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Inhaled Aztreonam improves symptoms of cough and sputum production in patients with bronchiectasis: a post-hoc analysis of the AIR-BX studies

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Short title: inhaled antibiotic treatment with aztreonam improve cough and sputum in bronchiectasis

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Abstract

Introduction: Inhaled antibiotics may improve symptom scores but it is not known which specific symptoms improve with therapy. Item-level analysis of questionnaire data may allow us to identify which specific symptoms respond best to treatment.

Methods: Post-hoc analysis of the AIR-BX1 and 2 trials of inhaled aztreonam vs placebo in bronchiectasis. Individual items from the quality of life bronchiectasis respiratory symptom scale (QOL-B) were extracted, as representing severity of 9 distinct symptoms. Generalized linear models were used to evaluate changes in symptoms with treatment vs placebo from baseline to end of first on treatment cycle and mixed models used to evaluate changes across the full 16 week trial.

Results: Aztreonam improved cough (difference 0.22; 95%CI 0.08-0.37,p=0.002), sputum production (0.30; 95%CI 0.15-0.44,p<0.0001) and sputum colour (0.29; 95%CI 0.15-0.43p<0.0001) vs placebo equating to a 20% improvement in cough and 25% improvement in sputum production and colour respectively. Similar results were observed for cough, sputum production and sputum purulence across the trial duration (all p<0.05). Patients with higher sputum production and sputum colour scores had a greater response on the overall QOL-B (difference 4.82; 95%CI 1.12-8.53,p=0.011) for sputum production and 5.02; 95%CI 1.19-8.86,p=0.01 for sputum colour. In contrast, treating patients who had lower levels of bronchitic symptoms resulted in shorter time to next exacerbations (HR 1.83; 95%CI 1.02-3.28,p=0.042).

Conclusion: Baseline bronchitic symptoms predict response to inhaled aztreonam in bronchiectasis. More sensitive tools to measure bronchitic symptoms may be useful to enrich for inhaled antibiotic responders and to evaluate patient response to treatment.

Introduction

Bronchiectasis significantly impacts upon patients quality of life, affecting physical, emotional and social aspects of wellbeing.(1,2) Disease progression is driven by recurrent exacerbations which are associated with reduced lung function and in severe cases can lead to respiratory failure and even death (1,3–5). Exacerbation frequency and long term mortality are increased in patients with chronic airway infection with bacterial pathogens.(6) *Pseudomonas aeruginosa* is the most common organism isolated from patients sputum worldwide and is strongly associated with worse quality of life, lower lung function, higher risk of hospitalisation and increased mortality rates.(7,8) This high burden of disease highlights the need to develop evidence based therapies that can reduce the burden of bacterial infection. Inhaled antibiotics deliver high concentrations of antibiotic to the site of infection resulting in marked reductions in airway bacterial load in patients with bronchiectasis.(9) It has been challenging, however, to clearly demonstrate that these reductions in bacterial burden translate into clinical benefits. A recent meta-analysis of 16 trials including 2597 patients with bronchiectasis showed strong antimicrobial efficacy with a pooled reduction of 2.3 log units in colony forming units, but this translated into only modest reductions in exacerbation frequency (rate ratio 0.8; 95% CI 0.67 to 0.97) and no clinically significant improvement in symptoms.(9) Despite these inconclusive data, inhaled antibiotics are still frequently used “off-label” with patient and clinician perception that they provide benefits.(10)

A possible explanation for this difference between “real life” and randomized studies is the tools used to measure symptoms.(11) The number of validated health related quality of life (HRQOL) tools used in bronchiectasis trials is limited, and the quality of life bronchiectasis questionnaire (QOL-B) is the only disease-specific tool that has been tested in multiple randomized trials.(11) Its respiratory symptom scale consists of 9 questions asking about congestion, cough, sputum production, sputum colour, shortness of breath, wheezing, chest pain, shortness of breath while talking, and nocturnal cough. Each of these symptoms is potentially important to patients with bronchiectasis, and each symptom is likely to be

associated with severity of disease and morbidity.(2,11) It is less certain, however, that a specific treatment would be expected to improve all of these symptoms.

Extensive prior research suggests that elevated bacterial load induces neutrophil inflammation including the release of myeloperoxidase which causes sputum purulence and neutrophil elastase which is a key mediator of cough and sputum production through direct stimulation of mucin production from epithelial cells and impairment of mucociliary clearance.(12–14) Inhaled antibiotic treatment has been shown to rapidly reduce neutrophilic inflammation and therefore might be expected to also reduce cough, sputum production and sputum purulence.(15) In contrast, breathlessness is associated with airflow obstruction, emphysema, cardiovascular fitness, anaemia conditioning and neuromuscular function, all of which may not be immediately modified with inhaled antibiotics.(3,16) In the recent meta-analysis inhaled antibiotics reduced, rather than improved FEV_1.(9)

We therefore hypothesised that by examining the effect of an inhaled antibiotic on individual symptom responses we would observe that inhaled antibiotics improve cough, sputum and sputum purulence while having much less effect on other symptoms. To investigate this, we used itemised QOL-B data collected during two large, previously published, randomised controlled trials of inhaled Aztreonam Lysine(17).

Methods

AIR-BX1 and AIR-BX2 were identical, double-blind, multicentre, randomised, placebo-controlled, phase 3 trials consisting of two, 4-week on 4-week off, treatment cycles. Patients received either inhaled aztreonam lysine (AZLI) 75mg or placebo three times daily. Eligibility criteria has previously been published (17). This analysis only included patients who gave additional consent for future exploratory analysis of their data and we have previously published that there were no significant differences in characteristics between the original trial cohort and those who consented to future analysis (18).

