International Journal of Chronic Obstructive Pulmonary Disease

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Sputum Microbiome, Potentially Pathogenic Organisms, and Clinical Outcomes in Japanese Patients with COPD and Moderate Airflow Limitation: The Prospective AERIS-J Study

Kazuhiro Yatera[1,*], Zhang Wang[2,*], Yoko Shibata[3], Nobuhisa Ishikawa[4], Tetsuya Homma[5], Kiyoyasu Fukushima[6], Osamu Hataji[7], Yoshikazu Inoue[8,9], Hiroki Kawabata[1], Keisuke Miki[10], Kazuhiro Sato[11], Kazunori Tobino[12], Makoto Yoshida[13], Takeo Ishii[14], Risako Ito[14], Tamami Kobayashi[14], Shinya Kawamatsu[14], Chris H Compton[15], Paul W Jones[15]

[1]University of Occupational and Environmental Health Japan, Fukuoka, Japan; [2]Institute of Ecological Sciences, School of Life Sciences, South China Normal University, Guangzhou, People's Republic of China; [3]Fukushima Medical University, Fukushima, Japan; [4]Hiroshima Prefectural Hospital, Hiroshima, Japan; [5]Showa University School of Medicine, Tokyo, Japan; [6]Japanese Red Cross, Nagasaki Genbaku Isahaya Hospital, Nagasaki, Japan; [7]Matsusaka Municipal Hospital, Mie, Japan; [8]Osaka Anti-tuberculosis Association, Osaka Fukujuji Hospital, Osaka, Japan; [9]NHO Kinki Chuo Chest Medical Center, Osaka, Japan; [10]NHO Osaka Toneyama Medical Center, Osaka, Japan; [11]Nagaoka Red Cross Hospital, Niigata, Japan; [12]Iizuka Hospital, Fukuoka, Japan; [13]NHO Fukuoka National Hospital, Fukuoka, Japan; [14]GSK K.K., Tokyo, Japan; [15]GSK, R&D Global Medical, Brentford, Middlesex, UK

*These authors contributed equally to this work

Correspondence: Zhang Wang, Institute of Ecological Sciences, School of Life Sciences, South China Normal University, Guangzhou, People's Republic of China, Tel +86-20-85211372, Fax +86-20-85211850, Email wangz@m.scnu.edu.cn

Background: In Western studies, lung microbiome changes are reported in patients with chronic obstructive pulmonary disease (COPD) and are associated with poorer outcomes, but similar studies in Asian patients or those with less severe COPD are limited.

Methods: The Acute Exacerbation and Respiratory InfectionS in COPD Japan (AERIS-J; jRCT1080224632/NCT03957577) was a prospective, non-interventional study to evaluate sputum microbiome diversity at baseline and after 12 months (V2; exploratory analysis), in patients aged 40–80 years with stable COPD (June 2019–June 2022). Baseline sputum potentially pathogenic organisms (PPOs) were identified. Blood cell counts and COPD Assessment Test (CAT) scores were collected at baseline and COPD symptoms measured over 12 months using the Evaluating Respiratory Symptoms in COPD and EXAcerbations of Chronic pulmonary disease Tool, collected by eDiary.

Results: Patients (N=63) had a mean age of 72.8 years, and percent predicted post-bronchodilator forced expiratory volume in 1 second was 58.3%; 92% were male. Across 62 baseline sputum samples, microbiome composition was similar between 16S rRNA/metagenomic datasets. Patients graded Global Initiative for Chronic Obstructive Lung Disease (GOLD) III versus GOLD I/II had minimal differences in their microbial taxonomic profile and no differences in microbial diversity (Wilcoxon P=0.71). Alpha diversity (Shannon index) positively correlated with blood basophils (rho=0.41; P=0.0019) and negatively correlated with CAT score (rho=0.36; P=0.0069). Alpha diversity and sputum (rho: −0.0637; P=0.7836) or blood (rho: 0.1739; P=0.2043) eosinophils were not correlated. No difference in alpha (P=0.5) or beta (P=0.3) diversity or Operational Taxonomic Unit (Anosim R=−0.024; P=0.892) was observed between PPO-positive or -negative sputum.

Conclusion: A less diverse microbiome correlated with poorer health status and lower blood basophils in patients with COPD and moderate airflow limitation. There was no relationship between PPO presence and microbiome diversity.

Plain Language Summary: Chronic obstructive pulmonary disease (COPD) is a progressive lung condition resulting in breathing difficulties. Research has shown that patients with COPD experience changes in the diversity of bacteria in their lungs, leading to a worsening of symptoms. The lung microbiome includes different types of bacteria, and is involved in important roles, such as

Received: 5 June 2024
Accepted: 21 March 2025
Published: 14 May 2025

regulating the immune system and protecting the lung from invading pathogens. Many studies on the microbiome have been based in western countries, and there are few studies among Asian patients and populations with moderate COPD.

The aim of this year-long study was to assess the diversity of the lung microbiome in Japanese patients with COPD and moderate airflow limitation, and how it affects patients' immunity and disease severity. Patients' sputum and blood samples were obtained at the start of the study and the different types of bacteria in the sputum and the number of immune cells in the blood were measured. Patients' symptoms were also assessed at study start. Results showed that a less diverse lung microbiome was associated with lower levels of blood immune cells and worse COPD symptoms.

These results improve our knowledge of the lung microbiome in an Asian population with COPD, providing insights into how lower bacterial diversity may worsen patient immunity and COPD severity.

