Oral Azithromycin Use and the Recovery of Lung Function from Pulmonary Exacerbations Treated with Intravenous Tobramycin or Colistimethate in Adults with Cystic Fibrosis

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Abstract

Rationale: The potential of azithromycin to alter the antimicrobial and clinical benefits of inhaled tobramycin in patients with cystic fibrosis (CF) has been previously reported. The potential interaction between azithromycin and intravenous antibiotics in the treatment of pulmonary exacerbations is unknown.

Objectives: To determine if chronic azithromycin use as a concomitant therapy is associated with change in lung function after receiving intravenous antibiotic regimens including tobramycin or colistimethate.

Methods: This was a retrospective cohort study evaluating the association of azithromycin with intravenous tobramycin or colistimethate in adult patients with CF treated for a pulmonary exacerbation. The primary outcome was relative lung function recovery (forced expiratory volume in 1 s [FEV₁]) after exacerbation treatment. Generalized estimating equations were applied to account for repeated events with independent correlation structures and robust standard errors, incorporating several confounders.

Results: A total of 220 exacerbation events occurred in 121 patients in the tobramycin group (47% using azithromycin), and 207 exacerbation events occurred in 86 patients in the colistimethate group (59% using azithromycin). Azithromycin use was associated with less FEV₁% recovery in patients treated with tobramycin (−3% relative FEV₁% recovery [95% confidence interval (CI), −7 to 0.2] and −2.64% absolute FEV₁% change [95% CI, −4.52 to −0.76]). Azithromycin use was associated with greater recovery of FEV₁% when treated with colistimethate (+3% relative FEV₁% recovery [95% CI, −0.1 to 7] and 2.00% absolute improvement in FEV₁% [95% CI, 0.13 to 3.87]). The odds of 90% or 100% recovery to baseline FEV₁% were lower with azithromycin use in the tobramycin cohort and higher with azithromycin use in the colistimethate cohort but were not statistically significant.

Conclusions: Azithromycin use was associated with a more favorable response in adult patients with CF treated with intravenous colistimethate but a less favorable response in those treated with intravenous tobramycin.

Keywords: cystic fibrosis; azithromycin; tobramycin; pulmonary exacerbation; lung function

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Chronic *Pseudomonas aeruginosa* airway infection is present in approximately two-thirds of adults with cystic fibrosis (CF) in the United States and is the most common

bacteria treated with intravenous antibiotics at the time of pulmonary exacerbation (1, 2). Chronic oral azithromycin (defined as daily or thrice weekly administration) and

inhaled antipseudomonal antibiotics are both commonly prescribed to people with CF (3). We have recently reported that azithromycin may selectively antagonize

inhaled tobramycin (4–6). During acute CF pulmonary exacerbations treated in the United States, intravenous tobramycin is used for *P. aeruginosa*-positive patients nearly 90% of the time (3, 7), but intravenous colistimethate is a common alternative in some centers. These intravenous antibiotics are rarely, if ever, used together and are seen as an anchor antibiotic to which a wide variety of β -lactam or fluoroquinolone antibiotics are added.

On the basis of U.S. CF Foundation Patient Registry data, we estimate that half or more of patients treated with intravenous tobramycin are chronically prescribed azithromycin (3, 8). *In vitro* studies by our group and others indicate that azithromycin may reduce the ability of tobramycin, but not several alternative antibiotics, including colistimethate, to kill *P. aeruginosa* (4, 9). This bacteria appears to respond to azithromycin by inducing antibiotic resistance mechanisms that also increase resistance to aminoglycosides (4, 10, 11). Although azithromycin has not been studied in the context of pulmonary exacerbation, current guidelines generally recommend continuing chronic therapies during pulmonary exacerbation treatment (12). In the case of azithromycin, it may require weeks for the drug to adequately eliminate from the pulmonary space, which complicates the ability to interrupt chronic azithromycin therapy during acute treatment of a pulmonary exacerbation (13–16). Virtually no data exist on the impact of chronic azithromycin use and its interaction with common intravenous antibiotics on pulmonary exacerbation recovery; thus, we conducted this study to determine if concomitant azithromycin use associated with the degree of lung function recovery in patients treated with intravenous tobramycin or intravenous colistimethate. The purpose of this study is to consider the impact of concomitant azithromycin in response to intravenous antibiotic treatment. It is not a study comparing different intravenous antibiotic regimens.

Part of this work was presented in abstract form at the North American Cystic Fibrosis Conference in October 2017 in Indianapolis, IN (17).