The primary endpoint used in the AIRBX studies was change in quality of life bronchiectasis respiratory symptoms score (QOLB-RSS) at week 4, the end of the first on-treatment cycle.(11) Secondary endpoints included the change in QOLB-RSS at week 12. The minimal important difference for the QOL-B-RSS as a whole is an 8-point change.(11)

Time to first protocol-defined exacerbation by week 16 was a secondary endpoint of special interest in the AIRBX studies. The exacerbation definition used was as follows; acute worsening of respiratory disease meeting $\geqslant$3 major criteria (or two major and at least two minor). Major criteria were increased sputum production, change in sputum colour, dyspnoea and cough while minor criteria were fever (>38°C) at clinic visit, increased malaise or fatigue, FEV_1 (L) or FVC decreased by more than 10% from baseline, and new or increased haemoptysis.(17)

Item level analysis

The 9 items of the QOLB-RSS are shown below in Table 1. The items are answered with a one week recall period and have four options. Lower scores reflect poor quality of life from increased respiratory symptoms while high scores show lesser impact. For individual item level analysis, the responses (table 1) were converted into numerical values for analysis with 1 representing the most severe response (a lot/always) and 4 representing the least severe response (not at all/never). For sputum colour, there are 6 possible

responses in the QOL-B. ‘Brownish-dark’ and ‘green with traces of blood’ are both rated the same severity and no score is given to the answer ‘Don’t know’.

Question	Responses			
29. Have you felt congestion (fullness) in your chest?	A lot	A moderate amount	A little	Not at all
30. Have you been coughing during the day	A lot	A moderate amount	A little	Not at all
31. Have you had to cough up sputum?	A lot	A moderate amount	A little	Not at all
32. Has your sputum been mostly	Green with traces of blood or Brownish- dark	Yellowish-green	Clear to yellow	Clear
33. Have you had shortness of breath when being active, such as when doing housework or gardening?	Always	Often	Sometimes	Never
34. Have you had wheezing?	Always	Often	Sometimes	Never
35. Have you had chest pain?	Always	Often	Sometimes	Never
36. Have you had shortness of breath when talking?	Always	Often	Sometimes	Never
37. Have you woken during the night because you were coughing?	Always	Often	Sometimes	Never

Table 1. Individual items completing the quality of life bronchiectasis respiratory symptom domain. These questions form the last domain of QOLB and are numbers 29 – 37.

Statistical analysis

Changes from baseline to visit 4 (end of first on-treatment period)

To test the hypothesis that AZLI treatment would improve specific symptoms linked to bacterial load we analysed the effect of AZLI treatment vs placebo on each individual item within the QOL-B RSS questionnaire as listed in table 1. Analysis was conducted using the intention to treat principle. Studies were initially evaluated separately (AIR-BX1 and AIR-BX2) and then pooled. The second hypothesis was that

patients with more cough, sputum production and sputum purulence at baseline would experience a superior response to inhaled antibiotic treatment. In the analysis of change from baseline to visit 4 we used a generalized linear model with the change in each individual symptom domain as the outcome in each of the 4 baseline severity categories. High symptom patients were those answering 1-2 per item and low symptom patients were those answering 3-4.

Repeated measures analysis across the entire study duration

For analysis of whether baseline symptoms predicted QOL-B RSS response across the entire trial duration we used a mixed effects model. The full model for each question contained the four treatment terms: 1) an immediate effect of treatment (visits 3,4 and 6 while patients were receiving either AZLI or placebo) and 2) a longer term (study duration incorporating data from visits when off-drug) placebo effect affecting both arms equally, as well as 3) an additional short-term and 4) a longer-term drug effect that only affected the patients receiving AZLI. For each analysis data are presented for full models, which contain all 4 treatment parameters and for a best model which, where all available parameters were statistically significant. The data from the two trials was combined, and an intercept plus a single common drift term included along with individual as a random effect. Interactions were included for baseline symptoms and AZLI response. Model selection and averaging was done by Akaike information criterion (AICc).

To evaluate treatment effects of individual symptoms across the entire study duration we used a similar mixed effects model with the response to each individual question as the outcome. As there are only four possible answers to each question, uncertainties around these models' estimates were generated by bootstrap resampling the data. The data from the two trials was pooled for simplicity and because preliminary analyses identified no differences between the response in the two trials. 15 sub-models were fitted, containing each combination of the four treatment terms. Terms were considered statistically significant where their 95% confidence interval from bootstrapping did not cross zero. The model for each

question, among those for which all parameters were statistically significant, that contained the largest number of parameters was considered best.

For interpretation of results we considered a p-value of less than 0.05 as statistically significant. In view of the multiple comparisons performed we also applied a Holm-Bonferroni correction to the primary results. All statistical analysis was performed using IBM SPSS Statistics version 25 or R version 3.5.1.

Results

AIR-BX1&2 Demographics

A combined 440 participants were included in the analysis. Table 2 shows the demographics for participants. More detailed patient characteristics have been previously published demonstrating no significant differences between the patient characteristics in AIR-BX1 and 2.(18) Patients had an average age of 64 years. Patients were predominantly female (70%) with moderate FEV_1 impairment. 131 protocol defined exacerbations were recorded throughout the study although 70% of participants did not experience an exacerbation during the 3 month study duration.

	N (%); mean (SD); median (IQR)	Range
Sample size	440	
Female Sex	306 (69.5%)	
Age	63.8 (12.9)	18 to 87
FEV_1 %predicted	62.3 (20.4)	18.0 to 115.5
QOLB-RSS score	55.9 (18.5)	0 to 96
- QOLB-RSS score change to visit 4 (n=385)	6.84 (17.49)	-50.9 to 77.8
Protocol defined exacerbations during study period	0 (0-1)	0 to 3
1 exacerbation	110 (24.8%)	
>1 exacerbation	21 (4.8%)	

Table 2. Combined AIR-BX1 and AIR-BX2 participant characteristics.

Treatment response for individual symptoms

First we examined the original trial primary endpoint which was change from baseline to visit 4, after 28 days of AZLI treatment or placebo. For the total score, the original trials reported an improvement of 0.8 points (95% CI -3.1 to 4.7, p=0.68) in AIR-BX1 and 4.6 points (95% CI 1.1 to 8.2, p=0.011) in AIR-BX2. For individual symptoms we observed clear improvements at week 4 in daily cough, sputum production and sputum purulence of 20, 25.7 and 25.2% respectively (all p<0.05, table 3). There were also non-significant improvements in congestion, but negative effect estimates for breathlessness, wheeze and chest pain which were not statistically significant. Table S1 online shows the results for each individual study. Consistent with the original trials, the effects were stronger in AIR-BX2 but were concordant in the sense that more patients achieved a 2 point or more improvement in individual symptoms with AZLI than with placebo by week 4 for congestion (p=0.015), sputum production (p=0.003) and sputum purulence (p=0.01). No significant differences were observed between groups for the other symptoms (table S2 online).

