**Effect of azithromycin on incidence of acute respiratory exacerbations in children with HIV taking antiretroviral therapy and co-morbid chronic lung disease: a secondary analysis of the BREATHE trial**

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**Abstract**

**Background:** In the BREATHE trial weekly azithromycin decreased the rate of acute respiratory exacerbations (AREs) compared to placebo among children and adolescents with HIV-associated chronic lung disease (CLD) taking antiretroviral therapy (ART). The aim of this analysis was to identify risk factors associated with AREs and mediators of the effect of azithromycin on AREs.

**Methods:** The primary outcome of this analysis was the rate of AREs by study arm up to 49 weeks. We analysed rates using Poisson regression with random intercepts. Interaction terms were fitted for potential effect modifiers.

**Findings:** We analysed data from 345 participants (171 allocated to azithromycin and 174 allocated to placebo). In the placebo arm, the rate of AREs were higher among those with an abnormally high respiratory rate at baseline (adjusted rate ratio (aRR) 2.90 95% CI 1.30-6.46 p-value 0.0068) and among those with a CD4 cell count <200 cells/mm3 (aRR 2.50; 95%CI 1.04-5.97; p-value 0.0387). We found some evidence for heterogeneity in the effect of azithromycin by sex (p-value for interaction=0.0660); males had a greater reduction in the rate of ARE with azithromycin treatment compared to females. There was weak evidence of azithromycin having a greater impact on reducing AREs in participants with chronic respiratory symptoms at baseline, those on 1st line ART, with a FEV1 score >-2 and participants without baseline resistance to azithromycin.

**Interpretation:** These may represent subgroups who may benefit the most from treatment with weekly azithromycin

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**Research in Context**

**Evidence before this study**

We conducted a literature search of PubMed, Medline and Embase of publications between January 2010 and June 2020 to review the previous research related to the clinical, lung function and radiological features of chronic lung disease (CLD) in children and adolescents with HIV in sub-Saharan Africa using the terms “Chronic lung disease”, “obliterative bronchiolitis”, “bronchiectasis”, “lymphocytic interstitial pneumonitis”, plus “human immunodeficiency virus”, “HIV” plus “children”, “adolescents”.

We identified 16 relevant studies, all of which were observational. Studies showed a high prevalence of respiratory symptoms (25-55%), with tachypnoea being the most common followed by chronic cough. Abnormal spirometry was a frequent finding (26-45% of participants). Bronchiectasis and obliterative bronchiolitis (OB) were the most frequently described pathologies in recent studies, with lymphocytic interstitial pneumonitis (LIP) described in studies carried out in the pre- antiretroviral therapy (ART) era. Risk factors for HIV-associated CLD included low CD4 count, stunting, wasting, previous tuberculosis, taking 2nd line ART, older age at ART initiation, previous hospitalisation and low body mass index . Studies showed a high prevalence of chest radiographic abnormalities (46-68%), and high resolution computed tomography studies were consistent with constrictive obliterative bronchiolitis being the most common cause of CLD. We identified no interventional studies, and little evidence exists on the management of CLD in children and adolescents with HIV.

Azithromycin has both anti-microbial and anti-inflammatory activity, which may help to suppress generalised immune activation, provide prophylaxis against respiratory tract infections. In studies in patients with cystic fibrosis, treatment with azithromycin resulted in improved lung function, reduced respiratory exacerbations and a reduced need for treatment with oral antibiotics. The BREATHE trial found that treatment with weekly weight-based azithromycin for 48 weeks did not result in improved lung function but did result in a significantly lower risk of fewer acute respiratory exacerbations (AREs).

**Implications of this study**

In this post hoc analysis of the BREATHE trial we examined risk factors for AREs in the placebo group, and factors that affect the association between weekly azithromycin and risk of AREs.We found that a baseline abnormally high respiratory rate and CD4 count <200 cells/mm3 was associated with developing AREs. We found evidence that azithromycin was more effective at reducing AREs in male than in female participants. Although not statistically significant, there was also evidence of azithromycin having a greater impact on reducing AREs in participants with chronic respiratory symptoms (cough or abnormally high respiratory rate), an FEV1Z-score ≥-2, those on 1st line ART, and participants without resistance to azithromycin at baseline.

**Implications of all the available evidence**

These may represent subgroups who may benefit the most from treatment with weekly azithromycin. The use of targeted treatment may reduce concerns regarding the emergence of antimicrobial resistance (AMR) Further studies to prospectively evaluate the sustainability of effect and the optimum dose and length of treatment to prevent AREs, the sustainability of effect and which groups would benefit most from azithromycin therapy are needed. These studies should also evaluate the risk of AMR with prolonged use.

**Introduction**

The scale-up of paediatric antiretroviral therapy (ART) has resulted in a dramatic increase in survival such that children who would have died in infancy or early childhood are now surviving to adolescence. However, children growing up with HIV experience a range of multisystem comorbidities despite ART, which may be sequelae of infections that occur as a result of immunosuppression caused by HIV or a consequence of HIV infection itself or its treatment. Chronic lung disease (CLD) is one of the most common comorbidities among older children and adolescents with HIV (1). In the pre-ART era, lymphoid interstitial pneumonitis (LIP) was the most common cause of CLD. LIP responds well to ART and is now a rare presentation to clinical practice (2). Recent studies from sub-Saharan Africa (sSA) have shown that constrictive obliterative bronchiolitis is now the predominant underlying cause for CLD in children and adolescents with HIV, and is associated with morbidity including chronic cough, hypoxia, reduced exercise tolerance and recurrent respiratory tract infections (1,3).

