Original Article

Respiratory Infections and Antibiotic Usage in Common Variable Immunodeficiency

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What is already known about this topic? Even with immunoglobulin replacement, respiratory tract infections remain the commonest clinical feature in common variable immunodeficiency (CVID) and impair quality of life. Encapsulated bacteria are thought to be the most common pathogens.

What does this article add to our knowledge? This is the first detailed description of respiratory exacerbations in CVID, capturing 6210 days of data. Viruses are commonly represented. There is a delay in commencing antibiotic therapy and the response to antibiotic therapy depends on the symptomatic presentation.

How does this study impact current management guidelines? Because viral infections are common in CVID, antibiotic therapy should be considered with caution. However, self-administered antibiotic therapy should be started more promptly with symptoms of cough and purulent sputum.

BACKGROUND: Patients with common variable immunodeficiency (CVID) suffer frequent respiratory tract infections despite immunoglobulin replacement and are prescribed significant quantities of antibiotics. The clinical and microbiological nature of these exacerbations, the symptomatic triggers to take antibiotics, and the response to treatment have not been previously investigated.

OBJECTIVES: To describe the nature, frequency, treatment, and clinical course of respiratory tract exacerbations in patients with CVID and to describe pathogens isolated during respiratory tract exacerbations.

METHODS: We performed a prospective diary card exercise in 69 patients with CVID recruited from a primary immunodeficiency clinic in the United Kingdom, generating 6210 days of symptom data. We collected microbiology (sputum microscopy and culture, atypical bacterial PCR, and mycobacterial culture) and virology (nasopharyngeal swab multiplex PCR) samples from symptomatic patients with CVID.

RESULTS: There were 170 symptomatic exacerbations and 76 exacerbations treated by antibiotics. The strongest symptomatic predictors for commencing antibiotics were cough, shortness of breath, and purulent sputum. There was a median delay of 5 days from the onset of symptoms to commencing antibiotics. Episodes characterized by purulent sputum responded more quickly to antibiotics, whereas sore throat and upper respiratory tract symptoms responded less quickly. A pathogenic virus was isolated in 56% of respiratory exacerbations and a potentially pathogenic bacteria in 33%.

CONCLUSIONS: Patients with CVID delay and avoid treatment of symptomatic respiratory exacerbations, which could result in structural lung damage. However, viruses are commonly represented and illnesses dominated by upper respiratory tract symptoms respond poorly to antibiotics, suggesting that antibiotic usage could be better targeted.   © 2017 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2018;6:159-68)

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Common variable immunodeficiency (CVID) is a heterogeneous primary immunodeficiency in which patients fail to produce adequate levels of immunoglobulins. With a prevalence

Abbreviations used
COPD- Chronic obstructive pulmonary disease
CVID- Common variable immunodeficiency
HR- Hazard ratio
IQR- Interquartile range
OAT- Oral antibiotic therapy
OR- Odds ratio
SGRQ- St George's Respiratory Questionnaire
TE- Treated exacerbation
TSE- Treated symptomatic exacerbation
USE- Untreated symptomatic exacerbation

TABLE I. List of symptoms collected in diaries and variables used for analysis

Variable	Values	Analysis group (all dichotomous)
Blocked nose	Present, not present	Upper respiratory tract symptoms
Nasal discharge	Present, not present	
Sinus pain	Present, not present	
Sore throat	Present, not present	Sore throat
Cough	Present, not present	Cough
Shortness of breath	Present, not present	Shortness of breath
Wheeze	Present, not present	Wheeze
Sputum color	White, yellow, green, not present	White sputum Purulent sputum
Sputum volume	Equivalent to teaspoon, egg cup, cup, not present	Increased sputum volume

Note. "Upper respiratory tract symptoms" are generated by a combination (inclusive disjunction) of blocked nose, nasal discharge, and sinus pain. Sputum color with 4 possible values was separated into 2 binary variables. Sputum volume with 4 possible values was reduced to a binary variable.

between 1 in 10,000 and 1 in 50,000, it is the most common symptomatic primary immunodeficiency.[1-4]

Despite adequate immunoglobulin replacement, recurrent respiratory tract infections are the commonest clinical feature in CVID[2,5] and can result in progressive bronchiectasis.[6-9] Respiratory tract infections were thought to be caused largely by encapsulated bacteria.[6,10] However, recent evidence shows that there may be a significant contribution from viral infections.[11,12]

Despite the high incidence of respiratory tract infections and their negative influence on quality of life in primary antibody deficiency syndromes,[13] the nature of symptoms during these episodes remains unknown. Patients are often prescribed antibiotics to mitigate respiratory tract infections, both as "rescue" courses to promptly self-administer for acute events and as prophylaxis to reduce infection frequency. However, the symptomatic triggers for taking breakthrough antibiotics and the clinical response to these treatments are not known.

In this prospective study, we sought to answer these questions by systematically recording daily symptoms and treatment in a cohort of patients with CVID over a winter period. In a parallel analysis, we also explored bacterial and viral pathogens encountered during acute respiratory symptoms in patients with CVID.

METHODS
Participants

Patients were recruited from the joint Immunology-Respiratory service at the Royal Free Hospital, London. Patients had a diagnosis of CVID made by a clinical immunologist following the definitions of the Pan-American Group for Immunodeficiency and the European Society for Immunodeficiencies.[14] All were receiving immunoglobulin replacement and were under regular (at least 6-monthly) clinical review. The only exclusion criterion was inability to provide informed consent. All participants provided written informed consent (REC 04/Q0501/119).

Study design

For this observational, prospective cohort study, patients completed daily checkbox symptom diaries for 90 days between December 2014 and February 2015, covering the UK winter season. Participants were asked to report new or increased respiratory symptoms from a predefined list (Table I). Chronic or stable symptoms were not to be reported. Definitions of symptoms and instructions for diary completion were clearly explained; further details are provided in this article's Online Repository at www.jaci-inpractice.org. We have previously used such methodology in other chronic respiratory diseases.[15] Participating patients were also asked to complete the St George's Respiratory Questionnaire (SGRQ), a validated measure of respiratory health status scored between 0 (best) and 100 (worst) quality of life.[16]

Simultaneously, but independently from the described study, we conducted a cross-sectional study in which patients experiencing acute respiratory symptoms provided samples (nasopharyngeal swabs and spontaneously expectorated sputum) for bacterial and viral testing. Sputum was considered purulent when more than 10 granulocytes per hpf were found. Samples were either collected by clinic staff or, after careful instruction on sampling, submitted directly from patients by mail. Figure 1 shows the 2 investigations undertaken on the cohorts.

Definition of exacerbations and variables

For preanalysis, we grouped clinically related symptoms as presented in Table I. For calculation of total symptom count, each symptom was counted individually for each patient and each day. Cumulative total symptom count is the sum over all days of an exacerbation period.

We used 2 definitions of exacerbation, based either on symptoms or health care utilization. Similar methodology has been reported and validated in chronic obstructive pulmonary disease (COPD).[17] For the first definition, we identified a symptomatic exacerbation as an event of 2 or more new symptoms lasting for 2 or more consecutive days as recorded by the patient in their diary, whether or not they received additional treatment. The start of a symptomatic exacerbation episode was the first day of 2 or more new symptoms lasting for 2 or more consecutive days. The end of the episode was the last consecutive day with 2 or more symptoms (allowing symptoms to change over time). If oral antibiotic therapy (OAT) was used during a symptomatic exacerbation episode, this was considered a treated symptomatic exacerbation (TSE). If not, it was an untreated symptomatic exacerbation (USE).