QOLB-RSS item (pooled)	**Difference (95% CI)**	**P-value**	**%Change in patient response (95% CI)**
Q29 – Congestion	0.13 (-0.03 to 0.29)	0.110	12.3 (-3 to 25.3)
Q30 – Daily cough	0.22 (0.08 to 0.37)	0.002*	20 (7.6 to 30.8)
Q31 – Sputum production	0.30 (0.15 to 0.44)	<0.0001*	25.7 (14.3 to 35.6)
Q32 – Sputum purulence	0.29 (0.15 to 0.43)	<0.0001*	25.2 (14.1 to 34.8)
Q33 – Breathlessness on daily activity	-0.03 (-0.17 to 0.11)	0.680	-3.1 (-18.9 to 10.7)
Q34 - Wheeze	-0.06 (-0.20 to 0.07)	0.347	-6.6 (-21.9 to 6.7)
Q35 – Chest pain	-0.04 (-0.14 to 0.05)	0.377	-4.3 (-14.5 to 5.0)
Q36 – Breathlessness on talking	0.02 (-0.11 to 0.15)	0.779	1.8 (-11.5 to 13.6)
Q37 – Nocturnal cough	0.04 (-0.1 to 0.18)	0.582	3.8 (-10.4 to 16.2)

Table 3. *Pooled item level response following 4 weeks of treatment with AZLI vs placebo.* The difference represents the treatment effect between AZLI and placebo on a 4 point scale with positive values indicating improvement.*indicates that p-value remains statistically significant after Holm-Bonferroni correction for multiple comparisons.

The mixed model repeated measures analysis supported the results of the week 4 analysis. Figure 1 shows the trajectories of individual symptoms from baseline to the end of trial. We observed improvements in symptoms for both AZLI and placebo treated subjects consistent with a placebo effect. A clear treatment benefit was evident in figures 1B, 1C and 1D consistent with improved cough, sputum production and sputum colour. Interestingly, inhaled antibiotic treatment appeared to provide a sustained improvement in cough and sputum production during the off-treatment period but sputum colour returned to baseline during the off-treatment period.

Results of the mixed models are shown in Table S3. The best mixed model found a statistically significant improvement in cough and sputum with AZLI treatment throughout the study – difference 0.11 (95% CI 0.007 to 0.23) for cough and 0.19 (95% CI 0.09 to 0.29) for sputum production, and a significant improvement in sputum colour on AZLI treatment at visits 3, 4 and 6 - difference 0.25 (95% CI 0.17 to 0.33). In contrast, while on AZLI treatment there was a significant deterioration in breathlessness (-0.09; 95% CI -0.17 to -0.002) and wheeze (-0.11; 95% CI -0.20 to -0.02), Table S3.

We conclude that examining both the primary endpoint of week 4 and the trajectory across the study, inhaled aztreonam treatment improved cough, sputum production and sputum colour but resulted in worsening of breathlessness and wheeze.

Do baseline symptoms predict treatment response?

QOL-B RSS

The improvement in QOL-B RSS from baseline to 4 weeks observed in the overall population was associated with baseline symptoms. Patients with more severe sputum production and sputum colour had a greater response in terms of QOL-B RSS. For sputum production, those reporting “a lot” and a “moderate amount” (n=309, 70.2%) had a statistically significant improvement (difference 4.82; 95% CI 1.12 to 8.53,

p=0.011) while those reporting “a little” or “not at all” had no treatment benefit (difference -2.61; 95% CI -7.58 to 2.37, p=0.30). Likewise, patients with green or brownish-dark sputum (n=240, 54.5%) had an improvement in QOL-B RSS overall, difference 5.02 (95% CI 1.19 to 8.86, p=0.01) while those with yellow or clear sputum did not, difference -0.78 (95% CI -5.88 to 4.33, p=0.77) results can be seen in table S4.

Intriguingly, patients reporting little to no wheezing (n=328, 74.5%) (difference 3.74; 95% CI 0.50 to 6.97, p=0.024), shortness of breath when talking (n=338, 76.8%) (difference 3.23; 95% CI 0.12 to 6.33, p=0.042 and nocturnal cough (n=319, 72.5%) (difference 3.62; 95% CI 0.27 to 6.97, p=0.034) at baseline also showed statistically significant beneficial effects of treatment on overall QOL-B RSS. No other significant effects were observed.

In the mixed models across all study visits the only statistically significant interaction was for sputum production, whereby higher sputum production at baseline was significantly associated with QOL-B RSS treatment response across the entire study duration difference 2.18 (95% CI 0.14 to 4.22, p=0.04), table S5 and S6.

Exacerbations

In the original AIR-BX analysis, AZLI was associated with a non-significant shortening of time to first exacerbation and a higher number of exacerbations in the AZLI arm. This was speculated to be due to adverse effects of inhalation and bronchospasm, however, our analysis showed a phenotypical relationship with patients having a lower severity of congestion (n=207, 47.0%) (HR 1.69; 95% CI 1.02 to 2.8, p=0.042), cough (n=152, 34.5%) (HR 1.65; 95% 0.95 to 2.90, p=0.078), sputum colour (n=200, 45.5%) (HR 1.83; 95% CI 1.02 to 3.28, p=0.042) and nocturnal cough (n=121, 27.5%) (HR 1.57; 95% CI 1.03 to 2.37, p=0.034) having a shorter time to first exacerbation. In contrast although not statistically significant patients with greater baseline wheeze (n=112, 25.5%) had a shorter time to first exacerbation (HR 1.75; 95% CI 0.92 to 3.33, p=0.086) (table 4).

