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The Role of Viral Infection in Pulmonary Exacerbations of Bronchiectasis in Adults

A Prospective Study

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BACKGROUND: Although viral infections are a major cause of exacerbations in patients with chronic airway diseases, their roles in triggering bronchiectasis exacerbations in adults remain unclear. Therefore, we prospectively investigated the incidence and clinical impacts of viral infection in adults with bronchiectasis exacerbations.

METHODS: The study cohort of 119 adults with bronchiectasis was followed up prospectively for 12 months. Nasopharyngeal swabs and sputum samples were assayed for 16 respiratory viruses, using polymerase chain reaction assays. Symptoms, spirometry, quality of life, bacterial cultures, and inflammatory markers were assessed during steady-state bronchiectasis and exacerbations.

RESULTS: A total of 100 exacerbations were captured from 58 patients during 1-year follow-up. Respiratory viruses were found more frequently in nasopharyngeal swabs and sputum during bronchiectasis exacerbations (49 of 100, 49.0%) than during steady state (11 of 58, 18.9%; $P < .001$). The most common viruses found in patients experiencing exacerbations were coronavirus (19 of 65, 39.2%), rhinovirus (16 of 65, 24.6%), and influenza A/B viruses (16 of 65, 24.6%). Virus-positive exacerbations were associated with a greater increase in markers of systemic and airway inflammation (serum IL-6 and tumor necrosis factor-α; sputum IL-1β and tumor necrosis factor-α) compared with virus-negative exacerbations, but the differences in spirometric indexes, quality of life, and bacterial density were unremarkable. In receiver operating characteristics analysis, serum interferon-γ-induced protein 10 yielded an area under curve of 0.67 (95% CI, 0.53-0.77; $P = .018$). Furthermore, a greater proportion of patients with virus-positive exacerbations received IV antibiotics.

CONCLUSIONS: Prevalence of viral infections, detected by polymerase chain reaction assay, is higher in cases of bronchiectasis exacerbations than in steady-state bronchiectasis, suggesting that respiratory viruses play crucial roles in triggering bronchiectasis exacerbations. The potential mechanisms of virus-induced bronchiectasis exacerbations merit further investigations.

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ABBREVIATIONS: BE = bronchiectasis exacerbation; BSI = Bronchiectasis Severity Index; CAT = COPD Assessment Test; CFU = colony-forming unit; CRP = C-reactive protein; HCoV = human coronavirus; HRCT = high-resolution CT; IP-10 = interferon-γ-induced protein 10; IQR = interquartile range; LCQ = Leicester Cough Questionnaire; NPS = nasopharyngeal swab; PCR = polymerase chain reaction; QoL = quality of life; SGRQ = St. George's Respiratory Questionnaire; TNF = tumor necrosis factor

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Patients with bronchiectasis frequently develop acute infective exacerbations characterized by increased cough frequency and sputum volume and purulence that readily lead to hospital admissions with high treatment costs.[1,2] More importantly, frequent exacerbations impose adverse effects on quality of life (QoL)[3] and might accelerate loss of lung function and increase mortality.[4,5] To date, however, triggers of bronchiectasis exacerbations (BEs) have been poorly elucidated. Because antibiotic treatment of BEs has been shown to mitigate clinical symptoms, restore lung function, and concurrently suppress systemic and airway inflammatory responses,[6,7] BEs have been postulated to result from airways bacterial infections of novel strains, increased bacterial density, or both.[8] However, Tunney et al[9] showed that total bacterial density and microbiota taxa, analyzed by culture and 16s ribosomal DNA pyrosequencing, did not change remarkably during antibiotic treatment of BEs, suggesting that changes in lung microbiota composition might not account significantly for BEs. This has led us to examine the roles of other potential pathogens, especially viruses, in adults with bronchiectasis.

It has been well established that respiratory viral infections are a major trigger of acute exacerbations of COPD,[10,11] asthma[12,13] and cystic fibrosis.[14,15] A recent study in children with bronchiectasis found that respiratory viruses were detected in 48% of 77 pediatric pulmonologist-defined BEs and were associated with worse clinical outcomes.[16] However, significance of viral infection in adults with BEs remains unknown. In addition, clinical characteristics and investigations regarding virus-associated BEs in adults are also lacking, and the interactions between respiratory viruses, bacteria, and host immune response, which might directly relate to pathogenesis of bronchiectasis, as shown in COPD,[17] deserve special attention from researchers and clinicians. Meanwhile, biomarkers such as serum interferon-γ-induced protein 10 (IP-10) have been proposed as surrogate markers for predicting virus-associated exacerbations of COPD,[18] but the utility in bronchiectasis has never been assessed. In this prospective study, we sought to (1) document the incidence of 16 common respiratory viruses in adults with bronchiectasis during steady-state bronchiectasis and BEs, (2) investigate the clinical differences between virus-positive and virus-negative BEs, and (3) assess the utility of serum IP-10 for predicting virus-related BEs.