Keywords: 16S rRNA, Asia, basophil, COPD assessment test, sputum, metagenomic

Introduction

The lung microbiome is complex and dynamic, with a diverse bacterial community comprised mainly of *Prevotella, Veillonella*, and *Streptococcus* genera.[1,2] These organisms may help protect the lung from invading pathogens, and are known to interact with the immune system.[2] The specific microorganisms which make up the microbiome vary between individuals, and the lung microbiome is also believed to play an important role across a variety of diseases, including chronic obstructive pulmonary disease (COPD).[1–7]

COPD is a heterogeneous condition caused by abnormalities in the airways that lead to persistent, often progressive, airflow obstruction and emphysema, resulting in chronic respiratory symptoms.[8] An in-depth multi-omic analysis of healthy people and patients with COPD in China reported disruptions in microbe–host interactions in the sputum of patients with COPD.[4] These findings have also been reported across European patient populations.[9] For example, the prospective Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) study in the UK reported an increase in *Haemophilus* with increasing COPD severity, paralleled by a decrease in *Prevotella* and *Veillonella* and an overall decrease in microbial diversity in sputum.[3] Similarly, bronchial brush samples from patients with mild-to-moderate COPD identified an inverse relationship between the abundance of the main lung microbe genera and COPD symptom severity, lung function, and exercise capacity.[1]

The lung microbiome is also associated with COPD exacerbations. One UK study identified a 3%–5% increase in the relative abundance of *Moraxella* and *Haemophilus* in patient sputum samples during a COPD exacerbation compared with stable-state disease.[10] A similar and significant increase in *Moraxella* in sputum samples was also reported in the AERIS study, with bacterial abundance becoming more distinct with greater exacerbation frequency.[3] While the association between exacerbation severity and worse lung function[11] highlights the need for a more in-depth understanding of the role the lung microbiome plays during COPD exacerbations, the perturbed microbiome profile reported in stable-state disease remains an important consideration to help understand longer-term disease trajectory.

A pooled analysis of sputum samples from three large UK-based longitudinal cohort studies provided important insights into the microbiome in COPD and its association with sputum neutrophils and eosinophils;[9] however, the studies included patients with severe airflow limitation, which is associated with lowered airway microbial diversity.[3,9] One study, performed in China, reported sputum microbiome in patients with moderate airflow limitation (forced expiratory volume in 1 second [FEV_1] 59.6% predicted),[12] and recently a Japanese study reporting the sputum microbiome in asthma, asthma-COPD overlap (ACO) and COPD found no difference in diversity between the three conditions.[13]

Patients in Japan are reported to have milder COPD compared with patients in western countries, although this may be due to under-reporting of symptoms and exacerbations by Japanese patients.[14,15] The AERIS-Japan (AERIS-J) study was a prospective, non-interventional study designed to evaluate the etiology of acute exacerbations of COPD (AECOPD) in a Japanese patient population. However, as the study coincided with the coronavirus disease 2019 (COVID-19) pandemic, during which a decrease in exacerbations was observed across Asia and worldwide,[16,17] the study design was restricted to the evaluation of the sputum microbiome in stable-state disease. Here, we report a detailed

analysis of the interplay between sputum microbiome, presence of potentially pathogenic organisms and clinical measures in patients with COPD, using baseline stable-state data from the AERIS-J study.

Methods

Study Design

This was a prospective, non-interventional cohort study in patients with COPD in Japan with a recent history of respiratory infection or exacerbations (GSK study 208636, jRCT1080224632, NCT03957577). The study design is illustrated in Figure 1. The study period was between June 2019 and June 2022. Patients attended a screening visit and were then enrolled at Visit 1 (baseline), followed by a further visit at Month 12 (V2).

Eligibility Criteria

Patient eligibility was assessed at baseline. Eligible patients included those 40–80 years of age with a confirmed diagnosis of COPD, defined as a post-bronchodilator FEV_1/forced volume capacity (FVC) ratio <0.7, FEV_1% predicted 30%–80%, and a COPD Assessment Test (CAT) score ≥10. Patients had to be current or former smokers (≥10 pack-years history), with ≥1 lower respiratory tract infection or exacerbation treated with antibiotics and/or oral/systemic corticosteroids (OCS/SCS) in the previous 24 months (excluding the 4 weeks prior to screening). Patients also had to be able to provide a spontaneous or induced (using 0.9% or 3% saline) initial sputum sample of ≥0.5 g at baseline (reduced to ≥0.2 g during the COVID-19 pandemic due to difficulties in collecting samples using a nebulizer under the pandemic conditions). The ability to complete the daily eDiary was also a requirement.

Patients were excluded if they had a respiratory disorder diagnosis other than COPD or chest imaging revealed evidence of clinically significant abnormalities not believed to be due to COPD. Additional exclusion criteria included antibiotic treatment within 4 weeks of screening or treatment for ≥30 days within the 90 days prior to screening (except for long-term treatment with low-dose macrolide), treatment with SCS for ≥14 consecutive days within 90 days prior to the screening visit, chemotherapy for cancer in the 12 months prior to the screening visit, or a diagnosis of α-1 antitrypsin