Baseline symptom	Symptom group	Hazard ratio (95%C)	P-value
29- Congestion	1-2 (severe)	0.98 (0.61-1.58)	0.94
	3-4 (mild)	1.69 (1.02-2.80)	**0.042**
30- Cough	1-2 (severe)	1.11 (0.71-1.71)	0.65
	3-4 (mild)	1.65 (0.95-2.90)	0.078
31- Sputum production	1-2 (severe)	1.20 (0.79-1.82)	0.39
	3-4 (mild)	1.40 (0.75-2.61)	0.29
32- Sputum colour	1-2 (severe)	1.10 (0.71-1.71)	0.68
	3-4 (mild)	1.83 (1.02-3.28)	**0.042**
33- Shortness of breath	1-2 (severe)	1.10 (0.68-1.76)	0.70
	3-4 (mild)	1.44 (0.87-2.39)	0.16
34- Wheezing	1-2 (severe)	1.75 (0.92-3.33)	0.086
	3-4 (mild)	1.19 (0.79-1.80)	0.42
35- Chest pain	1-2 (severe)	1.85 (0.48-7.15)	0.37
	3-4 (mild)	1.22 (0.85-1.75)	0.27
36- SOB when talking	1-2 (severe)	1.54 (0.80-2.97)	0.20
	3-4 (mild)	1.18 (0.78-1.77)	0.43
37- nocturnal cough	1-2 (severe)	0.78 (0.41-1.50)	0.46
	3-4 (mild)	1.57 (1.03-2.37)	**0.034**

Table 4. The effect of baseline symptoms on time to first exacerbation. Statistically significant effects are highlighted in bold, though none of these results would be regarded as statistically significant after Holm-Bonferroni correction for multiple testing.

FEV_1

No effect of treatment was observed on FEV1 when stratified by baseline symptoms (table S7).

Discussion

The aim of this post-hoc analysis was to identify which QOLB symptoms respond to treatment with inhaled aztreonam. We show clear differences in treatment response between different symptoms that make up the respiratory symptom scale of the QOL-B questionnaire. Treatment with AZLI resulted in clear improvements in cough, sputum production and sputum purulence while causing no effect on other symptoms except for shortness of breath and wheeze which were slightly worse with treatment. Given that these symptoms improve with therapy, it would be expected that patients with more severe cough, sputum production and sputum purulence at baseline would respond better to inhaled antibiotics and this was also what we observed. Interestingly, in the AIR-BX studies there was an increase in exacerbations in the treatment group which was driven by an increase in adverse events.(17) We show that patients treated with inhaled antibiotics that lack the above symptoms were more likely to experience exacerbations at an earlier timepoint.

A series of randomized clinical trials of inhaled antibiotics in bronchiectasis have failed to demonstrate a significant effect on their primary endpoints. This leads to a clear need to identify which patients may respond to inhaled antibiotics.(19–22) An understanding of the biology of how inhaled antibiotics work suggests they should modify some symptoms and not others and that our current methods of identifying treatment response may not be optimal.

Taken together, this study adds to our understanding of the role of inhaled antibiotics in bronchiectasis and, while the post-hoc nature of our study requires some caution in the interpretation of their details, the results are concordant with the reported pathophysiology of bronchiectasis. Bacteria and particularly *Pseudomonas aeruginosa* produce an intense neutrophil mediated inflammatory response that increases in proportion to airway bacterial load.(23,24) Patients with higher bacterial load and higher levels of neutrophilic inflammation measured using cell counts or markers of neutrophil activation such as neutrophil elastase (NE) have worse symptoms and a higher frequency of exacerbations.(25,26) NE in particular, is directly

linked to symptoms by provoking secretion of mucins from bronchial epithelial cells, particularly MUC5AC which is a key mucin in bronchiectasis airway secretions and is linked to disease severity.(27,28) NE has also been reported to impair mucociliary clearance through direct effects on ciliated epithelium.(14) NE is released from neutrophil primary granules along with myeloperoxidase (MPO), the concentration of which greatly determines the green colour of purulent sputum.(23,25) Our previous work showed that neutrophil markers reduce in parallel with reducing bacterial load.(15,29) Therefore our results verify this model whereby inhaled antibiotics reduce bacterial load, which reduces neutrophilic inflammation which therefore reduces the stimulus for mucin secretion, improves mucociliary clearance, reducing cough and sputum production, and reduces sputum purulence through a fall in MPO concentration.

We have recently shown that patients with higher bacterial load at baseline are more responsive to inhaled antibiotics.(18) Currently, bacterial load quantification is not routinely tested in most healthcare environments whereas identifying clusters of symptoms that predict response is simple and easily implemented into clinical practice. It is perhaps not surprising that if inhaled antibiotics reduce cough, sputum production and sputum purulence that patients with more of these symptoms respond better to treatment. Nevertheless, this is useful information for future design of trials. Despite the QOLB-RSS being used as the primary endpoint for the AIRBX trials, patients were not required to have any specific baseline symptoms for enrolment. When we analysed the baseline data we found as many as 64.8% of participants did not have the reported symptom at baseline (known as a ceiling effect) and therefore no measurable improvement is possible in that symptom. Despite the study enrolling patients with a history of Gram-negative infection, many patients were not symptomatic and therefore may not have been typical of the kind of patients being prescribed inhaled antibiotics in clinical practice.(17)

It is interesting that wheeze and shortness of breath somewhat worsened during treatment. This is consistent with the recent meta-analysis of Laska et al who showed a mean reduction in FEV_1 with inhaled antibiotic treatment across 16 trials and the results of individual studies which have shown that inhaled antibiotics are irritant to the epithelium and therefore can cause bronchoconstriction or bronchospasm.(9) While there has

been a lot of focus on the number of patients withdrawing from treatment as a result of bronchospasm our results suggest that even in patients that persist with therapy there can be a deterioration in shortness of breath.

How do these results affect our future approach to inhaled antibiotic trials? Approval of drugs by regulatory agencies such as the US Food and Drug administration requires demonstration that drugs improve how patients “feel, function or survive”. This means showing an effect on symptoms or quality of life. We suggest that future studies should aim to enrich for patients with a higher level of the symptoms that are likely to respond to inhaled antibiotics. Secondly, cough, sputum production and sputum purulence are the key symptoms of bronchiectasis but comprise only 1/3 of the weight of the respiratory symptom domain of the quality of life bronchiectasis questionnaire. A questionnaire that gives greater weight to these symptoms may be more relevant to inhaled antibiotic treatment. The difference in weighting as well as recall period may explain differences observed in the RESPIRE trials where the St Georges Respiratory Questionnaire improved with treatment but the QOL-B questionnaire did not.(20,21) Indeed the widespread use of inhaled antibiotics in clinical practice suggests that physicians and patients find them beneficial and that improvements in these 3 key symptoms are considered clinically important by patients.(30)