Materials and Methods

Patients and Study Procedure

Patients with bronchiectasis, diagnosed by compatible history combined with bronchial dilatation on high-resolution CT (HRCT) scan, were recruited from outpatient clinics of The First Affiliated Hospital of Guangzhou Medical University and a previous cohort assessing the effects of anxiety and depression on BEs[19] between February and March 2013. Patients were aged ≥ 18 years and remained clinically stable, defined as no BEs or antibiotic treatment of respiratory infection within 4 weeks. The study was approved by Ethics Committee of The First Affiliated Hospital of Guangzhou Medical University (approval No. Medical Ethics 2012 [the 29th file]). Written informed consent was obtained from all participants.

Within the span of 12 months, patients were assessed at baseline, when clinically stable, and at BEs. During follow-up, patients were required to attend chest clinics routinely every 3 months. Between the neighboring scheduled visits, patients experiencing symptoms of exacerbations were requested to contact investigators immediately, with an additional visit being scheduled within 48 h. BEs were defined as persistent (> 24 h) deterioration in at least three respiratory symptoms, including cough, dyspnea, hemoptysis, increased sputum purulence or volume, chest pain, febrile, radiographic deterioration, systemic disturbances, or changes in chest auscultation.[20] Spontaneous or induced sputum was collected at each visit for bacterial culture, viral assays, and measurement of airway inflammation. Paired serum samples were collected for assessment of systemic inflammation. All parameters were measured when clinically stable and at times of BE.

Clinical Assessments

A comprehensive history including age, sex, BMI, influenza vaccination in the preceding year, the number of infective exacerbations and hospitalizations in the previous 24 months, and current treatment were recorded at baseline. Chest HRCT scan within 12 months was used for radiologic scoring based on the number of bronchiectatic lobes (with the lingula being scored as a separate lobe) and severity of bronchial dilatation (tubular: 1 point, varicose: 2 points, cystic: 3 points), with the maximal score of 18.[21] Etiology of bronchiectasis was determined after meticulous testing recommended by British Thoracic Society guidelines[2] and group discussion (Yong-hua Gao, W. G., and G. X.). Upper and lower airway symptoms were recorded when clinically stable and at BEs. Bronchiectasis severity at baseline was quantified according to Bronchiectasis Severity Index (BSI).[4] QoL was assessed using St. George's Respiratory Questionnaire (SGRQ), Leicester Cough Questionnaire (LCQ), and COPD Assessment Test (CAT)

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for all patients when clinically stable and those with the first BEs who had one or more exacerbations.[6,22]

Assessment of Bacterial Density and Lung Function

Methods for assessing sputum bacterial density for *Pseudomonas aeruginosa*, *Haemophilus influenza*, and other potentially pathogenic microorganisms have been introduced previously.[23] Details are provided in e-Appendix 1. Details of lung function measurement are given in e-Appendix 1.

Assessment of Systemic and Airway Inflammatory Mediators

Levels of IL-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α in sputum, and IL-6, IL-8, TNF-α, and IP-10 in serum were measured using commercial multiplex bead-based assay kits (Bio-Plex Cytokines Assay; Bio-Rad Inc) (14). C-reactive protein (CRP) level and WBC counts were also measured as systemic inflammatory markers. Details are given in e-Appendix 1.

Virus Detection in Nasopharyngeal Swab and Sputum

Nasopharyngeal swab (NPS) and sputum were sampled for virology analysis. Sixteen common respiratory tract pathogens were tested using TaqMan reverse transcriptase-polymerase chain reaction (PCR) as previously reported,[24] including rhinovirus, influenza viruses A and B, parainfluenza viruses (1, 2, 3, and 4), human coronovirus (HCoV) (HCoV-229E, OC43, NL63, and HKU1), respiratory syncytial virus, adenovirus, enterovirus, bocavirus, and human metapneumovirus. Specimens with cycle threshold ≤35 were deemed positive, according to manufacturer's specifications (Guangzhou HuYanSuo Medical Technology Co, Ltd). Lower cycle threshold values indicated that fewer cycles of PCR amplifications were needed to detect viral nucleic acids and, hence, reflected a higher magnitude of PCR positivity. Details of virus detection are provided in e-Appendix 1.

