so).

- You will be given either the study drug or placebo after all other procedures during visit 1 as shown in the above table. You will be asked to take two capsules once a day for 3 days. This is the routine dose given if the drug is prescribed as part of routine care. You should take the study drugs at least 1 hour before or 2 hours after food. If you are taking antacids you should take the study drug at least 1 hour before or 2 hours after antacids. Your study doctor will check the other medication you may be taking and any medical condition you may have to make sure this does not conflict with taking azithromycin.
- On your next visit in 5 days time we will ask you to return the drug containers and any unused drugs and confirm if you have been able to take the drug as prescribed.

Pregnancy test

- If you are female and of child bearing potential we will ask you to undertake a urine pregnancy test to ensure you are not pregnant before starting study procedures.

- **What are the alternatives for diagnosis or treatment?**
- The alternative to participating in this research is to continue through the routine process for treatment of your exacerbation and not participate in the study or take the study drug/placebo.
- **Are there any disadvantages to taking part in this study?**

The disadvantages from taking part in this study are those associated with collecting the samples as well as the time inconvenience to you of attending the AZALEA research site for further visits. We will try to arrange the time of your study visits to be convenient with you.

Blood samples

Blood samples will be taken from a vein in your arm using a sterile needle. Taking blood samples may cause a little discomfort and bruising to your arm but this will resolve in a few days.

Nose and throat swab and collection of nasal secretion

The nose swab may be associated with mild discomfort and may tickle or make your eyes water slightly but should be painless. The throat swab is not painful, but you may find it briefly uncomfortable. Collection of nasal secretion will only involve you blowing into a tissue.

Sputum (spit) sample

Induced sputum as described above may lead to some shortness of breath but we will monitor this with breathing tests. You are free to ask to stop the procedure at any time or it may be stopped by the study doctor or nurse if they feel this is necessary.

What are the side effects of the study drug?

- Azithromycin is a licensed antibiotic with a good safety record which has been widely used for a long time. Side effects and allergic reactions are rare. The most common side effects that you may experience are gastrointestinal ie nausea, diarrhoea, vomiting and abdominal pain/cramps.
- There is a low risk of cardiovascular side effects. In rare incidences azithromycin can cause abnormal changes in the electrical activity of the heart particularly in patients who already have abnormal heart rhythms (arrhythmias) or are taking medication for these conditions. These patients will therefore be excluded from taking part in the study.
- If you suspect that you have these or any other symptoms you must inform the study doctor. If you experience severe side effects or an allergic reaction please contact the study doctor immediately on XXXXXXXXXXXX or attend A&E or phone for an ambulance if you are concerned.
-
- If you have reacted badly in the past to azithromycin or related antibiotics and their ingredients then you will not be eligible to participate in this study. Similarly if you are taking any other medications or have other disease conditions or infections that could interfere with the way the drug works or with our monitoring of how it works then you will not be included in the study.

Harm to an unborn child

We do not know if the study drug is safe for an unborn child so to protect the unborn child, pregnant women must not take part in this study and neither should women who plan to become pregnant during the study. Women who are breast feeding will also not be allowed to take part in the study. Women who are at risk of pregnancy will be asked to have a pregnancy test before taking part in the study to exclude the possibility of pregnancy.

If you are female and find out that you have become pregnant while taking part in the study, you should immediately tell your study doctor.

Are there any benefits to taking part in this study?

You may benefit from this study if you are randomised to receive azithromycin and it is found that this is effective in improving recovery from an asthma exacerbation. We do not know however whether or not it is effective so we cannot promise the study will help you. While this study will not necessarily benefit you directly, it may help us to understand more about the best ways of treating asthma exacerbations in future patients with this condition.

What happens when the research study stops?

You will return to the care of your local doctor (GP) and the hospital doctors you would see routinely.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?

Yes. The details are included in Part 2.

This completes part 1.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

PART 2

What if relevant new information becomes available?

Sometimes new information about a treatment being used in a study becomes available during the course of the study. If this happens, the study doctor will let you know. You can then make a decision about whether you want to continue in the study. If you decide not to continue in the study, the study doctor will ensure that your 'normal' care continues. If you decide to continue in the study, you will be asked to sign an updated consent form.

After receiving new information, the Study Doctor might want you to withdraw from the study. He may feel it is in your best interest to do this. If this happens, the doctor will explain the reason for this and arrange for your 'normal' care to continue. Your participation in the study may be stopped for any of the following reasons:

- Failure to comply with the study instructions
- A serious reaction, which may require treatment or observation
- The Doctor decides it is in the best interest of your health and welfare to discontinue

What will happen to the samples I give?

- The above research samples that we take from you will be used to find out the following information:
    - Whether azithromycin treatment is effective during an acute exacerbation of asthma
    - The type of viral and/or bacterial infection present in participants during an exacerbation
    - Why azithromycin might be an effective treatment ie how it works
    - Whether there are 'markers' in participants blood or spit that would be able to tell us in advance about the severity of and recovery from the acute exacerbation

- The results from testing your research samples will not affect the care that you receive as part of your routine treatment as we will not know the best treatment until the outcome of the study

- All the samples will be labelled with a code with no identifying information about you. The samples will then be tested in designated laboratories but the laboratory scientists will not be able to identify them as your samples. Your identifying information will only be accessible to authorised members of the research team.

- If you agree, we would also like to keep any of your samples that are left over after our research. These would be stored and used for future ethically approved research and only accessible to authorised members of the research team or regulatory bodies. At any time you want, you could ask for these samples to be destroyed.

Can I withdraw from participation in this study?

Your participation in this study is voluntary and you have the right to refuse to participate. You are free to withdraw at any time and do not have to give a reason for this, even after you have agreed to take part. Being part of this study will not affect your normal medical care, either now or in the future.

What will happen if I don't want to carry on with the study?

You are free to withdraw from the study at any time, without needing to give a reason why. If you withdraw from the study, we would like to keep the samples and data collected up to your withdrawal for our analysis. Any stored blood or tissue samples that can still be identified as yours can be destroyed though if you wish.

What if something goes wrong?

Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator on the below contact details. The normal National Health Service complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Office.

Who is organising and funding the research?

The research is co-ordinated by Imperial College, London and has been funded by the National Institute for Health Research (NIHR) and Medical Research Council. It has been reviewed and given a favourable opinion by the London - Bloomsbury Research Ethics Committee and has been given a favourable opinion by the Medicines and Healthcare Regulatory Agency (MHRA) – the agency that reviews and approves drug studies.

The study is being carried out by experts in asthma from around the country as a collaboration between NHS Trusts and universities (a list of these collaborators is available to you should you wish). None of the investigators performing the research or participants taking part will benefit financially from the study.

Will the information on me be kept confidential?

Yes, all personal information will be kept confidential and secure. Only people involved in the study will have access to your personal information. When we send your samples for analysis in designated laboratories they will be labeled with a code and have no identifying information on them. This study will be published in medical journals but it won't be possible to identify you from what is written. With your permission we would also like to retain any of your samples not used in this study for future research projects.

Involvement of the General Practitioner/Family doctor (GP)

With your permission we will inform your GP of your participation in this study.

Expenses and payments

A maximum payment of £50 will be available to you at visit 4 if you have completed all study visits and completed and returned all 10 symptom diaries (this payment is made up of

£10 for attending visit 1, £10 for visit 2, £10 for visit 3, £10 for visit 4 and £10 for returning the symptom diaries).

We will also reimburse the cost of your travel expenses for the additional 3 visits you will be asked to attend for research purposes.