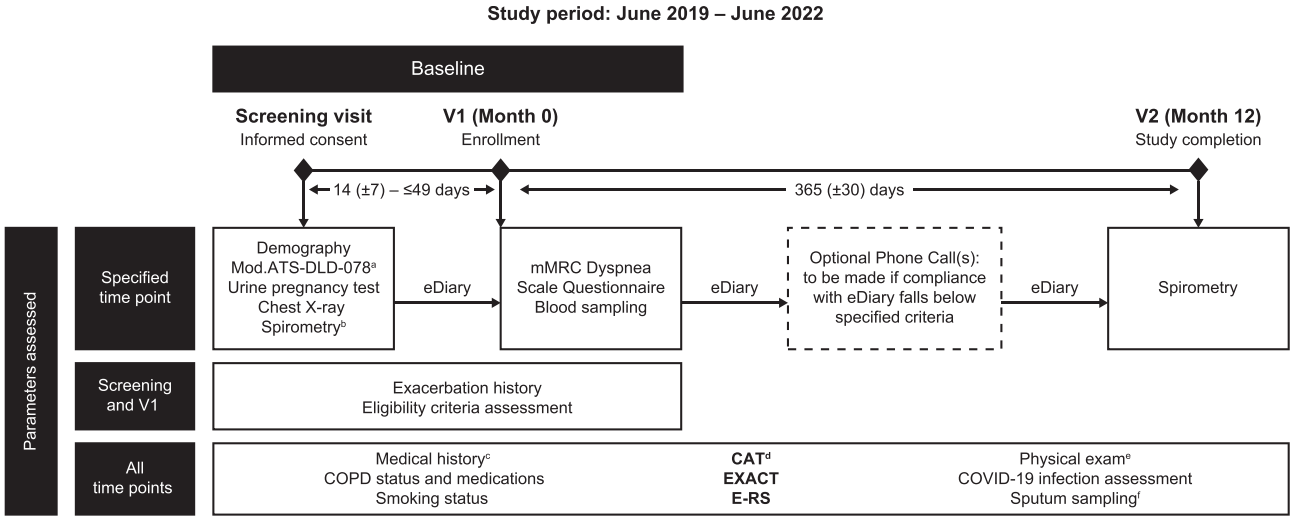

Figure 1 Study design. Patients completed a daily eDiary, which included screening questions for the detection of AECOPD during the study and the EXACT; the parameters captured in the eDiary are indicated in bold and all other parameters were captured using the eCRF, except for urine pregnancy test. Study visits were postponed or cancelled in the event of a positive COVID-19 diagnosis or presence of COVID-19 symptoms. If an AECOPD occurred at the date of study visit, the study visit was rescheduled, within the specified time frames, to ensure assessment of stable-state disease. [a]The mod.ATS-DLD-078 assessed patients' medical, family, smoking and occupational history; [b]spirometry data (in the 12 months prior to enrolment) in medical records was permitted to be used to assess patient eligibility; [c]including height and weight, medical conditions as specified in the eCRF, and pneumococcal and influenza vaccination status; [d]also completed between study visits every 2 months; [e]if deemed necessary by the investigator; [f]sputum was taken at V1 where a sputum sample of ≥0.2 g was unable to be collected at the screening visit. V2 sputum samples were taken where available.

Abbreviations: AECOPD, Acute Exacerbations of COPD; CAT, COPD Assessment Test; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; eCRF, electronic case report form; E-RS, Evaluating Respiratory Symptoms in COPD; EXACT, EXAcerbations of Chronic pulmonary disease Tool; mMRC, modified Medical Research Council; mod.ATS-DLD-078, modified American Thoracic Society and National Heart and Lung Institute-Division of Lung Disease Respiratory; V1, Visit 1; V2, Visit 2.

https://doi.org/10.2147/COPD.S481406

deficiency as the underlying cause of COPD. Patients were also excluded if they had lung surgery within 12 months prior to the screening visit, had plans to have lung surgery within 12 months after study entry, had a confirmed or suspected immunosuppressive/immunodeficient condition, or had any psychiatric disorder or condition that interfered with the ability to understand the procedures.

Objectives and Endpoints

The primary objective of the study was to evaluate the microbiome in patients with COPD using sputum samples, as in other large studies.[3,9] Sputum was collected at baseline and at V2. V2 samples were optional following the amendment of the study design due to the COVID-19 pandemic, resulting in possible differences between the V1 and V2 populations (eg, reduced exacerbations during the COVID-19 pandemic, stable-state disease only in V2 population). As this limited data collection, analyses assessing V2 data were therefore considered exploratory. Induced sputum was used if spontaneous sputum was not possible, or the sample was too small. Two data types were included: 1) genus-level taxonomic profile from 16S rRNA gene sequencing and 2) species-level taxonomic profile from metagenomic sequencing. Microbial taxonomic profiles and microbial diversity were compared between patients categorized according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria: mild-to-moderate (GOLD I/II) and severe (GOLD III).[8] The association between the sputum microbiome and blood cell counts and CAT score at baseline was explored as a post hoc analysis. Sputum cell counts were assessed using CytoSpin or similar methods to identify neutrophil, eosinophil, macrophage, lymphocyte, squamous cell, and bronchial epithelial cell counts. Blood cell counts identified white blood cell, differential white blood cell (neutrophil, lymphocyte, monocyte, eosinophil, basophilic leucocyte), red blood cell, and platelet count.

The relationship between potentially pathogenic organisms (PPOs) in sputum and the microbiome at baseline was examined. Bacterial pathogens were identified from sputum by quantitative polymerase chain reaction (qPCR) and/or bacterial culture, and viral pathogens were identified by PCR. The following seven PPOs were analyzed owing to their association with COPD: *Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus, Pseudomonas* species, *Klebsiella* species, and *Pneumocystis* species; sputum samples that were positive for PPO by qPCR and/or culture (any positive) were compared with those that were negative (all negative).