Methods

Study Sample

This was a retrospective cohort study of individuals aged 18 years or older with CF

followed at the Colorado Adult Cystic Fibrosis Program in Denver, Colorado from August 1, 2014 through July 31, 2017. All patients included had provided consent for collection of data and use in research studies. Subjects had a diagnosis of CF (18) and a clinically diagnosed pulmonary exacerbation requiring hospital admission during the study period. Subjects were excluded if they had previously undergone lung transplantation or if they had a positive test for a respiratory virus at the time of exacerbation. Pulmonary exacerbation episodes were excluded from the study if the antibiotic regimen included less than 5 days of intravenous tobramycin or intravenous colistimethate, if the antibiotic regimen included both tobramycin and colistimethate at any point, and if any of the following data parameters were missing: 1) baseline lung function (forced expiratory volume in 1 s [FEV₁%] predicted), 2) FEV₁ data at the start of antibiotics (representing acute drop at time of pulmonary exacerbation), 3) number of days prescribed tobramycin or colistimethate during the pulmonary exacerbation episode, and 4) FEV₁ at the end of treatment. Eligible pulmonary exacerbation events were stratified into cohorts for separate analysis on the basis of receipt of tobramycin or colistimethate and then compared within each cohort on the basis of concomitant use of chronic azithromycin therapy.

Variable Definitions

Baseline characteristics that were not time-varying included sex (male/female), race, and highest mutation functional class (class I–III/class IV–V/missing). Time-varying covariates included age (years), FEV₁% predicted using the Global Lung Function Initiative Equations (19), azithromycin use, *P. aeruginosa* infection status, and the number of pulmonary exacerbation episodes requiring hospitalization for intravenous antibiotic treatment. Patients were categorized as having chronic *P. aeruginosa* if 50% or more of cultures obtained in the prior 2 years identified *P. aeruginosa*. Baseline FEV₁ was defined as the best value measured at a clinical encounter over the preceding 24 months relative to the pulmonary exacerbation event date. Start of treatment FEV₁ represented the lung function measured within 48 hours of hospital admission. For all subjects, the FEV₁ obtained in the hospital at the initiation of treatment was used when

available. In the event that an FEV₁ measurement was not obtained in the hospital within 48 hours of the start of treatment, an FEV₁ measurement from clinic was used, if available. The recovery FEV₁% was obtained within 7 days of the end of intravenous antibiotic therapy. In the majority of pulmonary exacerbation events, the beginning and end of treatment FEV₁ was measured within 24 hours of starting and ending intravenous antibiotics. The magnitude of FEV₁ drop is the difference between 12-month baseline and start of treatment. Absolute recovery in FEV₁% is the difference between start and end of intravenous treatment values. Relative recovery in FEV₁% is calculated as a ratio on the basis of the comparison of the end of treatment and 24-month baseline values.

Statistical Analyses

Baseline demographic and clinical characteristics were summarized at the time of the first pulmonary exacerbation episode for a subject. Descriptive analyses were used to summarize the data, with medians and interquartile ranges for continuous variables and proportions for categorical variables.

Subjects were initially categorized into either the tobramycin or colistimethate arms on the basis of their antibiotic regimen. In nearly all cases, tobramycin was administered at 10 mg/kg every 24 hours, guided by peak serum concentration and drug-free interval calculated after the second dose. Rarely, when tobramycin clearance was delayed because of renal insufficiency, the frequency of administration was increased to 30 or 36 hours. Colistimethate was initiated at 2 mg/kg/d (colistimethate base equivalent) every 8 hours and increased over the next 72 hours to a goal of 4 mg/kg/d as tolerated. The primary exposure variable assessed within each arm was the use of azithromycin. Subjects in each arm were classified as azithromycin-positive or azithromycin-negative on the basis of report of azithromycin as a concomitant medication at the time of pulmonary exacerbation treatment. As azithromycin is not used as an acute medication to treat pulmonary exacerbation at the study center, its use during hospitalization is an accurate measure of chronic use; however, the duration of prior azithromycin use could not be obtained from available records.

The primary outcome was the relative magnitude of lung function (FEV₁%) recovery after treatment of a pulmonary

exacerbation with either tobramycin- or colistimethate-based regimens with or without concurrent azithromycin. Analyses focused on the azithromycin use within each arm and were not intended to compare tobramycin- and colistimethate-based regimens, as these groups reflect inherently different patient populations. We constructed multivariable generalized estimating equations (GEEs) accounting for repeated events with an independent correlation structure and robust variance to determine 1) the ratio of FEV₁% predicted at discharge relative to baseline, 2) absolute recovery of FEV₁% predicted during intravenous therapy compared with start of treatment, 3) recovery to 90% of baseline FEV₁% predicted, and 4) recovery to 100% of baseline FEV₁% predicted. We incorporated potential confounders of sex, age, highest cystic fibrosis transmembrane conductance regulator (*CFTR*) mutation class, methicillin-resistant *Staphylococcus aureus* status, CF-related diabetes status, baseline FEV₁% predicted, and drop in FEV₁% at time of exacerbation (as an absolute value) into the multivariable models. These were selected as plausible variables that may be associated with a decision to use chronic azithromycin, may influence lung function recovery during pulmonary exacerbation treatment, and are not believed to mediate a relationship between azithromycin exposure and lung function recovery (20).

In an effort to further consider confounding by indication wherein time-dependent FEV₁ measurements may affect the likelihood to be prescribed chronic azithromycin, we also conducted a sensitivity analysis for the primary outcome through marginal structural modeling (21, 22). Marginal structural models (MSM) can account for confounders that vary over time (i.e., lung function) and may affect the likelihood that a subject will receive a given treatment (i.e., azithromycin). Subjects with lower FEV₁ may be more likely to receive azithromycin, and this may be associated with a clinical outcome, such as FEV₁ recovery after pulmonary exacerbation. Marginal structural models thus allow for adjustment of time-varying confounders on the causal pathway of interest to examine the effect of azithromycin on lung function recovery while minimizing bias.

