Inflammatory Responses, Spirometry, and Quality of Life in Subjects With Bronchiectasis Exacerbations

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BACKGROUND: Bronchiectasis exacerbations are critical events characterized by worsened symptoms and signs (ie, cough frequency, sputum volume, malaise). Objectives: Our goal was to examine variations in airway and systemic inflammation, spirometry, and quality of life during steady state, bronchiectasis exacerbations, and convalescence (1 week following a 2-week antibiotic treatment) to determine whether potentially pathogenic microorganisms, including *Pseudomonas aeruginosa*, were associated with poorer conditions during bronchiectasis exacerbations. METHODS: Peripheral blood and sputum were sampled to detect inflammatory mediators and bacterial densities. Spirometry and quality of life (St George Respiratory Questionnaire [SGRQ]) were assessed during the 3 stages. RESULTS: Forty-eight subjects with bronchiectasis (43.2 ± 14.2 y of age) were analyzed. No notable differences in species and density of potentially pathogenic microorganisms were found during bronchiectasis exacerbations. Except for CXCL8 and tumor necrosis factor alpha (TNF-α), serum inflammation was heightened during bronchiectasis exacerbations and recovered during convalescence. Even though sputum TNF-α was markedly higher during bronchiectasis exacerbations and remained heightened during convalescence, the variations in miscellaneous sputum markers were unremarkable. Bronchiectasis exacerbations were associated with notably higher SGRQ symptom and total scores, which recovered during convalescence. FVC, FEV_1, and maximum mid-expiratory flow worsened during bronchiectasis exacerbations (median change from baseline of −2.2%, −0.8%, and −1.3%) and recovered during convalescence (median change from baseline of 0.6%, 0.7%, and −0.7%). Compared with no bacterial isolation, potentially pathogenic microorganism or *P. aeruginosa* isolation at baseline did not result in poorer clinical condition during bronchiectasis exacerbations. CONCLUSIONS: Bronchiectasis exacerbations are characterized by heightened inflammatory responses and poorer quality of life and spirometry, but not by increased bacterial density, which applies for subjects with and without potentially pathogenic microorganism isolation when clinically stable. (ClinicalTrials.gov registration NCT01761214.) *Key words: bronchiectasis; exacerbation; potentially pathogenic microorganism; inflammation; spirometry; quality of life.*
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Introduction

Bronchiectasis is a chronic respiratory disease characterized by repetitive exacerbations[1,2] associated with significantly worsened clinical symptoms[3] that impact daily life. They are common according to previous stud-

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ies,[4] and variation in bacterial species and/or density may play a role, as bacterial infection triggers airway inflammation[5-7] and induces epithelial biofilm formation,[8] leading to inflammatory mediator release[1] and oxidative stress.[9,10] Subjects with stable bronchiectasis who had higher bacterial density reportedly yielded higher serum intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin.

Short- and long-term antibiotics effectively diminish airway inflammation and have been effective in reducing bacterial load. Murray et al[11] reported high bacterial clearance rates and improved quality of life following intravenous antibiotic therapy. Courtney et al[12] documented substantial reduction in C-reactive protein, sputum inflammatory cell count, sputum inflammatory mediators (eg, tumor necrosis factor-α [TNF-α] and interleukin-8 [CXCL8]), and quality of life after antibiotic treatment. However, previous findings suffered from limited sample sizes ($N < 20$) and failure to monitor changes from steady state to exacerbations. This warranted elucidation of the changes in clinical parameters at different stages.

We hypothesized that bronchiectasis exacerbations in clinically stable subjects with potentially pathogenic microorganisms compared with those without would be associated with higher bacterial density and inflammatory biomarker levels, poorer lung function, and impaired quality of life. Because serum C-reactive protein has been shown to sensitively reflect the efficacy of antibiotic therapy, sample size was calculated based on C-reactive protein, the primary end point in our study. Our objectives were 2-fold: (1) to compare airway bacterial density, systemic and airway inflammation, spirometry, and quality of life when clinically stable and during bronchiectasis exacerbation and convalescence and (2) to compare the variations in these parameters between clinically stable subjects with and without potentially pathogenic microorganisms (in particular, *P. aeruginosa*).

QUICK LOOK

Current knowledge

Bronchiectasis is a chronic respiratory disease characterized by repetitive exacerbations and worsening quality of life. Bacterial infection is associated with airway inflammation, biofilm formation, and worsening clinical symptoms. Antibiotic treatment is associated with a reduction in inflammation and improved respiratory function.

