Bacterial Colonization Increases Daily Symptoms in Patients with Chronic Obstructive Pulmonary Disease

Himanshu Desai[1], Karen Eschberger[2], Catherine Wrona[3], Lori Grove[3], Aarti Agrawal[4], Brydon Grant[3,5], Jingjing Yin[3], G. Iyer Parameswaran[2,3], Timothy Murphy[3], and Sanjay Sethi[2,3]

[1]Chesapeake Pulmonary and Critical Care Medicine, Chesapeake, Virginia; [2]Veterans Affairs Western New York Healthcare System and [3]University at Buffalo, State University of New York, Buffalo, New York; [4]Infectious Disease Consultants of Hampton Roads, Norfolk, Virginia; and [5]Fayetteville Veterans Affairs Medical Center, Fayetteville, North Carolina

Abstract

Rationale: Respiratory pathogens are frequently isolated from the airways of patients with chronic obstructive pulmonary disease (COPD) in the absence of an exacerbation. This bacterial "colonization" by potential pathogens is associated with host inflammatory and immune responses, which could increase respiratory symptoms.

Objectives: To study whether bacterial colonization impacts daily respiratory symptoms in COPD.

Methods: In a longitudinal prospective observational study of COPD, patients recorded daily symptoms electronically on the Breathlessness, Cough, and Sputum Scale (BCSS). Sputum cultures and quantitative polymerase chain reaction (PCR) were performed every 2 weeks. The relationship of BCSS and bacterial colonization was analyzed with generalized linear mixed effects models, after controlling for exacerbations, weather conditions, lung function, and demographic variables.

Measurements and Main Results: A total of 41 patients recorded daily symptoms for 12,527 days. The average BCSS score was higher during the periods of colonization, determined by sputum culture with one or more of the following pathogens: nontypeable *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*, compared to periods without colonization (5.28 vs. 4.46; $P = 0.008$) after controlling for confounding variables. The finding did not change when colonization was defined by quantitative PCR (average BCSS, 4.77 vs. 4.25; $P = 0.006$). Sputum IL-8 levels were elevated with bacterial colonization.

Conclusions: Even in the absence of clinical exacerbation, colonization by bacterial pathogens in COPD was associated with a clinically significant moderate increase in daily symptoms, likely mediated by increased airway inflammation. Novel therapies that decrease bacterial colonization in COPD could improve daily symptoms and quality of life.

Keywords: bacterial infection; airway inflammation; chronic obstructive pulmonary disease symptoms; colonization

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Correspondence and requests for reprints should be addressed to Himanshu Desai, M.D., 713 Volvo Parkway, Suite 101, Chesapeake, VA 23320. E-mail: desaihd@outlook.com

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Chronic obstructive pulmonary disease (COPD) continues to be a major health problem and enormous economic burden worldwide, with significant morbidity and mortality (1–4). Infection, both bacterial and viral, is an important comorbidity in COPD and is clinically manifested as acute exacerbations (5). The clinical manifestations of COPD exacerbation result from the direct effects of viruses and bacteria and from the host inflammatory/immune response to the pathogen (6). In contrast to healthy individuals, bacterial pathogens are detected in the lower respiratory tract of 25–50% of patients with stable COPD when examined by analysis of sputum, bronchoalveolar lavage, bronchial brushings, or bronchial biopsies (7–10). Several studies have demonstrated that this bacterial "colonization" in stable COPD is associated with host inflammatory and

immune responses that parallel those seen during bacterial exacerbations but are of lesser magnitude (10–13). Bacterial colonization and associated airway inflammation develop early in the course of the disease in smokers with nonobstructive chronic bronchitis and also persist in former smokers with COPD (10, 13). Airway inflammation related to bacterial colonization is neutrophilic, with IL-8 as a major mediator. Both IL-8 production and airway neutrophilia have been associated with emphysema in asymptomatic smokers as well as with increased sputum production, worsening airflow obstruction, and peripheral airway dysfunction in COPD (14–16).

