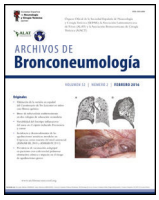
Arch Bronconeumol. 2020;56(10):621–629

Sociedad Española de Neumología y Cirugía Torácica SEPAR

ARCHIVOS DE Bronconeumología

www.archbronconeumol.org



Original Article

The Roles of Bacteria and Viruses in Bronchiectasis Exacerbation: A Prospective Study

Chun-Lan Chen[a,b], Yan Huang[a], Jing-Jing Yuan[a], Hui-Min Li[a], Xiao-Rong Han[a], Miguel Angel Martinez-Garcia[c], David de la Rosa-Carrillo[d], Rong-chang Chen[e], Wei-Jie Guan[a,*], Nan-Shan Zhong[a,*]

[a] *State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China*
[b] *Guangdong General Hospital (Guangdong Academy of Medical Sciences), Guangzhou, China*
[c] *Pneumology Department, University and Politechnic La Fe Hospital, Valencia, Spain*
[d] *Pulmonology Service, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain*
[e] *Shenzhen People's Hospital, Shenzhen, China*

ARTICLE INFO

Article history:
Received 12 September 2019
Accepted 9 December 2019
Available online 8 April 2020

Keywords:
Bronchiectasis
Acute exacerbation
Bacteria
Virus

ABSTRACT

Background: Exacerbations are crucial events during bronchiectasis progression.
Objectives: To explore the associations between bacterial, viral, and bacterial plus viral isolations and bronchiectasis exacerbations.
Methods: In this prospective study, we enrolled 108 patients who were followed up every 3–6 months and at onset of exacerbations between March 2017 and November 2018. Spontaneous sputum was split for detection of bacteria (routine culture) and viruses (quantitative polymerase chain reaction). Symptoms and lung function were assessed during exacerbations.
Results: The median exacerbation rate was 2.0 (interquartile range: 1.0–2.5) per patient-year. At any visit, viral isolations (V+) occurred more frequently during onset of exacerbations [odds ratio (OR): 3.28, 95% confidence interval (95%CI): 1.76–6.12], as did isolation of new bacteria (NB+) (OR: 2.52, 95%CI: 1.35–4.71) and bacterial plus viral isolations (OR: 2.24, 95%CI: 1.11–4.55). Whilst coryza appeared more common in exacerbations with V+ than in exacerbations with no pathogen isolations and those with NB+, lower airway symptoms were more severe in exacerbations with NB+ ($P < .05$). Sputum interleukin-1β levels were higher in exacerbations with NB+ than in exacerbations with no pathogen isolations and those with V+ (both $P < .05$). Significantly more coryza symptoms correlated with bacterial plus viral isolations at exacerbations ($P = .019$). Compared with V+ alone, bacterial with and without viral isolations tended to yield more severe lower airway symptoms, but not sputum cytokine levels at exacerbations.
Conclusions: Viral isolations, isolation of new bacteria and bacterial plus viral isolation are associated with bronchiectasis exacerbations. Symptoms at exacerbations might inform clinicians the possible culprit pathogens.

El papel de las bacterias y los virus en la exacerbación de bronquiectasia: un estudio prospectivo

Palabras clave:
Bronquiectasias
Exacerbación aguda
Bacterias
Virus

RESUMEN

Contexto: Las exacerbaciones son eventos cruciales durante la progresión de la bronquiectasia.
Objetivos: Analizar las asociaciones entre el aislamiento de bacterias, virus y virus y bacterias juntas y las exacerbaciones de las bronquiectasias.
Métodos: En este estudio prospectivo se incluyó a 108 pacientes a los que se siguió cada 3-6 meses y al comienzo de las exacerbaciones entre marzo de 2017 y noviembre de 2018. La muestra de esputo espontáneo se dividió para la detección de bacterias (cultivo de rutina) y virus (reacción en cadena de la polimerasa cuantitativa). Se evaluaron los síntomas y la función pulmonar durante las exacerbaciones.

* Corresponding author.
E-mail addresses: battery203@163.com (W.-J. Guan), nanshan@vip.163.com (N.-S. Zhong).

https://doi.org/10.1016/j.arbres.2019.12.010
0300-2896/

Resultados: La mediana de la tasa de exacerbación fue de 2,0 (rango intercuartil: 1,0-2,5) por paciente/año. En cualquier visita, los aislamientos de virus (V+) tuvieron lugar con mayor frecuencia durante el inicio de las exacerbaciones (*odds ratio* [OR]: 3,28; intervalo de confianza del 95% [IC 95%]: 1,76-6,12), al igual que el aislamiento de nuevas bacterias (NB+) (OR: 2,52; IC 95%: 1,35-4,71) y los aislamientos de bacterias y virus juntos (OR: 2,24; IC 95%: 1,11-4,55). Mientras que la coriza parecía más común en las exacerbaciones con V+ que en las exacerbaciones sin aislamientos de patógenos y en aquellas con NB+, los síntomas de las vías respiratorias inferiores fueron más graves en las exacerbaciones con NB+ ($p < 0,05$). Los niveles de interleucina-1β en el esputo fueron más altos en las exacerbaciones con NB+ que en las exacerbaciones sin aislamiento de patógenos, y aquellas con V+ (ambos $p < 0,05$). De manera significativa, más síntomas de coriza se correlacionaron con aislamientos de bacterias y virus juntos durante las exacerbaciones ($p = 0,019$). Comparados con los V+ en solitario, los aislamientos de bacterias con y sin virus tienden a producir síntomas más graves en las vías respiratorias inferiores, pero no alteran los niveles de citocinas en el esputo durante las exacerbaciones.
Conclusiones: Los aislamientos de virus, el aislamiento de nuevas bacterias y el aislamiento de bacterias y virus juntos están asociados a las exacerbaciones de las bronquiectasias. Los síntomas de las exacerbaciones pueden proporcionar información a los médicos sobre los posibles patógenos responsables.

Introduction

Bronchiectasis is a debilitating chronic airway inflammatory disease aggravated by bacterial and/or viral infections.[1–4] Acute exacerbations (AEs) are critical events associated with a considerable morbidity and mortality,[5] contributing to significantly impaired quality-of-life.[6–8] Understanding the roles of pathogens may help diagnose and identify targets for interventions.

Infections are frequently associated with AEs. The dilated bronchi become the niche for bacteria, viruses and fungi.[9–11] Viruses have frequently been isolated during AEs in bronchiectasis (detection rate: 30%–50%).[11,12] No significant changes in total bacterial density and microbial compositions were observed during AEs.[13] Nevertheless, antibiotics remain the principal effective management for AEs, suggesting that bacterial isolations might have aggravated the inflammatory responses.[14,15]

Accumulating evidence has demonstrated the interactions between pathogenic bacteria, viruses, and host-defense in chronic airway inflammatory diseases.[16] Bacterial plus viruses (e.g. nontypeable *Haemophilus influenzae* with rhinovirus isolation) were more frequently detected during chronic obstructive pulmonary disease (COPD) exacerbations than stable state, and correlated with more severe COPD exacerbations.[17–19] The roles of bacteria and viruses in bronchiectasis have been reported separately. No prospective study has investigated the impacts of bacterial plus viral isolations in adults with bronchiectasis. Moreover, symptoms that could differentiate bacterial from viral or bacterial plus viral isolation during exacerbations are not entirely clear.