The BREATHE (Bronchopulmonary function in response to azithromycin treatment for chronic lung disease in HIV-infected children) trial investigated whether adjuvant treatment with azithromycin results in improved lung function in children and adolescents with HIV taking ART who had CLD (4). The trial showed that azithromycin did not lead to a significant improvement in FEV1 Z-score (primary outcome), but did lead to a significant reduction in the risk of acute respiratory exacerbations (AREs) (secondary outcome) (5). Respiratory infections have been shown to be the most common cause of hospital admissions amongst adolescents with HIV established on ART, and AREs are likely to impact significantly on morbidity and quality of life (6).

In this *post hoc* analysis of the BREATHE trial, we investigated the impact of azithromycin on AREs, to identify potential subgroups in this population who would most benefit from azithromycin treatment. Specifically, we investigated the risk factors for AREs in the placebo group; crude rates of AREs in both the placebo and azithromycin group and factors that may modify the effect of azithromycin on risk of AREs.

**Methods**

**Study Design**

The BREATHE trial is a multicentre, individually randomised, placebo-controlled trial. A detailed study protocol has been published (4).

Participants were recruited from outpatient HIV clinics at the Harare Children´s Hospital (Zimbabwe) and Queen Elizabeth Central Hospital in Blantyre (Malawi). Ethical approval was provided by College of Medicine Research Ethics Committee (COMREC P.04/15/1719) (Malawi), the Medical Research Council of Zimbabwe and the Biomedical Research and Training Institute IRB (Zimbabwe xxxxx), the London School of Hygiene and Tropical Medicine Ethics Committee (UK xxxxxx) and the University of Tromso Ethics Committee (Norway xxxxxx). The trial is registered with ClinicalTrials.gov, NCT02426112.

**Participants**

Participants were eligible if they were aged 6-19 years, had been on any combination of ART for at least six months and had CLD, defined as an FEV1 Z-score less than -1.0 with no reversibility ( <12% improvement in FEV1 after 200mcg of salbutamol inhaled via a spacer), established by spirometry. Exclusion criteria included having a potentially fatal condition, tuberculosis (TB) or an acute respiratory infection (ARI) at the time of screening, pregnancy, breastfeeding, history of a cardiac arrythmia, a prolonged QTc interval (>440 milliseconds in males and >460 milliseconds females), creatinine clearance <30 mls/minute, elevated alanine aminotransferase (ALT) >2 times the upper limit of normal, known hypersensitivity to a macrolide and concomitant use of drugs known to cause QTc prolongation. TB screening was performed using the Xpert™ MTB/RIF (Cepheid, Sunnyvale, CA, USA) on one sputum sample obtained either spontaneously or through induction. Participants over 18 consented independently. For those aged <18 consent was sought from the guardian with age-appropriate assent from the participant.

Participants were randomised 1:1 by block randomisation to receive either an oral weekly weight-based dose of azithromycin or placebo.

**Procedures**

Weight-based oral azithromycin tablets (10–19.9 kg, 250 mg; 20–29.9 kg, 500 mg; 30–39.9 kg, 750 mg; > 40 kg, 1250 mg) or identical placebo tablets were given weekly under direct observation by a treatment monitor identified within the family for a total of 48 weeks. Characteristics recorded at baseline include socio-demographic and clinical history, symptom history, drug history, spirometry, shuttle walk test, height, weight, electrocardiogram (ECG), serum creatinine and ALT, pregnancy test, sputum sample for TB screening, CD4 count, HIV viral load (VL). Participants were followed up at two weeks and at three monthly intervals thereafter for a total of 49 weeks. Participants were asked to attend for an unscheduled visit if they developed acute symptoms. AREs were defined as new or worsening respiratory symptoms (cough with or without sputum production, breathlessness, chest pain) with or without fever as assessed by a clinician. For participants attending with AREs, sputum and nasal swabs were taken and participants were treated with co-amoxiclav for 10 days. If this resulted in no improvement than a CXR and culture for *Mycobacterium tuberculosis* (M. tb) was performed. Participants were also asked to contact the study team if they were admitted to hospital, and were asked about hospitalisation at each study visit.

Conventional culture was performed for common bacterial pathogens at baseline, 12 and 18 months on sputum and nasopharyngeal samples. Antibiotic susceptibility testing was performed on relevant cultured isolates using Vitek-2 (bioMerieux, France) or disk diffusion testing.

**Statistical Analysis**

Statistical analysis was performed using STATA software version 16.1 (STATA Corp, College Station, Texas, USA). The pre-specified analysis of the BREATHE trial has been described elsewhere (4,5). In this *post-hoc* analysis, incidence rate ratios of AREs by arm with 95% confidence intervals (CIs) were calculated. Poisson regression was used to create a model of the effect of azithromycin on AREs, with random effects to account for multiple events within a participant. Lexis expansion was used to allow for joint adjustment of time variables (including season, calendar time and follow-up time), to look for variation of rates of AREs within these. Season was defined as rainy (November to March?) and dry (April to October); calendar time was split into two groups, 2016-2017 versus 2018-2019; and follow-up time was split into four 12-week periods corresponding to the prescribing regimen. The relationship between ARE and time variables independent of other risk factors was explored. Collinearity among time variables was assessed by examining the change in standard errors between unadjusted and models adjusted for other time variables. All subsequent models were adjusted for those time variables which had Wald p-value <0.1 once adjusted for each other and where they did not exhibit co-linearity. Participants were censored at death, last study visit if lost to follow-up, date of withdrawal if withdrawn from the study or at 49 weeks after commencing study medication. Two participants missing HIV viral load at baseline were excluded from all analyses.