- **What if I have any problems or would like further information about the study?**

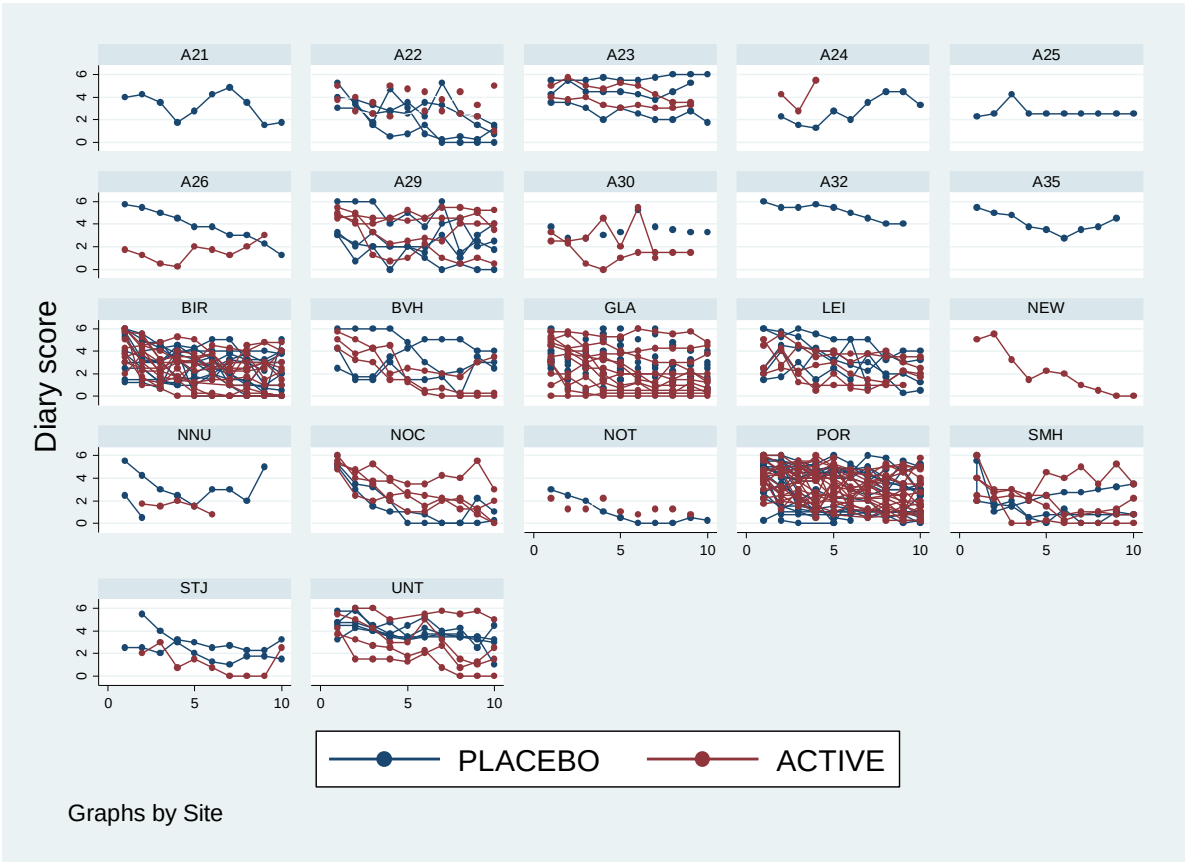
If you have a concern about any aspect of this study, you should ask to speak to the researchers on xxxxxxxxxxxx who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the normal NHS Complaints Procedure.

THANK YOU FOR TAKING THE TIME TO CONSIDER PARTICIPATING IN OUR RESEARCH

APPENDIX 2: STATISTICAL DETAILS

8.1 Observed diary scores for each centre by treatment arm

Figure 17: Observed diary scores for each centre by treatment arm



A21	Royal Berkshire Hospital
A22	Rowden Surgery
A23	East Surrey Hospital
A24	Countess of Chester
A25	Musgrove Park Hospital
A26	Worcester Acute Hospital
A29	New Cross Hospital, Royal Wolverhampton
A30	Ipswich Hospital, NHS Trust
A32	Telford
A35	Gloucestershire Royal Hospital
BIR	Heart of England NHS Foundation Trust
BVH	Blackpool Victoria Hospital
GLA	Western Infirmary Glasgow
LEI	University Hospitals of Leicester NHS Trust
NEW	The Newcastle upon Tyne Hospitals NHS Foundation Trust
NNU	Norfolk and Norwich University Hospital
NOC	Nottingham City Hospital
NOT	Queen's Medical Centre, Nottingham
POR	Portsmouth Hospitals NHS Trust
SMH	St Mary's Hospital, Imperial College Healthcare NHS Trust
STJ	St James's University Hospital
UNT	University Hospital of North Tees

8.2 The three main components of the model

Let DS_{id} represent the diary score for patient *i* on day d, d = 1, …, 10, and *t(i)* represent the treatment given to individual *i* (azithromycin or placebo). Then modelled DS_{id} as the sum of three components: an intercept term, a change over time term and a residual error term, i.e.

$$DS_{id} = intercept_i + change\ over\ time_{t(i)d} + residual\ error_{id}$$

Possible choices for each of these components are outlined below. The options explored for the primary analysis will be determined by the results of the exploratory analysis, and the final choice will be the simplest model that satisfies standard checks of model fit (e.g. residual plots).

8.2.1 Intercept term

The intercept term will estimate the diary score on day 1 (the day of randomisation and start of the study medication). This term will comprise an individual level random effect, which will be drawn from a distribution parameterised using the associated centre level random effect. Hence the unexplained variation in the diary scores will be split into three components corresponding to the three levels of the model, i.e. the variation attributable to the centre (between centre variation) and the individual (between individual variation), as well as the residual variation (within individual variation).

Additionally, baseline covariates can be incorporated into the model at the individual level. None will be incorporated for the initial analysis unless the baseline characteristics analysis reveals a substantial imbalance. Further analyses will examine the effect of incorporating baseline variables (age, gender, asthma severity, smoking history and asthma exacerbation).

8.2.2 Change over time (cot) term

This term will capture the change in the diary score from the start of the study medication (day 1), hence time will enter the model as day 1. The simplest assumption would be a linear change over the period, however alternatives may need to be considered as the rate of change may not be constant over the 10 day period. Alternatives are to include a quadratic term or use splines. The coefficients in this term will be dependent upon treatment.

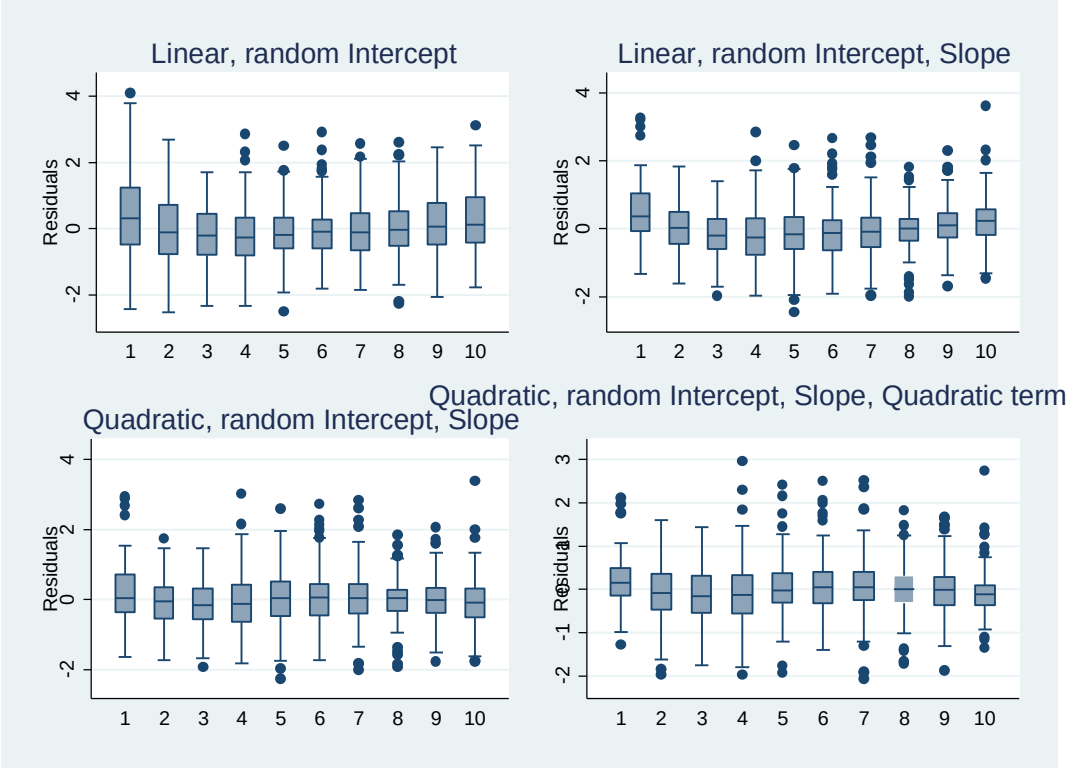
8.2.3 Residual error term

We were assuming that the residual errors have a Normal distribution. An alternative was to assume that these errors follow a heavier tailed distribution such as a t distribution with 4 degree of freedom, which will provide robustness to outliers. Normality of residual error was checked graphically.