What this paper contributes to our knowledge

Bronchiectasis exacerbations were characterized by markedly heightened inflammatory responses and poorer quality of life and spirometry, but not greater bacterial density. There was no relationship between changes in biomarkers and quality of life from baseline to exacerbations or convalescence regardless of bacterial infection status.

Methods

Subjects

Between September 2012 and October 2013, adults with clinically stable bronchiectasis (see bronchiectasis etiology in Table 1) were recruited from the First Affiliated Hospital of Guangzhou Medical University in Guangdong, China. Diagnosis of bronchiectasis was based on chest high-resolution computed tomography at 2-mm collimation within 12 months, compatible with typical symptoms.[13] Subjects with severe systemic diseases (ie, malignancy), antibiotic use within 4 weeks, or limited understanding were excluded. Approval was obtained from the ethics committee of the First Affiliated Hospital of Guangzhou Medical University, and all subjects provided written informed consent.

Study Design

This study consisted of 3 stages. At stage 1, subjects with clinically stable bronchiectasis (respiratory symp-

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Table 1. Baseline Levels

Parameter	All Subjects ($N = 49$)	Baseline Culture		P*
		Positive ($n = 28$)	Negative ($n = 21$)	
Anthropometry				
Age, y	43.2 ± 14.2	44.2 ± 15.6	41.9 ± 12.4	.71
Height, cm	161.1 ± 7.2	160.4 ± 6.9	162.1 ± 7.6	.70
Weight, kg	51.6 ± 8.6	49.7 ± 8.2	54.3 ± 8.7	.81
BMI, kg/m^2	19.8 ± 3.1	19.3 ± 2.6	20.7 ± 3.5	.70
Males	19 (38.8)	9 (32.1)	10 (47.4)	.27
Never-smoker	41 (83.7)	25 (89.3)	16 (76.2)	.22
Spirometry				
FVC, % predicted	79.5 ± 25.2	72.5 ± 25.6	88.7 ± 21.9	**.02**
FEV_1, % predicted	66.0 ± 25.0	58.9 ± 24.2	75.4 ± 23.3	**.02**
FEV_1/FVC	0.69 ± 0.13	0.68 ± 0.13	0.71 ± 0.13	.40
Maximum mid-expiratory flow, % predicted	46.6 ± 29.6	31.8 (27.9)	54.2 ± 30.9	.09
Disease-related clinical parameters				
No. of exacerbations within 2 y,	3.0 (3.0)	3.6 ± 2.4	3.0 (4.0)	.96
Chest HRCT score	8.3 ± 4.1	9.6 ± 4.3	6.6 ± 3.1	**<.01**
Leukocytes, $\times 10^9$/L	8.0 ± 2.4	8.3 ± 2.0	7.6 ± 2.8	.31
Neutrophils, %	61.7 ± 10.3	61.4 ± 10.7	62.1 ± 10.0	.81
C-reactive protein, mg/dL	0.3 (0.5)	0.3 (0.8)	0.3 (0.4)	.96
Medications used within 6 mo†				
Mucolytics	38 (77.6)	24 (88.9)	14 (66.7)	.11
Theophylline	33 (67.4)	18 (64.3)	15 (71.4)	.60
Macrolides	21 (42.9)	11 (39.3)	10 (47.6)	.56
Inhaled corticosteroids	13 (26.5)	7 (25.0)	6 (28.6)	.78
Comorbid conditions‡				
Post-infection	12 (24.5)	5 (17.9)	7 (33.3)	.21
Immunodeficiency	7 (14.3)	5 (17.9)	2 (9.5)	.68
Asthma	4 (8.2)	2 (7.2)	2 (9.5)	.82
Gastroesophageal reflux	3 (6.1)	3 (10.7)	0 (0.0)	.25
Miscellaneous	9 (18.4)	6 (21.4)	3 (14.3)	.79
Idiopathic	19 (38.8)	10 (35.7)	9 (42.9)	.61

Continuous data are expressed as mean ± SD for normal distribution or median (interquartile range). Categorical data are expressed as number (percent).
* Comparisons between positive- and negative-culture groups. Data in boldface indicate comparisons with statistical significance.
† No subjects had ever received domiciliary intravenous antibiotics or inhaled antibiotics.
‡ The detailed underlying conditions of the whole cohort included: post-infection ($n = 12$), immunodeficiency ($n = 7$), asthma ($n = 4$), gastroesophageal reflux disease ($n = 3$), lung maldevelopment ($n = 2$), rheumatoid arthritis ($n = 1$), yellow nail syndrome ($n = 1$), COPD ($n = 1$), Young's syndrome ($n = 1$), eosinophilic bronchiolitis ($n = 1$), and idiopathy ($n = 19$). No subjects were found to have primary ciliary dyskinesia. The sum of percentages was > 1 because a minority of subjects had dual etiologies. The underlying conditions of bronchiectasis were determined after meticulous testing recommended by British Thoracic Society guidelines and group discussion (WG, YG, and GX). Further details will be published elsewhere.
BMI = body mass index
HRCT = high-resolution computed tomography