Our post-hoc analysis of quality of life data has provided a more detailed insight to the true drug response experienced in the AIRBX trials and may provide an improved understanding to the ideal patient phenotype who should be recruited into antibiotic trials. Nevertheless, our study has important limitations. Only 81% of the original trial cohort was available for re-analysis, though we have previously shown that the cohorts of included/excluded patients had no significant differences.(18) Our post-hoc analysis is exploratory by definition and requires confirmation in further cohorts. In particular, we tested only one formulation of inhaled antibiotics in the form of aztreonam and validation with other antibiotics would be of interest. The effect of inhaled antibiotics on cough, sputum production and sputum purulence is mediated by the ability to reduce bacterial load, and the recent study by Laska et al of 16 inhaled antibiotics trials found no heterogeneity between aztreonam (pooled bacterial load reduction 2.6 log units 95% CI 2.1 to 3.1) and the

other antibiotics included (pooled reduction 2.3 units 95% CI 1.2 to 3.4) in both their ability to reduce bacterial load and to improve symptoms (heterogeneity I_2=1%).(9) This strengthens the view that these results will be generalizable. Only the central conclusion of the study, that inhaled antibiotics improve cough, sputum production and sputum colour remained statistically significant after adjustment for multiple comparisons. Therefore the results showing that baseline symptoms predict treatment response should be treated with caution as given the number of comparisons performed, some of the results may have arisen by chance. These are, however, not entirely independent analyses, and the number, consistency, and biological plausibility of our findings give confidence in their overall pattern. In the original AIR-BX studies a symptomatic benefit was seen in the first 4 week treatment period that was not evident during the second on treatment period.(17) The reasons for this are unknown but they also impacted on our analysis where the strength of associations with symptoms were strongest in the first 4 weeks and weaker when analysed over the full study duration. No specific minimum clinically important difference has been established for individual symptoms on the 4 point scale, and so while we found statistically significant differences favouring aztreonam vs placebo for cough, sputum purulence and sputum colour, further work is required to understand the clinical impact of these changes. Nevertheless change of 1 point on the 4 point scale is substantial, as it indicates a change from “a lot” to “a moderate amount”, “a little” to “not at all”, “often” to ”sometimes” or “sometimes” to “never” for individual symptoms to give some examples. The absolute changes observed in our study would generate a number needed to treat for a cough and sputum production of between 3 and 5 patients to achieve a 1 point change in each symptom.

In conclusion, our results suggest aztreonam improves cough, sputum production and sputum colour but does not significantly affect other symptoms in bronchiectasis. Inhaled antibiotic treatment may be most effective in patients with daily cough and producing discoloured sputum, and clinicians may wish to avoid treatment in patients with significant breathlessness and wheeze. Future trials should consider enrolling patients with a higher burden of bronchitic symptoms and develop symptom evaluation tools which give greater weight to these symptoms.

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Figure legends

Figure 1. Trajectories of individual symptoms comparing AZLI treatment vs placebo in the full model as described in the statistical analysis section. Solid lines show the response in the group receiving AZLI and broken lines show the placebo response. 7 visits are shown; visits 1 and 2 are screening and baseline prior to treatment, visit 3 is 14 days into the first treatment cycle, visit 4 at 28 days (end of the first treatment cycle), visit 5 is at the end of the off-treatment cycle (28-days off treatment). Visit 6 is the end of the second 28-day treatment cycle while visit 7 is the end of the final 28-day off-treatment cycle and is the end of the study.

ONLINE SUPPLEMENT FOR

Inhaled antibiotics improve symptoms of cough and sputum in patients with bronchiectasis: a post-hoc analysis of the AIR-BX studies

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Trial	QOL-B question	Difference (95% CI)	p-value	% change in patient response (95% CI)
AIRBX1	29- Congestion	0.04 (-0.21 to 0.29)	0.74	4.2 (-23.2 to 25.5)
	30- Cough	0.09 (-0.14 to 0.32)	0.43	8.8 (-14.5 to 27.4)
	31- Sputum production	0.22 (-0.01 to 0.46)	0.06	20.1 (-1.2 to 36.9)
	32- Sputum colour	0.18 (-0.03 to 0.39)	0.10	16.2 (-3.4 to 32.1)
	33- Shortness of breath	-0.01 (-0.24 to 0.22)	0.94	-0.9 (-26.4 to 19.5)
	34- Wheezing	-0.04 (-0.25 to 0.17)	0.68	-4.5 (-28.8 to 15.3)
	35- Chest pain	-0.07 (-0.19 to 0.06)	0.30	-6.8 (-21.1 to 5.8)
	36- SOB when talking	-0.05 (-0.27 to 0.17)	0.67	-4.9 (-30.7 to 15.9)
	37- nocturnal cough	0.14 (-0.10 to 0.37)	0.26	12.6 (-10.3 to 30.7)
AIRBX2	29- Congestion	0.20 (-0.01 to 0.41)	0.07	17.8 (-1.2 to 33.3)
	30- Cough	**0.32 (0.13 to 0.50)**	**0.001**	27.0 (12 to 39.5)
	31- Sputum production	**0.35 (0.18 to 0.53)**	**<0.0001**	29.7 (16.1 to 41.1)
	32- Sputum colour	**0.37 (0.18 to 0.55)**	**<0.0001**	30.6 (16.7 to 42.1)
	33- Shortness of breath	-0.05 (-0.23 to 0.14)	0.62	-4.8 (-26.0 to 12.8)
	34- Wheezing	-0.08 (-0.26 to 0.09)	0.35	-8.7 (-29.3 to 8.6)
	35- Chest pain	-0.02 (-0.15 to 0.11)	0.77	-2.0 (-16.2 to 10.5)
	36- SOB when talking	0.06 (-0.10 to 0.21)	0.48	5.4 (-10.2 to 18.8)
	37- nocturnal cough	-0.03 (-0.20 to 0.14)	0.75	-2.8 (-21.9 to 13.2)

Table S1 – Treatment response for individual symptoms in AIRBX 1 & 2. Statistically significant effects are highlighted in bold.