8.3 Model selection

The plots of level 1 and level 2 residuals (where appropriate) patients of these models, including the model with splines at day 3 and day 7 and the fitted and observed values were also investigated graphically. These plots are presented below. As it can be seen, the more complex alternative models gave more flexibility than the standard linear model, but overall the residuals were just barely lower and the pattern of residuals remain the same, so in order of simplicity a linear model was chosen to calculate the estimated scores.

Figure 18: Boxplot of residuals for linear and quadratic models



8.3.1 Linear Model, random intercept and slope

Figure 19: Observed and fitted values for randomly selected examples

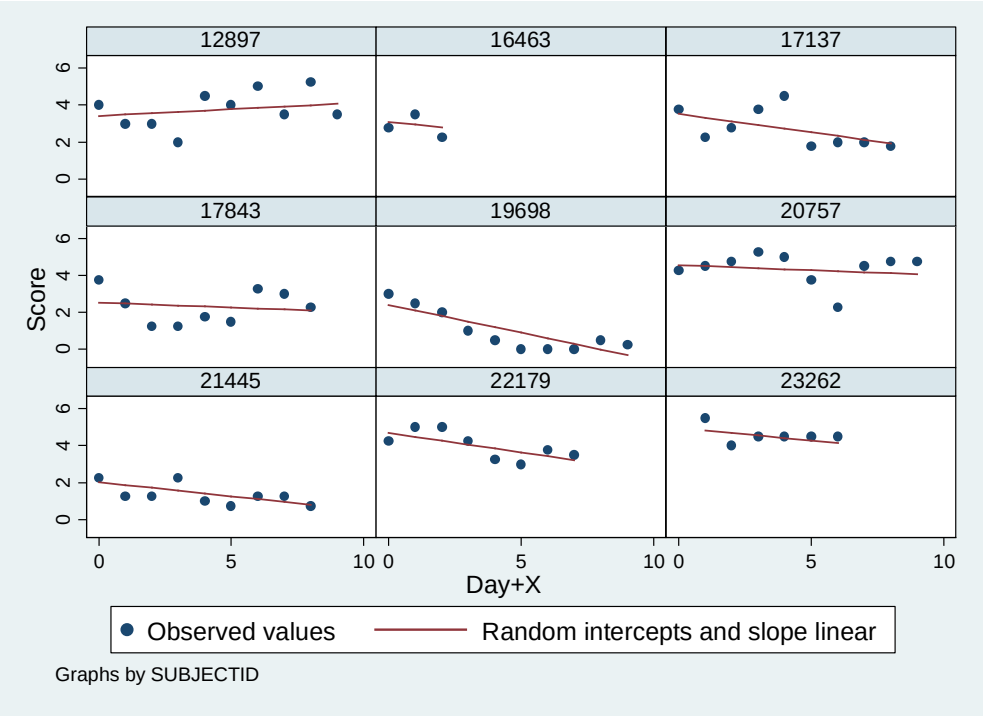


Figure 20: Observed and fitted values for randomly selected examples

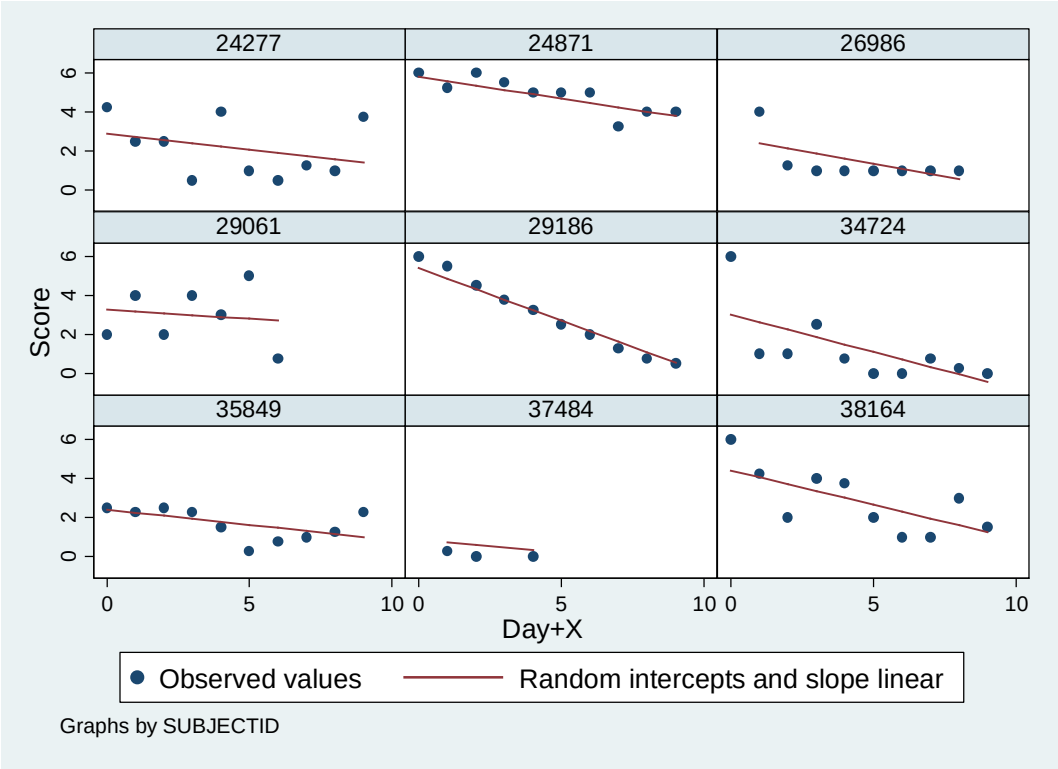


Figure 21: Residuals by day for linear model with random intercept and slope

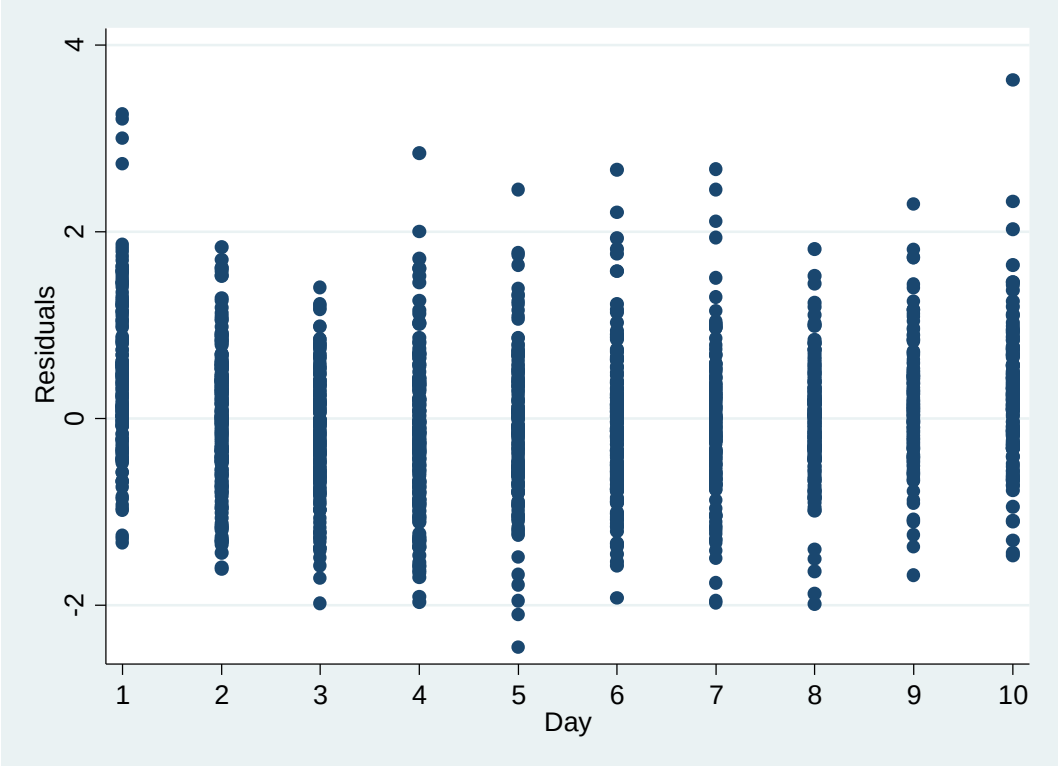


Figure 22: Level 2 Residuals (Slope) for linear model with random intercept and slope (by definition it is constant over time)