We conducted this sensitivity analysis separately in the tobramycin and colistimethate arms. In each arm, we assessed the probability of azithromycin

treatment on the basis of baseline lung function for each time point using logistic regression and calculated the inverse probability of receiving the given treatment. A regression model was constructed with unstabilized weights calculated for each pulmonary exacerbation event (on the basis of prior inverse probability calculations) and the confounders of age, sex, and FEV₁% predicted drop (baseline FEV₁ was excluded, as it was used to calculate the weights) were incorporated to examine the effect of azithromycin use on FEV₁ response. For the models for 90% and 100% FEV₁ recovery, the values derived from the MSM models represented population average effects.

Outcomes were predetermined, hypotheses were two-sided at α significance of 0.05, and analyses used STATA 14.2. The study was approved by the institutional review board of National Jewish Health/SCL Health-Front Range protocol 201522-NJH.

Results

Study Sample

A summary of the study sample and excluded exacerbation events is shown in the study flow diagram (Figure 1). Table 1 represents the clinical characteristics of the entire cohort at the time of study entry, stratified by patients who were treated with a tobramycin- or colistimethate-based antibiotic regimen. The tobramycin cohort consisted of 121 subjects with a total of 220 pulmonary exacerbation events (47% on azithromycin at time of first event); the colistimethate cohort consisted of 86 subjects with 207 exacerbation events (59% on azithromycin at time of first event). Thirty-four patients contributed data to both the tobramycin cohort and the colistimethate cohort. The percentage female, mutation class, and age at first exacerbation between the tobramycin and colistimethate groups were similar. Subjects in the colistimethate group had a lower median baseline FEV₁% predicted (55% vs. 69%) and a somewhat greater proportion of persons of Hispanic ethnicity (7% vs. 4.1%).

Clinical characteristics stratified by treatment group and azithromycin use are summarized in Tables 2 and 3. In the tobramycin cohort on azithromycin, there was a smaller proportion of females, more patients of Hispanic ethnicity, and a somewhat lower baseline FEV₁%, with median of 60% versus 67% (mean baseline FEV₁% was 65.5% vs. 66.4%). Statistical

comparisons of these characteristics were not used to select variables to include in the multivariable model. The relative drop in FEV₁% from baseline at the start of intravenous therapy was very similar (−24.7% vs. −24.9%).

In the distinct colistimethate cohort, subjects using azithromycin were slightly younger at the time of exacerbation (median, 26 vs. 28 yr) but were otherwise similar. Baseline FEV₁% in colistimethate-treated patients with azithromycin use was a median of 56%, versus 50% in those not using azithromycin (mean of 58.3% vs. 55.2%). The relative drop in FEV₁% from baseline at the start of intravenous therapy was similar (−27.4% vs. −24.3%).

Lung Function Recovery after Pulmonary Exacerbation

The median FEV₁% predicted at baseline, time of exacerbation (start of intravenous therapy), and end of intravenous therapy by group are summarized in Tables 2 and 3. Similar data are shown in Figures E2A and E2B in the online supplement. In our primary analyses using multivariable GEE models, azithromycin use in the tobramycin-treated cohort associated with a lower relative and absolute recovery of FEV₁% and lower odds ratio (OR) for recovery of 90% and 100% of baseline FEV₁ (Tables 4 and 5). Many of these effects were imprecise, and only absolute recovery of FEV₁% reached a statistical significance of $P < 0.05$. Azithromycin use in the colistimethate-treated cohort associated with a greater relative and absolute recovery of FEV₁% and greater OR for recovery of 90% and 100% of baseline FEV₁ (Tables 4 and 5). Of these analyses, again only absolute recovery of FEV₁% reached a statistical significance of $P < 0.05$. When comparing the association between azithromycin use and recovery of absolute FEV₁% predicted in those treated with tobramycin versus colistimethate, we observed a 2.64% (95% CI, −4.52% to −0.76%) lesser recovery in those treated with tobramycin (vs. no azithromycin use) and 2.00% (95% CI, 0.13–3.87%) greater recovery in those treated with colistimethate (vs. no azithromycin use). Variability in lung function recovery was observed in all cohorts, as demonstrated in Figure E1.

Sensitivity Analysis

Marginal structural models were conducted separately in both the tobramycin and

Figure 1. Study flow diagram for cohort study patient population. FEV₁ = forced expiratory volume in 1 second; IV = intravenous; PCR = polymerase chain reaction.

colistimethate cohorts. The associations with azithromycin use and recovery of FEV₁ were similar to those measured using GEE models in both tobramycin and colistimethate cohorts (Tables 4 and 5). This indicated that FEV₁ did not significantly influence the decision to use azithromycin during the timeframe of our analyses.