Clinical observation suggests that respiratory symptoms in the stable phase of COPD fluctuate on a daily basis. Underlying reasons for this fluctuation are not well understood. Because host inflammatory responses correlate with symptom intensity during exacerbation, we hypothesized that bacterial colonization during clinically stable COPD contributes to the daily respiratory symptoms of dyspnea, cough, and sputum production. Furthermore, we hypothesized that such bacterial colonization enhances airway inflammation, which then causes increased symptoms. Of the various bacterial pathogens cultured from the lower respiratory tract, we focused on four species that have been well characterized as etiologic agents in exacerbations (17, 18): nontypeable *Haemophilus influenzae* (NTHI), *Moraxella catarrhalis* (MC), *Streptococcus pneumoniae* (SP), and *Pseudomonas aeruginosa* (PA). Because environmental conditions and air pollution have been thought to contribute to respiratory symptoms in COPD, information collected on these variables was included in the analysis.

Methods

COPD Study Clinic

The study protocol was approved by the institutional review board of the Veteran Affairs Western New York Healthcare System (Buffalo, NY). This prospective longitudinal study of bacterial infection in COPD at the Buffalo Veterans Affairs Medical Center, ongoing since 1994, has been described previously (17, 19, 20), and details are provided in the online supplement. Fifty veterans with smoking-related COPD and chronic bronchitis were enrolled in this cohort initially, and ongoing recruitment is used to maintain the cohort. From this cohort, 41 subjects agreed to this substudy from October 2005 to January 2009. The patients were seen monthly, as well as whenever they had symptoms suggestive of an exacerbation. At each visit, clinical information and sputum and serum samples were obtained. Patients were questioned about the status of their respiratory symptoms at each clinic visit and a diagnosis of COPD exacerbation was made, using predetermined criteria as described previously (17, 20); details are provided in the online supplement. Additional sputum samples were obtained 2 weeks after every clinic visit by a courier sent to patients' homes. Hence, we had sputum samples for each patient at 15-day intervals.

Electronic Daily Diaries

All patients recorded peak expiratory flow and symptoms in the form of the Breathlessness, Cough, and Sputum Scale (BCSS) daily, using electronic diaries (Micro Diary; Micro Direct, Lewiston, ME). The BCSS scale is a widely used, validated daily symptom score in COPD (21–24). It is composed of three questions, one each for breathlessness, cough, and sputum (*see* Table E1 in the online supplement), with each answer ranging from 0 to 4. The total score can therefore be from 0 to 12, and higher scores reflect increased intensity of symptoms. A dramatic change in symptoms is associated with a change in mean BCSS score of greater than 1, whereas a moderate change is associated with a change of 0.6 to 0.7 in score (21). The best value of three attempts was used to record daily peak expiratory flow. The data were periodically transferred to a password-protected hard drive and were not reviewed until the end of the study.

Measurement of Weather Conditions, Air Pollution, and Environmental Factors

Daily levels/values of weather conditions and various environmental factors for the Buffalo area were obtained from the New York State Department of Environmental Conservation during the study period. Daily temperature, relative humidity, precipitation, relative wind speed, barometric pressure, particulate matter smaller than 2.5 μm ($PM_{2.5}$), carbon monoxide (CO), sulfur dioxide (SO_2), and nitrogen dioxide (NO_2) were recorded from the city of Buffalo site and ozone (O_3) levels were recorded from the town of Amherst site from October 1, 2005 to January 31, 2009. Levels of particulate matter were recorded every third day until December 2006, after which they were recorded daily. As a result, the reading from one day was used for the next 2 days until December 2006.

Sputum Samples

Study personnel who processed the sputum samples were unaware of the patients' clinical status. Spontaneously expectorated sputum samples were homogenized by incubation at 37°C for 15 minutes with an equal volume of 0.1% dithiothreitol. Serial dilutions of homogenized sputum in phosphate-buffered saline were placed on blood, chocolate, and MacConkey agar plates. Bacterial identification was performed by the use of standard techniques. Sputum supernatants and pellets were stored at −70°C.

Quantitative PCR

DNA was extracted from sputum pellets (details are provided in the online supplement) and used as a substrate for a single-step multiplex quantitative PCR assay. Primers and probes were designed to detect the *p6* gene of NTHI, the *copB* gene of MC, the *lytA* gene of SP, and the *ecfX* and *gyrB* genes of PA (details are provided in the online supplement). Bacterial pathogen was considered to be present when DNA corresponding to >0 CFU/ml for the NTHI *p6* gene, ⩾100 CFU/ml for the MC *copB* gene, ⩾1,000 CFU/ml for the SP *lytA* gene, or >0 CFU/ml for either the PA *ecfX* or *gyrB* gene was detected by quantitative PCR in the sputum pellet. These cutoffs were based on analyses of induced sputum samples from 10 healthy control subjects to determine background levels.