Statistical Analysis

Because studies investigating the roles of viral infections in bronchiectasis are lacking, by assuming that the detection rate of viruses in NPS and sputum in steady-state bronchiectasis was 19%, imputed from COPD,[11] 100 participants in the baseline subgroup and exacerbation subgroup during follow-up were required to detect a 20% difference in the proportion of viral infection with a two-sided significance level of $P = .05$ and power of 90%. Therefore, we planned to include 120 patients (assuming a dropout rate of 20% to capture sufficient exacerbation events during 1-year follow up). Data were presented as mean (SD), median (interquartile range [IQR]), or counts (proportion), as appropriate. Bacterial density (colony-forming units [CFU]/mL) was logarithmically transformed before analyses. Categorical variables were compared using χ^2 tests. For continuous variables, two-group comparisons were performed using unpaired *t* tests or Mann-Whitney tests when appropriate; one-way analysis of variance or Kruskal-Wallis test were applied for multiple-group comparisons as appropriate. Logistic regression analyses were performed to examine the relationship between independent variables and presence of viruses. The utility of IP-10 in predicting viral infection during BEs was investigated using receiver operating characteristics curve. Results were presented as area under the curve and 95% CI. A *P* value $< .05$ was considered statistically significant for all analyses. Statistical analyses were performed using SPSS 16.0 (IBM) and Graphpad Prism, version 5.0 (GraphPad Software Inc).

Results

Patients

Of 119 patients recruited (Fig 1), 58 with at least one reported BE during 1-year follow-up were included in the final analyses. These 58 patients experienced 127 BEs, with 100 reported BEs over 12-month follow-up. During the study period, 26 patients reported no BEs and 35 experienced BEs but failed to report them and, therefore, were not sampled. These 35 patients had a total of 77 unreported exacerbations. Thus, 119 patients had a total of 204 exacerbations (mean, 1.7 exacerbations per patient per year) during the whole study period. The characteristics and underlying causes of bronchiectasis of 58 included patients did not differ from those of the 61 patients who dropped out (Table 1, e-Table 1). Of the 58 patients, 24 (41.4%) had *Pseudomonas aeruginosa* colonization at baseline. Of these, only one received influenza vaccination in autumn before the study, and 34 patients (58.6%) had idiopathic/postinfective bronchiectasis in the baseline cohort. Baseline characteristics using different BSI scores are presented in the online supplement (e-Table 2).

Detection of Respiratory Viruses

Median time to sampling was 3 d (IQR, 2-5 d), which was unrelated to virus detection ($P = .79$). Respiratory viruses were detected in NPS or sputum in 49 of 100 reported BEs (49.0%) and 11 of 58 patients with bronchiectasis (18.9%) at baseline who then experienced exacerbations ($P < .001$) (Fig 2). None of the adults who were virus-positive at baseline had identical virus detected during subsequent exacerbations. Thirty (51.7%) were positive for a respiratory virus at the first BEs. Of the 24 patients with two or more reported BEs, only four had identical virus at two different BEs. In the 49 BE events, 65 viruses were detected, including 13 episodes of multiple viruses (e-Table 3). Three of 58 patients who reported exacerbations had multiple viruses isolated at steady-state bronchiectasis (one each of influenza with rhinovirus, influenza with HCoV-OC43, and influenza with HCoV-229E). The distribution of viruses detected is shown in Table 2 according to study group and type of specimens analyzed. The most common viruses were coronavirus, rhinovirus, and influenza A and B. The detected viruses in BEs according to bronchiectasis severity, assessed by BSI, are summarized in e-Table 4. Patients with moderate to severe bronchiectasis (BSI ≥ 5) had higher positivity of viral infection than those with mild bronchiectasis (BSI = 0-4; 56.0% vs 28.0%, respectively; $P = .015$).