Discussion

Our retrospective cohort study of adults with CF treated at a single U.S. CF center between 2014 and 2017 supports the

prediction on the basis of U.S. patient registry data that approximately half of patients with *P. aeruginosa* lung infection who are treated with intravenous antibiotics for a pulmonary exacerbation are prescribed concomitant chronic azithromycin at the time of treatment. Our analyses, although inconclusive and unable to infer causality, suggest that chronic azithromycin treatment may provide modest additional benefit during intravenous antibiotics to treat a pulmonary exacerbation in those treated with a colistimethate-based drug regimen but not when intravenous tobramycin is used. Whether or not azithromycin use

directly influenced the antimicrobial effects of intravenous colistimethate or intravenous tobramycin cannot be understood from these data. An association between azithromycin use and general lesser recovery of FEV₁ in patients treated with tobramycin occurred despite those patients trending to more days of intravenous therapy versus patients treated with colistimethate.

This work was inspired by our recent *post hoc* analyses of clinical trials wherein azithromycin use associated with less improvement in FEV₁ and other outcome measures in subjects receiving inhaled tobramycin but not an alternative inhaled

Table 1. Summary of patient characteristics

Patient Characteristic	TOB Subjects (n = 121)	COL Subjects (n = 86)	All Subjects (N = 173*)
Age at first exacerbation in time period, yr	28 (23–36)	29.5 (23.25–40)	28 (23–37)
Baseline FEV ₁ % predicted	69 (50–84)	55 (45–72.75)	60 (47–80)
No. of PEx/patient in study period	1 (1–2)	1 (1–3)	2 (1–3)
Female	69 (57.0)	51 (59.3)	120 (60.0)
Race or ethnicity			
White	112 (92.6)	75 (87.2)	187 (90.3)
Hispanic	5 (4.1)	6 (7.0)	11 (5.3)
Other	4 (3.3)	5 (5.8)	9 (4.4)
Mutation/functional class			
I–III	103 (85.1)	74 (86.1)	177 (85.5)
IV–V	10 (8.3)	8 (9.3)	18 (8.7)
Missing	8 (6.6)	4 (4.7)	12 (5.8)

Definition of abbreviations: COL = colistimethate; FEV₁ = forced expiratory volume in 1 second; PEx = pulmonary exacerbation; TOB = tobramycin.

Data presented as No. (%) or median (interquartile range). Patient characteristics of those contributing to pulmonary exacerbation events treated with TOB or COL are shown, along with characteristics of all subjects.

*Thirty-four patients contributed data to both the tobramycin analyses and the colistimethate analyses.

antibiotic (aztreonam) (4, 5). We have also recently reported *in vitro* studies demonstrating that azithromycin can selectively reduce tobramycin-mediated

killing of *P. aeruginosa* (4, 6). We and others have observed potential synergy when azithromycin is added to colistimethate *in vitro* (4, 10, 23). The data reported here

extend this line of inquiry to consider how azithromycin use at the time of intravenous antibiotic therapy associates with recovery in lung function. We again find a similar pattern of association suggesting a selective adverse drug interaction between azithromycin and tobramycin but acknowledge that several of our outcomes measured in this complex analysis were imprecise and did not reach the statistical significance threshold of $P < 0.05$, including the predetermined primary outcome of relative recovery of baseline FEV₁% (coefficient and 95% CI: tobramycin -0.03 [-0.07 to 0.002]; colistimethate $+0.03$ [-0.001 to 0.07]). The association between azithromycin use and absolute recovery of FEV₁% was statistically significant for both tobramycin and colistimethate groups (-2.64% and $+2.00\%$, respectively; Table 4).

Azithromycin came into routine use more than a decade ago and is one of the most highly used chronic pulmonary medications in CF (3, 8). It is most often prescribed for individuals with CF and chronic *P. aeruginosa* infection but is also prescribed to patients with CF without

Table 2. Characteristics of pulmonary exacerbation events by treatment group

Variable	Group			
	TOB (n = 126)	TOB/AZM (n = 94)	COL (n = 92)	COL/AZM (n = 115)
Age at exacerbation, yr	27 (23–33)	27 (24–36)	28 (25–34.5)	26 (23– 33)
Length of stay in hospital, d	13 (10–14)	14 (9–15)	14 (13–18.5)	14 (13– 20)
FEV ₁ % predicted				
Baseline	67 (47–83)	60 (49 –85)	50 (44–61)	56 (46–74)
Start of IV therapy	48.5 (29–65)	43.5 (31–61)	34 (29–45.5)	41 (29–56)
Female	80 (63.5)	49 (52.1)	55 (59.8)	66 (57.4)
Weight at admission, kg	54.1 (50.0–64.0)	55.3 (49.8–63.2)	55.5 (48.4–61.2)	54.3 (49.2–57.6)
Race				
White	120 (95.2)	84 (89.4)	85 (92.4)	95 (82.6)
Hispanic	2 (1.6)	8 (8.5)	4 (4.4)	10 (8.7)
Other	4 (3.2)	2 (2.1)	3 (3.3)	10 (8.7)
Chronic <i>Pseudomonas aeruginosa</i>	112 (88.9)	88 (93.6)	86 (93.5)	104 (90.4)
MRSA infection	33 (26.2)	27 (28.7)	22 (23.9)	23 (20.0)
Receiving vancomycin	23 (18.3)	21 (22.3)	16 (17.4)	16 (13.9)
<i>Burkholderia cepacia</i> complex infection	12 (9.5)	4 (4.3)	0	2 (1.7)
<i>Stenotrophomonas</i> species infection	5 (4.0)	7 (7.4)	6 (6.5)	8 (7)
(<i>Achromobacter</i>) <i>Alcaligenes</i> species infection	7 (5.6)	10 (10.6)	22 (23.9)	14 (12.2)
NTM pulmonary disease	11 (8.7)	12 (12.8)	8 (9)	30 (26.1)
Use of CFTR modulator, %				
Lumacaftor/ivacaftor	24.6	14.9	28.3	14.8
Ivacaftor	4	9.6	2.2	1.7