Risk factors for AREs among placebo group participants were examined. Risk factors at enrolment included FEV1 Z-score, CD4 cell count, being on second line ART, weight-for- age Z-score, height-for-age Z-score (7), presence of current cough, tachypnoea and a history of treatment for TB. An adjusted model included age group, sex, site and HIV viral load at enrolment, in alignment with the primary analysis, as the latter characteristics were found to differ between trial arms by chance and were also associated with loss to follow-up (5). The model estimates therefore accounted for missing data under a missing at random assumption. A second model was created which included the above mentioned characteristics as well as additional variables that were found to be associated with AREs in the initial model (p-value <0.2) (model 2).

Data from both trial arms was used to explore whether the reduced rate of AREs among those receiving azithromycin treatment, a pre-specified secondary outcome of the trial reported elsewhere (5), exhibited heterogeneity in effect by any of the risk factors assessed for their association with ARE in the placebo arm. Adjusted random effects Poisson regression models were used to explore whether the effect of azithromycin on AREs was modified by baseline risk factors including age group, sex, FEV1 Z-score, CD4 cell count, HIV VL, history of treatment for TB, weight-for-age Z-score, height-for-age Z-score, presence of cough and tachypnoea, and additionally carriage of azithromycin-resistant bacteria (in sputum or from nasal swab), time variables. P-values for interaction were determined using a likelihood ratio test (LRT) and indicated the strength of evidence that the treatment effect of azithromycin versus placebo was heterogeneous between risk factor levels. Age was regrouped in the interaction term into two levels (6-15 and 16+) but adjusted as three levels in other models. VL was adjusted for as a continuous variable but included in the interaction term as two levels, supressed (<1000 copies/ml) or unsuppressed (≥1000 copies/ml). Models were adjusted for *a priori* factors as per the primary analysis and time variables as previously stated. For risk factors where heterogeneity between the stratum specific estimates was observed unadjusted cumulative incidence curves, stratified by risk factor level and trial arm were generated.

**Role of the Funding Source**

The BREATHE trial was funded by the GLOBVAC Programme of the Medical Research Council of Norway. The funder had no role in study design, data collection, data analysis, data interpretation or manuscript writing. There was no funding source for this post hoc analysis.

**Results**

Participants were recruited between 15 June 2016 and 4 September 2018 and follow up ended in August 2019. Of the 347 participants included in the BREATHE trial, 345 participants had no missing information for all variables of interest and were included in this analysis, with 171 participants randomised to the azithromycin and arm and 174 participants randomised to the placebo arm. Baseline characteristic of participants stratified by trial arm and outcome (at least one ARE episode) are summarised in Table 1. In both trial arms, those who experienced at least one ARE were older, had lower FEV1 Z-score at baseline, had a lower CD4 cell count, higher HIV VL, and commenced ART at older age.

In the azithromycin arm more females developed at least one ARE compared to males (68.8% vs 31.2%), but this was not the case in the placebo arm where a similar proportion of females and males developed at least one ARE (46.7% vs 53.3% respectively). Participants in the azithromycin arm who experienced at least one ARE were more malnourished than those in the placebo arm who experienced at least one ARE. Overall, 9% of participants had a cough at baseline ; however, 26.7% of the participants in the placebo group who developed an ARE had a cough at baseline. An abnormal RR at baseline was observed in almost half of participants (43.8%), and this subgroup was also overrepresented in those who developed one or more AREs in the placebo arm, with 70% of these participants having a cough at baseline.

There were 38 episodes of AREs amongst placebo group participants and 19 episodes of AREs in the azithromycin arm, over a total of 154 and 157 person years respectively. In the azithromycin arm 14 participants had 1 ARE, 1 had 2 AREs and 1 had 3. In the placebo arm 24 participants had 1 ARE, 4 had 2 ARES and 2 had 3. There was evidence that the rate of AREs varied by season, calendar period and time in study in an unadjusted analysis (Tables S2 and S3). After adjusting for each other, season and calendar period remained associated with the rate of AREs both in the placebo arm (Wald p-value=0.0380 and p=0.0534 respectively) and in the azithromycin arm (Wald p-value= 0.0388 and p=0.0103). Rates of ARE were lower in the rainy season compared to the dry season and reduced over time (lower in 2018-19 compared to 2016-17). As a result, all subsequent analyses were adjusted for season and calendar time.

Among placebo arm participants, site, baseline CD4 cell count, presence of a cough and abnormal respiratory rate were associated with the rate of AREs (Table 2, p-values <0.1) after adjusting for age, sex, site, and baseline viral load. Effects were consistent after adjusting for the potential confounding effect of the identified risk factors, and follow up time. There was evidence of a lower rate of AREs in participants in Malawi compared to those in Zimbabwe (fully adjusted RR 0.33; 95% CI 0.12-0.92; p-value 0.0197). Having a CD4 count <200 cells/mm3 was associated with increased risk of AREs (fully adjusted RR 2.50; 95% CI 1.04-5.97; p-value 0.0387). Compared to those with a normal respiratory rate at baseline, those with an abnormal respiratory rate had a higher rate of AREs (fully adjusted RR 2.90 95% CI 1.30-6.46 p-value 0.0068). Presence of a cough at baseline was associated with higher rates of AREs in minimally adjusted analysis (model 1), but this association was attenuated in the fully adjusted model indicating some confounding (RR 2.16 95% CI 0.95-4.90 p-value 0.0837) (Table 2).

Stratum-specific ARE rates varied by trial arm and risk factor. However, statistical testing did not indicate heterogeneity in the effect of azithromycin versus placebo in the rate of ARE for risk factors measured at baseline (Table 3; Figure S1-S6). There was weak evidence that the effect of azithromycin versus placebo differed by sex (LRT test for interaction= 0.0660), with a reduced rate of AREs with azithromycin treatment compared to placebo among males (RR 0.23 95% CI 0.08-0.66) but not among females (RR 0.77 95% CI 0.35-1.68). Lower rates of AREs with azithromycin treatment compared to placebo were found among those with a cough and an abnormal respiratory rate, compared to those without these symptoms, those on 1st line versus 2nd line ART, those without carriage of azithromycin resistant bacteria, and those with better baseline lung function (FEV1 Z-score ≥-2 vs <-2). However, these findings did not reach statistical significance (Table 3).