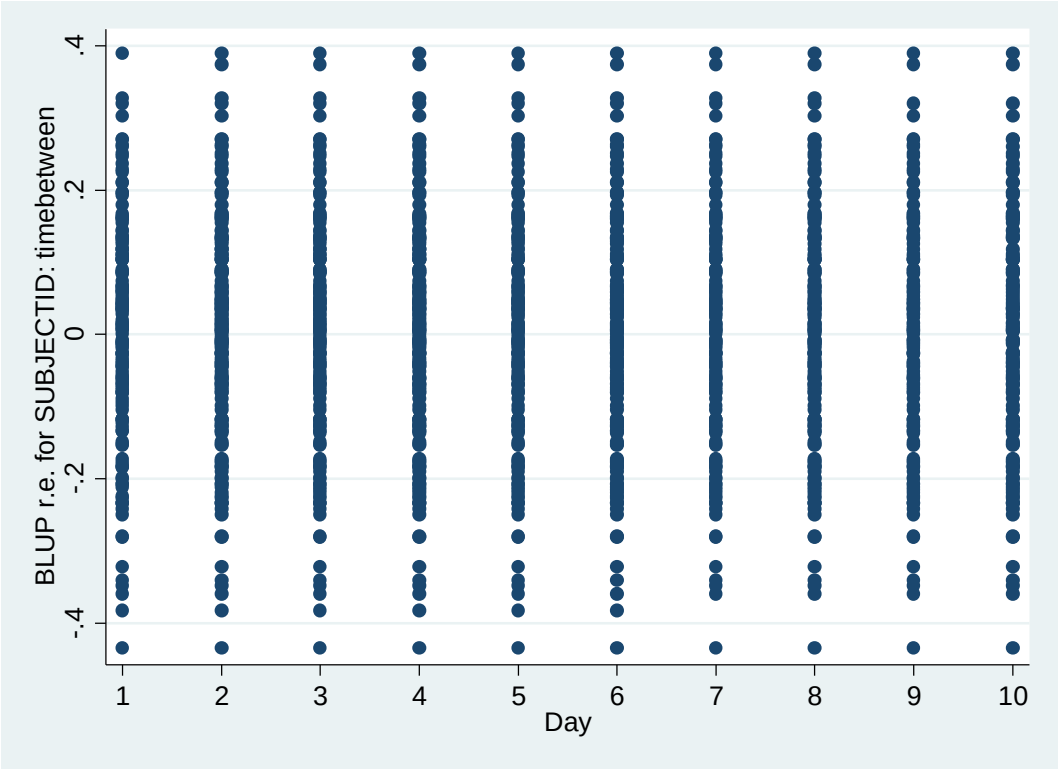


Figure 23: Level 2 Residuals (intercept) for linear model with random intercept and slope (by definition it is constant over time)