Definition of abbreviations: CFTR = cystic fibrosis transmembrane conductance regulator protein; COL = colistimethate; COL/AZM = colistimethate plus azithromycin; FEV₁ = forced expiratory volume in 1 second; IV = intravenous; MRSA = methicillin-resistant *Staphylococcus aureus*; NTM = nontuberculous mycobacteria; TOB = tobramycin; TOB/AZM = tobramycin plus azithromycin.

Data given as median (interquartile range) or No. (%) unless otherwise noted. Patient and clinical characteristics are shown for pulmonary exacerbation events.

Table 3. Lung function (forced expiratory volume in 1 s percent predicted) decline and recovery by treatment group

Variable	Group			
	TOB (n = 126)	TOB/AZM (n = 94)	COL (n = 92)	COL/AZM (n = 115)
Absolute change from baseline to start of IV therapy (i.e., “drop”)	−15 (−22 to −9)	−16 (−24 to −8)	−15 (−19 to −10)	−14 (−20 to −9)
Relative (%) change from baseline to start of therapy	−24.7 (−37.1 to −14.3)	−24.9 (−37.8 to −12.7)	−27.4 (−37.9 to −19.6)	−24.3 (−37.0 to −16.0)
Ratio of end of IV therapy to baseline	0.94 (0.84 to 0.99)	0.90 (0.83 to 0.97)	0.85 (0.75 to 0.93)	0.89 (0.80 to 0.98)
Absolute change in FEV ₁ % from start to end of IV therapy	9 (3.75 to 19)	7 (4 to 14)	7 (2 to 12)	8 (2 to 13)
Recovered 90% baseline	75 (59.5)	47 (50.0)	32 (34.8)	55 (47.8)
Recovered 100% baseline	31 (24.6)	18 (19.2)	11 (12.0)	24 (20.9)

Definition of abbreviations: COL = colistimethate; COL/AZM = colistimethate plus azithromycin; FEV₁ = forced expiratory volume in 1 second; IV = intravenous; TOB = tobramycin; TOB/AZM = tobramycin plus azithromycin.

Data given as median (interquartile range) or No. (%). Lung function recovery by group is shown for pulmonary exacerbation events.

P. aeruginosa, in whom it has been shown to reduce the risk of pulmonary exacerbations (12, 24). Data from the 2016 U.S. CF Foundation Patient Registry illustrate that 65% of U.S. patients aged 6 years and older who have *P. aeruginosa* are treated with chronic azithromycin (8). Appreciating that tobramycin is included in up to 90% of intravenous antibiotic regimens used to treat *P. aeruginosa*, we believe that this potential drug interaction deserves additional focused investigation.

As azithromycin tends to be introduced with lung disease progression, we investigated the potential for confounding

by time-varying change in FEV₁ through construction of marginal structural models for both the tobramycin and colistimethate cohorts. These results were similar when compared with our GEE models, suggesting that time-variable lung function did not significantly impact azithromycin use by clinical prescription in this adult study sample. Perhaps the decision to use chronic azithromycin was determined at an earlier age in our cohorts, which were nearly all categorized as infected with chronic *P. aeruginosa* and had a median age of 28 years and median FEV₁ of 60% predicted. CF registry data from countries outside of the United States indicate that azithromycin use

differs between CF populations, which may provide alternative approaches to test this hypothesis (25–27).

One of the primary strengths of our study was access to relatively large sample sizes of tobramycin and colistimethate cohorts treated at a single CF center in recent years. This provided accurate and generally more complete data around the pulmonary exacerbation events than could be obtained from the national registry database. We also included a large number of clinically relevant potential confounding variables in our GEE models and obtained consistent results when analyzing the data using multiple statistical approaches. This indicates that the association is less likely to be caused by indication bias or by use of any one particular statistical method, although the possibility of unmeasured confounders still exists.