We defined a health care utilization exacerbation as use of OAT for worsening respiratory symptoms. We call this a treated exacerbation (TE) event, and if it coincided with diary-defined symptoms it would be a TSE. The episode was considered to last from the first day on which a symptom occurred until recovery, defined as the last day of any symptom that was present when OAT was started. Additional details regarding exacerbation and variable
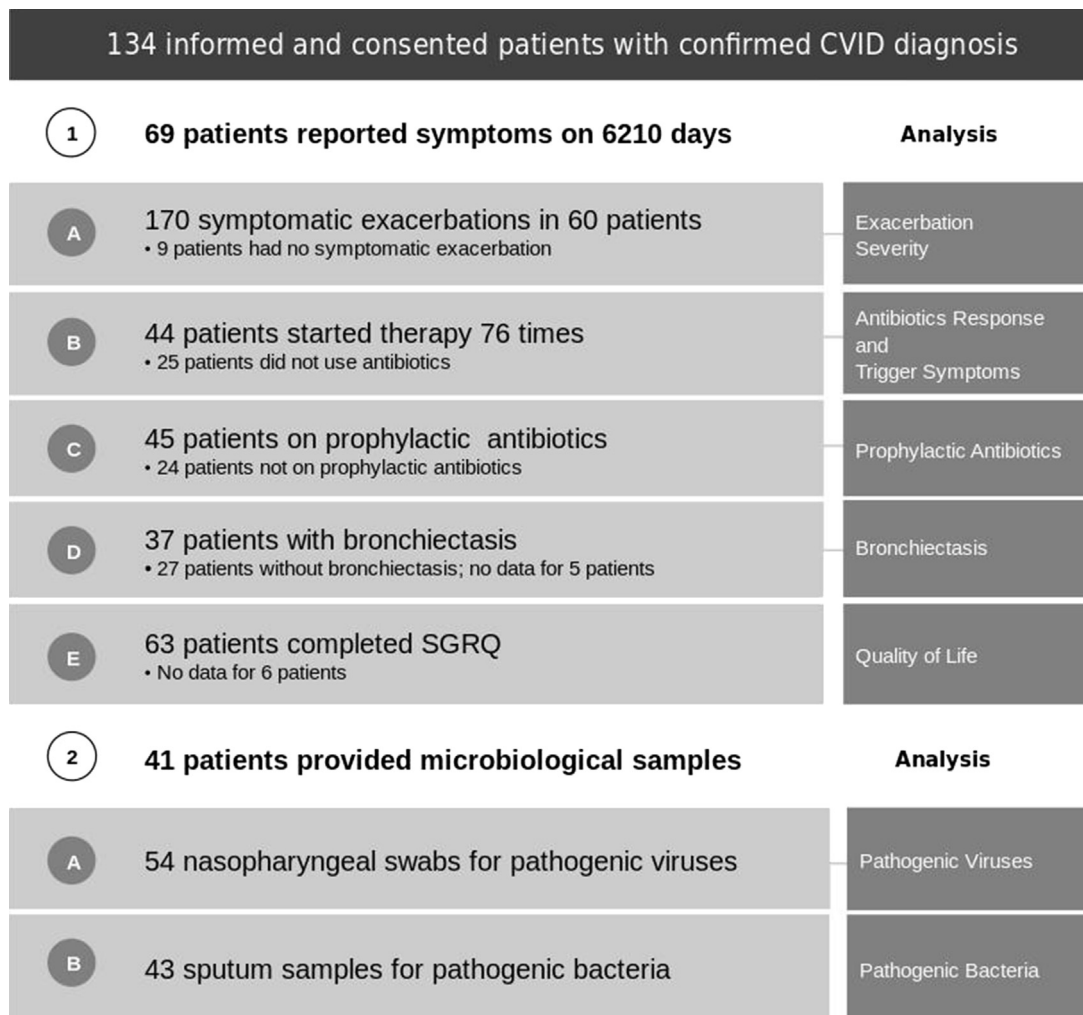
FIGURE 1. Study design and analysis flow chart. Out of 134 patients with CVID, 69 completed a symptom diary for investigation 1 and 41 provided microbiological samples for investigation 2. Details of further analyses and the numbers of participants included for each are provided.

definitions are provided in this article's Online Repository at www.jaci-inpractice.org.

Data handling and statistical analysis

Statistical analysis was performed using Stata version 14.0 (StataCorp LP, College Station, Tex). Continuous variables are presented as median and first and third quartiles or by mean and SD as appropriate. For categorical and binary variables, we present frequencies.

Missing data were not imputed. Results were considered statistically significant at a P value of less than .05. Data were analyzed with logistic regression for trigger symptom analysis, Cox regression for antibiotic response analysis, Pearson correlation, Wilcoxon rank sum test, and t tests as indicated. Further details are provided in this article's Online Repository at www.jaci-inpractice.org.

Analysis of microbial samples

A multiplex real-time PCR (RT-PCR) for adenovirus, coronavirus (HKU, NL63, OC43, and 229E), enterovirus, human metapneumovirus, influenza virus (A and B), parainfluenza virus (1, 2, 3, and 4), parechovirus, respiratory syncytial virus, and rhinovirus was performed in the National Health Service Virology Laboratories at the Royal Free Hospital.

Sputum samples were examined by microscopy and culture for bacteria and mycobacteria plus in-house multiplex RT-PCR for *Chlamydia pneumoniae*, *Legionella pneumophila*, and *Mycoplasma pneumoniae*. Further details are provided in this article's Online Repository at www.jaci-inpractice.org.

We included multiple samples from a single patient if separated by at least 2 weeks and the patient was asymptomatic between episodes. Airway colonization by pathogenic bacteria was diagnosed when the same organism had been isolated more than twice within the 2 years before our study.

RESULTS
Study population

A total of 134 patients with CVID were given a diary. Out of these, 69 (51%) patients returned a diary after completion of the study period, providing 6210 days of data (Figure 1). Demographic and clinical characteristics of included patients are presented in Table II. Patients who completed a diary were older

TABLE II. Patients' characteristics at study enrollment

Characteristic	Patients who completed symptom diaries (n = 69)	Patients who did not complete symptom diaries (n = 65)	P value
Age (y), median (IQR)	59.36 (46.74-68.22)	45.02 (36.33-53.79)	<.001
Female patients, n (%)	41 (59)	36 (55)	.73
IgG trough level* (g/L), median (IQR)	9.0 (8.0-10.0)	9.0 (7.8-10.6)	.99
Prophylactic antibiotic, n (%)	45 (65)		
Amoxicillin	7 (10)		
Azithromycin	22 (32)		
Ciprofloxacin	3 (4)		
Clarithromycin	3 (4)		
Co-amoxiclav	2 (3)		
Cotrimoxazole	2 (3)		
Doxycycline	4 (6)		
Lymecycline	1 (1)		
Penicillin	1 (1)		
Smoking status, n (%)			
Current smoker	6 (9)	4 (6)	.92
Past smoker	15 (22)	15 (23)	
Never a smoker	48 (70)	46 (71)	
Bronchiectasis on CT, n (%)	37 (57.81)	29 (49.15)	.22
BSI score†, median (IQR)	3.5 (2-6)	2 (1-4)	.01
FEV_1 (L), median (IQR)	2.24 (1.80-3.23)	2.63 (2.12-3.36)	.11
FEV_1 predicted‡ (%), median (IQR)	93.2 (73.3-102.9)	93.5 (74.6-105.4)	.95
SGRQ§ total score, median (IQR)	24.47 (8.41-45.54)		
SGRQ symptoms score, median (IQR)	39.28 (23.76-58.56)		
SGRQ activity score, median (IQR)	29.31 (5.96-59.46)		
SGRQ impact score, median (IQR)	14.90 (1.98-29.90)		

CT, X-ray computed tomography.

Note. P values were calculated using the Wilcoxon rank sum test for continuous variables and the Fisher exact test for categorical variables.

*Serum IgG level measured immediately before the next immunoglobulin replacement is administered.

†Bronchiectasis severity index, ranging from 0 (best) to 25 (worst), is a validated multicomponent score in bronchiectasis that predicts the future risk of exacerbations, hospitalizations, and mortality.

‡FEV_1 predicted is the proportion of actual FEV_1 vs predicted FEV_1 in accordance with the European Respiratory Society guidelines of 1993.

§The SGRQ is a validated measure of respiratory health status scored between 0 (best) and 100 (worst) quality of life.

(median [interquartile range, IQR], 59.36 [46.74-68.22] vs 45.02 [36.33-53.79] years; $P < .001$) and had a higher bronchiectasis severity index (median [IQR], 3.5 [2-6] vs 2 [1-4]; $P = .01$) than those who did not.