A secondary objective of the original AERIS-J design was to examine the relationship between COPD exacerbations and changes in COPD symptoms over a 1-year period. This was assessed using weekly CAT measurements and daily measurements of symptoms using the Evaluating Respiratory Symptoms in COPD (E-RS) tool,[18] and the EXAcerbations of Chronic pulmonary disease Tool (EXACT),[19] collected using a daily eDiary. The eDiary consisted of 20 questions, including screening questions for the detection of AECOPD as well as the EXACT, and patients completed the eDiary every evening. Patients completed the CAT in the eDiary every 2 months between study visits. The occurrence of an exacerbation was captured either as an EXACT event in the eDiary, or as a reported moderate or severe AECOPD. A moderate AECOPD was defined as requiring treatment with antibiotics and/or corticosteroids and a severe AECOPD as requiring an emergency department visit or hospitalization. An EXACT event was defined as a ≥12-point increase in EXACT score over 2 days or ≥9-point increase for 3 days, above the patient's EXACT baseline score (mean value over 7 days, with data for a minimum of 4 days).[20]

Statistical Analyses

Descriptive statistics were performed for outcomes with continuous measures and event counts. 16S rRNA gene datasets were processed using a standardized pipeline, and sputum microbial alpha diversity (Shannon index) was calculated using Quantitative Insights Into Microbial Ecology 2.0. Raw metagenomic reads were subject to the Sunbeam analysis pipeline. Sputum microbial alpha diversity comparisons between baseline and V2 samples were performed using the Wilcoxon test. Procrustes analysis was used to assess the microbiome similarity between baseline and V2 samples. Beta diversity was assessed using the Anosim R value. Spearman's rank correlation (rho) was performed to assess the relationship between sputum microbial alpha diversity and blood cell count and CAT score.

Spirometry data were stratified by EXACT events (0, ≥1), and change from baseline in CAT score, E-RS and EXACT scores were stratified by exacerbation status. For each of the CAT, EXACT, and E-RS component scores, patients were

categorized as "Improving", "Stable", or "Worsening", based on the 95% confidence intervals (CIs) of the slope of change over time, expressed as units per year. "Improving" was classified as data where lower and upper CIs were <0, "Worsening" where lower and upper CIs were >0, with the remaining combinations classified as "Stable". The difference in the mean slope for EXACT and E-RS score by PPO status was calculated as "any positive" minus "all negative" using analysis of variance.

Results

Patient Population

The demographics and clinical characteristics of the 63 patients recruited are reported in Table 1. Mean (standard deviation [SD]) age was 72.8 (7.0) years, 92% of patients were male, 19% were current smokers and 8% had current macrolide use. The highest proportion of patients (68%) were in GOLD grade II; 51% and 49% were categorized as GOLD group B and D, respectively. Mean (SD) post-bronchodilator (BD) FEV_1% predicted was 58.3 (14.3) and mean (SD) CAT score was 16.0 (5.4). Overall, a high proportion of patients (83%) had ≥1 exacerbation/lower respiratory tract infection that required OCS and/or antibiotics in the 12 months before recruitment; 19% required hospitalization.

Sputum Samples

From the 63 patients recruited, 62 stable-state sputum samples were collected at baseline and processed; 47% (n=29) were induced and one patient was unable to produce a sample. Of the 21 patients assessed at V2, 12 of the 13 sputum samples collected were processed for metagenomic data; 56 baseline samples and all the V2 samples had matched 16S

Table 1 Patient Demographics and Clinical Characteristics

Clinical Characteristics	Patient Population (N=63)
Age, years, mean (SD)	72.8 (7.0)
Male, n (%)	58 (92)
BMI, kg/m^2, mean (SD)	22.1 (2.9)
Current macrolide use, n (%)	5 (8)
Diagnosis, n (%)	
COPD	42 (67)
ACO	21 (33)
Type of COPD,[a] n (%)	n=42
Chronic bronchitis	17 (40)
Emphysema	38 (90)
Any vaccination,[b] n (%)	32 (51)
Smoking status	
Current smoker	12 (19)
Former smoker	51 (81)
Number of pack-years, mean (SD)	58.0 (31.0)
GOLD grade, n (%)	
I	1 (2)
II	43 (68)
III	18 (29)
IV	1 (2)

(*Continued*)

 https://doi.org/10.2147/COPD.S481406

Table 1 (Continued).

Clinical Characteristics	Patient Population (N=63)
GOLD group, n (%)	
A	0
B	32 (51)
C	0
D	31 (49)
COPD medications, n (%)	
Any	60 (95)
Anti-Ig-E/IL-5	2 (3)
Antibiotics/antiseptics	4 (6)
ICS	33 (52)
LTRA	10 (16)
Long-acting anticholinergic	50 (79)
LABA	
Once a day	48 (76)
Twice a day	6 (10)
Mucolytics	15 (24)
SABA	7 (11)
Theophylline	5 (8)
Xanthine	5 (8)
Other	53 (84)
Post-BD FEV_1% predicted, mean (SD)	58.3 (14.3)
Post-BD FEV_1/FVC ratio, mean (SD)	50.1 (11.5)
CAT score, mean (SD)	16.0 (5.4)
Exacerbations/LRTIs managed with oral corticosteroids and/or antibiotics,[c,d] n (%)	
0	11 (17)
1	37 (59)
≥2	15 (24)
Number of exacerbations/LRTIs requiring hospitalization,[d] n (%)	
0	51 (81)
1	10 (16)
≥2	2 (3)
Comorbidities at screening,[e] n (%)	
Hypertension	28 (44)
Gastro-esophageal reflux disease	10 (16)

Notes: Data were collected at baseline, unless otherwise specified; [a]patients could be diagnosed with both types of COPD; [b]including pneumococcal – polysaccharide vaccine and pneumococcal conjugate vaccine (full vaccination history) and influenza vaccine (in the prior year to enrolment to the end of the study); [c]no hospitalization; [d]in the past 12 months; [e]comorbidities with <10% prevalence are not shown.

