Azithromycin during Wheezing Illnesses among Preschool Children
Does the Airway Microbiota Provide Insights into Mechanism?

Young children often experience recurrent episodes of respiratory tract illnesses manifested by wheezing, cough, and breathlessness (1). A substantial fraction of these episodes can be quite impactful, leading to high rates of health care use along with frequent prescription of bronchodilators and systemic corticosteroids (which are not without consequences). Despite the commonplace use of these approaches, studies suggest that systemic corticosteroids may not be as effective in improving clinical symptoms as generally thought (2, 3), prompting research examining alternative treatment strategies.

The macrolide antibiotic azithromycin has been shown to impact such episodes, either by preventing symptom progression when given very early during an evolving episode (4) or by shortening illness duration when given after 3 days of symptoms (5). What remains uncertain is the mechanism(s) through which azithromycin exerts these effects. Potential explanations have included antiinflammatory actions (6, 7), antiviral effects (8), and/or classical antimicrobial effects. Although viruses have long been considered the dominant infectious driver of these episodes, recent culture-based and non–culture-based detection of bacteria in the upper airways of affected children have drawn significant attention to the role of these bacteria in illness pathogenesis.

In this issue of the *Journal*, Thorsen and colleagues (pp. 149–158) provide an examination of the relationship between the nasopharyngeal microbiome and response to azithromycin (9). This report is derived from a clinical trial involving a subset of children 12–36 months of age enrolled in the $COPSAC_{2010}$ (Copenhagen Prospective Studies on Asthma in Childhood) prospective birth cohort study (5). Children who had experienced recurrent asthma-like symptoms were randomized to receive either azithromycin (10 mg/kg/d for 3 d) or placebo at any subsequent episode of at least 3 consecutive days of parent-reported asthma-like symptoms. The primary outcome of the parent study was positive, with azithromycin therapy being associated with a 63.3% shortening of episode duration relative to placebo (3.4 vs. 7.7 d) (8). This effect was independent of the presence of pathogenic bacteria identified by the culture of hypopharyngeal samples, and there was no evidence for effect modification by the presence of a virus. For the current report, which used genomics to characterize the microbiome rather than culture-based techniques, the investigators sequenced the V4 region of the 16S rRNA gene in 139 hypopharyngeal aspirates obtained from 68 children at each initiation of study therapy (9).

Thorsen and colleagues identified a substantial microbial presence in hypopharyngeal samples, with 1,412 operational taxonomic units (OTUs) from 333 genera, a median richness of 45 OTUs (interquartile range, 33–61), and a median Shannon diversity index of 1.84 (interquartile range, 1.45–2.19). Not surprisingly, the four most commonly identified genera were typical respiratory tract organisms—*Moraxella*, *Haemophilus*, *Streptococcus*, and *Neisseria*—and these genera largely defined five discrete clusters, with each one dominating a cluster plus a fifth cluster without a dominant genus but comprised of several genera. There was no effect of azithromycin therapy on microbial richness or diversity during a subsequent illness. Greater richness and diversity were both associated with longer episode duration, with each 10-OTU increase associated with a 7.5% increase in episode duration. As sample richness increased, so too did the effect of azithromycin on episode duration, with each 10-OTU increase being associated with a 10% greater azithromycin response.

In addition to these overall microbiota effects, the presence of several specific OTUs was related to episode duration: 13 OTUs in the placebo-treated episodes and 3 in azithromycin-treated episodes. Fifteen of these OTUs were associated with longer episode duration, including several *Neisseria* and *Veillonella* OTUs, whereas only a *Moraxella* OTU was associated with shorter episode duration. The *Neisseria*-dominant cluster was associated with the longest episode duration, whereas the cluster without a dominant genus experienced the shortest episodes. In terms of effect on azithromycin response, nine OTUs, when present at high relative abundance, were associated with greater azithromycin effect. Finally, five individual OTUs were found to modify the effect of azithromycin, with three increasing (*Veillonella*, *Leuconostoc*, and *Vibrio*) and two decreasing (both *Neisseria*) the effect. The authors conclude that during acute episodes of asthma-like symptoms, the airway microbiota is associated with episode duration and modifies the effect of azithromycin during these illnesses (9).

What are we to conclude about these findings with respect to the mechanism(s) through which azithromycin reduces episode duration? These findings suggest that azithromycin is likely operating, at least in part, through its classic antimicrobial actions, as greater effects are seen during episodes with greater microbial content reflected by richness and abundance in hypopharyngeal samples. The presence of 15 specific OTUs was associated with longer episode duration, and greater relative abundance of 9 specific genera (including *Veillonella* and *Neisseria*) was associated with greater azithromycin effects. No data are presented in terms of antimicrobial resistance patterns of the identified OTUs, so it remains unknown whether the patterns of response (or lack thereof) are related to azithromycin resistance. The presence of a *Prevotella* was associated with longer episode duration and a diminished azithromycin response—could this have been because of azithromycin resistance among this genus?