Our study has several limitations inherent to these types of analyses. We acknowledge modest differences in baseline characteristics and, more importantly, the lack of randomization or standardized treatment regimens (e.g., additional antibiotics, airway clearance regimens, or other aspects of pulmonary exacerbation therapy). Indeed, many of these adult patients with CF had advanced lung disease and were treated with multiple combined antibiotics, which may have been changed during a course of therapy on the basis of a lack of clinical improvement or drug toxicity. Potential effects of these multiple additional variables cannot be adequately controlled for in a study of this size. At times, treatment was also delayed or interrupted for health reasons (e.g., nephrotoxicity), and the total length of

Table 4. Relative and absolute recovery of lung function

	Adjusted GEE Model*		Marginal Structural Model†	
	Coefficient (95% CI)	P Value	Coefficient (95% CI)	P Value
Tobramycin group				
Relative FEV ₁ % at end of IV therapy (vs. 6-mo baseline)				
AZM+	−0.03 (−0.07 to 0.002)	0.07	−0.03 (−0.07 to 0.008)	0.11
Absolute change in FEV ₁ % during IV therapy				
AZM+	−2.64% (−4.52 to −0.76)	0.006	−2.54% (−4.49 to −0.59)	0.011
Colistimethate group				
Relative FEV ₁ % at end of IV therapy (vs. 6-mo baseline)				
AZM+	0.03 (−0.001 to 0.07)	0.057	0.03 (−0.008 to 0.07)	0.11
Absolute change in FEV ₁ % during IV therapy				
AZM+	2.00% (0.13 to 3.87)	0.04	1.95% (−0.16 to 4.07)	0.07

Definition of abbreviations: AZM+ = study subjects with concomitant azithromycin compared to those without azithromycin; CI = confidence interval; FEV₁ = forced expiratory volume in 1 second; GEE = generalized estimating equation; IV = intravenous; MRSA = methicillin-resistant *Staphylococcus aureus*.

*Models adjusted for confounders of age, sex, baseline FEV₁%, drop in FEV₁%, MRSA infection, race, mutation functional class, and cystic fibrosis–related diabetes.

†Adjusted for age, sex, MRSA infection, and drop in FEV₁% after weighting based on baseline FEV₁%.

Table 5. Odds of recovery to 90% or 100% of baseline forced expiratory volume in 1 second

	Adjusted GEE Model* OR (95% CI)	P Value	Marginal Structural Model† OR (95% CI)	P Value
Tobramycin group				
Return to 90% of baseline				
AZM+	0.65 (0.34–1.24)	0.19	0.67 (0.34–1.29)	0.23
Return to 100% of baseline				
AZM+	0.69 (0.33–1.43)	0.32	0.67 (0.34–1.35)	0.26
Colistimethate group				
Return to 90% of baseline				
AZM+	1.74 (0.81–3.73)	0.15	1.61 (0.78–3.38)	0.20
Return to 100% of baseline				
AZM+	2.19 (0.95–5.05)	0.06	1.89 (0.73–4.88)	0.19

Definition of abbreviations: AZM+ = study subjects with concomitant azithromycin compared to those without azithromycin; CI = confidence interval; FEV₁ = forced expiratory volume in 1 second; GEE = generalized estimating equation; MRSA = methicillin-resistant *Staphylococcus aureus*; OR = odds ratio.

*Models adjusted for confounders of age, sex, baseline FEV₁%, drop in FEV₁%, methicillin-resistant *Staphylococcus aureus* infection, race, mutation functional class, and cystic fibrosis-related diabetes.

†Adjusted for age, sex, MRSA infection, and drop in FEV₁% after weighting based on baseline FEV₁%.

intravenous therapy commonly exceeded the treatment duration with tobramycin or colistimethate by several days. Some patients received inhaled antibiotics as well, which was not collected or considered in our analyses. Some of these concerns are mitigated by the fact that all patients were under the care of a small group of CF providers who have similar practice patterns and use agreed-on admission order sets. More so, there is no reason to suspect that selection of intravenous antibiotics or airway clearance routines were affected by concomitant azithromycin use, but we accept that the variability of treatment choices, practice patterns, or disease comorbidities may represent unmeasured confounding or contribute to the lack of precision in some of our analyses. Investigators are now working to understand

and standardize key variables in treatment of pulmonary exacerbation events (i.e., length of therapy), which will improve future prospective trials and retrospective analyses similar to ours (28, 29). We recognize that results from a single-center study may not generalize to other centers, and we encourage those engaged in multicenter prospective study of pulmonary exacerbations to consider our theory in their research.

Clearly, those treated with colistimethate in our dataset had more advanced pulmonary disease, and this work was not intended to compare tobramycin-versus colistimethate-based antibiotic regimens. Rather, including these two groups allowed us to contrast the association of azithromycin use on response to therapy. The beneficial association observed in colistimethate-treated patients further

challenges the idea that our observation is due to indication bias for azithromycin clinical use in those with more advanced disease. Despite this, the mean recovery of FEV₁% predicted at the end of treatment for the entire study sample was less than 90% of baseline FEV₁%, and just under half of the patients reached 90% of their baseline FEV₁% predicted by the end of intravenous therapy. From the available data, we cannot report additional recovery that may be seen after the end of pulmonary exacerbation treatment, but this finding is consistent with several other reports indicating that many people with CF do not fully regain baseline FEV₁ after pulmonary exacerbation events (30–33).