Measurement of IL-8

Levels of IL-8 were determined in sputum supernatants by a sandwich ELISA as described previously (10, 25). All tests were done in duplicate with appropriate controls, and the laboratory technician was unaware of the clinical condition (exacerbation, colonization, etc.) of the patients.

Statistical Analysis

Numeric variables are presented as means ± standard deviation or as medians (range) as

Table 1. Baseline characteristics of 41 patients with chronic obstructive pulmonary disease included in study

Characteristic	Value
Age, yr (mean ± SD)	64.6 ± 9.9
Male/female	41/0
White/African American	39/2
FEV_1, L (mean ± SD)	1.79 ± 0.62
FEV_1% predicted, mean ± SD	50.3 ± 13.5
FVC, L (mean ± SD)	3.22 ± 0.81
FVC% predicted, mean ± SD	70.1 ± 13.4
FEV_1/FVC ratio, mean ± SD	54.8 ± 10.4
Current smokers at enrollment in the study: n, percentage	23, 56.1%
Smoking pack-years, mean ± SD	71.6 ± 33.4

appropriate. For each clinic visit, the presence or absence of exacerbation was determined on the basis of predefined criteria (17, 20); details are provided in the online supplement. The presence or absence of major bacterial pathogens was determined on the basis of sputum culture or quantitative PCR. The unit of analysis was the clinic or courier visit. Averages for the BCSS score, peak expiratory flow, and each weather condition were calculated for each 15-day period (7 d before and 7 d after) around the clinic or courier visit. Colonization was defined as a visit with the absence of an exacerbation and with the presence of bacterial pathogen in sputum sample detected by culture or PCR. The relationship of bacterial colonization to average BCSS score was analyzed with a generalized linear mixed effects model (the GLIMMIX procedure) with random intercept to take repeated visits into account. Because the average BCSS score was not normally distributed, we grouped patients into four ordinal groups based on average BCSS score quartiles and used the cumulative logit link in the model (SAS statistical software version 9.2; SAS, Cary, NC). The adjusted odds ratio of a patient with bacterial colonization being in a higher BCSS group versus being in a lower BCSS group for absence of bacterial colonization was calculated by controlling for selected covariates of weather conditions, daily peak expiratory flow, exacerbation, and demographic variables (age, sex, baseline FEV_1, smoking status, and smoking pack-years). The selection of covariates was conducted by both forward and backward methods and both approaches identified the same group of potentially confounding covariates. The relationship of bacterial colonization to IL-8 level was analyzed with a linear mixed model using a random intercept and fixed colonization effects for the outcome, IL-8 level.