8.3.2 Quadratic Model with random intercept and Slope

Figure 24: Observed and fitted values for randomly selected examples

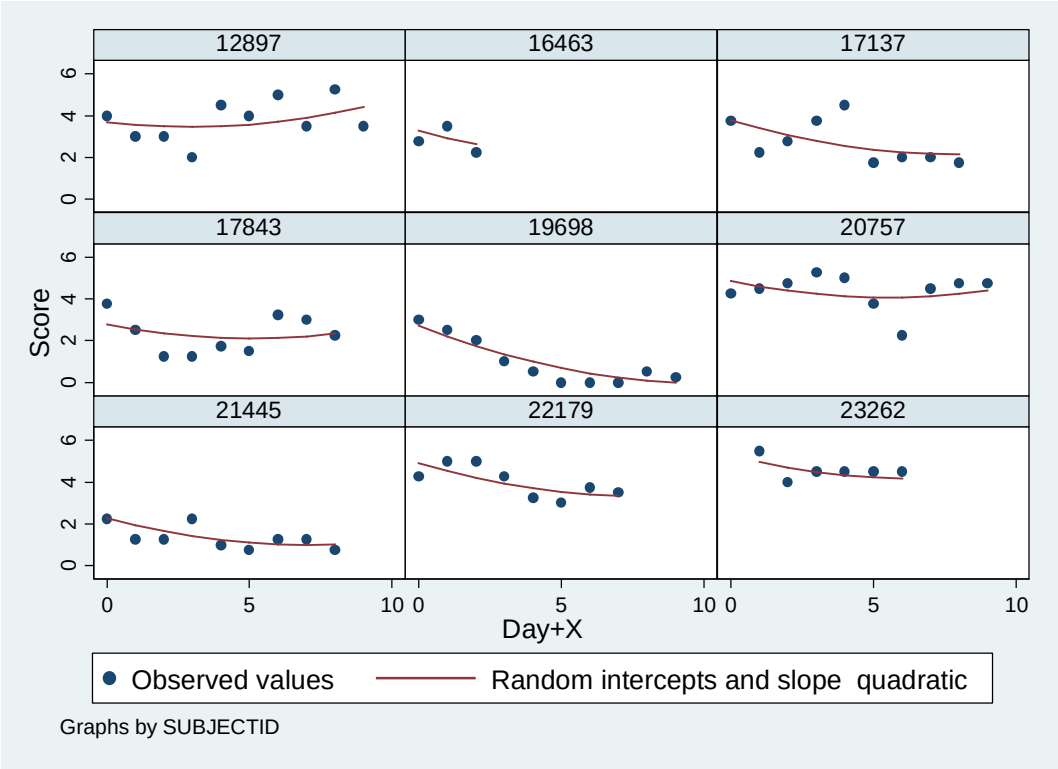


Figure 25: Observed and fitted values for randomly selected examples

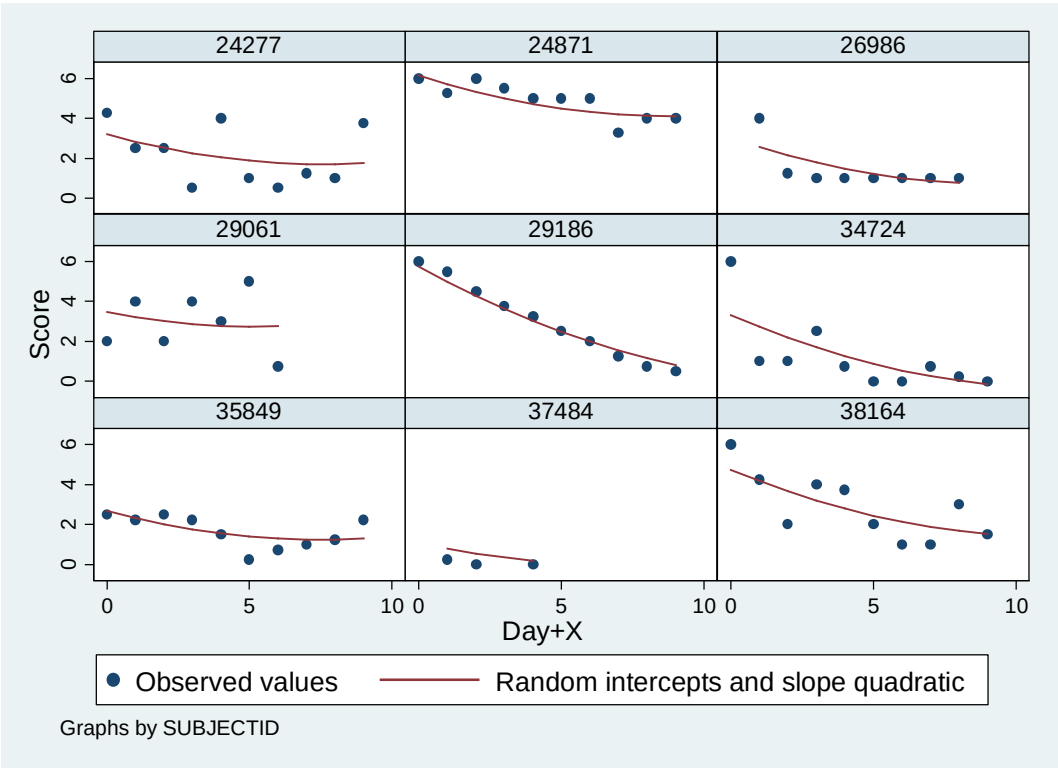


Figure 26: Residuals by day for quadratic model with random intercept and slope

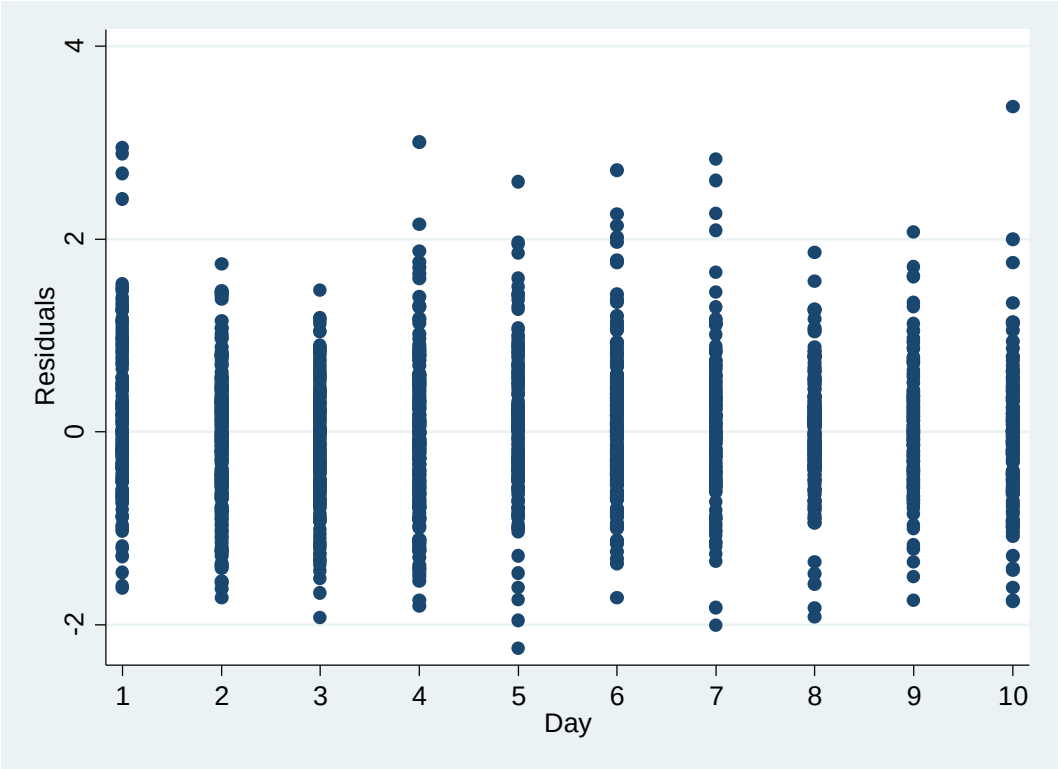


Figure 27: Level 2 Residuals (intercept) for quadratic model with random intercept and slope (by definition it is constant over time)

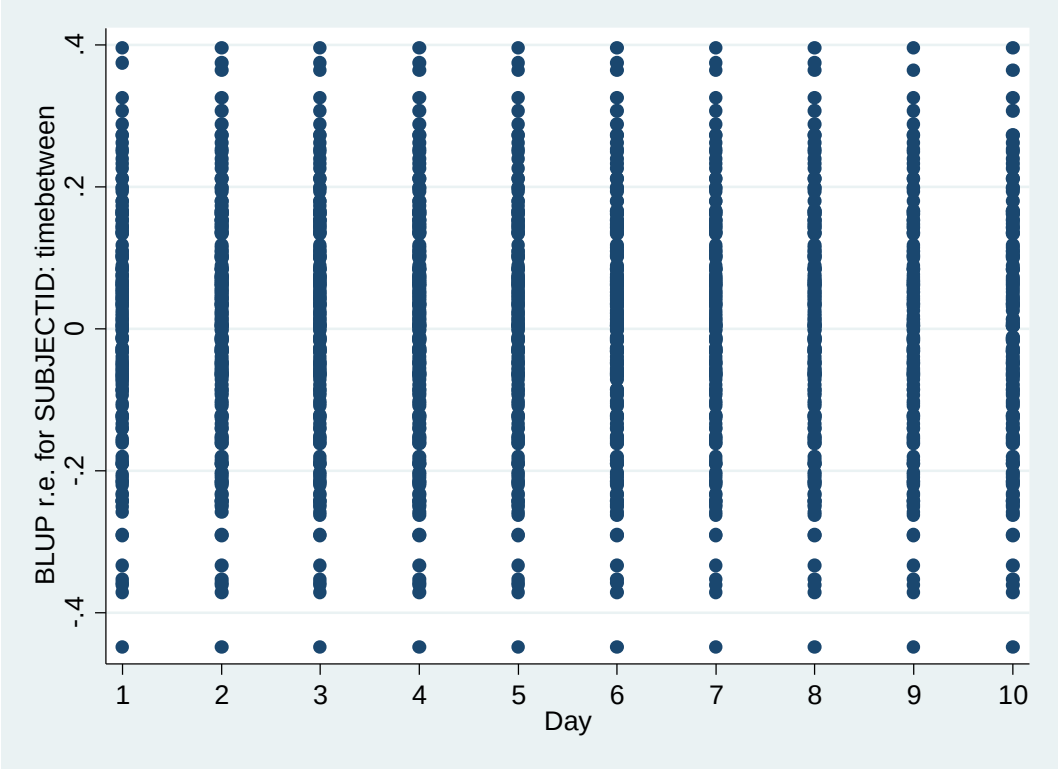
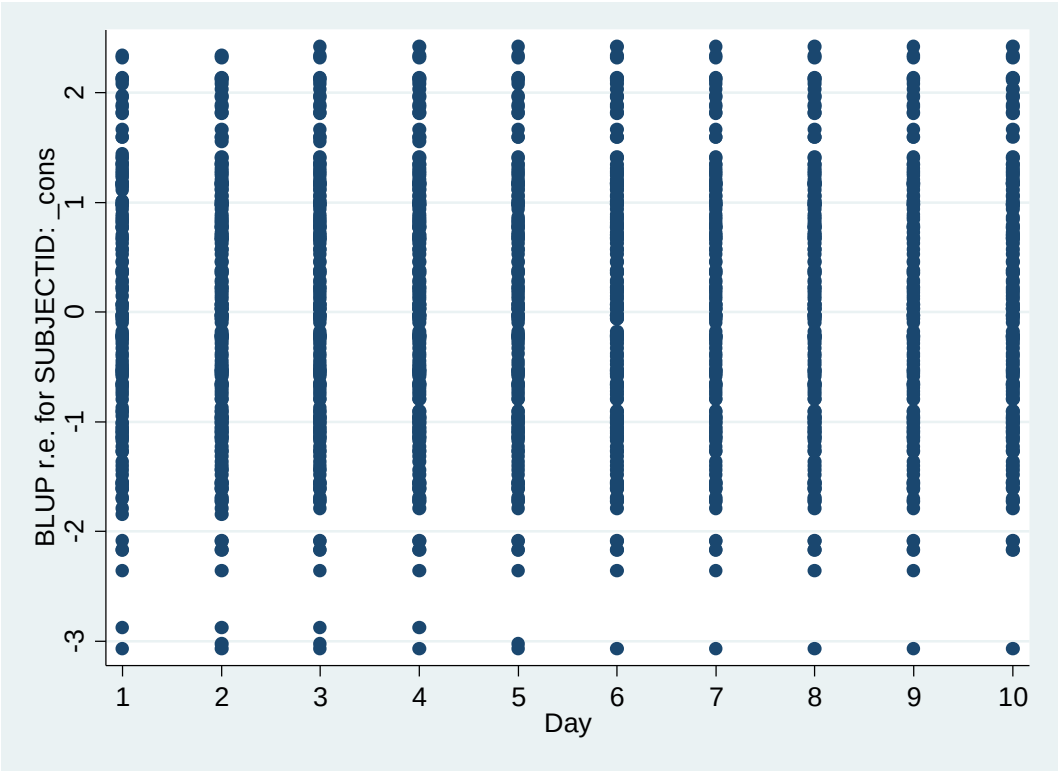