We aimed to explore the associations between bacterial and viral isolations and AEs, and further investigate the clinical characteristics which could indicate the possible pathogen isolations during AEs.

Methods

Study Population

In this observational single-center prospective study, we recruited bronchiectasis patients aged 18–75 years from outpatient clinics of The First Affiliated Hospital of Guangzhou Medical University between March 2017 and November 2018. Bronchiectasis was diagnosed according to chest high-resolution computed tomography (reviewed by an experienced radiologist) with compatible clinical symptoms (e.g. chronic cough, sputum production). Eligible patients remained clinically stable (respiratory symptoms not exceeding normal daily variations), and had no use of antibiotics (except for low-dose macrolides) for four weeks. Active tuberculosis, malignancy, acute respiratory tract infections within four weeks and asthma or COPD as the primary diagnosis were excluded. The study protocol was approved by The Ethics Committee of The First Affiliated Hospital of Guangzhou Medical University (Medical Ethics 2012, the 29th). All patients signed informed consent.

Study Design and Clinical Assessment

At initial visits, clinical evaluations included demography, clinical history, spirometry and exacerbation rate within the preceding 12 months. Blood and sputum were collected. Spirometry was performed according to international guidelines.[20] Radiologic severity was assessed with modified Reiff score.[21] Disease severity was calculated with bronchiectasis severity index (BSI)[8] and E-FACED score.[22] Patients were followed up at 3–6-month intervals until November 2018 (multiple visits), and were requested to contact investigators upon significant worsening of symptoms for an additional visit, scheduled within 48 h (antibiotic use, if any, did not exceed 24 h). The upper limit of duration from symptom onset was 7 days (5 days after confirming symptom onset) for AE visits. Symptom questionnaire (see *Online Supplement*) which queried upper and lower airway symptoms [rating the severity with visual analog scale (VAS, range: 0–10)], spirometry, sputum and blood specimens were obtained during each follow-up, including stable visits and AE visits.

AEs were defined as significant deterioration (>48 h) of ≥3 symptoms, including cough frequency, sputum volume and/or consistency, sputum purulence, breathlessness and/or exercise tolerance, fatigue and/or malaise, hemoptysis, which required immediate changes in treatment.[23] Treatment decisions were made before all testing results became available.

Sputum Collection and Processing

Details are shown in *Online Supplement*. Briefly, patients thoroughly rinsed their mouth, followed by deep cough for collecting spontaneous sputum. Sputum plugs (the most purulent portion) were selected from eligible samples (leukocytes/epithelial cells >2.5:1).[10,11] No uniform techniques of chest physiotherapy was employed. Sputum was immediately split for bacterial culture, viral detection with multiplex quantitative polymerase chain reac-

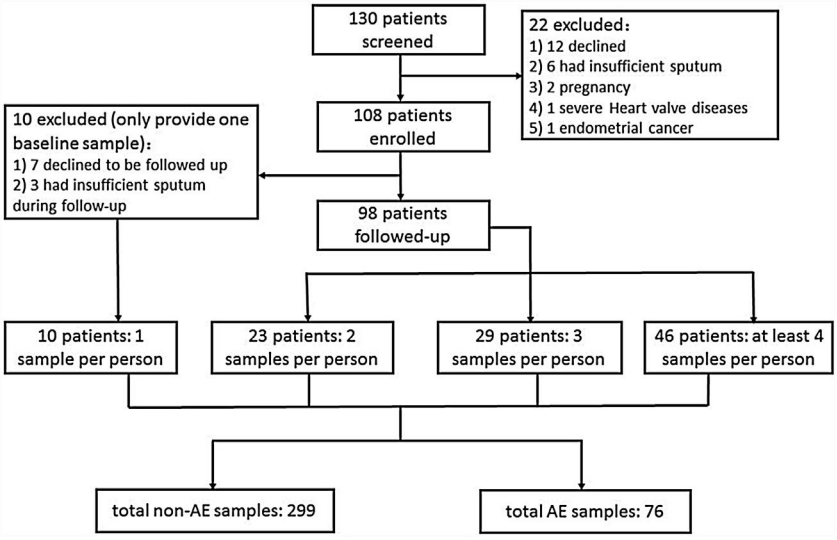


Fig. 1. Flow chart of patient recruitment.

tion (qPCR), and ultracentrifugation (20,000 × *g*) for 2 h at 4 °C for sputum sol preparation and storage at −80 °C for inflammatory biomarkers (interleukin-1β, CXC motif chemokine-8, tumor necrosis factor-α and interferon-γ) multiplex assays as described previously.[10,11]

Bacterial and Viral Detection

We did bacterial culture by homogenizing fresh sputum with SPUTASOL (Oxoid SR089A, UK), followed by inoculation in blood and chocolate agar plates (Biomeurix Inc., France) for overnight incubation.[11] Pathogenic bacteria included, but not limited to, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Haemophilus parainfluenzae, Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Streptococcus aureus* and *Escherichia coli.*[10] Isolation of new bacteria denoted sputum culture findings switching from negative to positive, or from any pathogenic bacterium to another pathogenic bacterium.

We extracted viral nucleic acids using extraction kit (TaKaRa MiniBEST Viral RNA/DNA Extraction Kit Ver. 5.0). We conducted qPCR based on TaqMan™ probes to identify sixteen common respiratory viruses: rhinovirus, influenza virus A/B, parainfluenza virus 1–4, human coronavirus (HCoV-229E, OC43, NL63 and HKU1), respiratory syncytial virus, adenovirus, enterovirus, bocavirus and human metapneumovirus. Validated viral detection kits were purchased from Guangzhou HuYanSuo Medical Technology Co., Ltd., Guangzhou, China.[11,24] The cycle threshold (Ct) of <40 was considered positive. Lower Ct indicated higher viral loads.

Statistical Analysis

No data exist regarding the proportion of patients with bacterial plus viral isolations in bronchiectasis. Assuming an equivalent proportion of patients with bacterial isolation during stable-states and AEs, and the difference of 20% in virus detection rate between AEs and stable-states,[11] 107 bronchiectasis patients would be needed based on the two-sided significance of 0.05 and power of 80%, taking into account a 25% drop-out rate.

Data were expressed as mean ± standard deviation or median (interquartile range, IQR) for continuous variables, and count (percentage) for categorical variables. Generalized estimating equations with logit link were used to explore the association between pathogen isolation and the odds of AEs compared with stable visits, taking into account repeated observations in individual participants. Continuous variables were analyzed with *t*-test, analysis-of-variance, Mann–Whitney or Kruskal–Wallis test depending on the variable distribution. Categorical variables were compared with Chi-square or Fisher's exact test. Missing values were not imputed. Statistical analysis was performed using SPSS 18.0 (SPSS Inc., Chicago, USA) and Graphpad Prism version 5.0 (Graphpad Inc., USA).