Patients with CVID suffer frequent respiratory exacerbations and often use antibiotics

During the study period, there were 170 symptomatic exacerbation events (mean, 0.82 per patient month). Of these events, 75 (mean, 0.36 per patient month) were treated by OAT but 95 (mean, 0.46 per patient month) were not. Nine patients had no symptomatic exacerbations during the period. Published literature suggests that 106 courses of antibiotics were prescribed per 1000 men and 155 per 1000 women for respiratory tract infections by general practitioners in the United Kingdom in 2014.[18] This corresponds to 2.3 courses of antibiotics in total (0.01 per patient month) prescribed to a group similar to our cohort in the general population during 3 months.

TSE episodes were more severe than USE in terms of cumulative total symptom count (median [IQR], 40 [24-82] vs 12 [6-30] symptoms; $P < .001$) and episode duration (median duration, 10 vs 4 days; hazard ratio [HR], 0.54; $P < .001$).

A total of 76 TEs were covered within our study period. One TE did not meet the criteria of TSE. The median (IQR) duration of TE episodes was 14 (9-19) days; median (IQR) time from the start of symptoms until OAT was 5 (2-7) days; and median (IQR) time until recovery was 6.5 (5-14) days. The median (IQR) duration of therapy was 14 (7-14) days. A detailed description of symptom prevalence is shown in Figure 2. As treatment, patients used co-amoxiclav for 23 exacerbations (30%), amoxicillin for 20 exacerbations (26%), doxycycline for 12 exacerbations (16%), ciprofloxacin for 10 exacerbations (13%), clarithromycin for 7 exacerbations (9%), and azithromycin, erythromycin, flucloxacillin, and levofloxacin for 1 exacerbation each (1%).

Cough, shortness of breath, and purulent sputum are the strongest triggers for patients to initiate antibiotic therapy

We compared 76 days on which OAT was started with 5370 days without OAT. The 764 days comprising the remainder of the antibiotic courses were ignored. In univariate analysis, all symptoms were positively and significantly associated with start of OAT. Cough (odds ratio [OR], 48.70; 95% CI, 24.02-111.47), purulent sputum (OR, 25.26; 95% CI, 15.25-42.49),
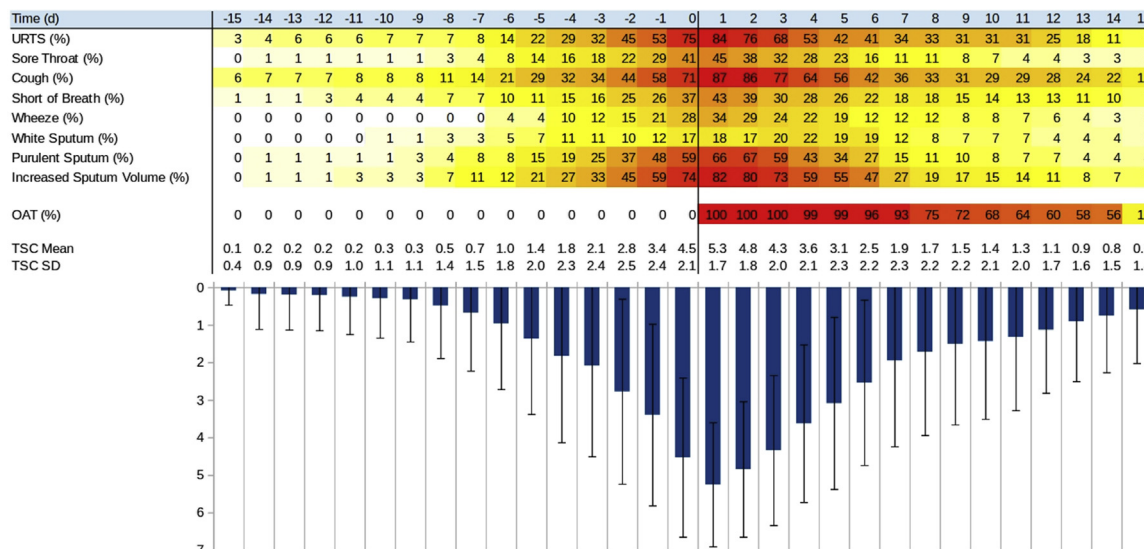
Time (d)	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
URTS (%)	3	4	6	6	6	7	7	7	7	8	14	22	29	32	45	53	75	84	76	68	53	42	41	34	33	31	31	31	25	18	11
Sore Throat (%)	0	1	1	1	1	1	1	3	4	8	14	16	18	22	29	41	45	38	32	28	23	16	11	11	8	7	4	4	3	3	3
Cough (%)	6	7	7	7	8	8	8	11	14	21	29	32	34	44	58	71	87	86	77	64	56	42	36	33	31	29	29	28	24	22	17
Short of Breath (%)	1	1	1	3	4	4	4	7	7	10	11	15	16	25	26	37	43	39	30	28	26	22	18	18	15	14	13	13	11	10	7
Wheeze (%)	0	0	0	0	0	0	0	0	0	4	4	10	12	15	21	28	34	29	24	22	19	12	12	12	8	8	7	6	4	3	3
White Sputum (%)	0	0	0	0	0	1	1	3	3	5	7	11	11	10	12	17	18	17	20	22	19	19	12	8	7	7	7	4	4	4	3
Purulent Sputum (%)	0	1	1	1	1	1	3	4	8	8	15	19	25	37	48	59	66	67	59	43	34	27	15	11	10	8	7	7	4	4	4
Increased Sputum Volume (%)	0	1	1	1	3	3	3	7	11	12	21	27	33	45	59	74	82	80	73	59	55	47	27	19	17	15	14	11	8	7	7
OAT (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	100	99	99	96	93	75	72	68	64	60	58	56	10
TSC Mean	0.1	0.2	0.2	0.2	0.2	0.3	0.3	0.5	0.7	1.0	1.4	1.8	2.1	2.8	3.4	4.5	5.3	4.8	4.3	3.6	3.1	2.5	1.9	1.7	1.5	1.4	1.3	1.1	0.9	0.8	0.6
TSC SD	0.4	0.9	0.9	0.9	1.0	1.1	1.1	1.4	1.5	1.8	2.0	2.3	2.4	2.5	2.4	2.1	1.7	1.8	2.0	2.1	2.3	2.2	2.3	2.2	2.2	2.1	2.0	1.7	1.6	1.5	1.4

FIGURE 2. Characterization of respiratory exacerbations treated with antibiotics. Symptom prevalence (%) and TSC are displayed over time (d) for 76 antibiotic-treated respiratory exacerbations in patients with CVID. Bar diagram reflects mean (SD) TSC. Day 1 is defined as start of OAT. *TSC*, Total symptom count; *URTS*, upper respiratory tract symptom.

increased sputum volume (OR, 23.85; 95% CI, 13.85-42.70), and shortness of breath (OR, 17.27; 95% CI, 10.54-28.22) showed the highest ORs (Figure 3, A). In multivariate analysis, only cough (OR, 13.00; 95% CI, 5.93-28.47), purulent sputum (OR, 6.30; 95% CI, 1.19-33.40), and shortness of breath (OR, 2.41; 95% CI, 1.31-4.46) remain significant when adjusted for other symptoms (Figure 3, A).

In univariate analysis, time since start of symptoms was not positively associated with start of OAT, and instead patients started OAT at a fairly constant rate over the first 12 days of symptoms (Figure 3, B). There was, however, a significant positive association between total symptom count and start of OAT (OR, 2.19; 95% CI, 1.96-2.43), suggesting an approximate doubling of the odds to start OAT for each additional symptom. The mean number of symptoms on days on which OAT was started was 4.97 ± 1.94 versus 0.86 ± 1.55 symptoms on days when antibiotics were not taken ($P < .001$).

Exacerbations characterized by purulent sputum respond rapidly to antibiotics, whereas those characterized by upper respiratory tract symptoms and sore throat respond more slowly

Median (IQR) time until recovery after start of OAT in all TEs was 6.5 (5-14) days (Figure 4, A). In 56% of TEs, time until recovery was 7 days or less; in 81% it was 14 days or less.