QOLB-RSS item	AIRBX1	AIRBX2	Pooled (improved MCID)	Pooled (worsening MCID)
Q29 – Congestion	0.499	**0.035**	**0.015**	0.171
Q30 – Daily cough	-	-	0.074	0.659
Q31 – Sputum production	0.053	**0.02**	**0.003**	0.143
Q32 – Sputum purulence	0.864	**0.004**	**0.01**	0.392
Q33 – Breathlessness on daily activity	-	-	0.687	0.126
Q34 – Wheeze	-	-	0.929	0.16
Q35 – Chest pain	-	-	0.657	0.334
Q36 – Breathlessness on talking	-	-	0.363	0.073
Q37 – Nocturnal cough	-	-	0.327	0.27

Table S2. Chi-squared test determining treatment effect with a MCID response following 4 weeks of treatment. Where a statistically significant effect was observed in the pooled data the analysis was repeated for each trial separately.

Table S3- Trajectories for answers to individual questions.

Models were separately fitted to the answers to each question. As there are only four possible answers to each question, uncertainties around these models' estimates were generated by bootstrap resampling the data and a pragmatic and empirical approach taken to model selection. The data from the two trials was pooled for simplicity and because preliminary analyses identified no differences between them. Each model contained an intercept term and a random effect for individual. The full model for each question also contained four terms: short (visit 3,4,6) and long (study duration) term placebo effects, affecting both arms equally, and equivalent drug effects that only affected the people on AZLI. 15 sub-models were fitted, containing each combination of the four treatment terms. Terms were considered statistically significant where their 95% confidence interval from bootstrapping did not cross zero. The model for each question, among those for which all parameters were statistically significant, that contained the largest number of parameters was considered best. Where this produced multiple candidates, parameters that were significant in the full model, or failing that, those with the largest absolute parameter value (effect size), were favoured.

			Placebo effect, affecting both arms equally		Drug effect on AZLI population only	
Q	model	Intercept	short term (visit 3,4,6)	long term (study duration)	short term (visit 3,4,6)	long term (study duration)
29	full	**1.40 (1.31, 1.48)**	0.06 (-0.03, 0.15)	**0.20 (0.10, 0.30)**	0.06 (-0.09, 0.21)	-0.06 (-0.19, 0.07)
	best	**1.40 (1.31, 1.48)**	**0.09 (0.02, 0.16)**	**0.17 (0.10, 0.25)**	-	-
30	full	**1.09 (1.02, 1.15)**	0.05 (-0.04, 0.13)	**0.16 (0.06, 0.26)**	0.06 (-0.07, 0.19)	**0.14 (0.02, 0.28)**
	best	**1.09 (1.02, 1.15)**	-	**0.19 (0.11, 0.27)**	**0.11 (0.01, 0.20)**	**0.11 (0.007, 0.23)**
31	full	**1.00 (0.94, 1.07)**	**0.09 (0.01, 0.17)**	**0.16 (0.07, 0.24)**	0.09 (-0.03, 0.21)	**0.14 (0.01, 0.26)**
	best	**1.00 (0.94, 1.07)**	**0.14 (0.08, 0.20)**	**0.13 (0.05,0.21)**	-	**0.19 (0.09, 0.29)**
32	full	**1.39 (1.33, 1.46)**	-0.01 (-0.09, 0.06)	**0.12 (0.04, 0.21)**	**0.27 (0.16, 0.39)**	-0.03 (-0.16, 0.09)
	best	**1.39 (1.33, 1.46)**	-	**0.11 (0.05, 0.16)**	**0.25 (0.17, 0.33)**	-
33	full	**1.48 (1.39, 1.57)**	0.06 (-0.01, 0.13)	**0.10 (0.02, 0.18)**	-0.07 (-0.17, 0.03)	-0.03 (-0.14, 0.08)
	best	**1.48 (1.39, 1.57**)	**0.07 (0.01, 0.13)**	**0.09 (0.02, 0.15)**	**-0.09 (-0.17, -0.002)**	-
34	full	**1.98 (1.90, 2.06)**	0.05 (-0.01, 0.12)	**0.11 (0.03, 0.19)**	-0.05 (-0.15, 0.05)	-0.03 (-0.13,0.08)
	best	**2.01 (1.93, 2.08)**	**0.11 (0.06, 0.17)**	-	**-0.11 (-0.20, -0.02)**	**0.08 (0.01, 0.15)**
35	full	**2.55 (2.48, 2.61)**	0.01 (-0.03, 0.06)	**0.09 (0.03, 0.16)**	-0.01 (-0.10, 0.07)	-0.03 (-0.11, 0.05)
	best	**2.55 (2.48, 2.61)**	-	**0.08 (0.04, 0.12)**	-	-
36	full	**2.05 (1.98, 2.13)**	0.05 (-0.01,0.11)	0.04 (-0.03, 0.11)	-0.03 (-0.13, 0.05)	-0.02 (-0.12, 0.08)
	best	**2.05 (1.98, 2.13)**	**0.05 (0.004, 0.10)**	-	-	-
37	full	**1.96 (1.88, 2.03)**	0.05 (-0.02,0.13)	0.08 (-0.01, 0.17)	0.03 (-0.09, 0.14)	-0.003 (-0.12, 0.11)
	best	**1.96 (1.88, 2.03)**	**0.07 (0.01,0.12)**	**0.07 (0.01, 0.14)**	-	-

Table S3: Models of answers to each question. Each cell contains the parameter estimate with the 95% confidence interval estimated by bootstrapping. Bold type indicates terms where the 95% CI does not include zero.

Table S4 and S5. Impact of baseline symptoms on overall QOL-B response.

Baseline symptom groups were associated with different responses in the overall QOL-B RSS scores from baseline to visit 4 as shown below in table S4.