Results

Recruitment and Clinical Characteristics

Of 130 patients screened, 108 patients were enrolled and 98 were followed-up (Fig. 1). The median follow-up duration was 13.0 months. The 108 patients provided 375 sputum samples (299 for stable-visits; 76 for AEs), with a median (IQR) of 3.0 (2.0–4.0) sputum specimens per patient. Seventy-three patients (74.5%) experienced at least one AE, and reported 169 AEs during follow-up (76 AEs sampled because 63.2% contacted too late, 21.1% declined due to no availability, 10.5% administered antibiotics for >2 days, and 5.2% yielded no sputum). Sputum was mostly sampled before antibiotic administration during AEs except that 2 samples were sampled within 24 h of antibiotic administration. The clinical characteristics did not differ between patients who did and did not provide sputum during AEs (Table E1).

Patient characteristics of the full and AE cohort are shown in Table 1. The mean age was 46.8 years. The median BSI was 7.0 (IQR: 4–9) and the E-FACED score was 2.5 (IQR: 1.0–4.0). The most common etiologies were post-infective and tuberculosis. Asthma was the primary etiology in eight (7.4%) patients.

Bacterial and Viral Compositions

The percentage of no pathogen detection, bacterial isolation, viral detection and bacterial plus viral detection was 35.8%, 52.8%, 4.4% and 7.0%, respectively during stable-visits, while the corresponding percentage was 23.7%, 47.3%, 14.5% and 14.5%, respectively during AEs ($P = .001$, Fig. 2A). 59.8% of stable-visits samples and 61.9% of AEs samples tested positive to bacteria ($P = .753$). The three prevalent species isolated in stable-visits and AEs samples were *Pseudomonas aeruginosa* (44.4% vs. 32.1%), *Haemophilus influenzae* (9.8% vs. 15.4%) and *Escherichia coli* (2.0% vs.

Table 1
Demographic and Clinical Characteristics of the Study Cohort.

Parameters	Full Cohort (n = 108)	AE Cohort (n = 49)	P Value
Age (yr)	46.8 (14.0)	46.1 (14.5)	.782
Body-mass index (kg/m²)	20.4 (3.3)	19.6 (3.3)	.179
Sex (% female)	65 (60.2%)	34 (69.5%)	.268
Smoking status			
Never smoke (No., %)	100 (92.6%)	46 (93.9%)	> .999
Ex-smoke (No., %)	8 (7.4%)	3 (6.1%)	
Current smoke (No., %)	0 (0.0%)	0 (0.0%)	
FEV_1% predicted	52.9	52.5	.792
IQR	(41.0–70.1)	(40.0–69.2)	
Number of exacerbations in the previous year	1.0 (1.0–2.0)	1.8 (1.0–3.0)	.066
Bronchiectasis severity index	7 (4–9)	8 (4–10)	.446
Mild (No., %)	32 (29.6%)	14 (28.6%)	.673
Moderate (No., %)	50 (46.3%)	20 (40.8%)	
Severe (No., %)	26 (24.1%)	15 (30.6%)	
E-FACED score	2.5 (1.0–4.0)	2.0 (1.0–4.0)	.929
Mild (No., %)	73 (67.6%)	33 (67.3%)	> .999
Moderate (No., %)	34 (31.5%)	16 (32.7%)	
Severe (No., %)	1 (0.9%)	0 (0%)	
Etiology			
Post-infective (No., %)	27 (25.0%)	15 (30.6%)	.966
Idiopathic (No., %)	26 (24.1%)	11 (22.4%)	
Post-tuberculous (No., %)	17 (15.7%)	7 (14.3%)	
Primary immunodeficiency (No., %)	11 (10.2%)	5 (10.2%)	
Others (No., %)[a]	27 (25.0%)	11 (22.4%)	
Medication			
Inhaled corticosteroids (No., %)	28 (25.9%)	14 (28.6%)	.729
Low-dose macrolides (No., %)	13 (12.0%)	8 (16.3%)	.464
Vaccine			
Influenza vaccination within 1 year	7 (6.5%)	5 (10.2%)	.518
Pneumococcal vaccination within 5 years	4 (3.7%)	3 (6.1%)	.678

Notes: yr = year; FEV_1 = forced expiratory volume in 1 s.
Data are presented as mean (standard deviation) or median (interquartile range) or n (%).
[a] Other aetiologies included: Kartagener's syndrome: 8 (7.4%), asthma-associated condition 8 (7.4%), gastro-oesophageal reflux disease: 3 (2.8%), diffuse panbronchiolitis: 3 (2.8%), connective tissue disease: 2 (1.9%), cystic fibrosis transmembrane conductance regulator-related disease: 1 (0.9%), congenital lung maldevelopment: 1 (0.9%).
None of the study participants was receiving inhaled antibiotics during the study. None of the study participants had physician-diagnosed cystic fibrosis.

3.8%). However, we noted a higher detection rate of *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae* during AEs. The overall bacterial compositions that took into account all bacterial species did not differ remarkably between stable-visits and AEs (P = .070, Fig. 2B).

The proportion of patients tested positive to virus increased from 11.4% at stable-visits to 29.0% at AEs (P = .003). Prevalent viruses included coronavirus (3.4%), herpes simplex virus (2.4%) and influenza A (2.0%) at stable-visits, and influenza B (7.7%), coronavirus (7.7%) and rhinovirus (6.4%) at AEs. The viral spectrum alone did not differ between stable-visits and AEs (P = .396). Dual viral species were detected during 4 (5.3%) AE episodes (Table E2).

Of the 49 patients, 17 (34.7%) provided ≥2 AE sputum samples, for which no identical virus was detected whereas an identical (colonized) bacteria was isolated in 8 patients.

Isolation of New Bacteria and Viral Isolation Occurred More Frequently During AEs

Among 375 sputum samples, isolation of bacteria alone did not correlate with AEs (P > .05, Fig. 3). Nonetheless, isolation of new bacteria occurred more frequently during AEs than stable-visits (OR = 2.52, 95%CI: 1.35–4.71), in which culture switching from negative to positive accounted for 66.7% (12/18) of episodes [from culture negative to *Haemophilus influenzae* (38.9%) and *Moraxella catarrhalis* (11.1%)], whereas bacterial class-switch accounted for 33.3% (6/18) of episodes [from *Pseudomonas aeruginosa* to *Haemophilus influenzae* (16.7%) and other bacteria (11.1%); *Pseudomonas aeruginosa* was not isolated at most AEs].

Viral isolations occurred more frequently during AEs than stable-visits (OR = 3.28, 95%CI: 1.76–6.12). The odds was highest for rhinovirus (OR = 8.14, 95%CI: 1.90–34.81), followed by influenza A/B (OR = 4.81, 95%CI: 1.64–14.13). However, isolation of coronavirus did not correlate with AEs (OR = 2.80, 95%CI: 0.96–8.21). Moreover, bacterial plus viral isolations occurred more frequently during AEs than stable-visits (OR = 2.24, 95%CI: 1.11–4.55).