Figure 28: Level 2 Residuals (intercept) for quadratic model with random intercept and slope (by definition it is constant over time)



8.3.3 Square root model

Figure 29: Observed and fitted values for randomly selected examples

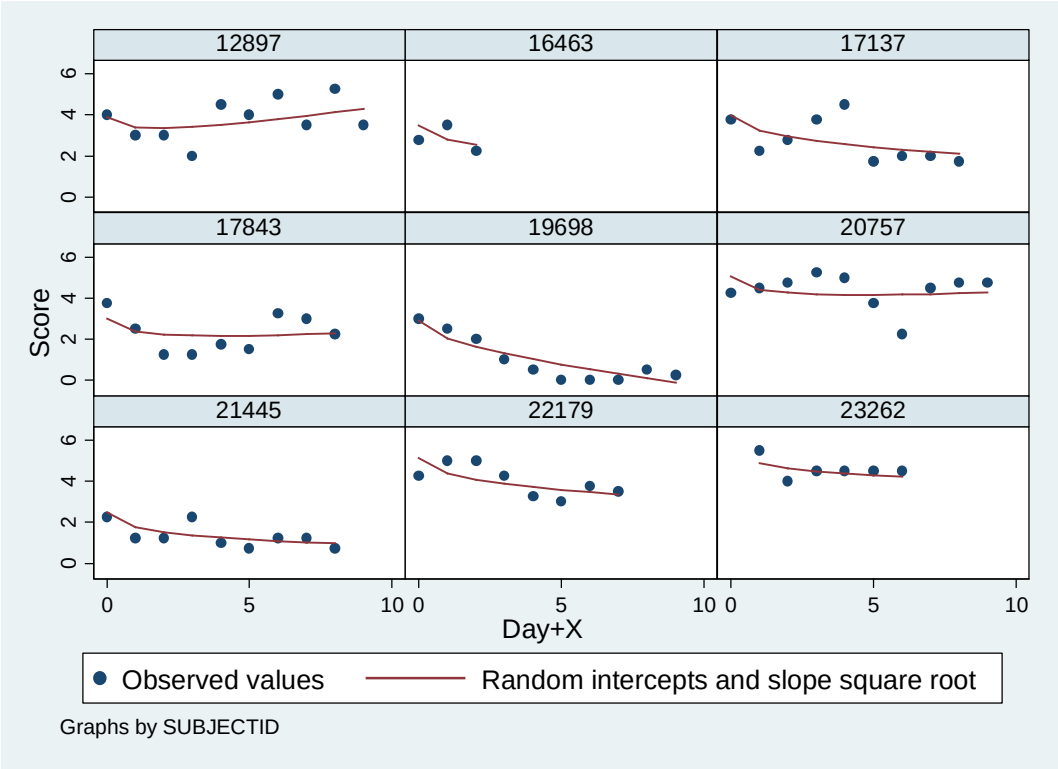


Figure 30: Observed and fitted values for randomly selected examples

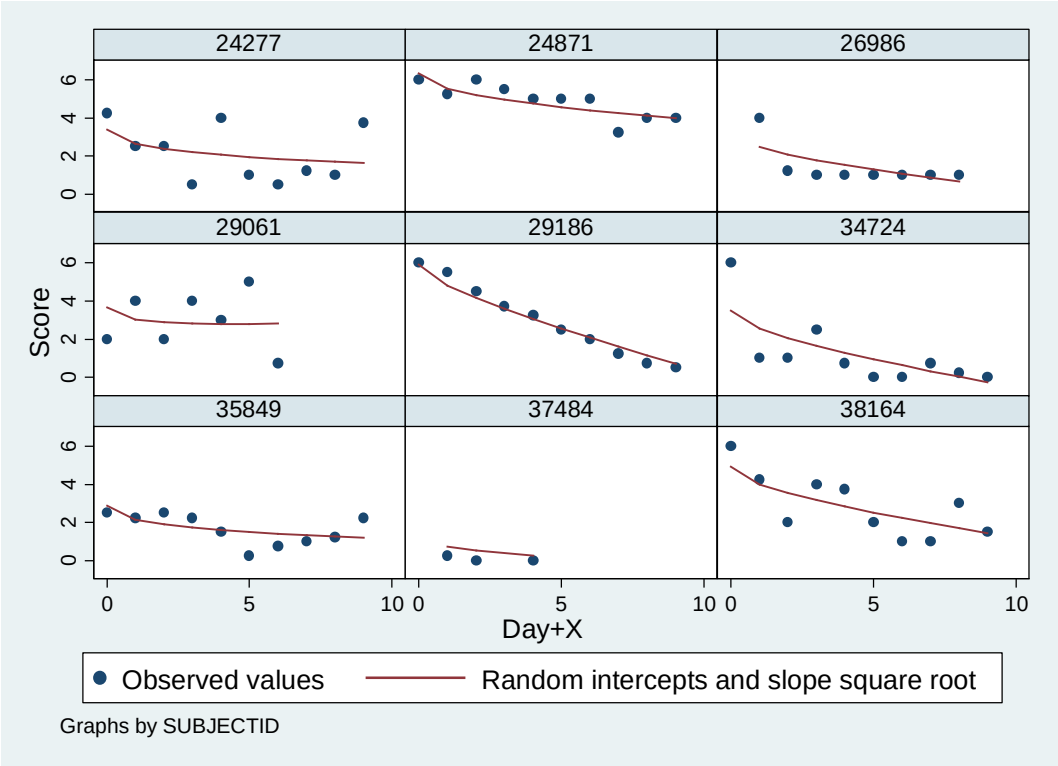


Figure 31: Residuals by day for quadratic model with random intercept and slope

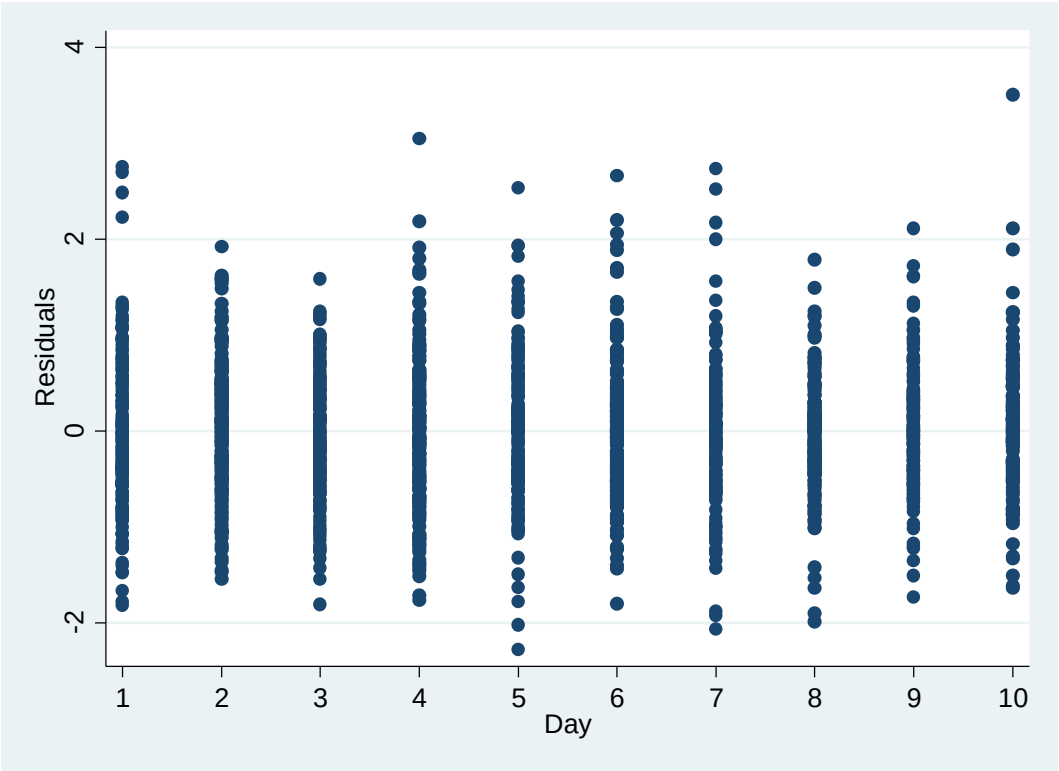


Figure 32: Level 2 Residuals (Slope) for square root model with random intercept and slope (by definition it is constant over time)