Table S4 - ***Effect of baseline symptoms on changes to total QOL-B RSS at week 4***

	Overall effect on QOLB	
Symptom	Effect estimate (95% CI)	p-value
Q31- sputum production		
1 - 2 (severe)	4.82 (1.12 to 8.53)	**0.011**
3 - 4 (mild)	-2.61 (-7.58 to 2.37)	0.305
Q32 colour		
1 - 2 (severe)	5.02 (1.19 to 8.86)	**0.01**
3 - 4 (mild)	-0.78 (-5.88 to 4.33)	0.766
Q34 Wheezing		
1 - 2 (severe)	-0.66 (-8.06 to 6.74)	0.861
3 - 4 (mild)	3.74 (0.50 to 6.97)	**0.024**
Q36 SOB when talking		
1 - 2 (severe)	0.66 (-7.67 to 8.99)	0.876
3 - 4 (mild)	3.23 (0.12 to 6.33)	**0.042**
Q37 Woken due to cough		
1 - 2 (severe)	-0.33 (-7.01 to 6.35)	0.923
3 - 4 (mild)	3.62 (0.27 to 6.97)	**0.034**

Nine sets of 194 mixed models were fitted to the data from visits 2-7 of both trials together looking at the effect of each question separately. These models allowed a common drift over time for all individuals, and potentially different long and short term treatment effects on placebo and AZLI and an interaction between each of these and the answer to the symptom question at baseline. The estimates for the effects of the baseline questions are shown in table S4 below, the other parameter values are uninteresting. In each case the full model contains all the parameters, the best model is the one with the lowest AICc, and the average is a model averaged result, that uses the AICc weight for each model. This stage was necessary because there would be over 10^{15} models if each combination of all these terms for all questions were considered.

Q	model	effect of baseline question answer	interaction of baseline question answer with:			
			Placebo effect, affecting both arms equally		Drug effect on AZLI population only	
			long term (study duration)	short term (visit 3,4,6)	long term (study duration)	short term (visit 3,4,6)
29	full	12.86 (0.66)	-3.34 (0.74)	-1.23 (0.71)	0.41 (1.08)	-1.02 (1.09)
	best	12.86 (0.66)	-3.17 (0.58)	-1.67 (0.54)	-	-
	average	12.86 (0.66)	-3.23 (0.63)	-1.32 (0.78)	-0.46 (0.83)	0.02 (0.36)
	p	***<0.001***	***<0.001***	*0.09*	*0.58*	*0.95*
30	full	13.74 (0.87)	-3.95 (0.90)	-0.90 (0.85)	0.01 (1.33)	-1.25 (1.32)

	best	13.74 (0.87	-4.33 (0.62)	-	-	-1.92 (0.87)
	average	13.74 (0.87	-4.17 (0.74)	-0.66 (0.82)	-0.02 (0.44)	-0.97 (1.14)
	p	***<0.001***	***<0.001***	*0.42*	*0.96*	*0.39*
31	full	12.63 (0.97)	-3.52 (0.99)	-0.85 (0.94)	0.57 (1.43)	-2.66 (1.41)
	best	12.63 (0.97)	-3.67 (0.67)	-	-	-3.07 (0.92)
	average	12.63 (0.97)	-3.52 (0.78)	-0.55 (0.89)	0.07 (0.56)	-2.49 (1.39)
	p	***<0.001***	***<0.001***	*0.53*	*0.90*	*0.07*
32	full	6.90 (1.02)	-2.06 (0.98)	-2.23 (0.93)	-2.27 (1.41)	0.13 (1.38)
	best	6.90 (1.02)	-3.10 (0.74)	-2.17 (0.69)	-	-
	average	6.86 (1.03)	-2.63 (1.20)	-1.90 (1.04)	-0.98 (1.47)	-0.43 (1.08)
	p	***<0.001***	***0.03***	*0.68*	*0.51*	*0.69*
33	full	11.22 (0.65)	-1.24 (0.74)	-1.67 (0.71)	2.42 (1.04)	-1.88 (1.03)
	best	11.22 (0.65)	-2.44 (0.47)	-	-	-
	average	11.22 (0.65)	-2.07 (0.83)	-0.57 (0.83)	-0.39 (0.96)	0.66 (1.12)
	p	***<0.001***	***0.01***	*0.50*	*0.69*	*0.56*
34	full	12.59 (0.79)	-3.49 (0.83)	-0.60 (0.80)	-0.97 (1.21)	-0.24 (1.20)
	best	12.59 (0.80)	-4.34 (0.53)	-	-	-
	average	12.59 (0.80)	-4.05 (0.65)	-0.20 (0.47)	-0.16 (0.55)	-0.39 (0.73)
	p	***<0.001***	***<0.001***	*0.68*	*0.77*	*0.59*
35	full	12.46 (1.09)	-4.83 (1.19)	0.89 (1.13)	0.65 (1.57)	-1.05 (1.55)
	best	12.46 (1.09)	-4.29 (0.69)	-	-	-
	average	12.46 (1.09)	-4.33 (0.78)	0.11 (0.48)	0.01 (0.42)	-0.07 (0.55)
	p	***<0.001***	***<0.001***	*0.82*	*0.90*	*0.99*
36	full	11.92 (0.83)	-2.86 (0.89)	0.77 (0.85)	-0.68 (1.27)	0.74 (1.26)
	best	11.92 (0.83)	-3.17 (0.68)	1.11 (0.63)	-	-
	average	11.92 (0.83)	-2.88 (0.73)	0.56 (0.72)	-0.06 (0.44)	0.29 (0.71)
	p	***<0.001***	***<0.001***	*0.44*	*0.88*	*0.68*
37	full	12.80 (0.76)	-5.01 (0.83)	-0.56 (0.79)	2.40 (1.17)	-0.61 (1.16)
	best	12.80 (0.76)	-4.84 (0.76)	-0.84 (0.58)	2.02 (0.92)	-
	average	12.80 (0.76)	-4.74 (0.86)	-0.37 (0.61)	1.29 (1.35)	-0.15 (0.72)
	p	***<0.001***	***<0.001***	*0.54*	*0.34*	*0.83*
total score	full	0.93 (0.03)	-0.26 (0.04)	-0.06 (0.04)	-0.01 (0.06)	-0.02 (0.06)
	best	0.93 (0.03)	-0.26 (0.03)	-0.07 (0.03)	-	-
	average	0.93 (0.03)	-0.27 (0.03)	-0.05 (0.04)	-0.002 (0.017)	-0.02 (0.04)
	p	***<0.001***	***<0.001***	*0.23*	*0.92*	*0.63*

Table S5: Parameter estimates in models of the effect of each question's answer at visit 1 on QOLB-RSS scores at visits 2-7. Each full model contained the terms shown plus separate short and long term effects of treatment, allowing these to be different for placebo and AZLI. The data from the two trials was combined, and an intercept plus a single common drift term included along with individual as a random effect.