Complete sets of virology samples (NPS and sputum) were collected at 100 episodes of BE without missing samples. Of these, 45 viruses could be detected in 39 (39.0%) compared with five viruses in five of 58 patients

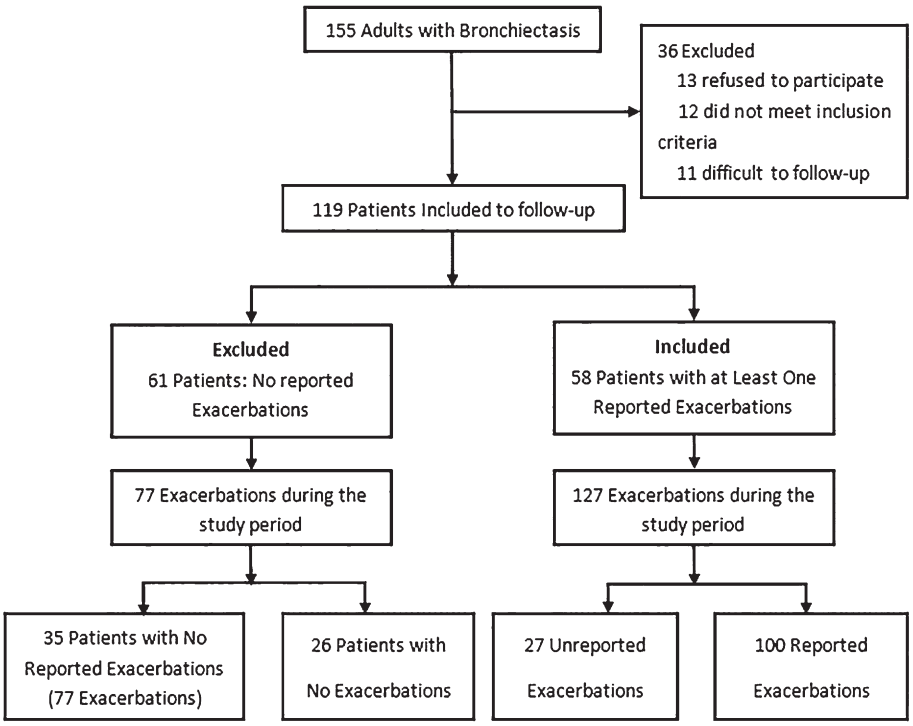

Figure 1 – *Flowchart of patient recruitment.*

during steady-state bronchiectasis in NPS (8.6%; $P < .001$) (Fig 2, Table 2). Forty-two viruses were detected in 35 of 100 episodes of BE (35.0%) compared with 11 viruses in eight of 58 patients during steady-state bronchiectasis in sputum (13.8%; $P < .001$) (Fig 2, Table 2). Comparing PCR results of the upper and lower airways

TABLE 1] Characteristics of All Enrolled Patients (Cohort A) and Those Who Were Sampled During Bronchiectasis Exacerbations (Cohort B)

Parameters	Cohort A (n = 119)	Cohort B (n = 58)	*P* Value[a]
Age, y	44.46 (14.34)	44.27 (14.35)	.93
Male sex, No. (%)	42 (35.29)	20 (34.48)	.92
BMI, kg/m^2	20.24 (3.16)	19.84 (2.98)	.43
Bronchiectasis severity index	6.73 (3.96)	7.14 (4.45)	.54
Baseline spirometry			
FEV_1, L	1.83 (0.74)	1.69 (0.69)	.24
FEV_1 % predicted	64.90 (23.04)	62.66 (23.02)	.55
FVC, L	2.50 (0.85)	2.42 (0.84)	.58
FVC % predicted	74.55 (19.79)	73.33 (21.06)	.95
FEV_1/FVC %	71.81 (14.06)	69.11 (13.13)	.23
Exacerbation frequency in the past 12 mo	2 (1.00-3.00)	2 (1.00-2.00)	.60
Hospital admission in the past 12 mo	0 (0.00-1.00)	0 (0.00-1.00)	.66
Modified Reiff score for chest HRCT scan	7.55 (3.89)	8.23 (3.95)	.28
Pseudomonas aeruginosa colonization, No. (%)	43 (36.13)	24 (41.38)	.50
Treatment, No. (%)			
Inhaled corticosteroids	15 (12.61)	9 (15.52)	.60
Macrolides	41 (34.45)	26 (44.83)	.18
Influenza vaccination in last 12 mo	1 (0.84)	1 (1.72)	.61

Data are presented as mean (SD) or median (IQR) unless otherwise indicated. HRCT = high-resolution CT; IQR = interquartile range.
[a]*P* values refer to comparisons between groups using χ^2 test (categorical data), analysis of *t* test, or Mann-Whitney test, when appropriate.