Abbreviations: ACO, asthma-COPD overlap; BD, bronchodilator; BMI, body mass index; CAT, COPD assessment test; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; ICS, inhaled corticosteroids; Ig, immunoglobulin; IL, interleukin; LABA, long-acting β_2-agonist; LRTI, lower respiratory tract infection; LTRA, leukotriene receptor antagonist; SABA, short-acting β_2-agonist; SD, standard deviation.

rRNA gene sequencing data. Sputum cell counts are reported in the Supplementary Materials (Table S1). Of note, most patients had sputum eosinophils ≤3% (median [interquartile range]: 1.8% [3.8]).

The Microbiome of Patients with COPD

A similar microbiome composition was observed between 16S rRNA and metagenomic datasets, as indicated from the Procrustes paired analysis (M^2=0.24, P<0.001) (Supplementary Materials, Figure S1a). Of the 52 bacterial genera with a relative abundance >0.001 in the 16S rRNA gene sequencing data, *Streptococcus* was the most abundant, followed by *Veillonella*; of the 99 bacterial species with a relative abundance >0.001 in the metagenomic data, the relative abundance of *Neisseria* and *Haemophilus* was low (Table 2). *Pseudomonas aeruginosa* was not found.

The beta diversity for baseline–V2 paired samples was not significantly different for either the 16S rRNA sequencing (Anosim R=0.0035, P=0.472) (Supplementary Materials, Figure S2a) or the metagenomic sequencing (Anosim R=0.0063, P=0.458) datasets (Supplementary Materials, Figure S2b). Repeatability of the relative abundance of major bacterial genera was demonstrated between baseline and V2 (Supplementary Materials, Figure S1b). In a subanalysis comparing patients with GOLD III versus GOLD I and II, there were minimal differences in microbial taxonomic profiles and no differences in microbial diversity (Wilcoxon P=0.71; Figure 2).

Association Between Microbiome and Baseline Blood Cell Count and CAT Score

Blood cell counts are reported in the Supplementary Materials (Table S2). At the genus level, a positive correlation was observed between microbial alpha diversity measured by the Shannon index and baseline blood basophils (rho=0.41; P=0.0019, Figure 3a); no correlations were observed with other blood cells (neutrophils: rho=−0.139, P=0.310;

Table 2 Relative Abundance of Bacterial Genera and Species Identified From Baseline and V2 Sputum Samples

Taxonomy	Baseline	V2
16S rRNA data	n=56	n=12
Streptococcus	0.210	0.221
Veillonella	0.143	0.148
Neisseria	0.118	0.077
Haemophilus	0.096	0.074
Prevotella	0.091	0.070
Porphyromonas	0.032	0.044
Fusobacterium	0.034	0.025
Actinomyces	0.033	0.049
Moraxella	0.015	0.006
Metagenomic data	n=62	n=12
Neisseria subflava	0.087	0.056
Streptococcus pneumoniae	0.048	0.031
Prevotella melaninogenica	0.047	0.027
Veillonella atypica	0.030	0.065
Streptococcus mitis	0.033	0.043
Rothia mucilaginosa	0.037	0.034
Prevotella jejuni	0.035	0.025
Streptococcus pseudopneumoniae	0.033	0.056
Veillonella parvula	0.036	0.023
Haemophilus influenzae	0.033	0.025
Moraxella catarrhalis	0.016	0.014

Notes: Genera and species with an average relative abundance >0.01 are shown.
Abbreviation: V2, Visit 2.