In univariate analysis, time until recovery was longer in the presence of upper respiratory tract symptoms (median, 8 vs 5 days; HR, 0.50; $P = .03$; Figure 4, B), sore throat (12 vs 6 days; HR, 0.54; $P = .007$; Figure 4, C), or white sputum (12 vs 6 days; HR, 0.63; $P = .03$) on the day before commencing OAT. However, time until recovery was shorter in exacerbations in which purulent sputum was present (6 vs 13 days; HR, 1.98; $P = .02$; Figure 4, D). In multivariate analysis, upper respiratory tract symptoms, sore throat, and purulent sputum were significant independent predictors for response to OAT (Figure 4, E).

There was no statistically significant correlation between time until starting OAT and time until recovery nor between total symptom count on the day before OAT was started and subsequent time until recovery. However, a longer time until starting OAT was associated with a longer episode duration (HR, 0.92; $P < .001$).

Patients taking prophylactic antibiotics have more untreated exacerbations and wait longer from the onset of symptoms to initiate breakthrough antibiotics

We proceeded to investigate whether the frequency and nature of exacerbations were affected by antibiotic prophylaxis or by the presence of bronchiectasis. The mean numbers of symptomatic exacerbations (total, treated, and untreated) were analyzed with t tests and interaction was tested with 2 (prophylactic antibiotics) by 2 (bronchiectasis) analyses of variance.

There were more symptomatic exacerbation events in patients on prophylactic antibiotics than in patients not on prophylaxis (mean, 2.87 ± 2.21 vs 1.71 ± 1.33; $P = .02$). However, there was no significant difference in the number of TSE events (mean, 1.09 ± 1.02 vs 1.08 ± 1.32; $P = .98$) and the difference was explained by more USEs in patients on prophylactic antibiotics (mean, 1.78 ± 2.14 vs 0.63 ± 0.88; $P = .01$). In contrast, there was no significant difference in the numbers of symptomatic exacerbations (mean, 2.46 ± 1.74 vs 2.63 ± 2.48; $P = .75$), USEs (mean, 1.19 ± 1.63 vs 1.81 ± 2.24; $P = .20$), and TSEs (mean, 1.27 ± 1.15 vs 0.81 ± 1.08; $P = .11$) between patients with or without bronchiectasis.

The higher numbers of symptomatic exacerbations and USEs in patients on prophylactic antibiotics did not depend on the presence or absence of bronchiectasis. We observed a mean difference of 0.93 in the number of symptomatic exacerbations between patients on and off prophylaxis in those with bronchiectasis and of 1.61 in those without. For USEs, the differences in
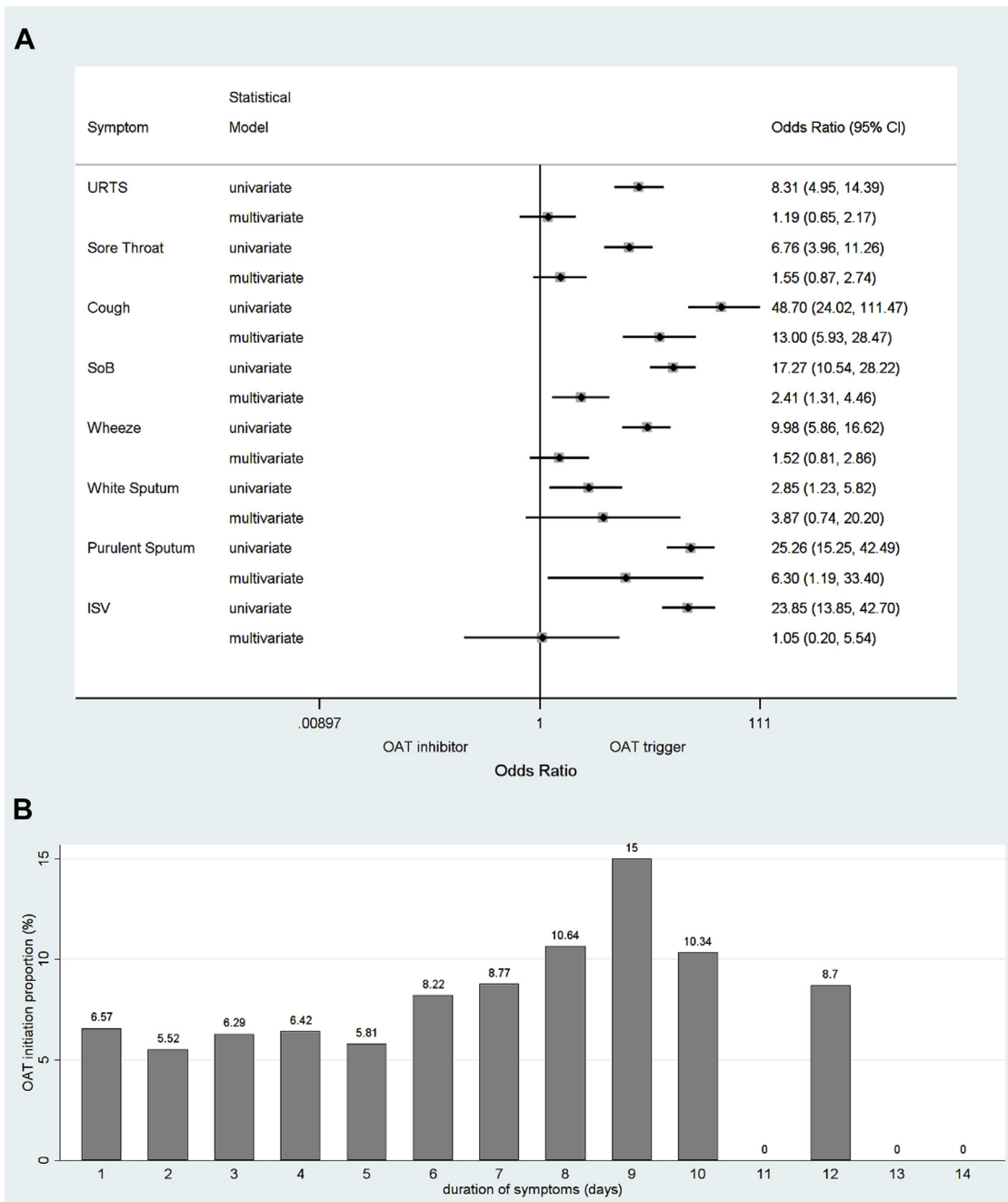
A

Symptom	Statistical Model	Odds Ratio (95% CI)
URTS	univariate	8.31 (4.95, 14.39)
	multivariate	1.19 (0.65, 2.17)
Sore Throat	univariate	6.76 (3.96, 11.26)
	multivariate	1.55 (0.87, 2.74)
Cough	univariate	48.70 (24.02, 111.47)
	multivariate	13.00 (5.93, 28.47)
SoB	univariate	17.27 (10.54, 28.22)
	multivariate	2.41 (1.31, 4.46)
Wheeze	univariate	9.98 (5.86, 16.62)
	multivariate	1.52 (0.81, 2.86)
White Sputum	univariate	2.85 (1.23, 5.82)
	multivariate	3.87 (0.74, 20.20)
Purulent Sputum	univariate	25.26 (15.25, 42.49)
	multivariate	6.30 (1.19, 33.40)
ISV	univariate	23.85 (13.85, 42.70)
	multivariate	1.05 (0.20, 5.54)

.00897   1   111
OAT inhibitor   OAT trigger
Odds Ratio

B

FIGURE 3. Trigger symptom analysis for patients with CVID to commence antibiotic therapy. **A,** Prospective diary data of respiratory symptoms and OAT usage were collected from 69 patients with CVID. The forest plot displays ORs and 95% CI as a measure of effect size for individual symptoms to trigger the start of OAT (higher ORs imply a strong association between the symptom and starting OAT). Results are derived from univariate and multivariate logistic regression on the basis of 5446 observations (d). **B,** The bar graph shows the proportion of patients initiating OAT on each of the first 14 d of consecutive symptoms. The time since start of symptoms is defined as the number of days for which 2 or more symptoms were present. The OAT initiation proportion is the proportion of OAT that was started after a specific time since start of symptoms. *ISV,* increased sputum volume; *SoB,* shortness of breath; *URTS,* upper respiratory tract symptom.

mean values were 1.27 and 0.98, respectively. Interaction effects were nonsignificant in all analyses.