toms and signs within normal daily variation for at least 4 weeks) underwent baseline assessment consisting of sputum culture, serum and sputum inflammatory marker measurement, and spirometry. Subjects were instructed to inform investigators by telephone if symptoms worsened. Following confirmation of bronchiectasis exacerbations, subjects had exacerbation visits at stage 2, within 5 d of symptom onset. They were treated with 14 d of antibiotics based on British Thoracic Society guidelines.[14] At 1 week after completion,[15] subjects had a convalescence visit (stage 3). The test items were identical throughout the 3 stages, including sputum culture, serum and sputum inflammatory marker measurement, quality-of-life assessment, and spirometry.

Bronchiectasis Exacerbations

Bronchiectasis exacerbations were defined as 3 or more of the following criteria that lasted for at least 24 h: significantly increased sputum purulence and/or volume; worsened tachypnea or dyspnea; increased cough frequency; temperature of > 37.5°C; fatigue, malaise, or exercise intolerance; new onset of wheezing; increased pulmonary

crackles; and radiologic findings (ie, increased pulmonary infiltration).[1,2,16-18]

Although other large-scale clinical trials[19-21] employed slightly different criteria for defining bronchiectasis exacerbations, it should be recognized that we still lack an accepted standard. Different definitions of bronchiectasis exacerbations might be associated with selection bias; however, most criteria relied on assessment of the cardinal items, including marked changes in cough frequency, sputum purulence, or color and other clinically important symptoms and signs. Therefore, different definitions of bronchiectasis exacerbations might have a limited influence on our data analyses.

Antibiotic prescriptions are shown in Table E1 in the supplementary materials at http://www.rcjournal.com. The doses of antibiotics were based mainly on British Thoracic Society guidelines.[14] For *P. aeruginosa* infection, levofloxacin at 500 mg was prescribed once daily for 14 d. Subjects with any known bacterial resistance to oral antibiotics or with exacerbations (necessitating hospitalization) were treated with intravenous antibiotics.

Sputum Sampling and Bacterial Culture

Sputum was sampled during hospital visits between 9:00 and 12:00 AM. Following removal of oral cavity contents and chest physical therapy for 15 min, subjects expectorated into a 60-mL sterile clear plastic container for bacterial culture and preparation of sol phase. Hypertonic saline (3–5%) induction was applied, as appropriate.[22] Samples with $\geq$25 leukocytes and $\leq$10 epithelial cells under microscopic field ($\times$100) were deemed eligible.

Within 2 h of sampling, sputum was split for bacterial culture and ultracentrifugation (50,000 $\times$ *g*) at 4°C for 90 min to prepare for sputum sol stored in −80°C freezers until measurements. Bacterial culture and inflammatory marker measurements were done on the same sputum sample. Sputum neutrophil count was not assessed per our protocol. See the supplementary materials at http://www.rcjournal.com for further details regarding sputum culture and the definition of potentially pathogenic microorganisms.

Inflammatory Biomarker Assessment

Serum CXCL8 and TNF-α and sputum sol interleukin (IL)-1β, CXCL8 and TNF-α were measured using Luminex bead-based chips (Bio-Rad, Hercules, California) following the manufacturer's instructions. Details are provided in the supplementary materials at http://www.rcjournal.com.

Spirometry

The Quark PFT spirometer (COSMED, Milan, Italy) was used. Between-maneuver variation was $<$ 5% or 200 mL in FVC and FEV_1, with maximum values reported. Maximum mid-expiratory flow was chosen from the best maneuver. Predicted values were selected using the reference model of Zheng and Zhong.[23]

Quality-of-Life Assessment

Quality of life was assessed by using the St George Respiratory Questionnaire (SGRQ),[24] which comprises 50 items categorized in 3 domains: symptoms, activity, and impacts. For domain and total scores, the lowest and highest values were 0 and 100, respectively, with higher scores indicating poorer quality of life. The Leicester Cough Questionnaire was used during bronchiectasis exacerbations only and therefore not included in analyses.