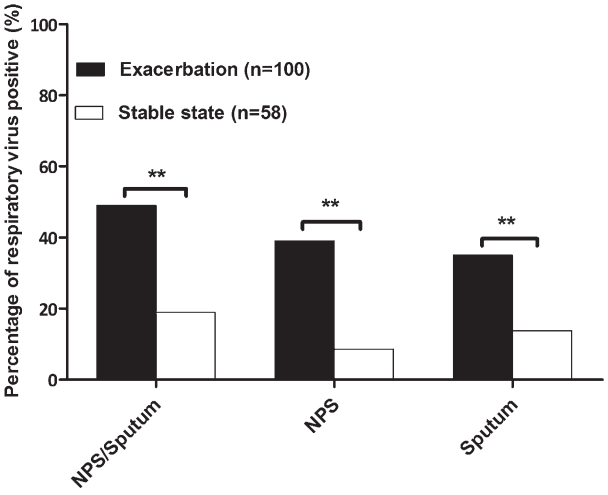

Figure 2 – *Detection of respiratory viruses in NPS and sputum at steady-state bronchiectasis and bronchiectasis exacerbations by polymerase chain reaction assays.* **P < .001. *NPS = nasopharyngeal swab.*

during BEs, we found respiratory viruses in 25 of 49 virus-positive BEs (51.0%) in both NPS and sputum (κ = 0.49), with the same type of viruses detected in 22 virus-positive BEs (44.9%). Additionally, the rates of virus detection in NPS only (14 of 49, 28.6%) were not different from those only in sputum (10 of 49, 20.4%) ($P = .35$). The mean cycle threshold value of PCR for virus-positive specimen at BEs was lower than that when clinically stable (BE, 28.44 [SD, 4.27]; steady-state bronchiectasis, 30.84 [SD, 2.44]; $P = .032$).

Impacts of Viral Infection on Clinical Indexes at BEs

The odds of virus-positive BEs being associated with individual symptoms are summarized in e-Table 5. Blocked nose and hoarseness were more common at virus-positive BEs. QoL was measured in 58 patients with the first BEs. Of these, 30 were in the virus-positive group and 28 in the virus-negative group. Changes in SGRQ, LCQ, and CAT total scores between BEs and steady-state bronchiectasis in patients in the virus-positive group were more significant (albeit not statistically significant) than those in patients in the virus-negative group (Table 3).

TABLE 2] Distribution of Viruses in NPS or Sputum During 49 of 100 Exacerbations Reported by 58 Patients With Bronchiectasis

	All		Sputum		NPS	
Virus	Exacerbation	Steady State	Exacerbation	Steady State	Exacerbation	Steady State
Cases, No.	100	58	100	58	100	58
Total viruses, No.	65	14	42	11	45	5
Coronavirus	19 (19.0)	5 (8.6)	9 (9.0)	3 (5.2)	15 (15.0)	3 (5.2)
229E	6 (6.0)	1 (1.7)	0 (0.0)	1 (1.7)	6 (6.0)	0 (0.0)
OC43	9 (9.0)	2 (3.4)	5 (5.0)	2 (3.4)	6 (6.0)	1 (1.7)
NL63	2 (2.0)	0 (0.0)	2 (2.0)	0 (0.0)	2 (2.0)	0 (0.0)
HKU1	2 (2.0)	2 (3.4)	2 (2.0)	0 (0.0)	1 (1.0)	2 (3.4)
Rhinovirus	16 (16.0)	2 (3.4)	14 (14.0)	2 (3.4)	10 (10.0)	1 (1.7)
Influenza	16 (16.0)	4 (6.9)	8 (8.0)	3 (5.2)	14 (14.0)	1 (1.7)
Influenza A	12 (12.0)	3 (5.2)	4 (4.0)	3 (5.2)	10 (10.0)	0 (0.0)
2009 Influenza A (H1N1)	3 (3.0)	0 (0.0)	3 (3.0)	0 (0.0)	3 (3.0)	0 (0.0)
Influenza B	1 (1.0)	1 (1.7)	1 (1.0)	0 (0.0)	1 (1.0)	1 (1.7)
Parainfluenza	3 (3.0)	0 (0.0)	2 (2.0)	0 (0.0)	1 (1.0)	0 (0.0)
Type 1	1 (1.0)	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
Type 2	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)
Type 3	1 (1.0)	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
Type 4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Respiratory syncytial virus	7 (7.0)	0 (0.0)	5 (5.0)	0 (0.0)	3 (3.0)	0 (0.0)
Metapneumovirus	1 (1.0)	0 (0.0)	1 (1.0)	0 (0.0)	1 (1.0)	0 (0.0)
Enterovirus	2 (2.0)	1 (1.7)	2 (2.0)	1 (1.7)	0 (0.0)	0 (0.0)
Adenovirus	1 (1.0)	2 (3.4)	1 (1.0)	2 (3.4)	1 (1.0)	0 (0.0)

Data given as No. (%) unless otherwise indicated. NPS = nasopharyngeal swab.