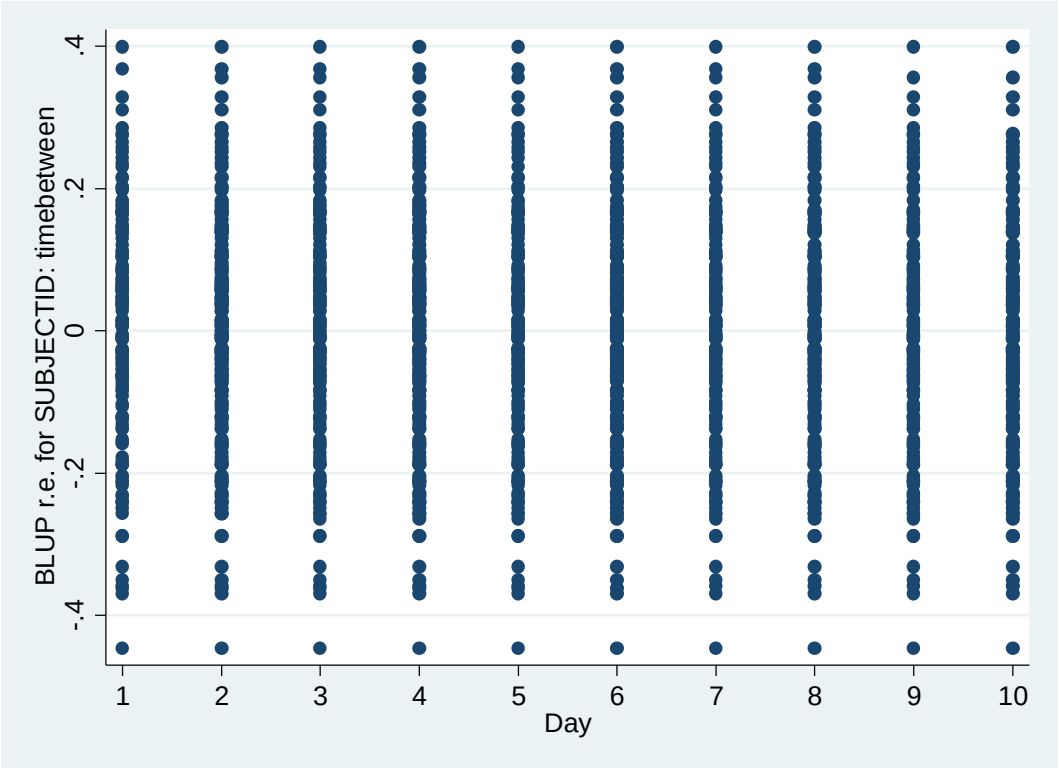
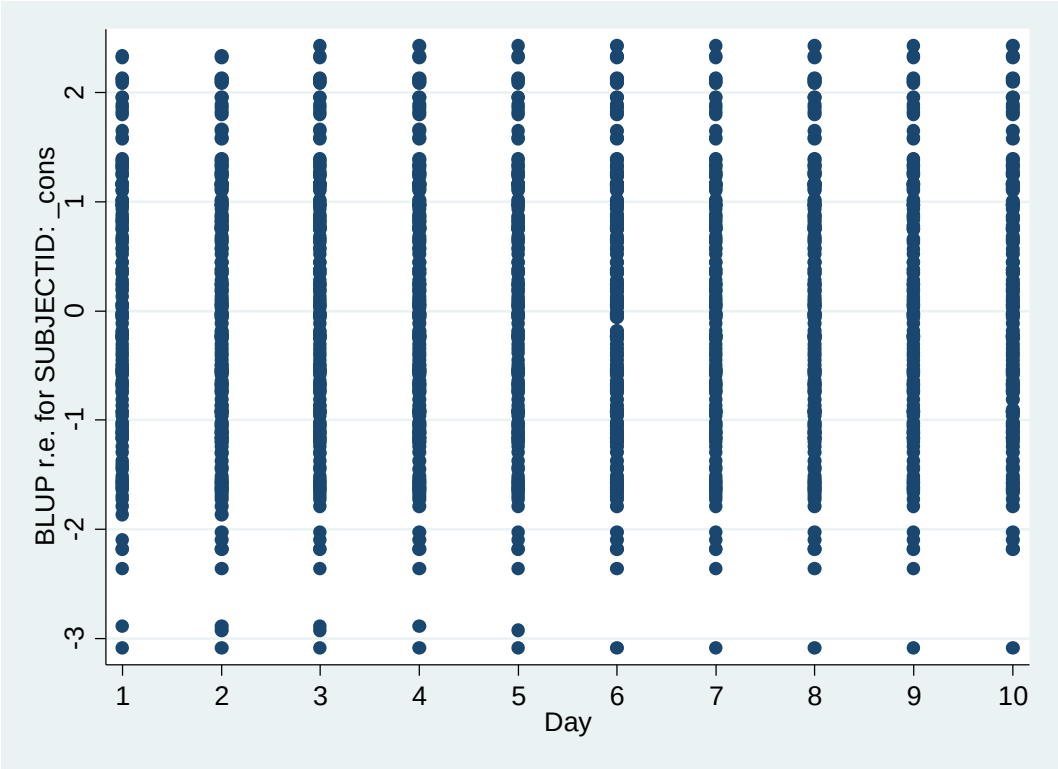


Figure 33: Level 2 Residuals (intercept) for square root model with random intercept and slope (by definition it is constant over time)