Statistical Analysis

C-reactive protein has been reported to be a useful parameter in reflecting the efficacy of antibiotic therapy following bronchiectasis exacerbations; therefore, we calculated the sample size according to the pre- and post-treatment C-reactive protein based on the study of Murray et al.[11] It has been shown that antibiotics lead to a significant reduction in C-reactive protein levels (6.7 $\pm$ 7.1 vs 0.7 $\pm$ 1.1 mg/dL). By assuming the levels of α and β to be 0.05 and 0.10 (2-sided tests), respectively, we estimated that 15 subjects ($N = \sigma^2 \times f[\alpha,\beta]/[\mu_1 - \mu_2]^2 = 7.1^2 \times 10.5/[6.7 - 0.7]^2$) were required to be randomized in each arm. Therefore, a total of 36 subjects would be included in the analysis when factoring a dropout rate of 20%.

Statistical analysis was performed using SPSS 16.0 (SPSS, Chicago, Illinois.). Dot plots were depicted using Prism 5.0 (GraphPad Software, La Jolla, California). Numerical data are expressed as mean $\pm$ SD or median (interquartile range) as indicated. Categorical data are presented as *n* (%) and were compared using chi-square tests. Two-sided pairwise *t* tests or non-parametric tests was adopted for between-group comparisons as appropriate. One-way analysis of variance or the Kruskal-Wallis test was applied for among-group comparisons as indicated. $P < .05$ was deemed statistically significant for all comparisons.

Results

Subject Recruitment

Subject recruitment is explained in Figure 1. The main reasons for dropouts were: (1) subjects did not report bronchiectasis exacerbations to investigators ($n = 59$), and (2) subjects received antibiotics for 2 d or longer ($n = 27$).

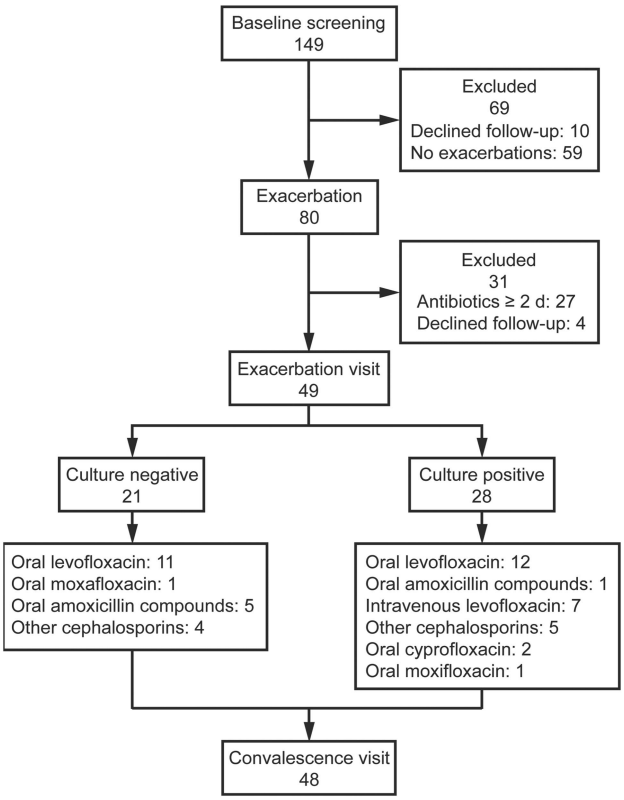

Fig. 1. Flow chart.

Baseline Levels

There was no significant difference in anthropometry between culture-positive and culture-negative subjects. The positive-culture group was associated with lower FVC and FEV_1 and higher high-resolution computed tomography scores. No remarkable between-group differences in leukocyte and neutrophil counts and C-reactive protein were noted. The most common medications used within 6 months were mucolytics (77.6%), followed by theophylline (67.4%). No subjects received domiciliary intravenous or inhaled antibiotics. Idiopathy, post-infection, and immunodeficiency were common underlying conditions (see Table 1).

Use of Antibiotics

The use of antibiotics is listed in Table E2 in the supplementary materials at http://www.rcjournal.com. Oral fluoroquinolones (ie, levofloxacin) constituted the most common antibiotics (49.0%), followed by intravenous fluoroquinolones (22.5%), oral β-lactamase inhibitors (20.4%), and intravenous β-lactamase inhibitors (6.1%). When stratified by sputum culture findings in clinically stable bronchiectasis, both groups demonstrated similar use of oral fluoroquinolones and intravenous β-lactamase inhibitors. The positive-culture group was associated with higher utilization of intravenous fluoroquinolones (32.1% vs 9.5%) and lower utilization of oral β-lactamase inhibitors (14.3% vs 28.6%).