Regarding the impact of antibiotic prophylaxis on exacerbation severity, there was no significant difference in episode duration or cumulative total symptom count during symptomatic exacerbations between patients on or off prophylactic antibiotics. Patients taking prophylactic antibiotics waited longer before starting OAT for breakthrough infections (median, 6 vs 3
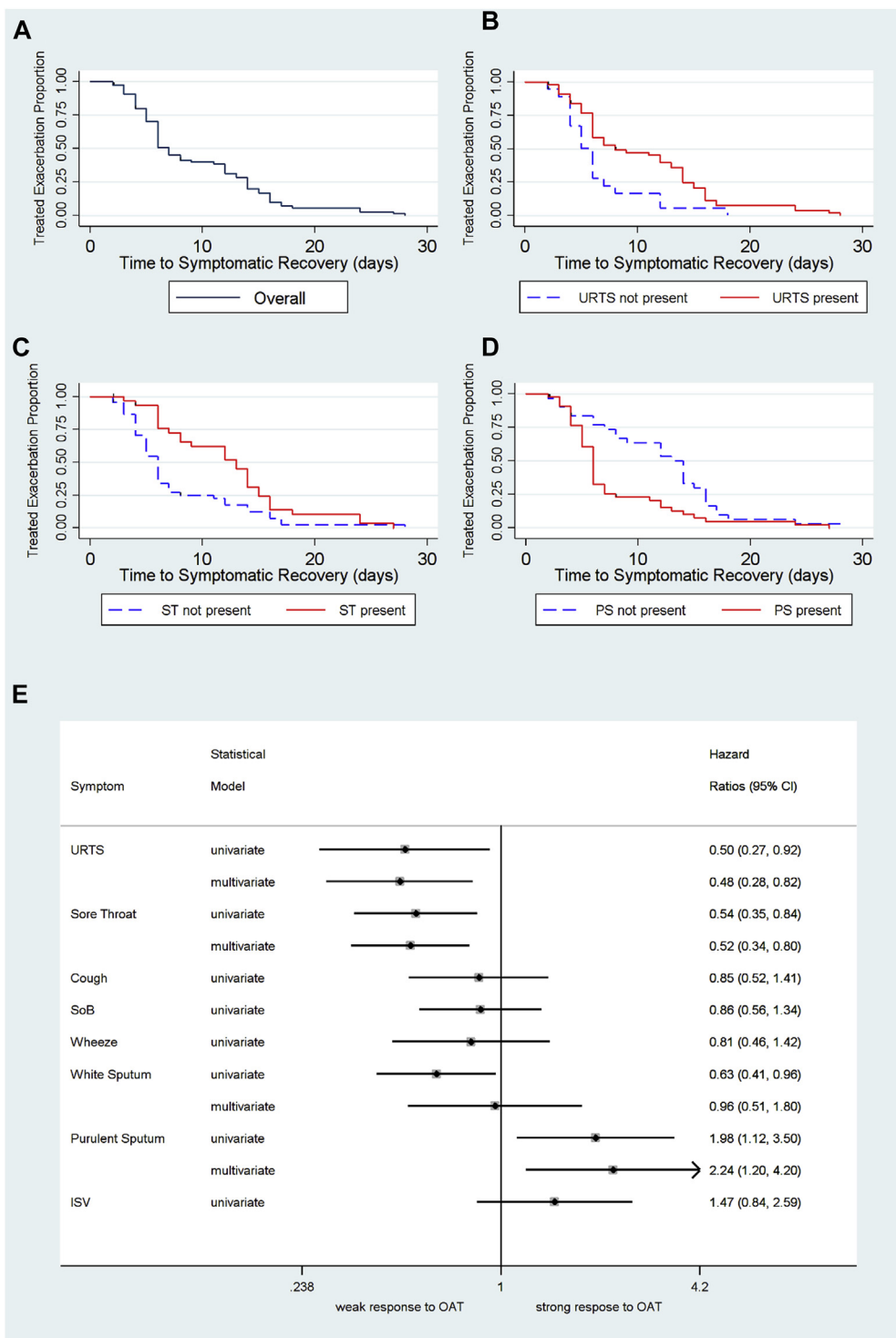
FIGURE 4. Antibiotic response analysis of predictor symptoms. Kaplan-Meier plots display time until recovery based on 76 antibiotic-treated respiratory exacerbations in patients with CVID: **A**, for all exacerbations; **B**, according to presence or absence of upper respiratory tract symptoms (URTSs); **C**, according to presence or absence of sore throat (ST); and **D**, according to presence or absence of purulent sputum (PS). **E**, Forest plot displays HRs for time until recovery after start of OAT depending on the presence of specific symptoms. A multivariate Cox model was used for all symptoms that proved to be significant in univariate analysis (only multivariate data are shown for these variables). HR reflects the "risk" for earlier complete symptomatic remission over time. *ISV*, increased sputum volume; *SoB*, shortness of breath.

days; HR, 0.55; P = .03). However, time until recovery after commencing OAT was not significantly different between patients on or off prophylactic antibiotics.

There were no significant differences between patients with and those without bronchiectasis in exacerbation severity, time until OAT, and time until recovery.

Prospective symptoms correlate modestly with cross-sectional analysis of quality of life

There was moderate correlation between the SGRQ total score and the number of days on which new cough (r = 0.29; P = .02), sore throat (r = 0.29; P = .02), shortness of breath (r = 0.38; P = .002), and wheeze (r 0.32; P = .01) were present. The cumulative total symptom count or cumulative number of days of symptomatic exacerbation episodes over the study period also correlated with SGRQ symptom score (r = 0.36; P = .004) and SGRQ total score (r = 0.28; P = .03).

Respiratory exacerbations in CVID demonstrate a high frequency of viral and bacterial pathogens

A total of 54 nasopharyngeal swabs were obtained from 41 patients with acute respiratory symptoms. Viruses were detected in 30 (56%) exacerbations (Figure 5). Rhinovirus was the most common virus detected (in 18 [33%] exacerbations), including 2 (4%) co-infections with respiratory syncytial virus, 2 (4%) co-infections with adenovirus, and 1 (2%) co-infection with human metapneumovirus.

A total of 43 spontaneously expectorated sputum samples were obtained from 34 patients with acute respiratory symptoms. Pathogenic bacteria were isolated in 14 (33%) exacerbations (Figure 5). The most common bacteria were *Haemophilus influenzae* in 8 (19%), *Streptococcus pneumoniae* in 2 (5%), and *Pseudomonas aeruginosa* in 2 (5%) exacerbations. Two patients accounting for 4 exacerbations were colonized with *H. influenzae* as defined earlier. All samples were negative for mycobacterial culture and PCR for atypical pneumonia organisms.

There was bacterial and viral co-infection in 25% of exacerbations; in 27.5% no pathogen was found. Microscopic evidence of purulence as measured by more than 10 granulocytes per hpf was found on microscopy in 41% of exacerbations positive for a pathogenic virus (whether or not patients produced sputum), in 69% of exacerbations positive for a pathogenic virus where contemporaneous sputum was collected, and in 64% of exacerbations positive for pathogenic bacteria.

DISCUSSION

This is the first prospective cohort study describing symptoms and treatment of respiratory tract infection in CVID. We discovered a clinically important delay in commencing antibiotic therapy and that many symptoms are untreated, especially in patients taking prophylactic antibiotics. Episodes characterized by purulent sputum respond more quickly to antibiotics, whereas sore throat and upper respiratory tract symptoms respond less quickly; perhaps correspondingly, in many respiratory exacerbations we detected a pathogenic virus.