**Discussion**

In this post hoc analysis of the BREATHE trial, an abnormal respiratory rate at baseline was associated with almost 3-times the rate of ARE compared to those with normal respiratory rate among participants in the placebo group. Presence of a cough, although rare, was a risk factor among those participants. Other studies have found tachypnoea to be strongly associated with HIV-associated CLD in children: a cross-sectional study from South Africa in 2005-2006 in children with HIV-associated CLD found that tachypnoea was associated with reduced FEV1, although their cohort was younger (mean age 5 years) and the predominant pathology was LIP, which is uncommon in the ART era (8). An abnormal respiratory rate may represent more symptomatic and severe CLD, which can in turn put patients at greater risk of more frequent and severe exacerbations. Presence of a cough may represent an underlying pathology, such as pulmonary hypertension which has been described in children and adolescents with HIV related CLD (9), which may predispose to more severe disease and AREs. It may also represent chronic inflammation or unresolved previous infections, which in turn increase the likelihood of subsequent AREs. In adults with chronic obstructive pulmonary disease (COPD) a chronic cough has been found to be associated with increased frequency of exacerbations, something which may also be true in this population (10).

A CD4 count <200 cells/mm3 was a risk factor for AREs in our fully adjusted model. In the context of adults living with HIV, higher CD4 counts are protective for community acquired pneumonia, and lower CD4 counts bring increased risk of many respiratory infections including pneumocystis pneumonia (PCP) and cytomegalovirus (CMV) pneumonia (11,12), so this is likely to play a role as a risk factor for AREs in this population.

Being recruited from Zimbabwe, compared to Malawi was also found to be a risk factor for AREs. There is no clear explanation for this although it could be due to differences in demographics or reporting between these two sites. Poverty and the multiple associated risk factors have long been associated with lower respiratory tract infections (13). According to the World Bank Poverty and Equity report, Zimbabwe has higher rates of national poverty at 70% compared to a national poverty rate of 51.5% in Malawi. The situation in Zimbabwe is also compounded by recent droughts and a deteriorating economic situation, which has made food security and health service delivery increasingly fragile (14), all of which may contribute to increased rates of AREs.

Stratification of rates of ARE by season showed seasonal variation with higher rates in the dry season compared to the rainy season. Seasonal variation of respiratory illnesses has been well documented, and in Zimbabwe ARIs are more common during the colder dry season (15). This is largely due to winter peaks of respiratory viruses including respiratory syncytial virus, which shows peak activity in June, July and August in Southern Africa and is one of the commonest causes of ARIs, both in sSA and globally (16).

The BREATHE trial found that rates of total AREs were approximately 50% lower in the group who received weekly weight based dosing of azithromycin compared to the placebo group, and this association remained after adjusting for *a priori* determined factors. In addition to trial arm, this post hoc analysis found that the crude rates of AREs differed by age, sex, site, FEV1 Z-score, CD4 count, HIV viral load, being on second line ART, history of TB treatment, resistance to azithromycin at baseline season and calendar year.

There was some evidence of effect modification in relation to sex (p=0.0660), with azithromycin appearing to reduce AREs by 77% in males but only 23% in females (stratum specific RR in the azithromycin group was 0.23 and 0.77 respectively). One possible explanation for heterogeneity by sex, is engagement with care. In this cohort most participants are older adolescents and so would likely take responsibility themselves for accessing care. Being male and under the age of 30 is a risk factor for disengaging from HIV care (17) and HIV related mortality in sSA is higher in males for this reason (18). In this cohort male adolescents may be less likely to seek acute care for AREs, and as a result may experience more repeated infections, or may only seek care when symptoms become severe, resulting in more severe lung damage, and an increased likelihood of experiencing severe AREs in the future. As a result, preventative treatment may have a greater impact in this subgroup.

Although the test for interaction was not significant, there is some evidence of heterogeneity in the impact of azithromycin with regards to presence or absence of a cough and an abnormal or normal respiratory rate. This may be because both a cough and an abnormal respiratory rate represent symptomatic and more severe CLD in this cohort and thus represent a group who are more likely to experience recurrent AREs. These participants may hence benefit more from preventative weekly treatment with azithromycin. Our findings also show that azithromycin was more effective at reducing AREs in participants with a baseline FEV1 Z-score ≥-2, compared to those with and FEV1 Z-score <-2 (RR 0.25 95%CI 0.08-0.77 and RR 0.62 95%CI 0.30-1.30 respectively), although again, the test for interaction did not reach statistical significance. This result may also be representative of disease severity and a predisposition to repeated and severe AREs. A prospective cohort study from South Africa by Githinji *et al* found that amongst adolescents with HIV a lower FEV1 score was associated with previous lower respiratory tract infections (19), and in children with cystic fibrosis respiratory exacerbations are associated with a reduction in FEV1 (20). In children and adolescents living with HIV it is also plausible that repeated chest infections will cause structural damage, and chronic inflammation which in turn reduces FEV1, whilst also increasing the risk of repeat infections, making prophylactic azithromycin less effective in this subgroup.

