Airway abundance of *Haemophilus influenzae* predicts response to azithromycin in adults with persistent uncontrolled asthma

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Short summary: Sputum *Haemophilus influenzae* load predicts response to azithromycin in adults with persistent uncontrolled asthma: Secondary findings from the AMAZES trial

Long-term azithromycin treatment reduced exacerbations in adults with persistent symptomatic asthma in the AMAZES trial (Australian and New Zealand Clinical Trials Registry (ANZCTR), number 12609000197235) [1]. However, response to treatment was variable between participants and the characteristics of those who experience most clinical benefit have not been determined. The inability to define a specific population who are more responsive limits the ability to personalise this therapy, which is the goal for airways disease management [2]. With recent studies identifying airway *Haemophilus influenzae* colonisation as a candidate marker for asthma subgrouping [3,4], we assessed whether *H. influenzae* abundance, measured using quantitative PCR (qPCR) [5,6], predicted the ability of azithromycin therapy to reduce the incidence of acute asthma exacerbations.

H. influenzae abundance was measured in triplicate in 96 available baseline (pre-intervention) induced sputum samples from the AMAZES trial [1], using a validated, species-specific qPCR assay with a standard curve of *H. influenzae* strain NTCC8468 of known quantities, as previously described [5,6]. Participants subsequently received oral azithromycin 500 mg (n=46) or placebo (n=50) three times weekly for 48 weeks, and total number of asthma exacerbations were recorded. All baseline characteristics were similar between treatment groups, as well as between this sub-group and the original AMAZES cohort. As per the prespecified trial subgroup analysis, negative binomial regression was performed for the analysis of asthma exacerbations. Length of intervention treatment was included as an offset and clustering for study site was adjusted for. The incidence rate ratio estimating the treatment effect and 95% confidence intervals were calculated.

H. influenzae abundance had a detection limit of 3×10^4 copies/mL of sputum and was similar between azithromycin and placebo groups at baseline (median [interquartile range]: 3×10^4 [1.89×10^5] vs 3×10^4 [3.13×10^5], respectively; p=0.99). There was a significant interaction between treatment group and baseline *H. influenzae* abundance (incidence rate ratio [95%

confidence interval]: 0.40 [0.23, 0.69]; p=0.001). Specifically, higher *H. influenzae* abundance was associated with lower exacerbation frequency in the azithromycin arm, whereas, in the placebo arm, higher *H. influenzae* abundance was associated with higher exacerbation frequency (Figure 1). This suggests that baseline *H. influenzae* abundance by qPCR can identify participants who will receive most benefit in terms of exacerbation reduction with add-on azithromycin therapy.

In conclusion, from this post-hoc analysis, culture-independent measurement of *H. influenzae* significantly predicted improved azithromycin efficacy in reducing total asthma exacerbation frequency in adults with persistent symptomatic asthma. Prospective measurement of *H. influenzae* abundance in patients prior to commencing azithromycin therapy will facilitate the identification of a clinically-informative *H. influenzae* cut-off value.

The following investigators constitute the AMAZES Investigators Group:

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Figure 1: Effect of add-on azithromycin treatment on asthma exacerbations according to *Haemophilus influenzae* levels. Predicted exacerbation rates from negative binomial regression and 95% confidence interval, adjusting for length of intervention treatment and study site.

References

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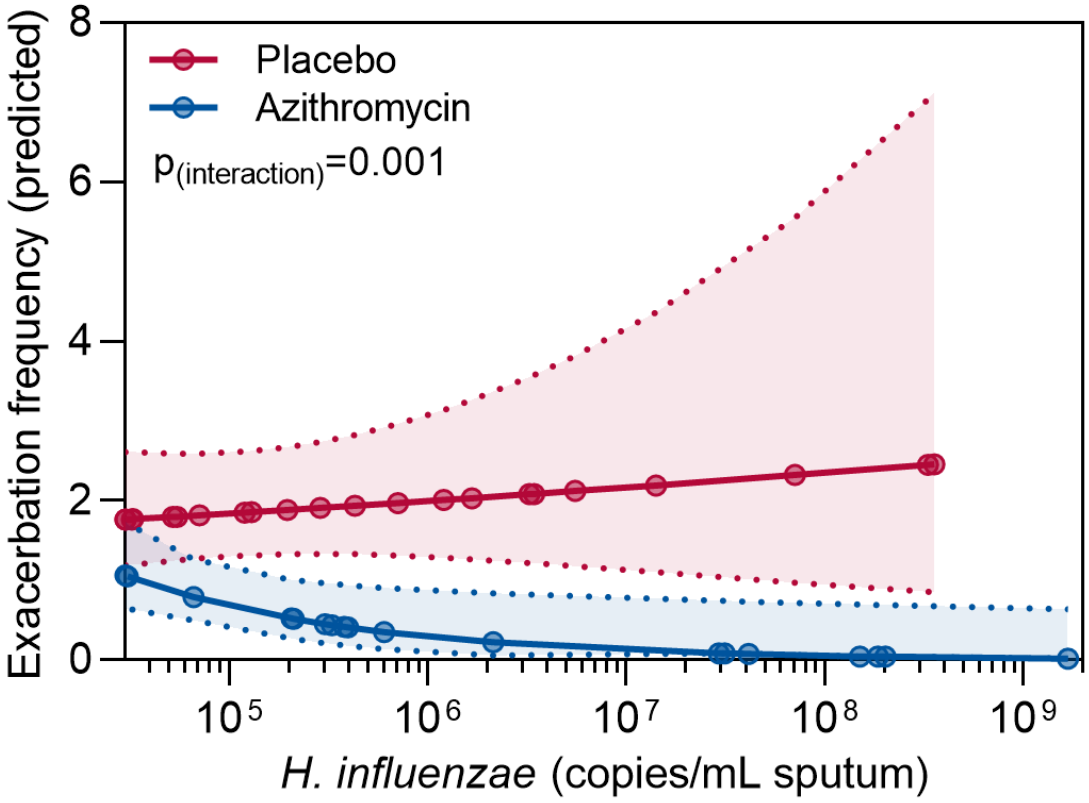
Placebo
Azithromycin
p(interaction)=0.001
Exacerbation frequency (predicted)
0
2
4
6
8
10^5
10^6
10^7
10^8
10^9
H. influenzae (copies/mL sputum)