Patients with CVID are frequently prescribed antibiotics and educated to promptly take them if they suffer "breakthrough" infections. However, their actual behaviors in relation to this therapy have not previously been documented. Here, across 6210 days of data, we discovered that individual "warning" symptoms (cough, shortness of breath, and purulent sputum) are

the most important triggers for patients to start OAT. Time since start of symptoms is a less important trigger, and the proportion of patients starting therapy each day is fairly constant across the first 12 days of symptoms. Consequently, and despite the fact that all patients should have antibiotics available for immediate usage, there is a median delay of 5 days in starting OAT. We are investigating whether delays to commencing treatment are explained more by patient choice or by access to health care.

A longer time to commencing therapy did not adversely impact subsequent response to antibiotics (measured by time until recovery), but inevitably increased the total length of an infectious episode. Because infections in CVID can lead to structural lung damage,[2,13] this delay may be clinically significant. Similarly, many exacerbations (95 across the study period) were untreated and may not have been reported without prospective data collection. Indeed, it is well documented that USEs often go unreported in COPD,[19] with up to 3 times more exacerbations collected by symptom diaries than by interview; patients with COPD also treat only half of all exacerbations recorded in diaries.[20]

Response to OAT, judged by time until recovery, did not correlate with delay to commencing therapy or total symptom count, but depended on individual symptoms. There was a slower response in patients with upper respiratory tract symptoms and sore throat, which we hypothesize may be explained by a purely viral etiology for some of these episodes. Conversely, exacerbations with purulent sputum resolved more quickly on antibiotics, perhaps indicating a dominant bacterial component.

The number of USEs, and delay to commencing OAT, was higher in patients on prophylactic antibiotics. This could imply a reluctance to start OAT in this group because of over-reliance on prophylaxis or as an increased tolerance of symptoms (generally prophylaxis is instituted only in patients with a background of high incidence of exacerbations). We found no difference in severity or duration of individual symptomatic exacerbations with or without prophylaxis; this could indicate effectiveness of prophylaxis, but conversely there is no evidence that prophylaxis attenuates the severity of breakthrough exacerbations.

There was a modest correlation between some acute symptoms reported in diaries and the SGRQ, which measures the impact of symptoms on health-related quality of life.[16] Cumulative total symptom count and cumulative number of days of symptomatic exacerbation over the study period also correlated with SGRQ scores, confirming that symptomatic exacerbations have a significant impact on patients. However, our study design included only new or worsening symptoms rather than chronic symptoms, which presumably explains only a moderate correlation between diary-derived parameters and SGRQ scores.

In our analysis of pathogens isolated during symptomatic exacerbations, we detected a virus in 56% of patients' samples. This is similar to other reports; for example, Kainulainen et al[11] reported positive viral PCR in 54% of 65 exacerbations in 12 patients. Bacterial pathogens, most commonly encapsulated organisms, were found in 33% of symptomatic exacerbations.

Interestingly, in exacerbations positive for a pathogenic virus but in which the patient also expectorated sputum, there was evidence of purulence as measured by high microscopic granulocyte count in 69% of samples. Although this may be partly explained by underlying bronchiectasis in some patients, we frequently observed co-infection with bacteria. Although this
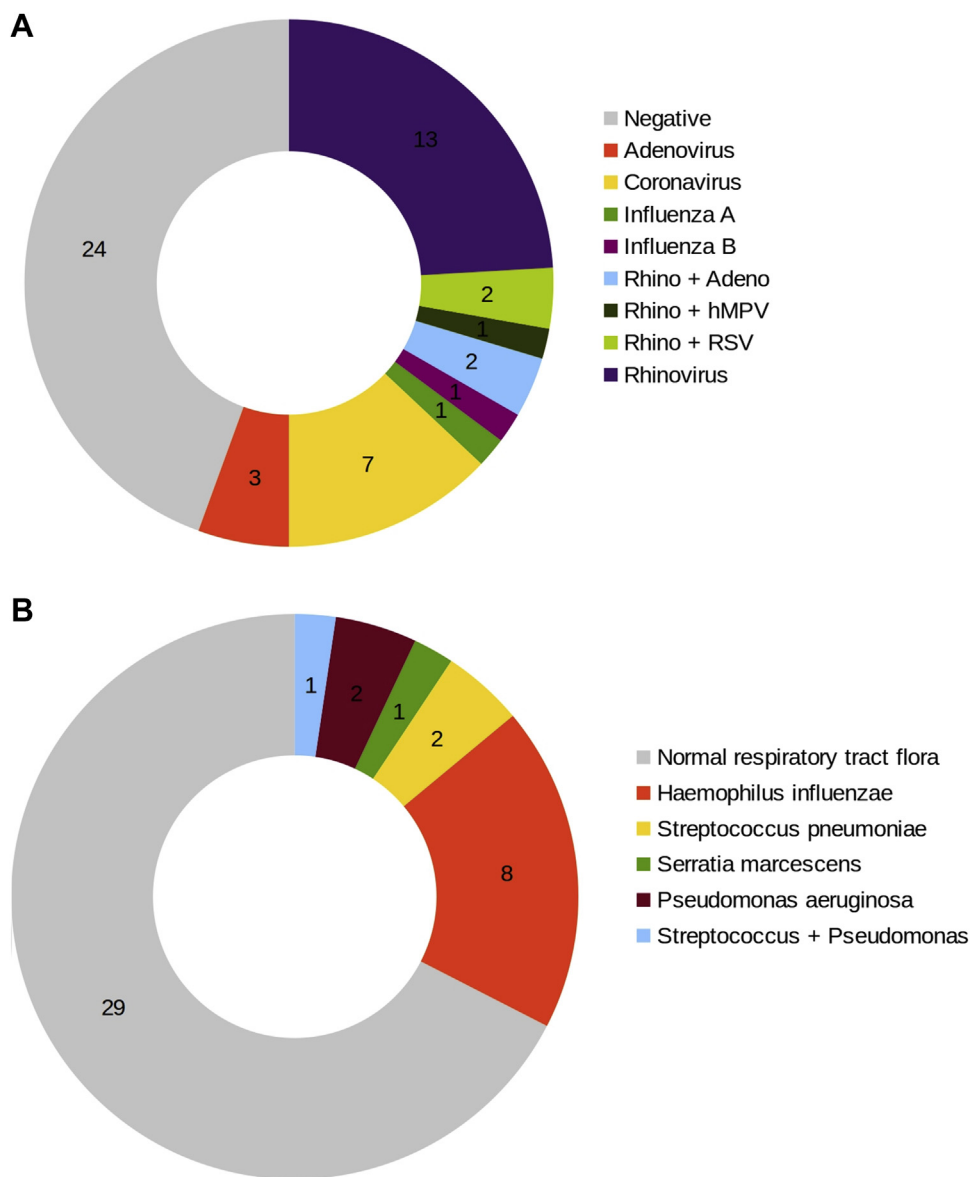
FIGURE 5. Pathogenic viruses and bacteria analysis. Viral and bacterial pathogens are frequently isolated in CVID-related respiratory exacerbations. **A**, Viral PCR was performed on nasopharyngeal swabs in 54 symptomatic respiratory exacerbations in patients with CVID. No pathogen (gray) was found in 24 (44%) exacerbations. A pathogenic virus was found in 30 (56%) exacerbations. Rhinovirus was found in 18 (33%) exacerbations, including 2 co-infections with adenovirus (Adeno), 2 with respiratory syncytial virus (RSV), and 1 with human metapneumovirus (hMPV). **B**, Bacterial culture was performed on spontaneously expectorated sputum in 43 symptomatic respiratory exacerbations in patients with CVID. No pathogen (gray) was found in 29 (67%) exacerbations. A pathogenic bacterium was found in 14 (33%) exacerbations. *Pseudomonas aeruginosa* was isolated in 3 (7%) exacerbations; among those was 1 co-infection with *Streptococcus pneumoniae*. Two patients (accounting for 4 exacerbations) were probably colonized with *Haemophilus influenzae*.

may represent simply colonizing bacteria in the presence of an acute viral exacerbation, there is evidence from COPD that rhinovirus infections adversely affect microbiome and the prevalence of pathogenic bacteria.[21,22] Our earlier results suggest that new or worsening purulent sputum predicts rapid response to antibiotic therapy, regardless of the organism isolated. Further research is required to investigate how the pathogens identified here influence the balance of other organisms in the respiratory tract and thereby the response to antibiotic therapy. However, our current recommendation would be to promptly treat exacerbations characterized by purulent sputum irrespective of virology results, not least because neutrophil elastase is significantly implicated in bronchiectasis pathogenesis.[22,23]

Our study has some limitations. It was performed at a single tertiary care center during the winter, when respiratory tract infections are more frequent.[24,25] The true incidence of symptomatic exacerbations and antibiotic use throughout the year thus cannot be extrapolated. We cannot exclude that factors particular to our geographic location and particular to the brief study period have influenced our results.