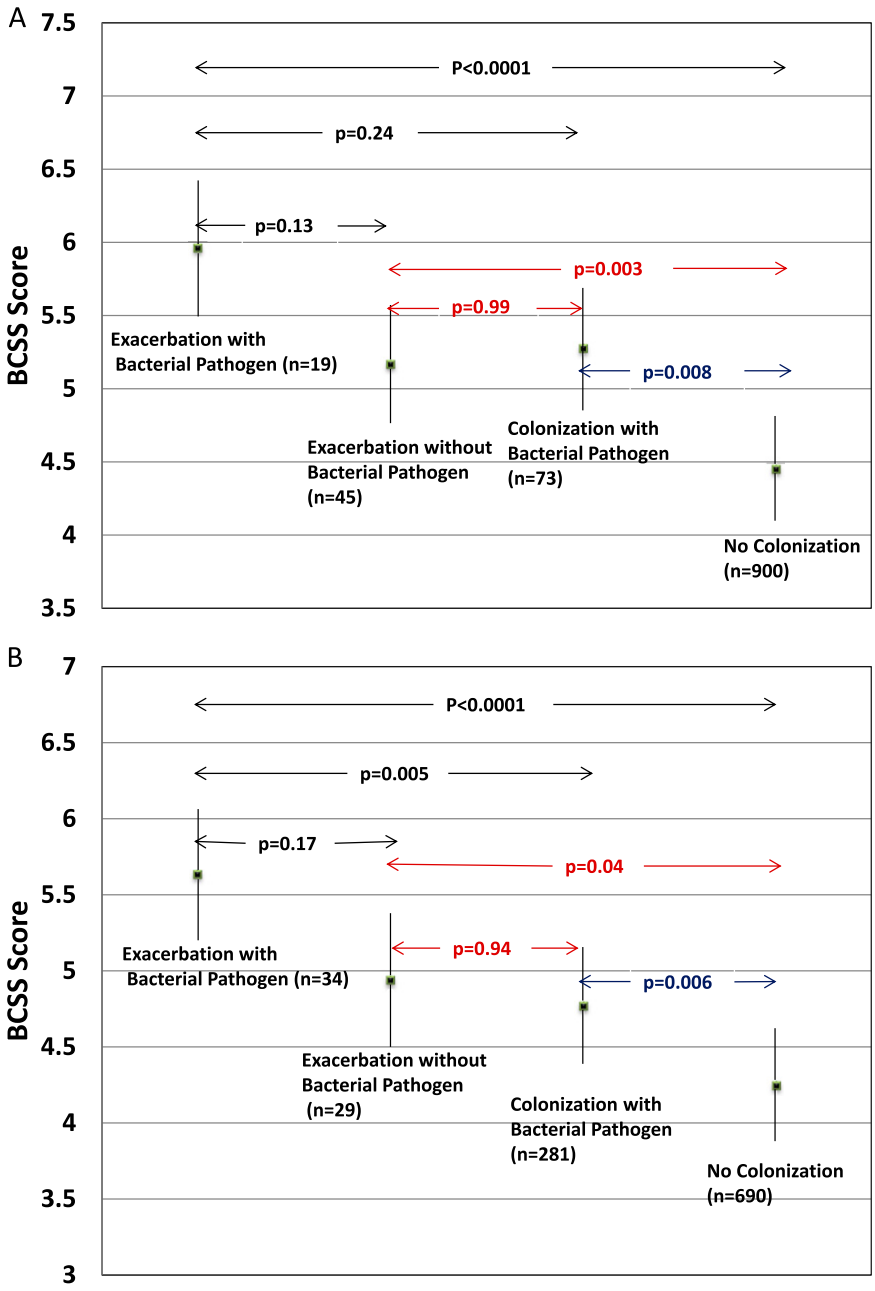

Figure 1. (*A*) Breathlessness, Cough, and Sputum Scale (BCSS) score (adjusted for age and peak expiratory flow) during exacerbation and colonization determined by sputum culture. The *central box* represents the average BCSS score and the *vertical lines* represent the standard error. (*B*) BCSS score (adjusted for age and peak expiratory flow) during exacerbation and colonization, using PCR to detect bacterial pathogens. The *central box* represents the average BCSS score and the *vertical lines* represent the standard error.

Results

Characteristics of Patients and Daily Diaries

All patients were elderly male veterans with moderate to severe COPD and significant smoking history (Table 1). These patients

recorded the BCSS score and peak expiratory flow with electronic daily diaries for an average of 306 (range, 1–770) days out of an average 446 (range, 1–917) days of participation. The median BCSS score was 5 (interquartile range, 3–7) with significant variations in daily symptoms among individual subjects.

Bacterial Colonization and Daily Symptoms

Of a total 1,037 sputum samples, 524 were clinic samples and 513 were courier samples. Four major bacterial pathogens (NTHI, MC, SP, or PA) were present in 19 (30%) of 64 sputum samples obtained during exacerbation. These same pathogens were isolated from 73 (8%) of 973 sputum samples during stable COPD.

After excluding exacerbation periods, but before adjusting for confounding variables, the average BCSS score was higher during periods of colonization with one or more of the four major bacterial pathogens compared to periods without colonization (5.31 vs. 4.26; $P < 0.001$, adjusted for multiple comparisons). In univariate analysis, other variables that were related to the BCSS score ($P < 0.1$) were barometric pressure, baseline FEV_1, baseline forced vital capacity, daily peak expiratory flow, pack-years of smoking history, and age (*see* Table E2). Of these variables, only age and daily peak expiratory flow were significant ($P < 0.05$) with multivariate logistic regression when added to the model that already had exacerbation and colonization as covariates (*see* Table E3). Presence of exacerbation, higher age, and lower daily peak expiratory flow were associated with higher average BCSS score (odds ratio [OR], 2.57; 95% CI, 1.30 to 5.07; $P < 0.007$; OR, 1.19; 95% CI, 1.07 to 1.33; $P = 0.045$ and OR, 0.38; 95% CI, 0.27 to 0.53; $P < 0.0001$, respectively). After excluding periods of exacerbation, and adjusting for age and daily peak expiratory flow, the average BCSS score remained higher during periods of bacterial colonization compared to periods without colonization (OR, 3.33; 95% CI, 1.41 to 7.9; $P = 0.008$, adjusted for multiple comparisons) (Figure 1A).