In conclusion, we have carried forward a line of inquiry regarding a suspected adverse drug interaction and here sought to determine if chronic azithromycin therapy, which is so widely used in the U.S. adult CF population, associates with a lesser benefit from intravenous tobramycin in the context of exacerbation treatment. Our findings, although inconclusive and unable to establish a causal relationship, suggest that concomitant azithromycin may be unhelpful when combined with intravenous tobramycin—a pattern of interaction that we have observed *in vitro*, in animal studies, and in the context of inhaled antipseudomonal therapies. Altogether, these data underscore a need to better understand the complexity of pulmonary exacerbation events and how CF medications work in combination. In this context, it appears important to determine whether or not long-term use of macrolides is beneficial and if and how azithromycin may alter the effects of other antibiotics used to treat *P. aeruginosa*. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

References

- 1 Cystic Fibrosis Foundation Patient Registry. 2017 Annual Data Report. Bethesda, MD: Cystic Fibrosis Foundation; 2018 [accessed 2018 Dec 1]. Available from: <https://www.cff.org/Research/Researcher-Resources/Patient-Registry/2017-Patient-Registry-Annual-Data-Report.pdf>.
- 2 Sanders DB, Solomon GM, Beckett VV, West NE, Daines CL, Heltshe SL, *et al*. Standardized Treatment of Pulmonary Exacerbations (STOP) study: observations at the initiation of intravenous antibiotics for cystic fibrosis pulmonary exacerbations. *J Cyst Fibros* 2017;16:592–599.
- 3 Cystic Fibrosis Foundation Patient Registry. 2015 Annual data report. Bethesda, MD: Cystic Fibrosis Foundation; 2016 [accessed 2018 Dec 1]. Available from: <https://www.cff.org/Our-Research/CF-Patient-Registry/2015-Patient-Registry-Annual-Data-Report.pdf>.
- 4 Nichols DP, Happoldt CL, Bratcher PE, Caceres SM, Chmiel JF, Malcolm KC, *et al*. Impact of azithromycin on the clinical and antimicrobial effectiveness of tobramycin in the treatment of cystic fibrosis. *J Cyst Fibros* 2017;16:358–366.
- 5 Nick JA, Moskowitz SM, Chmiel JF, Forssen AV, Kim SH, Saavedra MT, *et al*. Azithromycin may antagonize inhaled tobramycin when targeting *Pseudomonas aeruginosa* in cystic fibrosis. *Ann Am Thorac Soc* 2014;11:342–350.