https://doi.org/10.2147/COPD.S481406

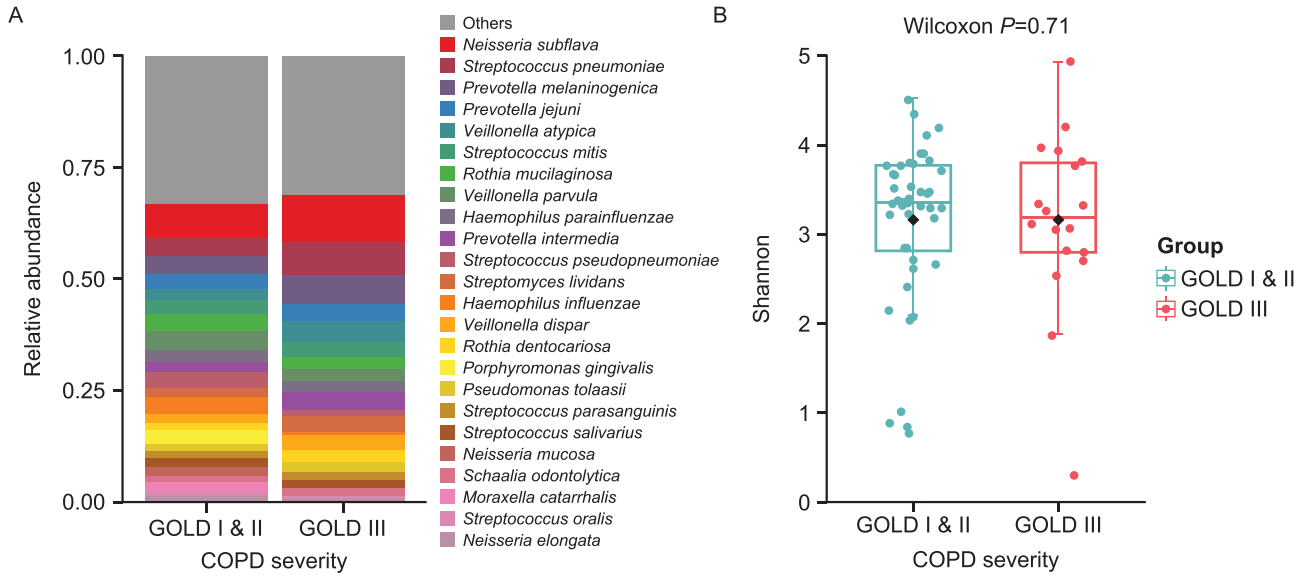

Figure 2 Taxonomic profiles (**A**) and alpha diversity between (**B**) GOLD I/II and III disease. Analysis of the metagenomic dataset, using baseline sputum samples. COPD severity according to GOLD guidelines.[8]
Abbreviations: COPD, chronic obstructive pulmonary disease; GOLD, Global Initiative for Chronic Obstructive Lung Disease.

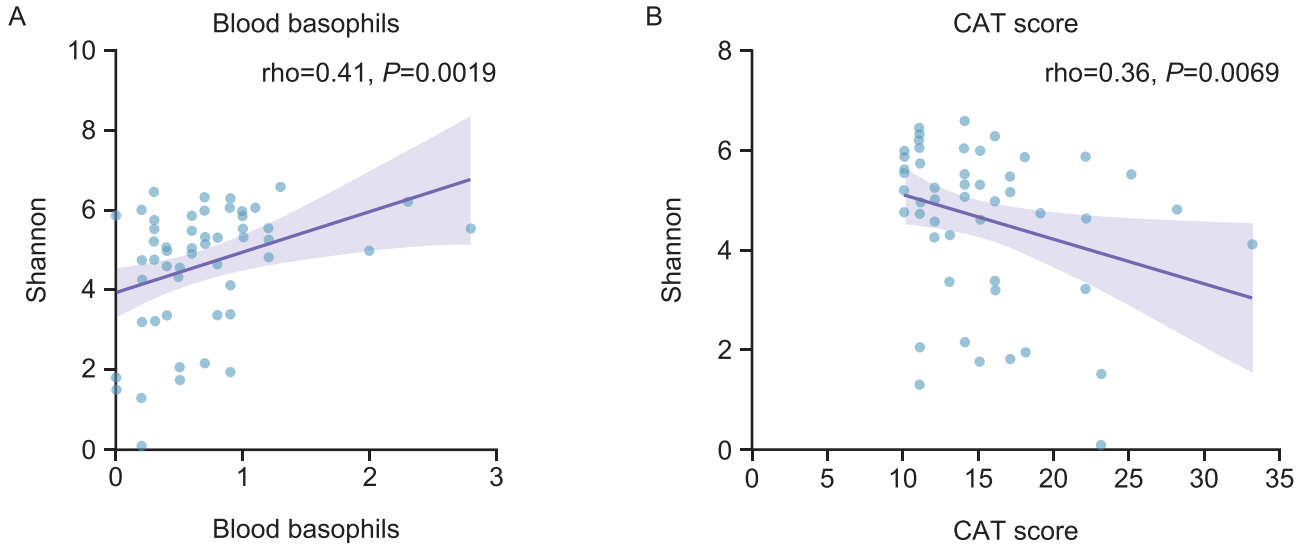

Figure 3 Correlation between Shannon diversity and baseline blood basophils (**A** and CAT score (**B**). Shannon diversity assessed by 16S rRNA gene sequencing using baseline sputum samples. CAT scores and blood basophil were assessed at baseline. Analysis performed using Spearman's rank correlation.
Abbreviations: CAT, COPD Assessment Test; COPD, chronic obstructive pulmonary disease.

eosinophils: rho=0.1739, *P*=0.2043) or sputum eosinophils (rho: −0.0637; *P*=0.7836) and there was a positive correlation between blood basophils and *Atopobium, Catonella, Eubacterium, Granulicatella*, and *Oribacterium*. The Shannon index was negatively correlated with baseline CAT score (rho=0.36; *P*=0.0069, Figure 3b), and there was a significant negative correlation between CAT score and *Aggregatibacter, Clostridia, Corynebacterium, Treponema, Tannerella*, and *TM7x* (Figure 4). After false discovery rate adjustment, the correlations between Shannon index and blood basophils and between Shannon index and CAT score remained statistically significant (*P*<0.008).

Inspection of the frequency histogram of Shannon scores showed a skewed distribution with 15 samples (27%) with scores 0–3.5 and the remaining 41 samples with scores ≥4 (Supplementary Materials, Figure S3a). On this basis, an exploratory analysis was carried out categorizing patients into low diversity and higher diversity. Compatible with the