We found evidence of effect modification with regards to resistance to azithromycin at baseline, but this did not reach statistical significance (LRT for interaction p=0.2570), although the stratum specific rate ratios did vary significantly between those with and without azithromycin resistance at baseline (RR 1.38 95% CI 0.19-9.93 and RR 0.42 95% CI 0.22-0.81 respectively). Resistance is a key concern when considering prophylactic antibiotic use, and previous studies have shown emergence of resistance associated with prophylactic use of azithromycin. A study investigating the effect of weekly azithromycin in indigenous children with non-cystic fibrosis bronchiectasis in Australia and New Zealand reported antibiotic resistance to be seven times higher in the treatment group compared to the placebo group (21). A Cochrane systematic review on the use of macrolides in patients with cystic fibrosis found that 2 of the 10 included studies reported a significant increase in macrolide resistant strains of *Staphylococcus aureus* in patients receiving azithromycin (22). This will not only remove the benefit of prophylactic azithromycin in this population, but is also an important public health concern.

Prevention of AREs in this population is likely to have benefits that extend beyond reducing symptoms. AREs are linked to worsening FEV1 in both children and adults with cystic fibrosis (20,23), and in children with non-cystic fibrosis bronchiectasis pulmonary exacerbations are the main risk factor for a decline in FEV1 (24). Prevention of AREs may hence protect against further decline in lung function. It is also important to consider the psychosocial impact of AREs. A study in children with cystic fibrosis in the US found that AREs are associated with worse psychosocial scores on the child health questionnaire, indicating that the impact of AREs goes beyond pure physical symptoms (25). A study in adults with bronchiectasis found that treatment with azithromycin was associated not only with reduced exacerbations and improvement in FEV1, but also improved quality of life (QOL) scores (26). Frequent AREs also impact schooling due to missed school days. A study from Zimbabwe showed that children with HIV miss more school days and are more likely to repeat a school year compared to their HIV negative peers (27). This likely has a significant impact on educational attainment, hence any means of reducing this disparity should be a priority.

In our cohort xx% of participants had been previously treated for TB. High prevalence of respiratory symptoms amongst children and adolescents with HIV leads to high levels of presumptive treatment for TB (9). Prevention of AREs and a possible subsequent reduction in the prevalence of respiratory symptoms has the potential to reduce presumptive TB treatment rates and thus reduce the treatment side effects and potentially economic burden and risk of AMR.

To date no other studies have evaluated potential treatments for HIV associated CLD in children and adolescents, and none have looked at the incidence or risk factors for AREs in this population. Strengths of this study include its robust design: as a double blinded randomized placebo-controlled control trial it is best placed to evaluate the impact of azithromycin on ARE in this cohort, minimizing confounding and many potential sources of bias.

However, this study also has important limitations. The definition of ARE was a clinical one and there may have been differences in ascertainment by trial site. However, a clinical definition was selected as a pragmatic approach as microbiological tests are often not available in these settings. The impact of azithromycin on AREs was a secondary outcome and the BREATHE trial was not powered for this outcome. Additionally, residual confounding may still exist, for example this study did not examine smoking status, something which may be an important confounder, especially amongst older children. However, smoking was not considered by the trial group because of the negligible prevalence of smoking in this group reported in other studies (28,29). Lastly this study focused on subgroup analyses and examined for effect modification using a statistical test for interaction. Tests for interaction are frequently underpowered and have known limitations (30). As a result there is a risk we may have failed to detect effect modification where it does exist, and these results require cautious interpretation.

In summary**,** risk factors for AREs in this population included presence of an abnormal respiratory rate at baseline and having a baseline CD4 count <200/mm3 . Azithromycin was more effective at reducing AREs in male than female participants. Although not statistically significant, there was also evidence of azithromycin having a greater impact on reducing AREs in participants with chronic respiratory symptoms at baseline, those on 1st line ART, those with a FEV1 score >-2 and participants without baseline resistance to azithromycin. These may represent subgroups who may benefit the most from treatment with weekly azithromycin. The use of targeted treatment may reduce concerns regarding the emergence of AMR, and adolescents with HIV-associated CLD should be screened for these respiratory symptoms and clinical characteristics to help identify those most at risk of AREs and to guide management.