At baseline, pathogen (including *Haemophilus influenzae*) isolation status failed to predict future risks of AEs during follow-up (Table E3). Neither bacterial nor viral isolations alone at baseline predicted a shorter time to the next AEs during follow-up (Fig. E1).

We collected 52 and 24 samples from warmer (May-October) and colder seasons (November-April), between which the detection rate of viruses differed significantly (36.5% vs. 12.5%, P = .032). However, we noted no significant difference in the rate of bacterial isolation (61.5% vs. 62.5%, P = .936), nor did the rate of isolating new bacteria (19.2% vs. 33.3%, P = .179) (Fig. E2).

Clinical Characteristics Differentiating AEs With Different Pathogens

Next, we stratified patients at AEs as: (1) New bacterial AE (50.0%): isolation of new bacteria; (2) Viral AE (21.1%): detection of any virus; (3) Unexplained AE (26.3%): AE without isolation of new bacteria or detection of viruses. Two AEs were not analyzed because

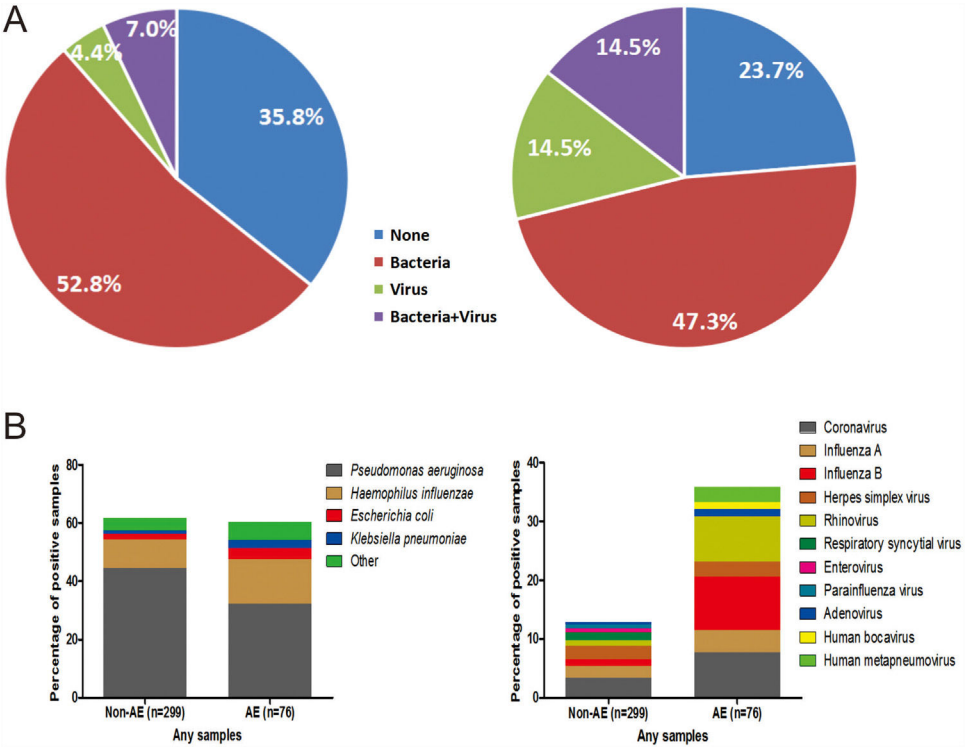


Fig. 2. Percentage and composition of pathogens in sputum samples at AEs and stable-visits. Percentage of pathogens in sputum samples at AEs and stable visits. Bacterial and viral composition in sputum samples at AEs and stable visits. AE: acute exacerbation of bronchiectasis. Other bacteria consisted of Proteus mirabilis ($n=4$), Acinetobacter baumannii ($n=2$), Moraxella catarrhalis ($n=2$), Pseudomonas ozanae ($n=1$), Staphylococcus aureus ($n=1$), Haemophilus haemolyticus ($n=1$), Haemophilus parahaemolyticus ($n=1$), Streptococcus pneumoniae ($n=1$), Shewanella algae ($n=1$), Actinomyces ureae ($n=1$), Pasteurella multocida ($n=1$), Enterobacter aerogen ($n=1$) and Serratia marcescens ($n=1$). There were more patients isolated with two bacteria when clinically stable. Hence, the overall percentage of patients isolated with pathogenic bacteria appeared higher when clinically stable compared with AE onset.

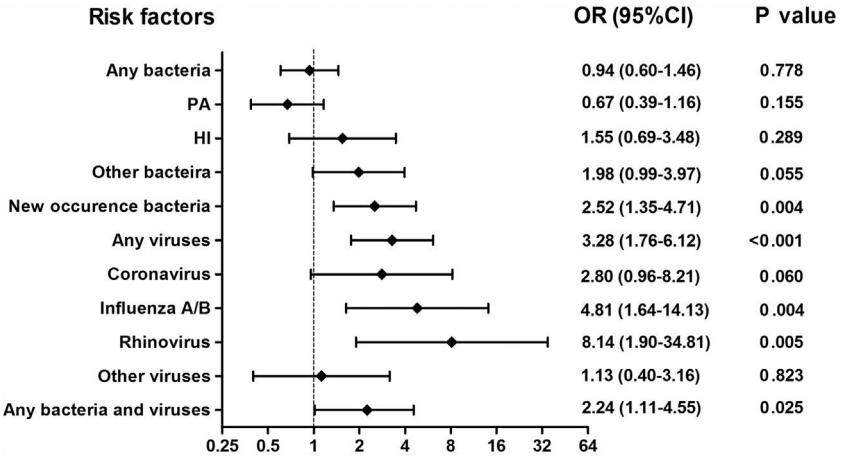


Fig. 3. Association between the detection of different pathogens and the risks of AEs. Notes: OR= Odds Ratio, PA= *Pseudomonas aeruginosa*, HI= *Haemophilus influenzae;* AEs: acute exacerbations of bronchiectasis. Any bacteria denotes bacterial culture positive for any bacteria; Isolation of new bacteria denotes sputum culture switching from negative to positive, or sputum culture positive switching from one pathogenic bacterium to other pathogenic bacterium.

of simultaneous detection of virus and new bacteria. Demographic and clinical characteristics did not differ among these subgroups (Table E4).

Symptom questionnaires were obtained from 58 (78.4%) AEs. Viral AEs tended to yield more coryza symptoms than unexplained AEs and new bacterial AEs ($P=.053$, Table 2). Neither the number nor the VAS of lower airway symptoms differed among the three groups during stable-visits (Fig. 4A). However, increased sputum purulence (mean difference: 2–4 for VAS) and breathlessness (mean difference: 1.6 for VAS) deteriorated most notably during new bacterial AE (Fig. 4B and C).

Spirometry was assessed in 46 (62.2%) AEs. Lung function decline did not differ among viral, new bacterial and unexplained AEs (Figure E3). Sputum cytokines and total leukocyte count were detected in 53 (71.6%) and 39 (51.3%) AEs, respectively. Median interleukin-1β levels increased significantly in new bacterial AEs than in unexplained AEs ($P=.006$) and viral AEs ($P=.005$), as did tumor necrosis factor-α levels except for the comparison with viral AEs ($P=.138$). New bacterial AEs trended toward higher blood neutrophil counts, while viral AEs yielded higher monocyte counts (Fig. 5).