Sputum Bacteriology

Subjects had similar isolation of individual bacterial species at different stages. *P. aeruginosa* was the most common potentially pathogenic microorganism (~30.0%). *Haemophilus influenzae* and *Haemophilus parainfluenzae* yielded similar positivity from sputum cultures during steady state and convalescence. Miscellaneous potentially pathogenic microorganisms comprised *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter* subspecies, and *Pseudomonas* subspecies. Commensals were isolated in 42.9% of cases when clinically stable. The isolation rate of potentially pathogenic microorganisms tended to be higher during bronchiectasis exacerbations. However, this trend seemed unremarkable for *P. aeruginosa*. For further details, see Table E3 in the supplementary materials at http://www.rcjournal.com.

Bacterial Density

No marked variations in bacterial density were noted at different stages, which applied to individual bacterial species, despite the trend toward a reduction during convalescence (Fig. 2 and Table E4 in the supplementary materials at http://www.rcjournal.com).

Systemic and Airway Inflammation

Apart from serum CXCL8 and TNF-α, there was an increase in leukocyte count and serum biomarkers during bronchiectasis exacerbations, followed by regression toward baseline levels during convalescence. Sputum sol TNF-α significantly increased during bronchiectasis exacerbations and remained high during convalescence (all $P < .05$). However, this trend was unremarkable for miscellaneous sputum biomarkers (Table 2).

Spirometry

During bronchiectasis exacerbations, there were significant reductions in FVC, FEV_1, and maximum mid-expiratory flow ($P = .01$, $<.01$, and .04, respectively, for comparisons between bronchiectasis exacerbations and stable state), but not FEV_1/FVC. Despite the trend toward decline during bronchiectasis exacerbations and improvement during convalescence, median changes in spirometric parameters were within 5% of baseline levels (Table 3).

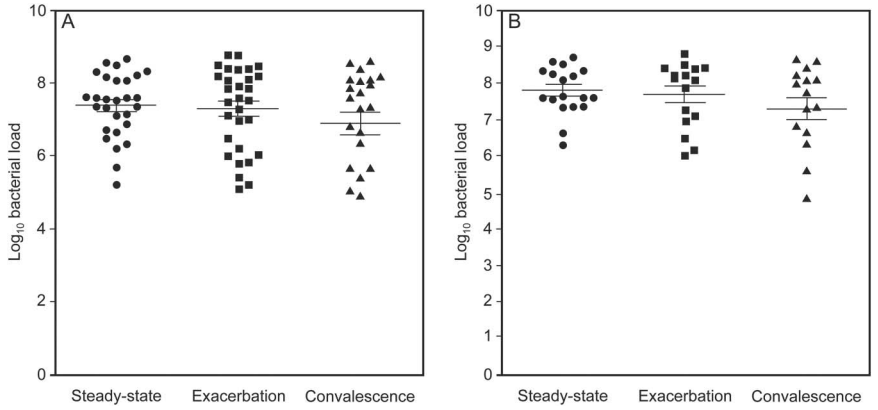

Fig. 2. A: Comparison of the bacterial density of all potentially pathogenic microorganisms at different clinical stages. B: Comparison of the bacterial density of *P. aeruginosa* at different clinical stages.

Table 2. Comparison of Inflammatory Biomarkers at Different Clinical Stages

Parameter	Steady State	Exacerbation	Convalescence	*P**	*P*†	*P*‡
Serum						
IL-6, pg/mL	0.3 (3.7)	3.5 (11.2)	0.1 (0.6)	**.04**	**<.01**	**<.01**
CXCL8, pg/mL	6.9 (5.1)	8.2 ± 3.9	7.1 (4.8)	.59	.59	.53
TNF-α, pg/mL	0.8 (2.0)	0.3 (9.7)	0.8 (7.6)	.58	.45	.52
WBCs, $\times 10^9$/L	7.9 ± 2.3	9.5 ± 2.8	7.7 ± 2.5	**<.01**	**<.01**	**<.01**
Neutrophils, %	61.5 ± 10.5	69.0 ± 9.7	61.8 (8.7)	**<.01**	**<.01**	**<.01**
C-reactive protein, mg/dL	0.3 (0.5)	2.3 (4.0)	0.2 (0.5)	**<.01**	**<.01**	**<.01**
Sputum sol phase						
IL-1β, ng/mL	23.7 (46.0)	40.2 (77.2)	19.0 (37.4)	**.04**	**.02**	.13
IL-6, ng/mL	6.1 (12.6)	5.7 (13.5)	8.1 (11.8)	.21	.26	**.02**
CXCL8, ng/mL	106.0 (19.8)	117.0 ± 35.2	134.0 (58.9)	.94	**<.01**	**<.01**
TNF-α, ng/mL	11.4 (19.6)	26.6 (22.4)	10.6 (23.2)	**<.01**	**<.01**	**<.01**

Continuous data are expressed as mean ± SD for normal distribution or median (interquartile range). Categorical data are expressed as number (percent). For the readings of serum tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, and CXCL8 below the lower detection limit, we arbitrarily assigned the values to be one tenth of the detection limit: 0.30 pg/mL for TNF-α, 0.11 pg/mL for IL-6, and 0.05 pg/mL for CXCL8. Data in boldface indicate the comparisons with statistical significance.