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**Tables and Figures**

**Table 1: Baseline characteristics of participants by trial arm and whether they experienced at least one ARE**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **All participants** | **Azithromycin arm** | | **Placebo arm** | |
|  |  | **No ARE** | **At least one ARE** | **No ARE** | **At least one ARE** |
| **Total, n (%)** | 345 | 155 (90.6) | 16 (9.4) | 144 (82.8) | 30 (17.2) |
| **Median (IQR) age in years** | 15.3 (12.7-17.7) | 14.7 (12.2-16.9) | 15.2 (14.2-17.0) | 15.1 (13.0-18.10) | 16.1 (12.7-18.6) |
| **Female sex, n (%)** | 168 (48.7) | 67 (43.2) | 11 (68.8) | 76 (52.8) | 14 (46.7) |
| **Site: Zimbabwe, n (%)** | 241 (69.9) | 106 (68.4) | 14 (87.5) | 95 (66.0) | 26 (86.7) |
| **Mean (SD) FEV1****Z-score at enrolment** | -2.00 (0.8) | -2.0 (0.7) | -2.4 (0.8) | -1.9 (0.7) | -2.3 (1.0) |
| **Mean (SD) FEV1 in litres at enrolment** | 1.66 (0.5) | 1.6 (0.5) | 1.5 (0.3) | 1.7 (0.5) | 1.7 (0.6) |
| **Median (IQR) CD4 count cells/mm3** | 572 (356-784) | 611 (418-788) | 566.5 (360.5-856) | 561.5 (343-807.5) | 456 (248-659) |
| **Median (IQR) HIV viral load copies/ml** | 400 (39-13200) | 309 (39-17700) | 398 (59.5-2200) | 286.5 (42-11180) | 1835 (400-52700) |
| **No (%) with HIV viral load <1000 copies/ml** | 194 (56.2) | 91 (58.7) | 9 (56.2) | 81 (56.3) | 13 (43.3) |
| **Mean (SD) duration of ART use in years**  **No (%) Missing** | 6.3 (3.2)  11 (3.2) | 6.4 (3.3)  4 (2.5) | 6.7 (2.8) | 6.3 (3.2)  5 (3.5) | 6.2 (3.1)  2 (6.7) |
| **Mean (SD) age in years at ART initiation**  **No (%) Missing** | 8.6 (4.0)  10 (2.9) | 8.1 (4.2)  3 (1.9) | 8.6 (3.2) | 8.8 (3.7)  5 (3.5) | 10.1 (3.4)  2 (6.7) |
| **No (%) on second line ART regimen** | 87 (25.5) | 38 (24.5) | 8 (50.0) | 31 (21.5) | 10 (33.3) |
| **Mean (SD) weight-for-age Z-score** | -2.2 (1.5) | -2.2 (1.4) | -2.3 (1.5) | -2.1 (1.5) | -2.15 (1.3) |
| **No (%) underweight** | 180 (52.2) | 87 (56.1) | 10 (62.5) | 68 (47.2) | 15 (50.0) |
| **Mean (SD) height-for-age Z-score** | -2.1 (1.2) | -2.1 (1.2) | -2.4 (1.1) | -2.1 (1.3) | -1.9 (1.1) |
| **No (%) stunted** | 174 (50.4) | 84 (54.2) | 10 (62.5) | 67 (46.5) | 13 (43.3) |
| **No (%) with history of treatment for TB** | 97 (28.2) | 50 (32.5) | 8 (50.0) | 30 (20.9) | 9 (30.0) |
| **No (%) with cough** | 31 (9.0) | 12 (7.8) | 1 (6.3) | 10 (6.9) | 8 (26.7) |
| **Mean (SD) respiratory rate** | 22.4 (3.1) | 22.1 (3.0) | 23.2 (3.4) | 22.5 (3.3) | 23 (3.0) |
| **No (%) with abnormal RR** | 151 (43.8) | 59 (38.0) | 7 (43.8) | 64 (44.4) | 21 (70.0) |
| **No (%) with resistance to AZM** | 34 (9.9) | 12 (7.7) | 2 (12.5) | 18 (12.5) | 2 (6.7) |

**Table 2: Risk factors for AREs among placebo arm participants**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Variable categories** | **Number of participants (%)** | **Total episodes of AREs/100 person-years** | **Model 1 RR (95% CI) 1** | **p-value2** | **Model 2 RR3 (95% CI)** | **p-value2** |
| **Total** |  | 174 | 38/ 154 |  |  |  |  |
| **Age (years)** | 6-15 | 92 (52.9) | 17/82 | 1 | 0.7618 | 1 | 0.4061 |
|  | 16+ | 82 (47.1) | 21/72 | 1.12 (0.54-2.31) |  | 0.73 (0.35-1.53) |  |
| **Sex** | Male | 84 (48.2) | 20/76 | 1 | 0.7942 | 1 | 0.4003 |
|  | Female | 90 (51.7) | 18/78 | 0.91 (0.44-1.86) |  | 0.75 (0.38-1.47) |  |
| **Site** | Zimbabwe | 121 (69.5) | 33/107 | 1 | 0.0372 | 1 | 0.0197 |
|  | Malawi | 53 (30.5) | 5/47 | 0.36 (0.13-1.01) |  | 0.33 (0.12-0.92) |  |
| **FEV1 Z-score** | ≥-2 | 97 (55.7) | 17/84 | 1 | 0.3226 | 1 | 0.5933 |
|  | <-2 | 77 (44.3) | 21/70 | 1.46 (0.70-3.04) |  | 1.22 (0.59-2.50) |  |
| **CD4 count**  **(cells/mm3)** | ≥ 200 | 156 (89.7) | 29/138 | 1 | 0.0641 | 1 | 0.0387 |
|  | <200 | 18(10.3) | 9/16 | 2.51 (0.99-6.34) |  | 2.50 (1.04-5.97) |  |
| **HIV VL**  **(copies/ml)** | <1000 | 94 (54) | 16/83 | 1 | 0.2232 | 1 | 0.5485 |
|  | ≥1000 | 80 (46) | 22/71 | 1.58 (0.76-3.28) |  | 1.25 (0.60-2.61) |  |
| **OART line ART** | 1st | 133(76.4) | 26/119 | 1 | 0.7053 | 1 | 0.7858 |
|  | 2nd | 41(23.6) | 12/35 | 1.17 (0.51-2.67) |  | 0.90 (0.44-1.88) |  |
| **Weight for age Z-score** | Not underweight | 91(52.3) | 20/79 | 1 | 0.6648 | 1 | 0.4880 |
|  | Underweight | 83(46.7) | 18/75 | 0.84 (0.38-1.86) |  | 0.78 (0.38-1.63) |  |
| **Height for age Z-score** | Not stunted | 94(54.0) | 22/82 | 1 | 0.7219 | 1 | 0.3290 |
|  | Stunted | 80(46.0) | 16/72 | 0.86 (0.40-1.89) |  | 0.68 (0.32-1.44) |  |
| **Presence of a cough** | No | 156 (89.7) | 28/139 | 1 | 0.0161 | 1 | 0.0837 |
|  | Yes | 18 (10.3) | 10/15 | 3.20 (1.34-7.67) |  | 2.16 (0.95- 4.90) |  |
| **Abnormal RR** | No | 89 (51.5) | 10/79 | 1 | 0.0093 | 1 | 0.0068 |
|  | Yes | 85 (48.9) | 28/75 | 2.87 (1.27- 6.47) |  | 2.90 (1.30- 6.46) |  |
| **History of TB** | No | 135 (77.6) | 27/119 | 1 | 0.9526 | 1 | 0.4687 |
|  | Yes | 39 (22.4) | 11/35 | 1.02 (0.46-2.30) |  | 0.76 (0.37-1.60) |  |
| **Season** | Rainy |  | 13/78 | 1 | 0.0620 | 1 | 0.0607 |
|  | Dry |  | 25/76 | 1.85 (0.94-3.62) |  | 1.86 (0.95-3.65) |  |
| **Calendar Period (years)** | 2016-2017 |  | 23/59 | 1 | 0.0164 | 1 | 0.0510 |
|  | 2018-2019 |  | 15/95 | 0.44 (0.22-0.90) |  | 0.52 (0.25-1.07) |  |