Table 2
Symptoms of AEs With Different Pathogen Detection.

Symptoms	Unexplained AE (*n* = 27)	New Bacterial AE (*n* = 15)	Viral AE (*n* = 16)	*P* Value
Number of coryza symptoms, Mean (SD)	2.5 (2.0)	2.2 (1.6)	3.9 (2.7)	.053
Fever and or shivery, *n* (%)	12 (44.4%)	11 (73.3%)	9 (55.2%)	.196
Headache, *n* (%)	10 (37.0%)	1 (6.7%)	8 (50.0%)[a]	**.030**
Ocular itching, *n* (%)	2 (7.4%)	0 (0.0%)	6 (37.5%)[a,b]	**.009**
Other systemic pain, *n* (%)	5 (18.5%)	6 (40.0%)	7 (43.8%)	.147
Runny nose, *n* (%)	12 (44.4%)	2 (13.3%)[c]	10 (62.5%)[a]	**.019**
Blocked or stuffy nose, *n* (%)	10 (37.0%)	1 (6.7%)	8 (50.0%)[a]	**.030**
Sneezing, *n* (%)	7 (25.9%)	3 (20.0%)	2 (12.5%)	.649
Sore throat, *n* (%)	5 (18.5%)	6 (40.0%)	8 (50.0%)	.082
Hoarseness, *n* (%)	4 (14.8%)	3 (20.0%)	5 (31.3%)	.450
Lower airway symptoms, Median (IQR)	4 (4–5)	4 (4–5)	3.5 (3–5)	.175
Increased cough frequency, *n* (%)	25 (92.6%)	13 (86.7%)	13 (81.3%)	.522
Increased sputum volume, *n* (%)	25 (92.6%)	14 (93.3%)	13 (81.3%)	.535
Increased sputum purulence, *n* (%)	18 (66.7%)	11 (73.3%)	9 (56.3%)	.598
Aggravated breathlessness, *n* (%)	20 (74.1%)	11 (73.3%)	11 (68.8%)	.931
Fatigue/malaise, *n* (%)	20 (74.1%)	12 (80.0%)	10 (62.5%)	.560
Hemoptysis, *n* (%)	10 (37.0%)	2 (13.3%)	3 (18.8%)	.230

Notes: AE: acute exacerbations of bronchiectasis; New bacterial AE: AE with isolation of new bacteria, including situations of sputum culture switching from negative to positive, or sputum culture positive switching from one pathogenic bacterium to another pathogenic bacterium; Viral AE: AE with virus detection positive; Unexplained AE: AE without new occurrence of bacteria or virus detected.
Data are presented as mean (SD) or median (IQR) or *n* (%).

[a] Symptoms of viral AE compared with those of new bacterial AE, $P < .05$.

[b] Symptoms of viral AE compared with those of unexplained AE, $P < .05$.

[c] Symptoms of new bacterial AE compared with those of unexplained AE, $P < .05$.

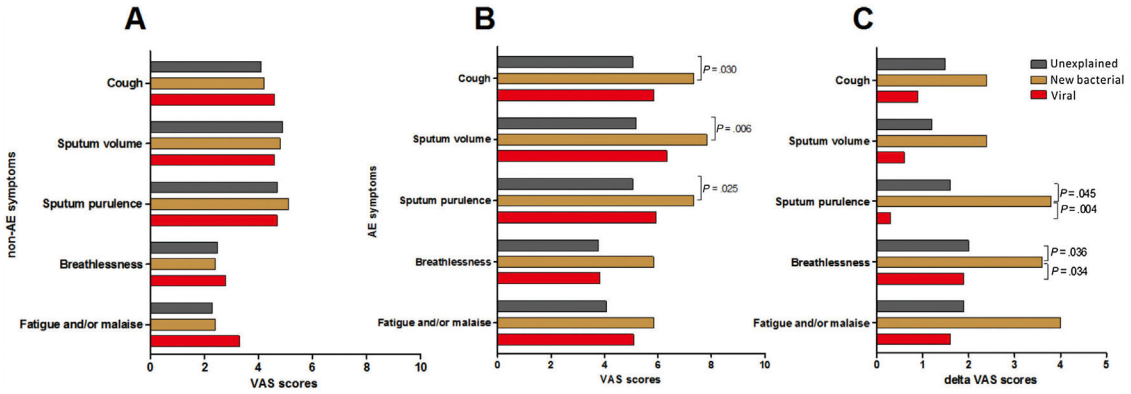


Fig. 4. The severity of lower airway symptoms assessed with the visual analog scale. (A) The VAS during stable visits. (B) The VAS during AEs. (C) The difference in VAS between AEs and stable visits. *Notes:* AE: acute exacerbation of bronchiectasis; VAS: visual analog scale.

Characteristics of AEs With Bacterial plus Viral Isolations

To further investigate the characteristics of bacterial plus viral isolations, AEs were divided into: no bacteria/viruses detected (B−V−, *n* = 18); Bacteria detected alone (B+V−, *n* = 36); Viruses detected alone (B−V+, *n* = 11); both bacteria and viruses detected (B+V+, *n* = 11).

There were more coryza symptoms in B+V+ group than in B−V− group (median: 4.6 vs. 1.6, $P = .019$). Ocular itching appeared more frequent in B+V+ group (42.9%) than in B−V− (0%) and B+V− group (7.1%, Table 3). The VAS for cough, sputum and sputum purulence tended to be higher in B+V+ and B+V− groups ($P_{trend} < 0.05$, Fig. E4). The greatest lung function decline ($P_{trend} = 0.043$ for forced vital capacity) was noted in B−V− group (Fig. E5). However, there was no notable among-group difference in sputum inflammatory biomarker levels (all $P > .05$) (Fig. E6).

Discussion

This is the first study that evaluates bacterial plus viral infections in adults with bronchiectasis. Isolation of new pathogenic bacteria and viral isolations were associated with AEs. Pathogen isolations when clinically stable did not predict future risks of AE. Bacterial plus viral isolations occurred more frequently during AE. Coryza symptoms were more frequent in viral AEs. Bacterial plus viral isolations did not correlate with greater respiratory symptom burden, airway inflammation or lung function impairment compared with bacterial or viral isolations alone.

Next-generation sequencing has been applied in bronchiectasis.[13,25,26] Here, we applied routine culture for detection of bacteria and PCR assay for detecting virus because these methods are simple, accurate and reliable, and has been widely used in clinical practice. Furthermore, the bacteria detected with culture indicate the viability and/or virulence. Hence, these routine techniques could be applied as the point-of-care tests in real-world practice.