8.3.4 Splines (at day 3, day 7)

Figure 34: Observed and fitted values for randomly selected examples

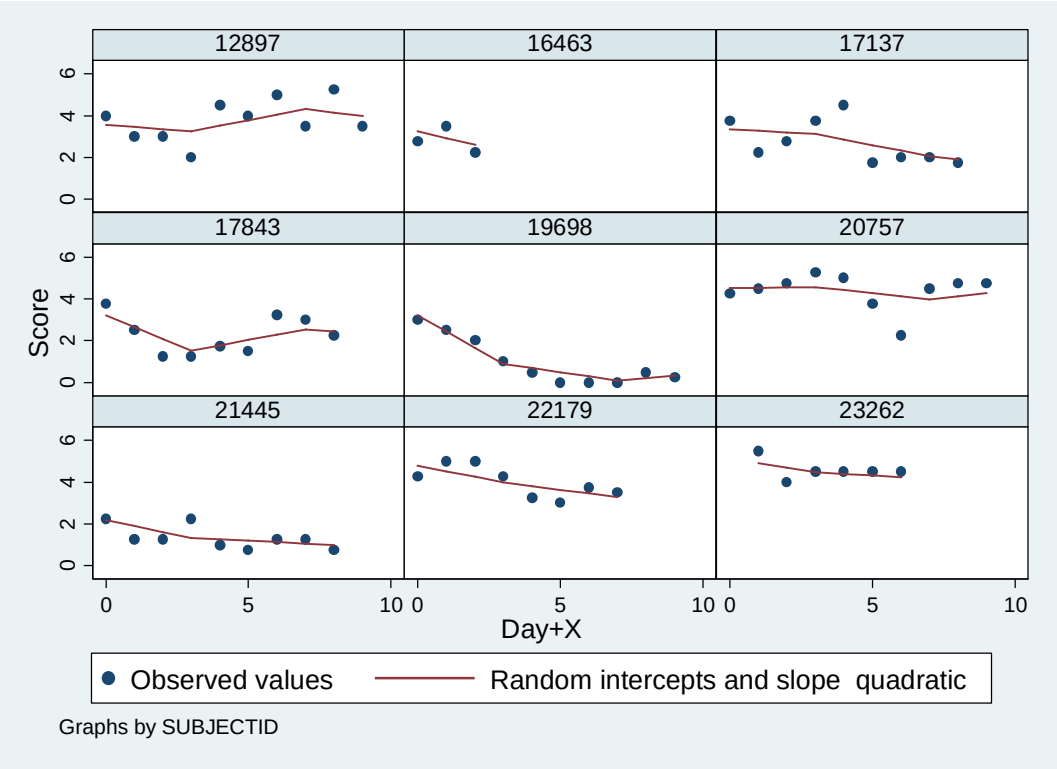


Figure 35: Observed and fitted values for randomly selected examples

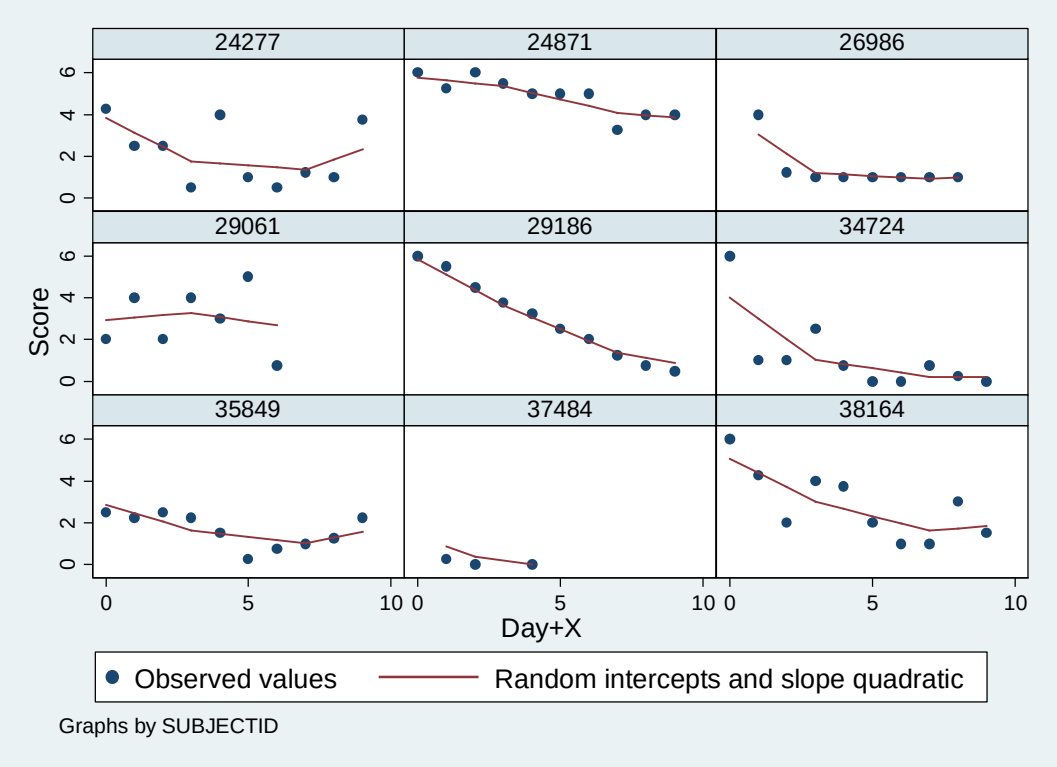
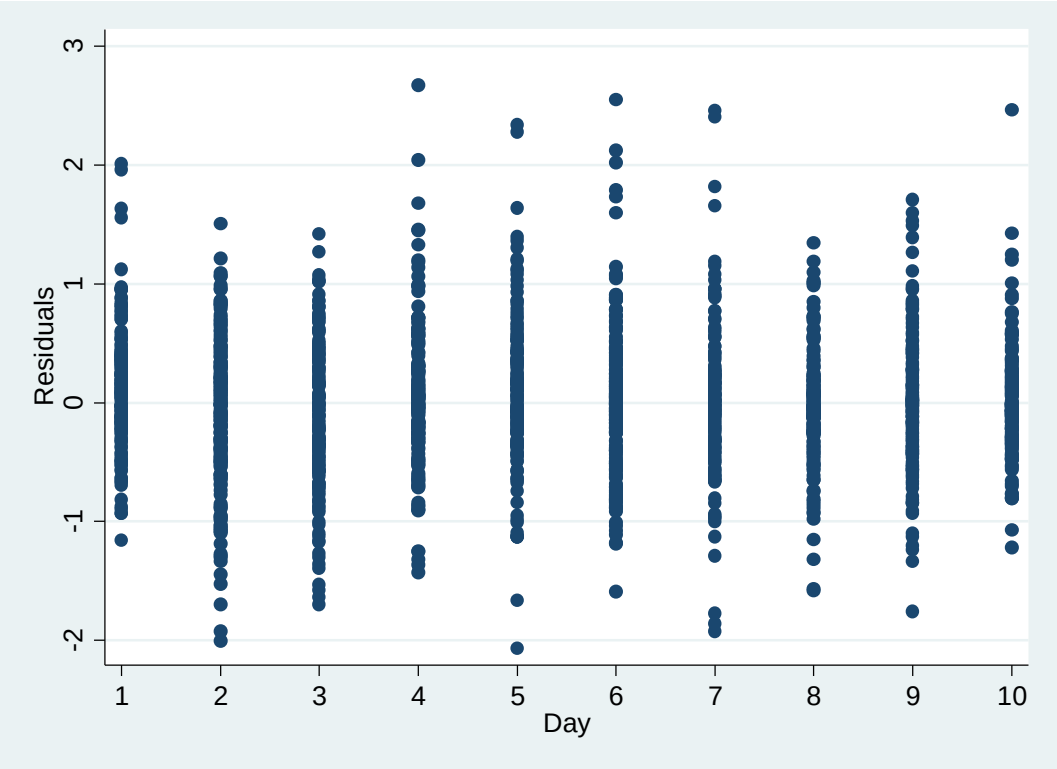


Figure 36: Residuals by day for Splines model with random intercept and slope



8.4 Details of the models for diary and AQLQ Scores

Table 38: Diary score

Fixed-effects Parameters					
Covariates		Coefficient	95% CI		P value
Constant	Mean score at baseline in the Placebo group	3.6595	3.4169	3.9022	0.000
Days (centered)	Daily change in Placebo group	-0.1792	-0.2217	-0.1367	0.000
Treatment #Day (interaction)	(Treatment effect) Difference in daily change compared to the Placebo group	-0.0185	-0.0744	0.0374	0.517

Random-effects Parameters				
Level	variance	Estimate	95% CI*	
Site	Constant (intercept)	0.0412	0.0012	1.4372
Subject	Constant (intercept) Days (slope)	1.6863 0.0334	1.3063 0.0251	2.1769 0.0443
	Covariance Days - Constant	-0.0957	-0.1461	-0.0453
	residuals	0.6941	0.6415	0.7510

***95% confidence intervals presented for the variance parameters should not be used to test** the significance of the variance parameters as the lower limits of these intervals can never be smaller than zero since variances are strictly positive quantities

LR test vs. linear regression: p < 0.0001

Table 39: AQLQ

Fixed-effects Parameters				
Covariates		Coefficient	95% CI	P value
Constant	Mean score at baseline in the Placebo group	4.727	4.491 4.962	0.000
Visits (centered)	Per visit change in Placebo group	0.429	0.275 0.583	0.000
Treatment #Visit (interaction)	(Treatment effect)	0.065	-0.138 0.269	0.530

Random-effects Parameters			
Level	variance	Estimate	95% CI*
Site	Constant (intercept)	0.063	0.009 0.450
Subject	Constant (intercept) Visits (slope)	0.888 0.165	0.583 1.353 0.059 0.464
	Covariance Visits - Constant	-0.074	-0.272 0.125
	residuals	0.903	0.727 1.123

Table 40: Mini AQLQ

Fixed-effects Parameters (MINI)				
Covariates		Coefficient	95% CI	P value
Constant	Mean score at baseline in the Placebo group	3.355	3.196 3.514	0.000
Visits (centered)	Per visit change in Placebo group	0.350	0.214 0.486	0.000
Treatment #Visit (interaction)	(Treatment effect)	-0.021	-0.204 0.163	0.823

Random-effects Parameters (MINI)			
Level	variance	Estimate	95% CI*
Site	Constant (intercept)	0.000	0.000 0.000
Subject	Constant (intercept) Visits (slope)	0.803 0.185	0.569 1.133 0.097 0.350
	Covariance Visits - Constant	-0.076	-0.220 0.069
	residuals	0.566	0.457 0.703