1 adjusted for age, sex, site, season, calendar time, and HIV VL (continuous); 2 p-value from LRT ; 3 adjusted for age, sex, site and season, calendar time, follow up time, HIV VL (continuous), abnormal RR, cough and CD4 count at baseline.

**Table 3: Rates of ARE stratified by arm and explanatory variables and stratum specific estimates of effect of azithromycin on total episodes of AREs**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Explanatory variable** | | **Placebo arm** | | **Azithromycin arm** | |
| **Total episodes of AREs 1** | **Rate of total AREs2 (95 % CI)** | **Total episodes of AREs 1** | **Rate of total AREs2 (95 % CI)** | **Adjusted RR for effect of azithromycin on total AREs 3**  **(95% CI)** | **P- value for interaction4** |
| **Total** | | 38/ 154 | 24.66 (17.29-36.44) | 19/155 | 12.27 (7.42-21.83) | 0.50 (0.29-0.87) | 0.0111 |
| **Age (years)** | 6-15 | 17/82 | 20.79 (12.00-39.39) | 13/100 | 12.93 (6.92-27.10) | 0.60 (0.27-1.33) | 0.3969 |
| 16+ | 21/72 | 29.0 (18.23-49.17) | 6/54 | 11.04 (4.51-34.61) | 0.35 (0.13-0.94) |  |
| **Sex** | Male | 20/76 | 26.44 (16.37-45.64) | 5/85 | 5.88 (2.55-16.88) | 0.23 (0.08-0.66) | 0.0660 |
| Female | 18/78 | 22.95 (13.50-42.28) | 14/70 | 20.04 (10.90-41.08) | 0.77 (0.35-1.68) |  |
| **Site** | Zimbabwe | 33/107 | 30.78 (21.11-46.70) | 17/108 | 15.68 (9.17-29.18) | 0.49 (0.25-0.93) | 0.8542 |
| Malawi | 5/47 | 10.67 (3.80-41.84) | 2/47 | 4.30 (0.96-40.09) | 0.41 (0.07-2.24) |  |
| **FEV1 Z-score** | ≥-2 | 17/84 | 20.21 (11.28-39.96) | 4/84 | 4.79 (1.85-16.53) | 0.25 (0.08-0.77) | 0.1738 |
| <-2 | 21/70 | 30.01 (19.41-49.03) | 15/71 | 21.04 (11.82-41.29) | 0.62 (0.30-1.30) |  |
| **CD4 count**  **(cells/mm3)** | <200 | 9/16 | 57.01 (25.75-151.80) | 5/15 | 34.37 (7.68-321.78) | 0.53 (0.14-1.98) | 0.8371 |
| ≥200 | 29/138 | 20.97 (14.16-32.44) | 14/140 | 9.98 (6.16-17.28) | 0.46 (0.23-0.90) |  |
| **HIV VL (copies/ml)** | <1000 | 16/83 | 19.22 (11.05-36.58) | 9/92 | 9.73 (5.33-19.82) | 0.49 (0.21-1.14) | 0.9808 |
| ≥1000 | 22/71 | 31.06 (19.54-52.45) | 10/62 | 16.02 (7.18-43.44) | 0.50 (0.21-1.16) |  |
| **ART line** | 1st | 26/119 | 21.78 (13.95-36.02) | 9/113 | 7.95 (4.00-18.20) | 0.35 (0.16-0.79) | 0.3173 |
| 2nd | 12/35 | 34.56 (19.42-67.44) | 10/42 | 24.00 (11.49-58.94) | 0.67 (0.25-1.77) |  |
| **Weight for age Z-score** | Underweight | 18/75 | 24.04 (14.53-42.80) | 10/90 | 11.09 (6.29-21.51) | 0.44 (0.19-1.03) | 0.8133 |
| Not underweight | 20/79 | 25.25 (15.27-44.90) | 9/65 | 13.90 (5.76-42.62) | 0.51 (0.21-1.22) |  |
| **Height for age Z-score** | Stunted | 16/72 | 22.30 (12.85-42.27) | 10/86 | 11.68 (6.64-22.60) | 0.49 (0.21- 1.17) | 0.8925 |
| Not stunted | 22/82 | 26.72 (16.75-45.36) | 9/69 | 12.99 (5.37-39.95) | 0.45 (0.19-1.06) |  |
| **Presence of a cough** | No | 28/139 | 20.16 (13.16-32.53) | 17/143 | 11.92(7.09-21.68) | 0.55 (0.29- 1.06) | 0.5151 |
| Yes | 10/15 | 65.67 (37.38-126.21) | 2/12 | 16.38 (4.10-65.50) | 0.30 (0.06-1.68) |  |
| **Abnormal RR** | No | 10/79 | 12.66 (6.74-26.75) | 11/95 | 11.55 (5.67-26.75) | 0.81 (0.33-2.01) | 0.1882 |
| Yes | 28/75 | 37.30 (24.59-59.40) | 8/60 | 13.42 (6.50-32.48) | 0.35 (0.15-0.82) |  |
| **History of TB treatment** | No | 27/119 | 22.74 (14.73-37.04) | 9/101 | 8.94 (4.51-20.39) | 0.42 (0.19-0.95) | 0.7112 |
| Yes | 11/35 | 31.12 (16.92-63.72) | 10/53 | 18.77 (8.88-46.93) | 0.55 (0.20-1.44) |  |
| **Resistance to AZM at baseline** | No | 36/137 | 26.35 (18.25-39.49) | 16/142 | 11.29 (6.56-21.17) | 0.42 (0.22-0.81) | 0.2570 |
| Yes | 2/17 | 11.47 (2.75-94.52) | 3/13 | 22.82 (4.41-280.93) | 1.38 (0.19-9.93) |  |
| **Season** | Rainy | 13/78 | 16.72 (9.68-31.56) | 8/78 | 10.26 (5.33-22.48) | 0.58 (0.23-1.45) | 0.5768 |
| Dry | 25/76 | 32.75 (22.00-51.02) | 11/77 | 14.30 (7.78-29.36) | 0.42 (0.20-0.89) |  |
| **Calendar Period (years)** | 2016-2017 | 23/59 | 39.00 (26.66-59.29) | 12/62 | 19.30 (10.03-42.25) | 0.48 (0.22-1.05) | 0.9484 |
| 2018-2019 | 15/95 | 15.78 (8.52-32.74) | 7/95 | 7.40 (3.28-20.46) | 0.46 (0.18-1.20) |  |