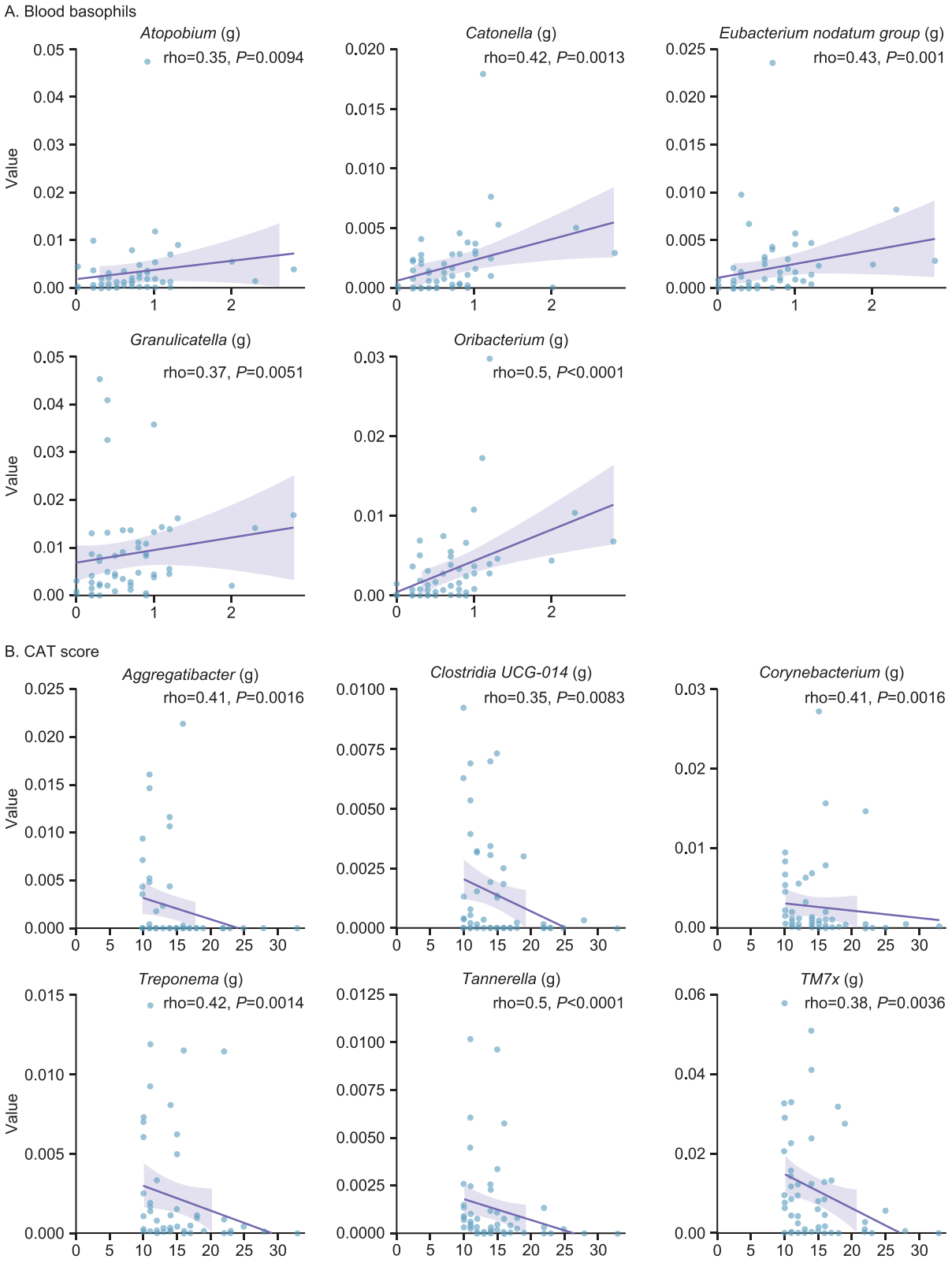

Figure 4 Correlation between microbial genera and baseline blood basophils (**A**) and CAT score (**B**). Microbial genera assessed by 16S rRNA gene sequencing, using baseline sputum samples. Blood basophils and CAT scores were assessed at baseline. Analysis performed using Spearman's rank correlation. Significant correlations ($P<0.01$) between each genus and blood basophils and CAT score.

Abbreviations: CAT, COPD Assessment Test; COPD, chronic obstructive pulmonary disease.

https://doi.org/10.2147/COPD.S481406

Table 3 Baseline Sputum Samples by PPO Species and Genera in Patients With Matched 16S Data and PPO Status by PCR or Culture

Species/Genera	Sputum Samples Negative for Specified PPO, n	Sputum Samples Positive for Specified PPO, n
Haemophilus influenzae	46	9
Moraxella catarrhalis	53	3
Streptococcus pneumoniae	50	6
Staphylococcus aureus	44	12
Pseudomonas species	27	–
Klebsiella species	55	1
Pneumocystis species	51	5

Notes: In one patient, presence or absence of *Haemophilus influenzae* by culture/PCR was not available. Data for *Pseudomonas* species were only available in culture in 27 patients, none were positive, PCR was not available.
Abbreviations: PCR, polymerase chain reaction; PPO, potentially pathogenic organism.

correlation analysis, patients with lower diversity had lower basophil counts and higher CAT scores (Supplementary Materials, Figure S3b and c). Patients with higher diversity had a wide range of basophil levels and CAT scores, whereas the distribution in the lower diversity group was narrower and more consistent around the median.

The Relationship Between PPOs and the Microbiome

In the 56 baseline sputum samples with matched 16S rRNA data, *S. aureus* and *H. influenzae* were the most prevalent PPOs (Table 3). *Pseudomonas* species were available in culture for 27 patients, though none were positive, and PCR data were not available. In a comparison of sputum samples stratified by PPO status, no difference was observed between the subgroups for alpha *(P*=0.5) diversity and observed operational taxonomic unit (*P*=0.3), nor beta (Anosim R=−0.024, *P*=0.99) microbial diversity (Figure 5); the genus-level bacterial profile was also similar (Supplementary Materials, Figure S4).