Similar results were obtained with quantitative PCR despite the increased sensitivity of PCR to detect bacterial pathogens (Table 2, Figure 1B, and Table E4). Of a total 1,034 sputum samples with PCR results, 63 were obtained during exacerbation, with the four major bacterial pathogens present in 34 (54%) sputum samples. Nontypeable *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, or *Pseudomonas aeruginosa* was detected in 281 of 971 (29%) sputum samples during stable COPD.

Table 2. Detection of bacterial pathogen; average Breathlessness, Cough, and Sputum Scale score; and sputum IL-8 levels with sputum culture and polymerase chain reaction

	Sputum Culture (*n = 1,037*)	Polymerase Chain Reaction (*n = 1,034*)
Exacerbations with bacterial pathogen	19/64 (30%)	34/63 (54%)
Bacterial colonization	73/973 (8%)	281/971 (29%)
BCSS during exacerbation with bacterial pathogen, mean (SEM)	6.54 (0.54)	5.63 (0.43)
BCSS during exacerbation without bacterial pathogen, mean (SEM)	5.62 (0.47)	4.94 (0.44)
BCSS during bacterial colonization, mean (SEM)	5.31 (0.41)	4.77 (0.38)
BCSS in stable COPD without bacterial colonization, mean (SEM)	4.26 (0.35)	4.25 (0.37)
Sputum IL-8 during exacerbation with bacterial pathogen, mean (SEM)	11.18 (1.85)	8.19 (1.4)
Sputum IL-8 during exacerbation without bacterial pathogen, mean (SEM)	4.88 (1.21)	4.89 (1.52)
Sputum IL-8 during bacterial colonization, mean (SEM)	8.06 (1.49)	4.81 (0.88)
Sputum IL-8 in stable COPD without bacterial colonization, mean (SEM)	3.28 (0.55)	3.28 (0.65)

Definition of abbreviations: BCSS = Breathlessness, Cough, and Sputum Scale; COPD = chronic obstructive pulmonary disease.

Bacterial Colonization and Sputum IL-8

Sputum IL-8 levels were higher during the periods of colonization with one or more of the major bacterial pathogens compared to periods without colonization with these pathogens (8.06 vs. 3.28 ng/ml; $P = 0.0002$) (Figure 2A). We found similar results with sputum IL-8 levels when quantitative PCR was used to detect bacterial pathogens (4.81 vs. 3.28 ng/ml; $P = 0.026$) (Table 2 and Figure 2B).

Discussion

The results of this study support our hypothesis that patients with COPD have higher daily respiratory symptoms during periods of bacterial colonization, due to increased host inflammatory response in the airway. Furthermore, this effect was seen after controlling for other host and environmental variables that are associated with daily symptoms, and also when colonization was determined by sensitive molecular techniques. The effect size of the average differences in BCSS seen with bacterial colonization as compared to the noncolonized state was 0.82. This has been assessed to be a moderate effect size, well above the minimal clinically important difference of 0.3, and just below the effect size of 1 seen with clinically reported exacerbations of COPD (21). We conclude that the increase in respiratory symptoms during periods of bacterial colonization was not only statistically but clinically significant.

A large proportion of COPD exacerbations remain unreported (26–30) when exacerbations are defined by change in symptoms based on daily diaries. These unreported exacerbations are associated with worse health status in COPD (26, 27). The causes of these unreported exacerbations have been unclear to date and, as is the case with reported exacerbations, are likely to vary. In this study, average BCSS scores during periods of bacterial colonization were in the same range as those during COPD exacerbation without isolation of bacterial pathogen in sputum. This observation strongly suggests that a proportion of unreported exacerbations described in previous studies