1events/ total person years; 2 per 100 person years; 3 adjusted for age (categorized as 6-10, 11-15 and 16+), sex, site, season, calendar time and HIV VL; 4p-value from LRT.

**Supplementary Tables**

**Table S2: Rate of AREs by time-varying variables among placebo arm participants**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **Events/**  **person-years** | **Rate/ 100 person-years (95% CI)** | **Unadjusted** | | **Adjusted** | |
| **RR (95% CI)** | **Wald test p-value** | **RR (95% CI) 2** | **LRT p-value** |
| **Season** |  |  |  | 0.0471 |  | 0.0380 |
| **Rainy** | 13/78 | 16.7 (9.7-31.6) | 1 |  | 1 |  |
| **Dry** | 25/76 | 32.8 (22.0-51.0) | 1.93 (0.99-3.79) |  | 2.01 (1.02-3.96) |  |
| **Year** |  |  |  | 0.0106 |  | 0.0534 |
| **2016-2017** | 23/59 | 39.0 (26.7-59.3) | 1 |  | 1 |  |
| **2018-2019** | 15/95 | 15.8 (8.5-32.7) | 0.40 (0.20-0.81) |  | 0.48 (0.23-1.01) |  |
| **Time in study** |  |  |  | 0.0966 |  | 0.1914 |
| **0-12 weeks** | 14/42 | 33.1 (18.9-63.5) | 1 |  | 1 |  |
| **13-25 weeks** | 14/41 | 34.1 (20.1- 62.3) | 1.04 (0.50-2.19) |  | 1.21 (0.56-2.57) |  |
| **26-38 weeks** | 5/40 | 12.4 (5.3-36.2) | 0.38 (0.14-1.05) |  | 0.44 (0.16-1.27) |  |
| **39- 52 weeks** | 5/30 | 16.4 (7.0-48.0) | 0.50 (0.18-1.39) |  | 0.63 (0.22-1.82) |  |

2 adjusted for all other time variables in table,

**Table S3: Rate of AREs by time-varying variables among participants in both arms**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Time** | **Events/person-years** | **Rate/ 100 person years (95% CI)** | **RR (95% CI)** | **Adjusted RR (95% CI) 2** |
| **Season** |  |  | P= 0.04101 | P= 0.03883 |
| **Rainy** | 21/157 | 13.4 (8.8-21.5) | 1 | 1 |
| **Dry** | 36/154 | 23.3 (16.7-33.7) | 1.74 (1.01-2.97) | 1.76 (1.02-3.03) |
| **Calendar period** |  |  | P= 0.00171 | P=0.01033 |
| **Years 2016-2017** | 35/121 | 28.9 (20.7-41.7) | 1 | 1 |
| **Years 2018-2019** | 22/188 | 11.7 (7.2-20.5) | 0.40 (0.22-0.71) | 0.45 (0.25-0.83) |
| **Time in study (weeks)** |  |  | P=0.04721 | P= 0.11093 |
| **0-12** | 20/84 | 23.7 (15.1-39.7) | 1 | 1 |
| **13-25** | 20/83 | 24.2 (15.7-39.5) | 1.03 (0.55-1.91) | 1.20 (0.64-2.26) |
| **26-38** | 7/81 | 8.6 (4.2- 20.6) | 0.37 (0.15-0.87) | 0.44 (0.18-1.07) |
| **39- 52** | 10/61 | 16.4 (9.1-32.7) | 0.69 (0.32-1.48) | 0.90 (0.41-1.99) |

1 p- value from Wald test; 2 adjusted for all other time variables in table, 3 p-value from LRT

**Supplementary Figures**

**Figure S1: Kaplan Meier survival curve by trial arm and sex**

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**Figure S2: Kaplan Meier survival curve by trial arm and FEV1 Z-score**

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**Figure S3: Kaplan Meier survival curve by trial arm and ART line**

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**Figure S4: Kaplan Meier survival curve by trial arm and presence of a cough at baseline**

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**Figure S5: Kaplan Meier survival curve by trial arm and presence of an abnormal respiratory rate at baseline**

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**Figure S6: Kaplan Meier survival curve by trial arm and resistance to AZM at baseline**

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