There are several limitations to consider in interpreting these results. The study population was a subset of the entire clinical trial (5)

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and exhibited greater asthma risk and more severe disease than the overall population, limiting the generalizability of the findings. Samples were taken from the hypopharynx and, although clearly informative and correlated to the clinical response, may not precisely reflect microbial community structure in the lower airways. The samples were obtained after 3 days of symptom development, so it is not known whether these patterns of microbiome structure would have been detectable even earlier in the course or whether they reflect the potential effects of an intercurrent viral infection on the microbiome. Unfortunately, there are no data presented on the direct effect of azithromycin on the microbiome structure after treatment, which would help to determine if the antimicrobial effects paralleled clinical response. Although the initial study report demonstrated no effect modification by viral infection (5), there are no data presented with quantification of the effect of azithromycin treatment on viral load or immune markers after treatment.

Our understanding of the infectious components of these lower respiratory tract illnesses in young children continues to evolve. Viral infections remain important triggers of episodes, but there is a clear interaction with nonviral airway microbes that influence episode development and likely impact treatment responses. Additional research is needed to further disentangle these factors and ultimately allow for more effective and targeted therapies that reduce the substantial morbidity associated with these episodes. ■

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References

1. Bisgaard H, Szefler S. Prevalence of asthma-like symptoms in young children. *Pediatr Pulmonol* 2007;42:723–728.
2. Beigelman A, Durrani S, Guilbert TW. Should a preschool child with acute episodic wheeze be treated with oral corticosteroids? A pro/con debate. *J Allergy Clin Immunol Pract* 2016;4:27–35.
3. Beigelman A, King TS, Mauger D, Zeiger RS, Strunk RC, Kelly HW, *et al.*; Childhood Asthma Research and Education Network of National Heart, Lung, and Blood Institute. Do oral corticosteroids reduce the severity of acute lower respiratory tract illnesses in preschool children with recurrent wheezing? *J Allergy Clin Immunol* 2013;131: 1518–1525.
4. Bacharier LB, Guilbert TW, Mauger DT, Boehmer S, Beigelman A, Fitzpatrick AM, *et al.* Early administration of azithromycin and prevention of severe lower respiratory tract illnesses in preschool children with a history of such illnesses: a randomized clinical trial. *JAMA* 2015;314: 2034–2044.
5. Stokholm J, Chawes BL, Vissing NH, Bjarnadóttir E, Pedersen TM, Vinding RK, *et al.* Azithromycin for episodes with asthma-like symptoms in young children aged 1-3 years: a randomised, double-blind, placebo-controlled trial. *Lancet Respir Med* 2016;4:19–26.
6. Fonseca-Aten M, Okada PJ, Bowlware KL, Chavez-Bueno S, Mejias A, Rios AM, *et al.* Effect of clarithromycin on cytokines and chemokines in children with an acute exacerbation of recurrent wheezing: a double-blind, randomized, placebo-controlled trial. *Ann Allergy Asthma Immunol* 2006; 97:457–463.
7. Simpson JL, Powell H, Boyle MJ, Scott RJ, Gibson PG. Clarithromycin targets neutrophilic airway inflammation in refractory asthma. *Am J Respir Crit Care Med* 2008;177:148–155.
8. Gielen V, Johnston SL, Edwards MR. Azithromycin induces anti-viral responses in bronchial epithelial cells. *Eur Respir J* 2010;36: 646–654.
9. Thorsen J, Stokholm J, Rasmussen MA, Mortensen MS, Brejnrod AD, Hjelmsø M, *et al.* The airway microbiota modulates effect of azithromycin treatment for episodes of recurrent asthma-like symptoms in preschool children: a randomized clinical trial. *Am J Respir Crit Care Med* 2021;204:149–158.

Are Adults with Chronic Obstructive Pulmonary Disease Vulnerable to Air Pollution and Cardiovascular Risk?

Substantial research has provided evidence that long-term exposure to air pollution, especially fine particulate matter (particles ⩽2.5 μm in aerodynamic diameter [$PM_{2.5}$]), contributes to cardiovascular disease (CVD) (1, 2). Key to this evidence is the growing number of cohort studies that have found long-term exposures to $PM_{2.5}$ air pollution to be associated with increased risk of mortality, including CVD, nonmalignant respiratory disease, and lung cancer mortality (3, 4).

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$PM_{2.5}$–mortality relationships have been observed mostly in broad, population-based cohorts. A few specific, susceptible subpopulations that may be especially vulnerable to air pollution exposures have been identified. For example, relatively large $PM_{2.5}$–mortality associations have been observed in cohorts of patients who received a cardiac transplant (5) and survivors of myocardial infarction (MI) (6). Also, relatively large associations between $PM_{2.5}$ air pollution and CVD mortality risk have recently been observed in a cohort of U.S. patients with cancer and cancer survivors (7).

Another identifiable subpopulation that may be especially vulnerable to CVD risk from exposure to air pollution consists of adults with chronic obstructive pulmonary disease (COPD). There is substantial CVD comorbidity in adults with COPD, and those with COPD are at greater risk of CVD and death (8, 9). A recent cohort study