Because of its design, this study lacks a healthy control group. We therefore cannot discuss differences in quality or quantity of exacerbations between CVID and nonimmunocompromised patients, but available data from other sources[18] suggest that the usage of antibiotics in our cohort is many times higher than in the general population.

We have data only from patients who agreed to complete a diary (69 patients) and not the entire CVID cohort (134 patients). This may result in a selection bias, especially because these patients are older and have more clinically severe bronchiectasis. Although exacerbations did not differ in number or severity between patients with or without bronchiectasis, generalizability may be affected by variation in the prevalence of bronchiectasis throughout centers.[2]

The symptomatic definition of a respiratory exacerbation in CVID is not standardized and we therefore operated with a simplified definition, which has been validated in COPD.[17]

Although patients were carefully instructed to record only new or worse symptoms, we cannot exclude the possibility that some reported chronic morbidity. We note that the mean number of "new" symptoms even on days without antibiotic therapy was 0.86; however, this includes the period before and after antibiotic therapy in exacerbations and may also reflect a genuinely high frequency of acute symptoms.

Because many patients resided at a significant distance from the hospital, we were unable to perform microbiology and virology tests on diary-defined exacerbations and thus performed 2 parallel studies (Figure 1).

CONCLUSIONS

We have demonstrated that respiratory exacerbations are extremely common in CVID, but that patients delay starting antibiotics and ignore symptoms. Although viruses were identified commonly, patients should nevertheless be educated to take antibiotics promptly if they develop purulent sputum.

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ONLINE REPOSITORY
METHODS
Study design

For this observational, prospective cohort study, patients completed daily checkbox symptom diaries for 90 days between December 2014 and February 2015. Participants were asked to report new or increased respiratory symptoms from a predefined list (Figure E1). Chronic or stable symptoms were not to be reported. Patients reported daily the use of OAT to treat breakthrough infections and once a month the use of prophylactic antibiotics. Patients discontinued prophylactic antibiotics if they started OAT for breakthrough infections.

Participating patients were also asked to complete the SGRQ, a validated measure of respiratory health status scored between 0 (best) and 100 (worst) quality of life and on which 4 points is considered the minimum clinically important difference.[E1] The SGRQ was conducted at the end of the study period so that its 3-month recall period covered the period during which the diary was competed.

Baseline information on spirometry, computed chest tomography, previous bacterial isolates on sputum culture, Medical Research Council breathlessness score, and smoking status was collected from medical records and the departmental database. From these data, we calculated the bronchiectasis severity index, a validated multicomponent score in bronchiectasis that predicts the future risk of exacerbations, hospitalizations, and mortality.[E2]

Simultaneously, we conducted an observational cross-sectional study in which patients experiencing acute respiratory symptoms provided samples for bacterial and viral testing. A total of 54 nasopharyngeal swabs were collected from 41 patients if patients were able to tolerate the procedure. A total of 43 spontaneously expectorated sputum samples were obtained from 34 patients if patients were able to produce a sufficient quantity of sputum. These samples were either collected by clinic staff or, after careful instruction on sampling, submitted directly from patients by mail.

Recruitment

Patients were recruited from outpatient clinics at the Royal Free Hospital, London. All patients with a diagnosis of CVID and with written informed consent were included in the study (134 patients). There were no other exclusion criteria. We sent a copy of the diary and instructions to all 134 patients. An example is shown in Figure E1. Patients who decided to participate had at least 3 follow-up contacts during the study period via telephone, via post, or at clinical visits to provide counseling on the diary and to improve adherence. A total of 69 (51%) patients returned a fully completed diary.

At scheduled or ad-hoc visits to our outpatient clinics, patients in the cohort of consented patients with CVID were also asked to provide a microbiological sample at times of acute exacerbation. In all, 41 (31%) patients provided samples.

Definition of exacerbations

For preanalysis, we grouped clinically related symptoms as presented in Table I. Blocked nose, nasal discharge, and sinus pain were combined as "upper respiratory tract symptoms." Yellow and green sputum were combined as "purulent sputum," whereas any reported increase in sputum volume was analyzed as a single parameter. For the calculation of total symptom count, each symptom was counted individually, giving a theoretical range up to 9 total symptoms on each day.

For the purpose of analysis of exacerbations, we distinguish exacerbation "events" from exacerbation "episodes." *Events* are occurrences at one time point, whereas *episodes* are time periods with a defined start and ending. Each exacerbation event was associated with an exacerbation episode. For events, we calculate incidence rates, association with other parameters, and time to events. For episodes, we calculate time-dependent characteristics such as duration and total symptom count, and we plot symptoms prevalence over time.

We used 2 different definitions of exacerbation (Figure E2), based either on symptoms or health care utilization. For the first definition, we identified a symptomatic exacerbation event as 2 or more new symptoms lasting for 2 or more consecutive days as recorded by the patient in their diary, whether or not they received additional treatment for that.

Each symptomatic exacerbation event was associated with a symptomatic exacerbation episode. The start of a symptomatic exacerbation episode was the first day of 2 or more new symptoms lasting for 2 or more consecutive days. The end of a symptomatic exacerbation episode was defined as the last consecutive day with 2 or more symptoms, allowing symptoms to change over time. The duration of an episode is thus given as the time between the first day on which at least 2 symptoms occurred until the last day on which 2 or more symptoms were present.

If OAT was used during a symptomatic exacerbation episode, this was considered a TSE. If not, it was a USE.

On the basis of treatment only, we defined a health care utilization exacerbation as use of OAT for worsening of respiratory symptoms. We call this a TE, and if this event coincided with diary-defined symptoms it would be a TSE. In our study, 1 TE did not fulfill the criteria of a TSE.

In analogy to the definition of symptomatic exacerbation episodes, each TE event was associated with a TE episode. Each TE episode lasted from the first day on which a symptom occurred until the last day a symptom was still present out of all symptoms present on the day OAT was started. Time until OAT was defined as the period between the first day of symptom occurrence and the first day of OAT. Time until recovery was defined as the period between the day OAT was started and the day all symptoms ended. Together, time until OAT and time until recovery represent TE duration. If a TE was only partially covered within the study period, we used censored data for time until OAT and time until recovery.

Data handling and statistical analysis

Statistical analysis was performed using Stata version 14.0 (StataCorp LP, College Station, Tex). We summarized continuous variables by median and IQR or by mean and SDs as appropriate. For categorical and binary variables, we present frequencies. Results were considered to be statistically significant at a P value of less than .05.

Missing data were not imputed. Six patients in the diary group did not complete the SGRQ. Data for these patients were ignored for the quality-of-life Analysis. Because of missing data, we were unable to calculate the bronchiectasis severity index for 19 patients (7 in the diary group). Computed tomography scans were not available for 11 patients (5 in the diary group). Data on lung function were missing for 13 patients (3 in the diary group).

Instructions: You fill in the boxes every single day. Please tick the box with a check-mark (√) or the according symbol, only if symptoms are new or worse than before. If your symptoms have stayed the same as always or if you do not have them on that day, please write in -.