- 6 Nichols DP, Caceres S, Caverly L, Fratelli C, Kim SH, Malcolm K, *et al*. Effects of azithromycin in *Pseudomonas aeruginosa* burn wound infection. *J Surg Res* 2013;183:767–776.
- 7 Wagener JS, Rasouliyan L, Vandevanter DR, Pasta DJ, Regelman WE, Morgan WJ, *et al*. Oral, inhaled, and intravenous antibiotic choice for treating pulmonary exacerbations in cystic fibrosis. *Pediatr Pulmonol* 2013;48:666–673.
- 8 Cystic Fibrosis Foundation Patient Registry. 2016 Annual data report. Bethesda, MD: Cystic Fibrosis Foundation; 2017 [accessed 2018 Dec 1]. Available from: <https://www.cff.org/research/researcher-resources/patient-registry/2016-patient-registry-annual-data-report.pdf>.
- 9 Lin L, Nonejuie P, Munguia J, Hollands A, Olson J, Dam Q, *et al*. Azithromycin synergizes with cationic antimicrobial peptides to exert bactericidal and therapeutic activity against highly multidrug-resistant gram-negative bacterial pathogens. *EBioMedicine* 2015;2:690–698.
- 10 Buyck JM, Plésiat P, Traore H, Vanderbist F, Tulkens PM, Van Bambeke F. Increased susceptibility of *Pseudomonas aeruginosa* to macrolides and ketolides in eukaryotic cell culture media and biological fluids due to decreased expression of *oprM* and increased outer-membrane permeability. *Clin Infect Dis* 2012;55:534–542.
- 11 Morita Y, Sobel ML, Poole K. Antibiotic inducibility of the MexXY multidrug efflux system of *Pseudomonas aeruginosa*: involvement of the antibiotic-inducible PA5471 gene product. *J Bacteriol* 2006;188:1847–1855.
- 12 Mogayzel PJ Jr, Naureckas ET, Robinson KA, Mueller G, Hadjiladis D, Hoag JB, *et al*. Pulmonary Clinical Practice Guidelines Committee. Cystic fibrosis pulmonary guidelines: chronic medications for maintenance of lung health. *Am J Respir Crit Care Med* 2013;187:680–689.
- 13 Sampson MR, Dumitrescu TP, Brouwer KL, Schmith VD. Population pharmacokinetics of azithromycin in whole blood, peripheral blood mononuclear cells, and polymorphonuclear cells in healthy adults. *CPT Pharmacometrics Syst Pharmacol* 2014;3:e103.
- 14 Beringer P, Huynh KM, Kriengkauykit J, Bi L, Hoem N, Louie S, *et al*. Absolute bioavailability and intracellular pharmacokinetics of azithromycin in patients with cystic fibrosis. *Antimicrob Agents Chemother* 2005;49:5013–5017.
- 15 Aubert JD, Juillerat-Jeanneret L, Fioroni P, Dayer P, Plan PA, Leuenberger P. Function of human alveolar macrophages after a 3-day course of azithromycin in healthy volunteers. *Pulm Pharmacol Ther* 1998;11:263–269.
- 16 Olsen KM, San Pedro G, Gann LP, Gubbins PO, Halinski DM, Campbell GD Jr. Intrapulmonary pharmacokinetics of azithromycin in healthy volunteers given five oral doses. *Antimicrob Agents Chemother* 1996;40:2582–2585.
- 17 Nichols DP, Somayaji R, Goss CH, Heltshe S, Nick S, Saavedra MT, *et al*. Chronic azithromycin associates with less improvement after IV tobramycin but not IV colistin during treatment for pulmonary exacerbation [abstract]. *Pediatr Pulmonol* 2017;52:A390.
- 18 Farrell PM, Rosenstein BJ, White TB, Accurso FJ, Castellani C, Cutting GR, *et al*. Cystic Fibrosis Foundation. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. *J Pediatr* 2008;153:S4–S14.
- 19 Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, *et al*. ERS Global Lung Function Initiative. Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012;40:1324–1343.
- 20 Lederer DJ, Bell SC, Branson RD, Chalmers JD, Marshall R, Maslove DM, *et al*. Control of confounding and reporting of results in causal inference studies: guidance for authors from editors of respiratory, sleep, and critical care journals. *Ann Am Thorac Soc* 2019;16:22–28.
- 21 Hernan MA. A definition of causal effect for epidemiological research. *J Epidemiol Community Health* 2004;58:265–271.
- 22 Robins JM, Hernán MA, Brumback B. Marginal structural models and causal inference in epidemiology. *Epidemiology* 2000;11:550–560.
- 23 Lin L, Nonejuie P, Munguia J, Hollands A, Olson J, Dam Q, *et al*. Azithromycin synergizes with cationic antimicrobial peptides to exert bactericidal and therapeutic activity against highly multi-drug resistant gram-negative bacterial pathogens. *EBioMedicine* 2015;2:690–698.
- 24 Mayer-Hamblett N, Retsch-Bogart G, Kloster M, Accurso F, Rosenfeld M, Albers G, *et al*. OPTIMIZE Study Group. Azithromycin for early *Pseudomonas* infection in cystic fibrosis: the OPTIMIZE randomized trial. *Am J Respir Crit Care Med* 2018;198:1177–1187.
- 25 Chen K, Xu W, Wilson M, He B, Miller NW, Bengten E, *et al*. Immunoglobulin D enhances immune surveillance by activating antimicrobial, proinflammatory and B cell-stimulating programs in basophils. *Nat Immunol* 2009;10:889–898.
- 26 Chugani SA, Whiteley M, Lee KM, D'Argenio D, Manoel C, Greenberg EP. QscR, a modulator of quorum-sensing signal synthesis and virulence in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* 2001;98:2752–2757.
- 27 UK Cystic Fibrosis Registry Annual Data Report 2013. 2014 July [accessed 2017 Aug 10]. Available from: <http://www.muco-ucl.be/documents/pdf/E173.pdf>.
- 28 Heltshe SL, West NE, VanDevanter DR, Sanders DB, Beckett VV, Flume PA, *et al*. Study design considerations for the Standardized Treatment of Pulmonary Exacerbations 2 (STOP2): a trial to compare intravenous antibiotic treatment durations in CF. *Contemp Clin Trials* 2018;64:35–40.
- 29 West NE, Beckett VV, Jain R, Sanders DB, Nick JA, Heltshe SL, *et al*. STOP investigators. Standardized Treatment of Pulmonary Exacerbations (STOP) study: physician treatment practices and outcomes for individuals with cystic fibrosis with pulmonary Exacerbations. *J Cyst Fibros* 2017;16:600–606.
- 30 Heltshe SL, Goss CH, Thompson V, Sagel SD, Sanders DB, Marshall BC, *et al*. Short-term and long-term response to pulmonary exacerbation treatment in cystic fibrosis. *Thorax* 2016;71:223–229.
- 31 VanDevanter DR, Morris NJ, Konstan MW. IV-treated pulmonary exacerbations in the prior year: an important independent risk factor for future pulmonary exacerbation in cystic fibrosis. *J Cyst Fibros* 2016;15:372–379.
- 32 de Boer K, Vandemheen KL, Tullis E, Doucette S, Fergusson D, Freitag A, *et al*. Exacerbation frequency and clinical outcomes in adult patients with cystic fibrosis. *Thorax* 2011;66:680–685.
- 33 Sanders DB, Bittner RC, Rosenfeld M, Redding GJ, Goss CH. Pulmonary exacerbations are associated with subsequent FEV1 decline in both adults and children with cystic fibrosis. *Pediatr Pulmonol* 2011;46:393–400.