TABLE 3] Changes in Quality of Life at Virus-Positive and Virus-Negative BEs

Parameter	Virus-Positive BEs (n = 30)	Virus-Negative BEs (n = 28)	*P* Value
△SGRQ score[a]	10.10 (13.59)	6.75 (13.26)	.35
△LCQ score[a]	−2.50 (3.13)	−1.41 (3.29)	.20
△CAT score[a]	8.13 (7.83)	5.11 (7.48)	.14

Data given as parameter score (SD) unless otherwise indicated.
BE = bronchiectasis exacerbation, CAT = COPD Assessment Test; △ = change in; LCQ = Leicester Cough Questionnaire; SGRQ = St. George's Respiratory Questionnaire.
[a]Mean change between BEs and steady-state bronchiectasis.

Spirometry was assessed in 43 (87.8%) virus-positive and 46 (90.2%) virus-negative BEs. Virus-positive BEs were not associated with greater decline in FEV_1 (mean, −0.12 L [SD, 0.31 L] vs −0.08 L [SD, 0.23 L]; $P = .48$) compared with virus-negative BEs.

Bacterial density was measured in 45 (91.8%) virus-positive and 48 (94.1%) virus-negative BEs. Patients with *P aeruginosa* infection at baseline and BEs were included. Of these, 19 (42.2%) were in virus-positive BEs and 12 (25.0%) in virus-negative BEs. Changes in mean total bacterial density in virus-positive BEs were not different from those in virus-negative BEs (0.01 $\log_{10}$ CFU/mL [SD, 0.93] vs 0.10 $\log_{10}$ CFU/mL [SD, 0.99]; $P = .78$).

CRP level and total leukocyte count were obtained in 44 (89.8%) virus-positive and 48 (94.1%) virus-negative BEs. The levels of IL-6, IL-8, TNF-α, and IP-10 were obtained in 41 (83.7%) virus-positive and 43 (84.3%) virus-negative BEs. For all BEs, median values for serum IL-6 and TNF-α increased to 1.66 pg/mL (IQR, 0.00-9.91) and 0.27 pg/mL (IQR, 0.00-5.68), respectively, during virus-positive BEs, which were greater than those in virus-negative BEs ($P = .042$ and $P = .022$, respectively) (Table 4). However, there were no significant changes in other systemic inflammatory parameters, including CRP level, WBC count, and IL-8 level, between virus-positive and virus-negative BEs (Table 4). Sputum samples at BEs (n = 89) were available to measure airway inflammation, with 42 (85.7%) in the virus-positive and 47 (92.2%) in the virus-negative group. Virus-positive BEs were associated with greater changes for sputum IL-1β and TNF-α levels (median, 19.37 ng/mL [IQR, 0.64-33.86] and 12.42 ng/mL (IQR, 1.00-39.46), respectively) than virus-negative BEs ($P = .03$ and $P = .024$, respectively), but not sputum IL-6 and IL-8 levels (Table 4).

Furthermore, virus-positive exacerbations were associated with greater likelihood of receiving IV antibiotics than virus-negative exacerbations (33 of 49 [67.3%] vs 22 of 51 [43.1%]; $P = .015$), but not hospitalization (eight of 49 [16.3%] vs six of 51 [11.8%]; $P = .51$].

Diagnostic Value of Serum IP-10 for Viral Infection at BEs

Median values for serum IP-10 were significantly increased in patients with respiratory viruses detected at BEs (virus-positive, 2.27 ng/mL [IQR, 1.28-3.79]; virus-negative, 1.43 ng/mL [IQR, 1.02-2.46] ; $P = .018$). The area under the receiver operating characteristic curve for serum IP-10 to identify virus-positive BEs was 0.65 (95% CI, 0.53-0.77) with the threshold of 2,049.64 pg/mL, corresponding to the sensitivity of 0.60 (95% CI, 0.43-0.74) and specificity of 0.64 (95% CI, 0.48-0.78) (Fig 3). The positive and negative predictive values were 0.60 and 0.64, respectively.