* Comparisons between exacerbation and steady state.

† Comparisons between exacerbations and convalescence.

‡ Among-group comparisons.

WBCs = white blood cells

Quality of Life

Apart from the activity domain ($P = .15$), bronchiectasis exacerbations elicited increased SGRQ symptom domain and total scores, followed by significant reductions during convalescence, even when compared with baseline levels. Changes in SGRQ total scores were greater than minimal clinically important differences (4.0 points) (Table 4).

Clinical Parameters Stratified by Baseline Sputum Bacteriology

There was a reduction in FEV_1/FVC during bronchiectasis exacerbations and an increase in serum CXCL8 during convalescence in subjects isolated with commensals compared with subjects isolated with potentially pathogenic microorganisms. Overall, no notable differences in changes in serum/sputum inflammatory biomarkers, spirometry, or quality-of-life measures were observed when comparing subjects isolated with potentially pathogenic microorganisms and those with commensals throughout the 3 stages (Fig. 3 and Figure E1 and Table E5 in the supplementary materials at http://www.rcjournal.com).

Clinical Parameters Stratified by *P. aeruginosa* Isolation

Similar results were shown when stratified by isolation of *P. aeruginosa* at baseline (Fig. 3 and Table E6 in the supplementary materials at http://www.rcjournal.com). Al-

Table 3. Comparison of Spirometry at Different Clinical Stages

Parameter	Steady State	Exacerbation			Convalescence		
		Measured Value	P*	Change from Baseline (%)	Measured Value	P	Change from Baseline (%)
FVC, % predicted	79.2 ± 25.4	75.9 ± 25.0	**.01**	−2.2 (10.4)	79.2 ± 23.8	.98	0.6 (10.3)
FEV_1, % predicted	65.6 ± 25.1	62.4 ± 25.1	**<.01**	−0.8 (10.8)	66.3 ± 23.5	.67	0.7 (8.1)
FEV_1/FVC	0.69 ± 0.13	0.68 ± 0.14	.53	−0.1 (9.5)	0.70 ± 0.12	.35	−0.8(5.8)
MMEF, % predicted	46.4 ± 29.9	39.7 (37.8)	**.04**	−1.3 (10.9)	45.5 ± 26.0	.67	−0.7 (12.2)

Continuous data are expressed as mean ± SD for normal distribution or median (interquartile range). Categorical data are expressed as number (percent).
* Comparisons between steady state and convalescence. Data in boldface indicate comparisons with statistical significance.
MMEF = maximum mid-expiratory flow

Table 4. Comparison of Quality of Life at Different Clinical Stages

Domain	Steady State	Exacerbation	Convalescence	P*	P†	P‡
SGRQ						
Symptom	38.4 (31.7)	52.3 ± 24.7	23.8 (30.6)	**<.01**	**<.01**	**<.01**
Activity	33.6 ± 22.5	38.2 ± 23.2	35.3 (48.0)	.13	**<.01**	.15
Impact	36.6 ± 25.0	42.2 ± 21.0	26.6 ± 22.3	.10	**<.01**	**<.01**
Total	36.6 ± 21.0	42.7 ± 20.4	28.6 ± 20.6	**.02**	**<.01**	**<.01**

Continuous data are expressed as mean ± SD for normal distribution or median (interquartile range). Data in boldface indicate comparisons with statistical significance.
* Comparisons between exacerbation and steady state.
† Comparisons between exacerbations and convalescence.
‡ Among-group comparisons.
SGRQ = St George Respiratory Questionnaire