Consistent with previous findings in COPD exacerbation,[27,28] isolation of new bacterial strain (class-switch) was associated with AEs (accounting for 20% of AEs), which warranted antibiotics treatment. However, the total bacterial load and microbiota taxa, analyzed by 16s rDNA sequencing, changed unremarkably before and after antibiotic treatment for AEs.[13] Intriguingly, isolation of new bacterial strain (*Haemophilus influenzae*, *Moraxella catarrhalis*, or *Streptococcus pneumoniae*) was associated with increased risks of COPD exacerbations.[28-30] Our study showed that isolation of new bacteria (mainly *Haemophilus influenzae*) was associated with

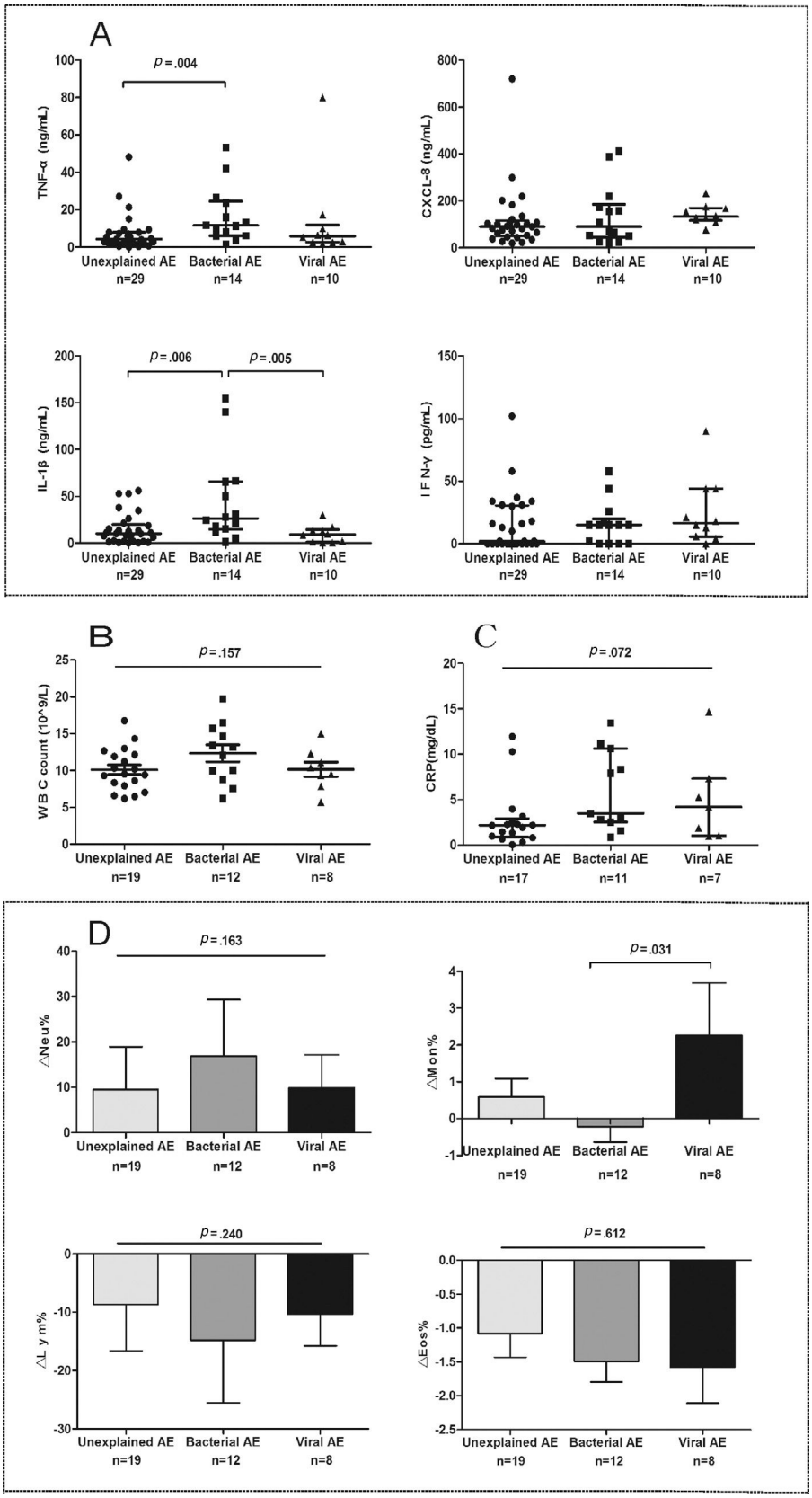


Fig. 5. The airway and systemic inflammations in different groups. (A) The level of sputum cytokines during AEs. (B) White blood cell count during AEs. (C) The level of C-reactive protein during AEs. (D) The difference in inflammatory cell count between AEs and stable visits. *Notes:* AE: acute exacerbation of bronchiectasis.

Table 3
Symptoms of AEs With Bacterial and or Viral Infection.

Symptoms	B−V− (*n* = 14)	B+V− (*n* = 28)	B−V+ (*n* = 9)	B+V+ (*n* = 7)	*P* Value
Number of coryza symptoms, Mean (SD)	1.6 (1.5)	2.8 (2.0)	3.4 (2.4)	4.6 (3.2)[a]	**.020**
Fever, *n* (%)	7 (50.0%)	16 (57.1%)	5 (55.6%)	4 (57.1%)	.978
Headache, *n* (%)	2 (14.3%)	9 (32.1%)	3 (33.3%)	5 (71.4%)	.082
Ocular itching, *n* (%)	0 (0%)	2 (7.1%)	3 (33.3%)	3 (42.9%)	**.009**
Other systemic pain, *n* (%)	1 (7.1%)	10 (35.7%)	3 (33.3%)	4 (57.1%)	.082
Running nose, *n* (%)	5 (35.7%)	9 (32.1%)	6 (66.7%)	4 (57.1%)	.245
Nasal congestion, *n* (%)	4 (28.6%)	7 (25.0%)	5 (55.6%)	3 (42.9%)	.342
Sneezing, *n* (%)	1 (7.1%)	9 (32.1%)	1 (11.1%)	1 (20.7%)	.262
Sore throat, *n* (%)	1 (7.1%)	10 (35.7%)	4 (44.4%)	4 (57.1%)	.057
Hoarseness, *n* (%)	1 (7.1%)	6 (21.4%)	1 (11.1%)	4 (57.1%)	.073
Lower airway symptoms, Median (IQR)	4.2 (1.2)	4.4 (0.9)	3.2 (1.6)	4.3 (0.8)	.063
Increased cough frequency, *n* (%)	13 (92.9%)	25 (89.3%)	7 (77.8%)	6 (85.7%)	.737
Increased sputum volume, *n* (%)	12 (85.7%)	27 (96.4%)	6 (66.7%)	7 (100.0%)	.053
Increased sputum purulence, *n* (%)	9 (64.3%)	20 (71.4%)	7 (77.8%)	2 (28.6%)	.181
Aggravated breathlessness, *n* (%)	9 (64.3%)	22 (78.6%)	4 (44.4%)	7 (100.0%)	.069
Fatigue/malaise, *n* (%)	11 (78.6%)	21 (75.0%)	4 (44.4%)	6 (85.7%)	.286
Hemoptysis, *n* (%)	5 (35.7%)	7 (25.0%)	1 (11.1%)	2 (28.6%)	.658

Notes: AE: acute exacerbations of bronchiectasis; B−V−: no bacteria and viruses detected; B+V−: any pathogenic bacteria detected but no viruses detected; B−V+: viruses detected but no pathogenic bacteria detected; B+V+: both bacteria and viruses detected.
Data are presented as mean (SD) or median (IQR) or *n* (%).
Kruskal–Wallis comparison with Bonferroni test was applied.