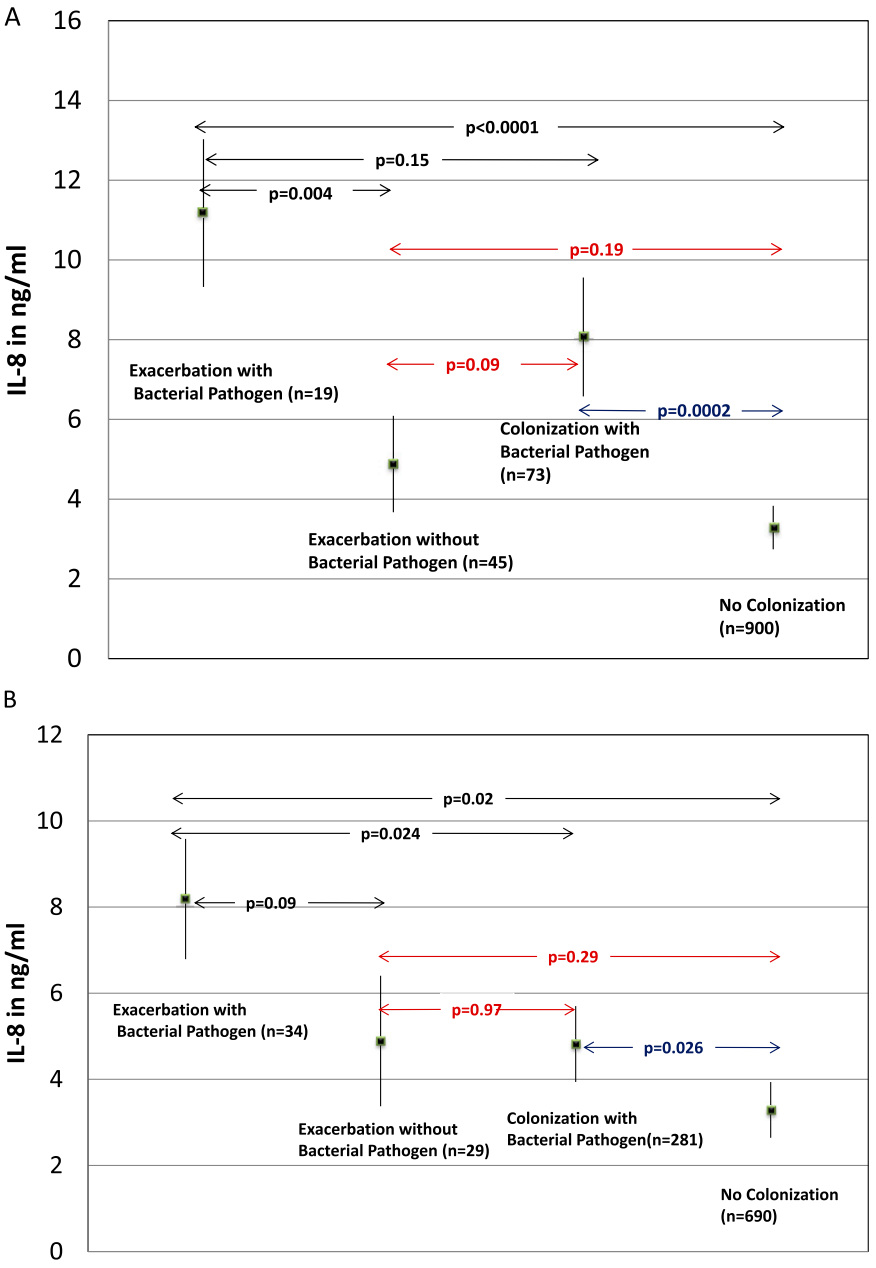

Figure 2. (*A*) Sputum IL-8 levels during exacerbation and colonization determined by sputum culture. The *central box* represents the average Breathlessness, Cough, and Sputum Scale (BCSS) score and the *vertical lines* represent the standard error. (*B*) Sputum IL-8 levels during exacerbation and colonization, using PCR to detect bacterial pathogens. The *central box* represents the average BCSS score and the *vertical lines* represent the standard error.

could be associated with periods of bacterial colonization. In a study by Mallia and colleagues, secondary bacterial infection followed a significant proportion of experimental exacerbations with rhinovirus infection in volunteers with moderate COPD (23). These infections led to protracted symptoms but did resolve spontaneously. Although we did not study viral infections in our patients, it is quite likely that mild viral infections also account for a proportion of these unreported exacerbations.