Time (d)	31 Dec 14	1 Jan 15	2 Jan 15	3 Jan 15	4 Jan 15	5 Jan 15	6 Jan 15	7 Jan 15	8 Jan 15	9 Jan 15	10 Jan 15	11 Jan 15	12 Jan 15	13 Jan 15	14 Jan 15
Blocked Nose	-	-	-	√	√	√	√	-	-	-	-	-	-	-	-
Nasal discharge	-	√	√	√	√	√	√	√	√	-	-	-	-	-	-
Sinus pain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sore Throat	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cough	-	-	-	√	√	√	√	√	-	-	-	-	-	-	-
Short of Breath	-	-	-	√	√	√	√	√	-	-	-	-	-	-	-
Wheeze	-	-	-	√	√	√	√	√	-	-	-	-	-	-	-
Sputum color	-	-	-	g	g	g	g	g	-	-	-	-	-	-	-
Sputum Volume	-	-	-	eg	eg	eg	eg	eg	-	-	-	-	-	-	-
OAT	-	-	-	-	-	√	√	√	√	√	√	√	√	√	√

FIGURE E1. Example of a section of the symptom diary. Check marks (√) indicate presence of the respective symptom or OAT. Dashes (-) indicate absence of specific symptom or OAT. Participants recorded their sputum color as absent/unchanged (-), white (w), yellow (y), or green (g). Sputum volume was recorded as absent/unchanged (-), increase equivalent in volume to a teaspoon (ts), egg cup (eg), or cup (c). Participants were asked to record new or increased symptoms only.

Trigger symptom analysis and antibiotic response analysis. When analyzing which symptoms trigger patients to start antibiotic treatment, only days without OAT and the first day after starting OAT were included in analysis. All other days in a consecutive course of OAT were ignored. First, to examine the association of each symptom separately with the start of OAT we calculated ORs and CIs for each symptom in univariate analysis. Next, we included all symptoms that proved to be significant in univariate analysis in a logistic regression model to analyze their effect adjusted for the other symptoms.

Similarly, ORs were calculated to test the association between total symptom count or time since start of symptoms and the start of OAT. The time since start of symptoms is defined as the number of days for which 2 or more symptoms have been present.

To assess response to OAT we analyzed the time until recovery after the start of OAT and the cumulative total symptom count after the start of OAT. The cumulative total symptom count is the sum of all symptoms on all days from start of OAT until the last day a symptom was still present. Because the results using either parameter were similar, we report time until recovery only.

To analyze the predictive value of each symptom for the response to OAT separately, we first used univariate Cox regression models. Next, we used a multivariate Cox regression model for all symptoms that proved to be significant predictors in the univariate setting to examine their effect adjusted for the other symptoms. To analyze the predictive value of time until OAT and total symptom count on the day before start of OAT for the response to OAT, we used univariate Cox models.

Exacerbation severity analysis. As indicators for exacerbation severity, we used exacerbation duration and cumulative total symptom count over the exacerbation episode. Cumulative total symptom count is defined as the sum of all symptoms over the duration of an exacerbation episode. We used Cox models to compare durations and Wilcoxon rank sum tests to compare cumulative total symptom counts.

Bronchiectasis and prophylactic antibiotics analysis. To analyze the impact of prophylactic antibiotics and bronchiectasis, we compared patients on or off prophylactic antibiotics and with or without radiological evidence of bronchiectasis in regard to number of exacerbations, exacerbation severity in symptomatic exacerbations, time until OAT, and time until recovery. We used t tests to compare means of the number of symptomatic exacerbations, the number of TSEs, and the number of USEs. The significance of the difference in the difference between mean values was assessed by an interaction test in a 2-way ANOVA. Time until OAT, time until recovery, and episode duration in symptomatic exacerbations were compared using univariate Cox regression models.

Quality-of-life analysis. The SGRQ is a validated and widely used measurement of respiratory health based on a 3-month recall period.[E1] Its total score is a continuous variable ranging from 0 to 100, where 0 indicates low and 100 indicates high impairment of respiratory health.[E1]

On the basis of our diary data, we calculated the number of days on which a specific symptom was present over the entire study period of 90 days, the cumulative total symptom count summing up the total symptom count over the entire study
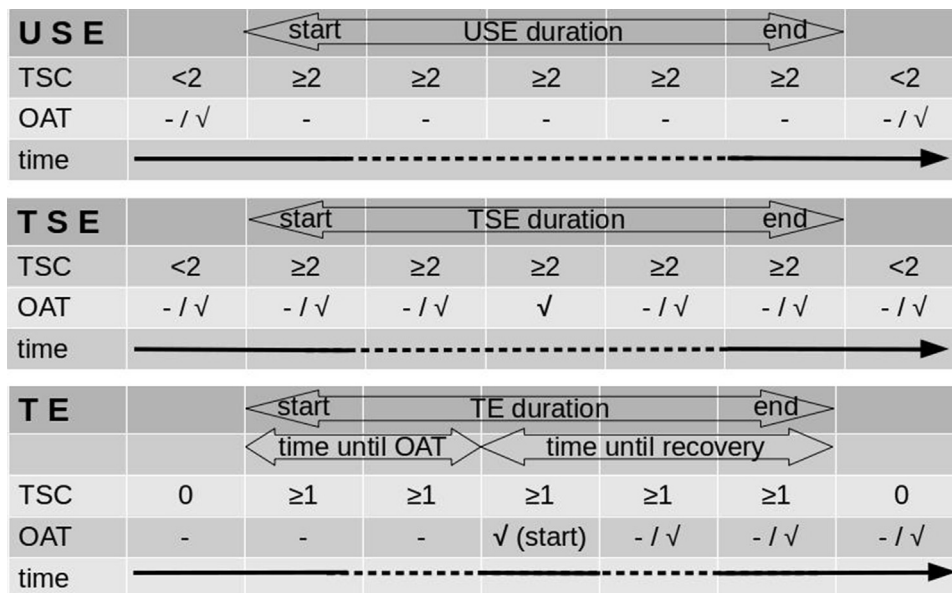
FIGURE E2. Illustration of different exacerbation definitions. Check marks (√) indicate the use of OAT on the day marked, dashes (-) indicate OAT was not used, and "-/√" indicates either use or nonuse. USE is defined as an event with a total symptom count (TSC) equal or greater than 2 for 2 or more consecutive days in absence of OAT during the episode. TSE is defined as an event with a total symptom count equal or greater than 2 for 2 or more consecutive days and use of OAT during the episode. TE is defined as use of OAT. The start of a TE episode is defined as the first day a symptom occurs and which is still present on the day OAT is started. The end of a TE episode is defined as the last day a symptom is present and which is also present on the day OAT is started. Time until OAT is defined as the period between TE start and OAT start. Time until recovery is defined as the period between OAT start and TE end.

period, and the number of days fulfilling the criteria for symptomatic exacerbation over the entire study period.

We then calculated correlation coefficients between the SGRQ total score and the number of days on which specific symptoms were present, cumulative total symptom count, and cumulative number of days of symptomatic exacerbation.

Analysis of microbial samples

An in-house multiplex RT-PCR was used for detection of 15 RNA viruses (influenza A, influenza B, parainfluenza virus [PIV] 1, PIV 2, PIV 3, PIV 4, rhinovirus, enterovirus, human metapneumovirus, respiratory syncytial virus, parechovirus, human coronavirus OC43, NL68, 229E, HKU) and 1 DNA virus (adenovirus). Phocine distemper virus was added as internal control to each sample and a primer pair and probe were designed to amplify K-Ras oncogene. Cloned complementary DNA (cDNA) positive controls for each of the targeted viruses were included; a negative control consisting of the reaction mixture and PCR-grade water was included in each run. Nucleic acid was extracted from 0.5 mL of nasopharyngeal swab using the automated extraction platform NucliSENS(r) easyMAG(r) (Biomerieux, Marcy l'Etoile, France). The Superscript III platinum one-step reverse transcription kit (Invitrogen, Paisley, UK) was used to generate and amplify cDNA. RT-PCR was

performed on the TaqMan 7500 sequence detection system (Applied Biosystems, Foster City, Calif).

Sputum samples were examined by microscopy and culture for bacteria and mycobacteria plus an in-house multiplex RT-PCR for *Chlamydia pneumoniae*, *Legionella pneumophila*, and *Mycoplasma pneumoniae*. Techniques used in our laboratories for sputum processing, microbiological culture, and multiplex PCR detection of bacteria have been described in greater detail elsewhere.[E3]

We included multiple samples from a single patient if the samples were separated by at least 2 weeks and the patient was asymptomatic between episodes. Airway colonization by pathogenic bacteria was considered possible if the same organism had been isolated more than twice within the 2 years before our study.

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