[a] The number of coryza symptoms in B+V+ subgroup compared with those in B−V− subgroup, $P < .05$.

AEs in bronchiectasis, implicating that *Pseudomonas aeruginosa* was not the culprit of AEs and that antibiotic therapy targeting at the emerging bacterial species would be warranted. Hence, rapid microbiological assessments are important to clinical practice because they may help avoid over-treatment or under-treatment.

Viral isolations occurred more frequently in AEs. The viral detection rate was lower (30%) than previously reported (∼48%).[11,12] The sources of samples (sputum vs. nasopharyngeal aspirates vs. sputum plus nasopharyngeal swab) and the characteristics of bronchiectasis patients might have contributed to the differences. The most prevalent viruses were influenza A/B, coronavirus and rhinovirus in our population, congruent with the findings from COPD patients in Hong Kong.[31] Influenza A/B and rhinovirus, but not coronavirus, played crucial roles in AEs. However, the mechanisms underlying these observations are unclear and warrant further investigations. Overall, our findings mirrored those reported in adults and children.[11,12] Compared with the study by Mitchell et al.,[32] the difference in viral spectrum could have resulted from the differences in: (1) detection methods and assay kits; (2) the positive threshold of CT values; (3) the geographic regions.

We have confirmed that bacterial plus viral isolations occurred more frequently during AEs than stable-visits. Interestingly, bacterial plus viral isolations were not associated with greater lung function impairment or airway inflammation than bacterial or viral isolations alone during AEs. By contrast, bacterial plus viral isolations yielded greater lung function impairment and heightened inflammatory responses during COPD exacerbations.[17,18] This might be because bacterial plus viral isolations frequently comprised *Pseudomonas aeruginosa* and viral isolations in bronchiectasis as opposed to *Haemophilus influenza*e and viral isolations in COPD. Indeed, viral isolation may induce secondary bacterial isolation and further aggravated inflammation.[33,34] However, the load of *Pseudomonas aeruginosa* did not increase significantly regardless of viral isolation status or disease status (exacerbation vs. steady-state) in cystic fibrosis.[35,36] Moreover, respiratory syncytial virus isolation reportedly enhanced *Pseudomonas aeruginosa* biofilm growth, rendering *Pseudomonas aeruginosa* infection more sustainable.[37] Collectively, *Pseudomonas aeruginosa* isolation was unlikely the trigger of AEs despite co-existing viral isolations in bronchiectasis.

This is the first study which analyzed the association between symptoms and AEs in bronchiectasis because this would help clinicians more rapidly identify the possible culprits. Coryza symptoms were indicators of viral isolations. Fever was ubiquitous in different types of AEs, hence viral AEs cannot be judged based on fever alone. AE associated with new occurrence of bacteria yielded more severe lower airway symptoms and heightened airway inflammatory responses, therefore clinicians should be vigilant for the identification and management with antibiotics if appropriate.

Some limitations should be considered. We did not recruit 'dry' bronchiectasis patients who still might have AEs attributable to pathogen infections. Our sample size was insufficient to power subgroup analyses. Some blood tests, spirometry were not available because some patients declined due to repeated assessments and poor overall well-being during AEs. We've only captured half AE episodes although the clinical characteristics of these patients did not differ from those whose AEs were not captured. Furthermore, we did not measure bacterial loads which reportedly changed insignificantly during AEs.[11] The AEs were managed at out-patient clinics, hence our findings might not be extrapolated to severe AEs needing hospitalization. Some viruses detected during AEs might not be pathogenic; however, the GEE model did reveal the association between pathogen isolation and AEs. Finally, findings of the symptoms associated with viral or bacterial isolations were not specific to bronchiectasis. However, our study would still be informative because our findings help clinicians to infer from possible culprit pathogens before further assays became available.

In summary, building on our previous publication,[11] the current study has further provided important clinical insights. Isolation of new bacteria, viral isolations, and bacterial plus viral isolations are associated with AEs in bronchiectasis. Further study determining the causes of unexplained AEs are needed.

Funding

This work was supported by National Natural Science Foundation No. 81870003, Pearl River S&T Nova Program of Guangzhou No. 201710010097 and Guangdong Province Universities and Colleges Pearl River Scholar Funded Scheme 2017 (to Prof. Guan), The Impact and Mechanisms of Physical, Chemical and Biological Interventions on the Development and Outcome of Acute Lung

Injury No. 81490534, National Key Technology R&D Program No. 2018YFC1311902, Guangdong Science and Technology Foundation No. 2019B030316028 (to Prof. Zhong).

Authorship

C. L. C., W. J. G. and N. S. Z. participated in study design; C. L. C. performed laboratory experiments and data analysis; C. L. C., H. M. L., J. J. Y., Y. H., W. J. G., X. R. H., R. C. C. and N. S. Z. recruited patients; C. L. C., H. M. L., J. J. Y., and Y. H. performed follow-up; C. L. C., W. J. G., D. R. C. and M. A. M. drafted the manuscript; W. J. G., N. S. Z., D. R. C. and M. A. M. were responsible for study conception and provided critical review of the manuscript. C. L. C., H. M. L., J. J. Y., Y. H., W. J. G., X. R. H., R. C. C., D. R. C., M. A. M. and N. S. Z. approved the final draft for publication. W. J. G. and N. S. Z. were the guarantors of the study.

Acknowledgments

We thank Dan-Hong Su (Department of microbiology, The First Affiliated Hospital of Guangzhou Medical University), for her assistance in sputum bacterial culture, Wen-Kuan Liu, Shi-Guan Wu and Shu-Yan Qiu (Department of virology, State Key Laboratory of Respiratory Diseases, Guangzhou Medical University) for their assistance in viral detection.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.arbres.2019.12.010.