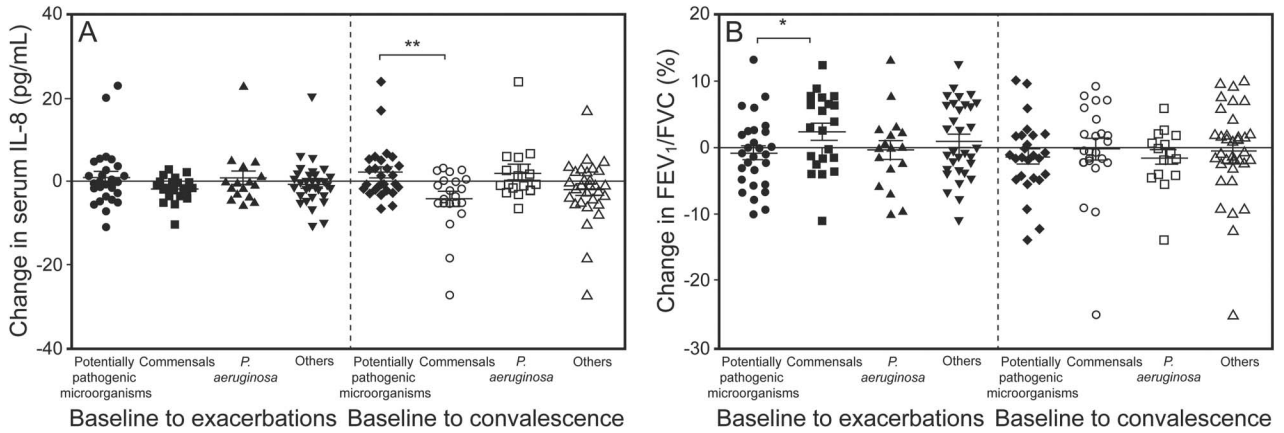

Fig. 3. Changes in FEV_1/FVC from baseline to exacerbation and from baseline to convalescence. No missing value was recorded in all subgroups from baseline to exacerbation visits. The number of missing values was 1 in the potentially pathogenic microorganisms and *P. aeruginosa* subgroups from baseline to convalescence visits. * $P < .05$; ** $P < .01$. IL-8 = interleukin-8.

though subjects isolated with *P. aeruginosa* had a significant recovery of IL-1β during convalescence, we did not observe notable differences in changes in other serum/sputum inflammatory biomarkers, spirometry, and quality-of-life measures when comparing subjects isolated with *P. aeruginosa* and non-*P. aeruginosa* counterparts throughout the 3 stages.

Discussion

Bronchiectasis exacerbations elicited augmented inflammatory responses, poorer quality of life, and worsened spirometry. In our study, clinically stable subjects isolated with potentially pathogenic microorganisms did not demonstrate greater variations in clinical parameters during

bronchiectasis exacerbations or convalescence than those without.

Our major findings are consistent with literature reports. Using anaerobic culturing and 16S ribosomal ribonucleic acid pyrosequencing, Tunney et al[22] found that microbiome taxa (predominantly Proteobacteria) abundance remained relatively constant during bronchiectasis exacerbations and convalescence, suggesting that changes in bacterial density are unlikely to account for bronchiectasis exacerbations. The increased systemic inflammation during exacerbations could be abrogated by antibiotics. In the study by Murray et al,[11] 24-h sputum volume, C-reactive protein, and SGRQ scores were responsive to therapeutic outcomes, but were unrelated to bacterial clearance. Courtney et al[12] reported a notable decline in serum C-reactive protein, sputum inflammatory cell counts, and biomarkers (TNF-α, CXCL8, and neutrophil elastase), but not spirometry or SGRQ scores, at day 14 following antibiotic therapy. Furthermore, bronchiectasis with higher bacterial density was associated with higher serum ICAM-1, VCAM-1, and E-selectin, leading to increased risks of bronchiectasis exacerbations.[4] These results collectively indicate the possible roles of bacterial infection in bronchiectasis exacerbations.

Positive sputum cultures for potentially pathogenic microorganisms (especially *P. aeruginosa*) were expected to be associated with significantly augmented inflammatory responses and poorer spirometry and quality of life during bronchiectasis exacerbations and greater pronounced recovery during convalescence. However, our findings reaffirmed that bacteria might not be solely responsible for bronchiectasis exacerbations. Intriguingly, antibiotics significantly ameliorate symptoms in most subjects. We therefore postulated that bacterial migration, antigen epitope shift, virulence factors, and altered host-pathogen immunologic balance[25] might be implicated in the pathogenesis of bronchiectasis exacerbations. Furthermore, viral infection (ie, adenovirus, coronavirus, and rhinovirus) could play crucial roles in bronchiectasis exacerbations.[26] Viral infections might also lead to enhanced bacterial virulence, resulting in augmented inflammation. It is likely that enhanced bacterial virulence, anaerobic bacterial infection, or viral-bacterial interactions also account for bronchiectasis exacerbations.