Discussion

To our knowledge, this was the first large-scale, prospective study to determine the incidence and clinical impact of viral infection, by using PCR-based methods, on BEs in adults. We found that the following: (1) BEs were significantly associated with the presence of viruses in NPS and sputum, with coronavirus, rhinovirus, and influenza virus being most common; (2) virus-positive BEs were associated with significant increase in systemic and airway inflammatory markers, and prone to require IV antibiotic therapy compared with virus-negative BEs. (3) Viral infection at BEs did not have significant effects on QoL (albeit greater deterioration in virus-positive BEs), lung function, and bacterial density; and (4) serum IP-10 levels had limited significance for predicting viral infection at BEs.

We detected respiratory viruses significantly more often in BEs (49.0%) than in steady-state bronchiectasis (18.9%). The overall incidence of viral infection in BEs was consistent with previous studies regarding pediatric bronchiectasis, COPD, and cystic fibrosis.[11,15,16] Additionally, mean PCR CT value for BEs was markedly lower (28.44 [SD, 4.27] vs 30.84 [SD, 2.44]; $P = .032$), indicating higher magnitude of PCR positivity. These findings unanimously support a pivotal role for respiratory viruses in BEs, as evidenced in COPD and cystic fibrosis.[11,15,25] Coronavirus, rhinovirus, and influenza virus were commonly identified at BEs, which was in keeping with the study of COPD conducted in Hong Kong, China.[10] The higher detection rate of influenza virus might be associated with the extremely low influenza

TABLE 4] Changes in Inflammatory Mediators[a] at Virus-Positive and Virus-Negative BEs

Inflammatory Markers	Virus-Positive Exacerbation (n = 42)	Virus-Negative Exacerbation (n = 43)	*P* Value
Changes in systemic inflammatory markers			
WBC,[b] mean (SD), $\times 10^9$/L	1.47 (2.84)	1.80 (2.50)	.55
CRP,[b] mg/dL	0.97 (0.22 to 3.46)	0.74 (0.08 to 2.55)	.37
IL-6, pg/mL	1.66 (0.00 to 9.91)	0.27 (0.00 to 5.68)	.042[c]
IL-8, pg/mL	1.50 (−0.16 to 5.45)	1.36 (−1.35 to 2.71)	.26
TNF-α, pg/mL	0.23 (−0.03 to 8.23)	0.00 (−0.51 to 1.17)	.022[c]
Changes in airway inflammatory markers	n = 42	n = 47	
IL-1β, ng/mL	19.37 (0.64 to 33.86)	4.76 (−4.16 to 17.25)	.03[c]
IL-6, ng/mL	2.92 (−1.71 to 10.01)	2.90 (−0.02 to 5.30)	.84
IL-8, ng/mL	7.21 (−31.10 to 26.39)	3.93 (−39.67 to 42.81)	.72
TNF-α, ng/mL	12.42 (1.00 to 39.46)	2.70 (−4.35 to 15.50)	.024[c]

Data given as median (IQR) unless otherwise indicated. CRP = C-reactive protein; TNF = tumor necrosis factor. See Table 3 legend for expansion of other abbreviation.

[a]Changes between BEs and steady-state bronchiectasis.

[b]N = 44 and 48 in virus-positive and virus-negative BEs, respectively.

[c]Statistically significant ($P \leq .05$).

vaccination rate of 0.84% in our study, compared with 74% in the East London cohort of COPD.[25]

Pointedly, the detection rate of virus in clinically stable bronchiectasis resembled that of healthy subjects (13.4% in a Guangzhou COPD exacerbation study [unpublished data]). Furthermore, viral carriage rate in steady-state bronchiectasis (11 of 58, 18.9%) was similar with that in cystic fibrosis (91 of 380, 24%).[15] Therefore, the rate of viral carriage in stable chronic airway disease, including bronchiectasis, might not be significantly different from that in the healthy adult population.