References

1. Amalakuhan B, Maselli DJ, Martinez-Garcia MA. Update in bronchiectasis 2014. Am J Respir Crit Care Med. 2015;192:1155–61.
2. McShane PJ, Naureckas ET, Tino G, Strek ME. Non-cystic fibrosis bronchiectasis. Am J Respir Crit Care Med. 2013;188:647–56.
3. Chang AB, Bilton D. Exacerbations in cystic fibrosis: 4 – non-cystic fibrosis bronchiectasis. Thorax. 2008;63:269–76.
4. Seitz AE, Olivier KN, Steiner CA, Montes de Oca R, Holland SM, Prevots DR. Trends and burden of bronchiectasis-associated hospitalizations in the United States, 1993–2006. Chest. 2010;138:944–9.
5. Roberts ME, Lowndes L, Milne DG, Wong CA. Socioeconomic deprivation, readmissions, mortality and acute exacerbations of bronchiectasis. Intern Med J. 2012;42:e129–36.
6. Wilson CB, Jones PW, O'Leary CJ, Hansell DM, Cole PJ, Wilson R. Effect of sputum bacteriology on the quality of life of patients with bronchiectasis. Eur Respir J. 1997;10:1754–60.
7. Murray MP, Turnbull K, MacQuarrie S, Pentland JL, Hill AT. Validation of the Leicester Cough Questionnaire in non-cystic fibrosis bronchiectasis. Eur Respir J. 2009;34:125–31.
8. Chalmers JD, Goeminne P, Aliberti S, McDonnell MJ, Lonni S, Davidson J, et al. The bronchiectasis severity index an international derivation and validation study. Am J Respir Crit Care Med. 2014;189:576–85.
9. Mac Aogáin M, Chandrasekaran R, Lim AYH, Low TB, Tan GL, Hassan T, et al. Immunological corollary of the pulmonary mycobiome in bronchiectasis: the CAMEB study. Eur Respir J. 2018;52:1800766.
10. Guan WJ, Gao YH, Xu G, Lin ZY, Tang Y, Li HM, et al. Sputum bacteriology in steady-state bronchiectasis in Guangzhou, China. Int J Tuberc Lung Dis. 2015;19:610–9.
11. Gao YH, Guan WJ, Xu G, Lin ZY, Tang Y, Li HM, et al. The role of viral infection in pulmonary exacerbations of bronchiectasis in adults: a prospective study. Chest. 2015;147:1635–43.
12. Kapur N, Mackay IM, Sloots TP, Masters IB, Chang AB. Respiratory viruses in exacerbations of non-cystic fibrosis bronchiectasis in children. Arch Dis Child. 2014;99:749–53.
13. Tunney MM, Einarsson GG, Wei L, Drain M, Klem ER, Cardwell C, et al. Lung microbiota and bacterial abundance in patients with bronchiectasis when clinically stable and during exacerbation. Am J Respir Crit Care Med. 2013;187:1118–26.
14. Hill AT, Sullivan AL, Chalmers JD, De Soyza A, Elborn SJ, Floto AR, et al. British Thoracic Society Guideline for bronchiectasis in adults. Thorax. 2019;74:1–69.
15. Chalmers JD, Smith MP, McHugh BJ, Doherty C, Govan JR, Hill AT. Short- and long-term antibiotic treatment reduces airway and systemic inflammation in non-cystic fibrosis bronchiectasis. Am J Respir Crit Care Med. 2012;186:657–65.
16. Dickson RP, Martinez FJ, Huffnagle GB. The role of the microbiome in exacerbations of chronic lung diseases. Lancet. 2014;384:691–702.
17. Wilkinson TMA, Aris E, Bourne S, Clarke SC, Peeters M, Pascal TG, et al. A prospective, observational cohort study of the seasonal dynamics of airway pathogens in the aetiology of exacerbations in COPD. Thorax. 2017;72:919–27.
18. Wilkinson TM, Hurst JR, Perera WR, Wilks M, Donaldson GC, Wedzicha JA. Effect of interactions between lower airway bacterial and rhinoviral infection in exacerbations of COPD. Chest. 2006;129:317–24.
19. Papi A, Bellettato CM, Braccioni F, Romagnoli M, Casolari P, Caramori G, et al. Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. Am J Respir Crit Care Med. 2006;173:1114–21.
20. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. ATS/ERS Task Force. Standardisation of spirometry. Eur Respir J. 2005;26:319–38.
21. Reiff DB, Wells AU, Carr DH, Cole PJ, Hansell DM. CT findings in bronchiectasis: limited value in distinguishing between idiopathic and specific types. AJR Am J Roentgenol. 1995;165:261–7.
22. Martinez-Garcia MA, Athanazio RA, Girón R, Máiz-Carro L, de la Rosa D5, Olveira C, et al. Predicting high risk of exacerbations in bronchiectasis: the E-FACED score. Int J Chron Obstruct Pulmon Dis. 2017;12:275–84.
23. Hill AT, Haworth CS, Aliberti S, Barker A, Blasi F, Boersma W, et al. Pulmonary exacerbation in adults with bronchiectasis: a consensus definition for clinical research. Eur Respir J. 2017;49:1700051.
24. Liu WK, Chen DH, Liu Q, Liang HX, Yang ZF, Qin S, et al. Detection of human bocavirus from children and adults with acute respiratory tract illness in Guangzhou, southern china. BMC Infect Dis. 2011;11:345.
25. Rogers GB, van der Gast CJ, Cuthbertson L, Thomson SK, Bruce KD, Martin ML, et al. Clinical measures of disease in adult non-CF bronchiectasis correlate with airway microbiota composition. Thorax. 2013;68:731–7.
26. Guan WJ, Yuan JJ, Li HM, Gao YH, Huang Y, Chen CL, et al. Proteobacteria community compositions correlate with bronchiectasis severity. Int J Tuberc Lung Dis. 2018;22:1095–105.
27. Han MK, Huang YJ, Lipuma JJ, Boushey HA, Boucher RC, Cookson WO, et al. Significance of the microbiome in obstructive lung disease. Thorax. 2012;67:456–63.
28. Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. N Engl J Med. 2008;359:2355–65.
29. Rogers GB, Zain NM, Bruce KD, Burr LD, Chen AC, Rivett DW, et al. A novel microbiota stratification system predicts future exacerbations in bronchiectasis. Ann Am Thorac Soc. 2014;11:496–503.
30. Sethi S, Evans N, Grant BJ, Murphy TF. New strains of bacteria and exacerbations of chronic obstructive pulmonary disease. N Engl J Med. 2002;347:465–71.
31. Ko FW, Ip M, Chan PK, Chan MC, To KW, Ng SS, et al. Viral etiology of acute exacerbations of COPD in Hong Kong. Chest. 2007;132:900–8.
32. Mitchell AB, Mourad B, Buddle L, Peters MJ, Oliver BGG, Morgan L. Viruses in bronchiectasis: a pilot study to explore the presence of community acquired respiratory viruses in stable patients and during acute exacerbations. BMC Pulm Med. 2018;18:84.
33. George SN, Garcha DS, Mackay AJ, Patel AR, Singh R, Sapsford RJ, et al. Human rhinovirus infection during naturally occurring COPD exacerbations. Eur Respir J. 2014;44:87–96.
34. Rynda-Apple A, Robinson KM, Alcorn JF. Influenza and bacterial superinfection: illuminating the immunologic mechanisms of disease. Infect Immun. 2015;83:3764–70.
35. Chin M, Gaudet DZMSE. VKCF. Acute effects of viral respiratory tract infections on sputum bacterial density during CF pulmonary exacerbations. J Cyst Fibros. 2015;14:482–9.
36. Asner S, Yau WVSMY. RSGH. Role of respiratory viruses in pulmonary exacerbations in children with cystic fibrosis. J Cyst Fibros. 2012;11:433–9.
37. Hendricks MR, Lashua LP, Fischer DK, Flitter BA, Eichinger KM, Durbin JE, et al. Respiratory syncytial virus infection enhances Pseudomonas aeruginosa biofilm growth through dysregulation of nutritional immunity. Proc Natl Acad Sci U S A. 2016;113:1642–7.