Similar to subjects with cystic fibrosis, subjects with bronchiectasis reportedly yield higher levels of airway inflammation (mucus hypersecretion,[27] matrix metalloproteinases,[28-30] tissue inhibitor of matrix metalloproteinase imbalance,[30] and neutrophil infiltration[28,30-32]) compared with healthy subjects, which could be directly reflected by sputum color.[30-32] In our study, sputum purulence was increased during bronchiectasis exacerbations (Table E7 in the supplementary materials at http://www.rcjournal.com), suggesting greater proteolytic activities because of matrix metalloproteinases release. The increased levels of inflammatory biomarkers further confirmed aggravated inflammatory responses during bronchiectasis exacerbations. It remains unknown whether the variation in matrix metalloproteinases and neutrophil infiltration would be significantly different between subjects with and without potentially pathogenic microorganisms isolated from sputum at baseline. Further studies regarding the utility of matrix metalloproteinases and neutrophil infiltration in bronchiectasis exacerbations are of merit.

Our findings regarding changes in spirometry mirrored literature reports of bronchiectasis.[11,33] However, changes in spirometry during COPD and asthma exacerbations were greater than those during bronchiectasis, suggesting that bronchiectasis exacerbations are pathophysiologically distinct events compared with COPD[34] and asthma[35,36] exacerbations.

We also aimed to determine changes in quality of life using the SGRQ. However, the SGRQ was initially designed for COPD subjects whose symptoms were predominantly dyspnea, which contrasted with cough and sputum production in bronchiectasis. This might partially explain the underestimation of changes in symptoms during bronchiectasis exacerbations. The unremarkable changes in activity scores indicate that exercise limitation was not the cardinal complaint during bronchiectasis exacerbations. However, changes in SGRQ scores were greater than the minimally clinical significant difference and did reflect poorer quality of life during bronchiectasis exacerbations, which was restored to baseline levels by administration of antibiotics.

We sought to evaluate changes in clinical parameters, but not effectiveness of individual antibiotics. British Thoracic Society guidelines[14] recommend appropriate selection of antibiotics based on baseline/previous sputum microbiology. Therefore, it would be impractical and unethical to prescribe identical antibiotics for observational purposes.

We also compared changes in different clinical parameters in subjects with mild and moderate-to-severe bronchiectasis (determined by the Bronchiectasis Severity Index) at the 3 stages. Despite the greater increase in sputum CXCL8 and SGRQ impact scores during bronchiectasis exacerbations, we did not observe more significant changes in clinical parameters in subjects with moderate-to-severe bronchiectasis compared with mild bronchiectasis (Table E8 in the supplementary materials at http://www.rcjournal.com). Therefore, the disease severity also seemed to contribute little to the magnitude of variation in clinical parameters.

We found very weak or no correlation between the changes in biomarkers and the quality of life from baseline to bronchiectasis exacerbations or convalescence regardless of bacterial infection status. Furthermore, changes in biomarkers were heterogeneous in subjects reporting significantly impaired quality of life. These findings suggest the complementary significance of biomarkers and quality

of life in measuring the effects of bronchiectasis exacerbations on a subject's well-being. The mechanisms of the discrepancy of their utility to reflect a subject's conditions are unclear, but might be associated with the different aspects they measure. For example, in our companion study,[33] we found that subjects elicited a statistically but not clinically significant reduction in FVC and FEV_1 during bronchiectasis exacerbations. The dissociation between airway and systemic inflammation has also been demonstrated in our sister study.[16] Therefore, it would not be surprising that changes in quality of life correlated poorly with other biomarkers of bronchiectasis. This again called for comprehensive assessment of subjects' conditions during exacerbation visits.

The significance of our findings is that baseline sputum bacteriology might not be a useful predictor of worsening clinical conditions during bronchiectasis exacerbations. Physicians should also be aware of viral infections, *P. aeruginosa* infection, or concomitant diseases that might alternatively be candidate predictors to warrant more intensive treatment and dynamic follow-up.

Some study limitations should be addressed. First, viral infection was not analyzed. Second, the Quality of Life Questionnaire-Bronchiectasis was not used because it was not available at the time of this study. Third, the effects of miscellaneous bacteria on bronchiectasis exacerbations were unclear because we did not conduct 16S ribosomal ribonucleic acid analysis or anaerobic culture.

Conclusions

In summary, bronchiectasis exacerbations elicit augmented airway and systemic inflammation and poorer quality of life, but do not significantly alter sputum bacteriology or spirometry. Clinically stable subjects isolated with potentially pathogenic microorganisms do not experience dramatic worsening of clinical conditions during bronchiectasis exacerbations.

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