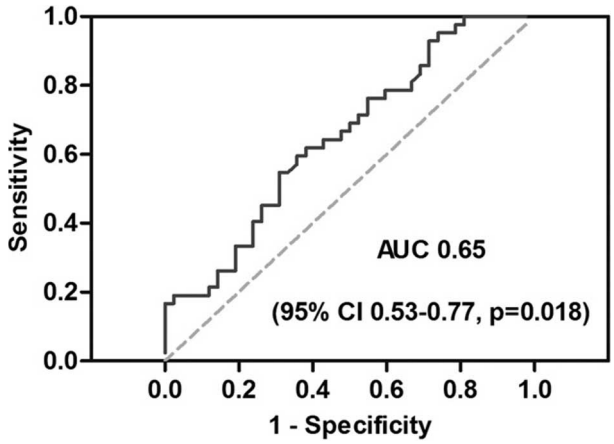

Figure 3 – *Receiver operating characteristic curve for serum interferon-γ-induced protein 10 in predicting viral infection at bronchiectasis exacerbation. AUC = area under the curve.*

An intriguing finding was the detection rate of respiratory viruses during BEs was higher in those with moderate to severe bronchiectasis (ie, BSI ≥ 5) (e-Appendix 1). The underlying interpretations remain entirely unclear but could be the following: (1) Participants with moderate or severe bronchiectasis were prone to virus-induced BEs, due to impaired innate interferon production following viral infection, as evidenced in patients with asthma[26-28]; (2) participants with moderate or severe bronchiectasis were more likely to harbor bacterial colonization, which might increase the susceptibility to virus by up-regulation of intercellular cell adhesion molecule-1[29,30]; and (3) the relatively small number of BEs might have biased our estimations of the underlying relationship between viral infection and bronchiectasis severity.

We have shown that viral infection at BEs did not impact significantly on the QoL, which might be explained by the fact that systemic and lower airway symptoms were not significantly different between virus-positive and virus-negative BEs, or that the discriminative ability of the aforementioned, unspecific questionnaires was limited. Additionally, viral infections during BEs were not associated with marked changes in FEV_1 from baseline, implying that FEV_1 was not a sensitive marker of BEs.[8] Disease-specific questionnaires and more sensitive indicators of spirometry, such as the lung clearance index, merit further investigations to determine whether viral infection at BEs is associated with worse QoL and greater reduction in lung function.[31,32]

Bacteria were frequently isolated from patients with steady-state bronchiectasis. Respiratory viruses have been postulated to disturb the balance between chronic bacterial infection and host-defense response, leading to outgrowth of bacteria and heightened inflammatory responses, which, in turn, resulted in BEs. Although virus-positive BEs were not associated with increased bacterial density in our study, there was a significant increase in systemic and airway inflammatory markers (including serum IL-6, TNF-α, and sputum IL-1β). Changes in bacterial communities or virulence caused by viral infection, but not abundance, might result in significant increase in inflammatory responses, which, in turn, stimulate exacerbation of symptoms. Furthermore, patients with virus-positive BEs were more likely to require IV antibiotics, implicating greater adverse impacts of viral infection on BEs.

Serum IP-10 has been a promising indicator of virus-associated exacerbation in COPD.[18,33] Although serum IP-10 was increased in virus-positive BEs, we found that the diagnostic accuracy was poor for predicting viral infection at BEs. Given the low positive and negative predictive values, our findings suggested that serum IP-10 was not an ideal surrogate of laboratory-confirmed viral infection at BEs in clinical practice and further researches. Novel biomarkers for identifying virus-associated BEs are urgently needed to guide future antiviral therapy, for antiviral agents are becoming increasingly available.[34]

The potential clinical implications of our study are as follows: (1) Determination of the contributions of various respiratory virus types in BEs would offer rationales for antiviral therapy; (2) supplementation of virologic data would better guide appropriate antibiotic use in clinics in the future; (3) provision of evidence for influenza vaccination in populations at high risk for bronchiectasis; (4) inspiration for future investigations regarding mechanisms of virus-induced exacerbations, especially deficient interferon production in bronchiectasis; and (5) extension of our understanding of other pathophysiologic mechanisms relevant to bronchiectasis, not just relying on extrapolating evidence from cystic fibrosis.

This study has some inherent limitations. Patients were requested to contact investigators once BEs occurred; therefore, milder BEs that mirrored upper respiratory tract infections might have been underreported. The scheduled follow-up investigations to determine effects of viral infection on symptom recovery could not be performed because of poor compliance and the lack of validated electronic diary cards. This reflected real-life practice, where patients have been frequently unwilling to record their symptoms using the daily paper diary in China. Because our setting was a single medical center, some of our findings need to be further validated in multicenter studies.

In summary, we have shown that respiratory viruses are a major trigger of BEs in adults. Further studies determining the mechanisms of virus-induced BEs and how viruses impact lower-airway microbial communities are merited.

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Additional information: The e-Appendix and e-Tables can be found in the Supplemental Materials section of the online article.

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