The severity of symptoms of acute exacerbations of COPD parallels sputum and serum inflammatory markers (31). Several cross-sectional and observational studies have also shown increased airway inflammation with bacterial colonization in patients with stable COPD (10–13, 32–35). However, none of those studies performed a longitudinal assessment of symptoms with a well-validated symptom assessment tool and controlled for confounding variables. Bacterial colonization of the respiratory tract is associated with increased markers of systemic inflammation in some studies (11, 34), whereas a few other studies failed to show such association (32, 34). We observed a doubling of the IL-8 levels in sputum with bacterial colonization in our study. Increased levels of IL-8 in bronchoalveolar lavage fluid distinguished smokers with emphysema from those without emphysema (16). Fuke and colleagues used laser-capture microdissection and found increased IL-8 expression in the bronchiolar epithelium in early COPD (14). Increased levels of IL-8 and neutrophils in bronchoalveolar lavage fluid were found to be inversely related to FEV_1% predicted in a study by Soler and colleagues (13). IL-8, produced by alveolar macrophages, lymphocytes, epithelial cells, and neutrophils, functions to recruit and activate neutrophils (36, 37). We speculate that increased inflammation as suggested by elevated IL-8 levels is the major driver of enhanced symptoms during periods of bacterial colonization, and contributes to disease progression.

We assessed the effects of colonization with only four major pathogens on daily symptoms, as these pathogens are known to play a major role in the pathogenesis in COPD, both during exacerbation and during the stable state (6, 9, 17, 19). Whether other bacterial pathogens prevalent in COPD, such as *Staphylococcus aureus* and Enterobacteriaceae, have similar effects remains unknown. We also used quantitative PCR to detect bacterial pathogens in sputum samples. The rate of bacterial colonization increased from 8 to 29% with PCR, which is consistent with prior studies (38, 39). Our findings of increased daily symptoms with bacterial colonization persisted when we defined colonization by PCR; however, there were some interesting differences. As compared to culture-defined groups, the average BCSS score was lower in both the PCR-defined colonization-negative stable and the colonization-positive stable groups. The increase in BCSS with colonization was also

smaller with the change in BCSS now being 0.52. This finding likely reflects the lower bacterial concentrations detected with PCR as compared to culture, and that these lower concentrations may not be as proinflammatory as the higher concentrations detected by culture. Similarly, the change in IL-8 between the colonization-negative and colonization-positive stable groups was less compared to culture-defined groups.

Limitations of this study include lack of full compliance with recording of daily symptoms and unequal duration of participation by various patients. The average compliance with daily diaries was 68%, and it varied among patients. However, the long follow-up period, the longitudinal study design, and the use of mixed models still provided us with sufficient power to draw reliable conclusions. Another limitation was the lack of detection of viral and atypical bacterial pathogens in sputum samples, as intermittent infection with these agents may have contributed to daily symptoms. However, the prevalence of viral carriage in stable COPD is quite low, even with molecular detection, and the appropriate method to determine chronic infection with atypical bacteria, for example, *Chlamydophila*, is controversial (40, 41). Patient populations to which the results of this study will need to be applied with caution include women and patients with COPD without chronic bronchitis, as these populations were not included in this study. The exclusion of women was related to the Veterans Affairs medical center–based nature of the study clinic, and the exclusion of patients without chronic bronchitis was related to the need for repeated spontaneous sputum sampling. Finally, the relationships between bacterial colonization, inflammation, and symptoms observed in this study are associations and cannot be presumed to be causal.

If bacterial colonization increases symptoms in stable COPD, one would expect a reduction in daily symptoms with treatment that could reduce colonization, such as prophylactic antibiotics. Interestingly, in studies with a macrolide and intermittent fluoroquinolone prophylaxis in COPD, the symptom component of the St. George's Respiratory Questionnaire score was impacted to the largest degree (42, 43). Although our findings provide the basis for future studies that could examine the effect of antibiotics on daily symptoms in patients with stable COPD, concerns about the emergence of antibiotic resistance should temper such an approach. Therapies that augment innate lung defense mechanisms, making the airway milieu less hospitable to bacterial colonization, could have significant impact on the daily symptom burden and unreported exacerbations of COPD. Development of such therapies would be welcome and could have these additional benefits besides reducing clinical exacerbations.

In summary, this study has demonstrated that colonization with four major bacterial pathogens is associated with a clinically significant increase in daily symptoms in patients with COPD in the absence of exacerbation. This study used a longitudinal design, accounted for demographic variables and weather conditions that could affect daily symptoms, and used sensitive molecular detection of pathogens in addition to culture to provide reliable observations. This work provides the basis for the development of novel therapies to improve COPD symptoms beyond the traditional approaches of bronchodilation and antiinflammatory agents. ■

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

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