The 25 question-based parameters that had p-values less than 0.5 were put into a model along with the four treatment terms and the models nested within it refitted. At this stage, all the individual question main effects had values close to 3, so they were replaced with the total score at visit 1, and the model fitting was redone. Separate terms were included for the interaction between being on the trial and the answer to each question, as well as with the total score at baseline. The main effects for the baseline score and the short term (visit=3,4,6) and longer term (duration of study) placebo effects were required to be in all of the 98,304 models. Table S5 shows the results:

parameter	estimate (SE) in models:	p

		full (A)	best (B)	averaged	
	Intercept	4.75 (1.62)	4.76 (1.58)	4.88 (1.62)	**0.003**
	drift	0.015 (0.009)	0.015 (0.009)	0.009 (0.01)	0.35
	baseline score	0.93 (0.03)	0.93 (0.03)	0.93 (0.03)	**<0.0001**
on trial (study duration)	main (placebo) effect	21.12 (2.42)	20.18 (1.97)	20.5 (2.32)	**<0.0001**
	drug effect	-0.09 (0.98)	-	-0.02 (0.51)	0.97
	interaction with Q29	-0.12 (0.67)	-	-0.16 (0.45)	0.73
	interaction with Q30	-1.51 (0.63)	-1.63 (0.54)	-1.48 (0.69)	0.03
	interaction with Q31	-0.43 (0.68)	-	-0.18 (0.48)	0.71
	interaction with Q32	-3.42 (0.52)	-3.39 (0.48)	-3.37 (0.49)	**<0.0001**
	interaction with Q33	0.43 (0.59)	-	0.10 (0.31)	0.75
	interaction with Q34	-2.31 (0.55)	-2.11 (0.49)	-2.20 (0.52)	**<0.0001**
	interaction with Q35	-1.63 (0.63)	-1.53 (0.60)	-1.40 (0.74)	0.06
	interaction with Q36	-0.25 (0.57)	-	-0.02 (0.30)	0.94
	interaction with Q37	-1.33 (0.65)	-1.40 (0.48)	-1.33 (0.65)	**0.04**
being treated (visit 3,4,6-short term)	Main (placebo) effect	2.70 (2.17)	3.91 (0.94)	3.44 (1.57)	**0.03**
	drug effect	4.69 (1.39)	4.64 (1.14)	4.48 (1.37)	**0.001**
	interaction with total baseline score	0.04 (0.08)	-	0.003 (0.034)	0.93
	interaction with Q29	-1.70 (0.87)	-1.44 (0.45)	-1.37 (0.72)	0.06
	interaction with Q33	-0.25 (0.74)	-	0.05 (0.31)	0.88
	interaction with Q37	-0.28 (0.83)	-	-0.05 (0.43)	0.90
	interaction with Q31 and on AZLI	-2.31 (0.93)	-2.36 (0.83)	-2.18 (1.04)	**0.04**
	AICc	20799.0	20784.6		
	ΔAICc	+14.4	0		
	AICc weight	0.00002	0.03		

Table S6: parameters in models of total score that allow effects from answers to individual questions at visit 1. The baseline QOL-B score is out of 100, while each question is scored 1-4, complicating the comparison of their effect sizes.

The intercepts for the models of different questions differ, reflecting the pattern of baseline symptoms among the participants, but the placebo estimated effects are remarkably similar. This suggests that such reported improvements were distributed across the range of symptoms. There seems to be more variability in the reported effects of AZLI itself, with answers to some questions (30,31,32,34) being more affected than the remaining ones.

Table S7- Effect of baseline symptoms on changes in FEV_1 at week 4

	Overall effect on FEV_1	
Symptom	Effect estimate (95% CI)	p-value
Overall effect	-0.01 (-0.12 to 0.09)	0.85
Q29- congestion		
1	-0.113 (-0.32 to 0.09)	0.277
2	0.036 (-0.14 to 0.22)	0.696
3	0.035 (-0.19 to 0.26)	0.754
4	-0.096 (-0.28 to 0.08	0.296
Q30- cough		
1	-0.095 (-0.26 to 0.07)	0.266
2	-0.072 (-0.27 to 0.13)	0.483
3	0.083 (-0.09 to 0.25)	0.339
4	-0.147 (-0.46 to 0.17)	0.348
Q31- sputum production		
1	-0.156 (-0.33 to 0.02)	0.082
2	0.11 (-0.16 to 0.18)	0.902
3	0.114 (-0.10 to 0.33)	0.289
4	0.036 (-0.56 to 0.64)	0.906
Q32 colour		
1	-0.274 (-0.46 to 0.02)	0.07
2	0.108 (-0.05 to 0.27)	0.181
3	-0.085 (-0.28 to 0.11)	0.382
4	0.022 (-0.39 to 0.44)	0.916
Q33 daily activity		
1	0.038 (-0.18 to 0.25)	0.729
2	-0.049 (-0.25 to 0.16)	0.637
3	0.018 (-0.16 to 0.20)	0.842
4	-0.098 (-0.35 to 0.15)	0.437
Q34 Wheezing		
1	-0.029 (-0.51 to 0.45)	0.904
2	-0.066 (-0.25 to 0.12)	0.484
3	0.063 (-0.09 to 0.22)	0.419
4	-0.065 (-0.27 to 0.14)	0.532
Q35 chest pain		
1	-0.332 (-1.1 to 0.44)	0.396
2	0.164 (-0.08 to 0.41)	0.195

3	0.105 (-0.10 to 0.31)	0.327
4	-0.064 (-0.19 to 0.07)	0.331
Q36 SOB when talking		
1	0.085 (-0.30 to 0.47)	0.661
2	-0.018 (-0.28 to 0.25)	0.896
3	-0.032 (-0.19 to 0.13)	0.69
4	-0.004 (-0.19 to 0.18)	0.968
Q37 Woken due to cough		
1	0.338 (-0.01 to 0.68)	0.053
2	0.046 (-0.18 to 0.27)	0.69
3	0.03 (-0.16 to 0.16)	0.968
4	-0.124 (-0.32 to 0.07)	0.213

Table S7. No effect of AZLI on FEV_1 change from baseline to week 4 regardless of baseline symptoms. As no effect was seen for any individual symptoms no further analysis was performed.