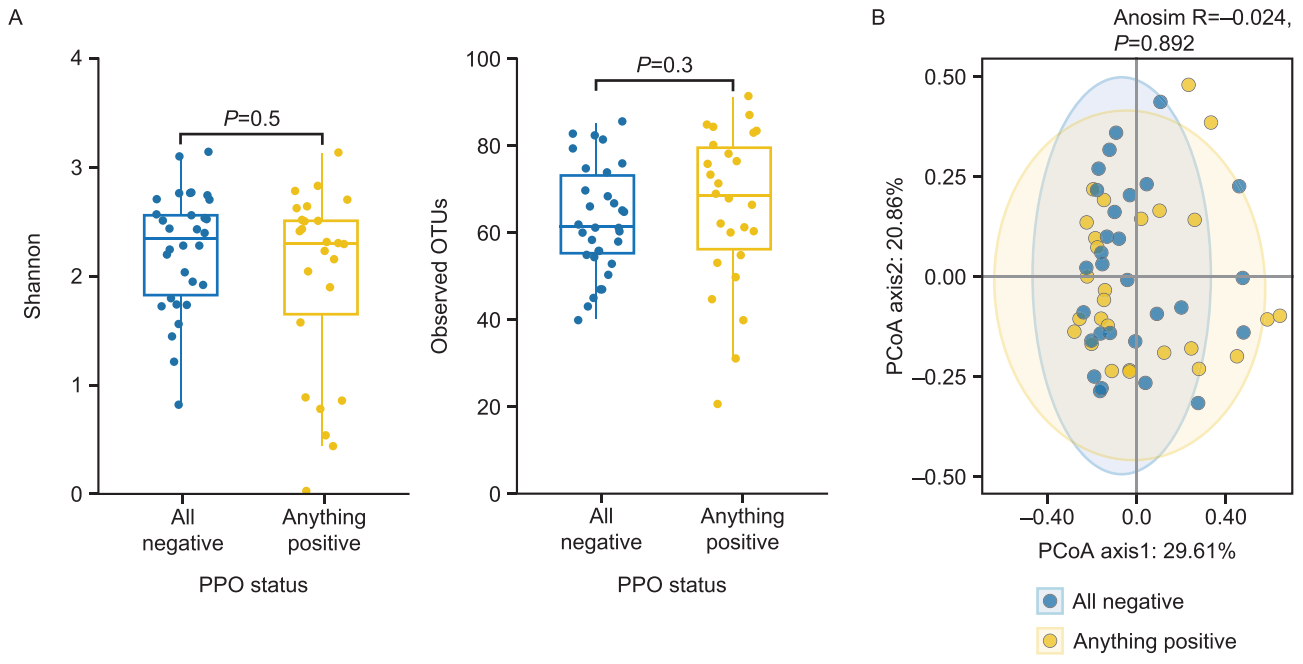

Figure 5 Baseline alpha (**A**) and beta (**B**) microbial diversity stratified by baseline PPO status. Alpha and beta diversity from 16S rRNA gene sequencing. PPO status, identified by qPCR or bacterial culture, was stratified by all negative (n=30) or any positive (n=26), using baseline sputum samples. Alpha diversity was assessed by Wilcoxon test. Beta diversity was assessed using Anosim R value.
Abbreviations: PPO, potentially pathogenic organism; qPCR, quantitative polymerase chain reaction; OTU, operation taxonomic unit; PCoA, principal coordinates analysis.

Table 4 Change From Baseline Over Time in CAT, E-RS, and EXACT Scores at V2

	All Patients (N=63)	No Event (n=43)	EXACT Event (n=13)	AECOPD Event (n=10)	AECOPD and/or EXACT Event (n=20)
CAT score	n=47	n=31	n=10	n=9	n=16
Mean (SD) slope year	−0.003	0.003	−0.017	−0.014	−0.015
	(0.016)	(0.015)	(0.012)	(0.011)	(0.012)
State, n (%)					
Improving	6 (10)	3 (7)	1 (8)	2 (20)	3 (15)
Stable	39 (62)	26 (60)	9 (69)	7 (70)	13 (65)
Worsening	2 (3)	2 (5)	0	0	0
No slope calculated[a]	16 (25)	12 (28)	3 (23)	1 (10)	4 (20)
E-RS					
Breathlessness	n=61	n=41	n=13	n=10	n=20
Mean (SD) slope year	0.002	0.003	−0.001	0	0
	(0.011)	(0.013)	(0.008)	(0.006)	(0.007)
State, n (%)					
Improving	22 (35)	11 (26)	8 (62)	5 (50)	11 (55)
Stable	17 (27)	14 (33)	2 (15)	2 (20)	3 (15)
Worsening	22 (35)	16 (37)	3 (23)	3 (30)	6 (30)
No slope calculated[a]	2 (3)	2 (5)	0	0	0
Cough and sputum	n=61	n=41	n=13	n=10	n=20
Mean (SD) slope year	0	0.001	−0.002	−0.001	−0.002
	(0.005)	(0.005)	(0.005)	(0.007)	(0.006)
State, n (%)					
Improving	21 (33)	10 (23)	7 (54)	6 (60)	11 (55)
Stable	21 (33)	16 (37)	3 (23)	3 (30)	5 (25)
Worsening	19 (30)	15 (35)	3 (23)	1 (10)	4 (20)
No slope calculated[a]	2 (3)	2 (5)	0	0	0
Chest symptoms	n=61	n=41	n=13	n=10	n=20
Mean (SD) slope year	0.002	0.003	−0.001	0.001	0
	(0.013)	(0.016)	(0.005)	(0.009)	(0.007)
State, n (%)					
Improving	23 (37)	12 (28)	8 (62)	6 (60)	11 (55)
Stable	16 (25)	14 (33)	1 (8)	1 (10)	2 (10)
Worsening	22 (35)	15 (35)	4 (31)	3 (30)	7 (35)
No slope calculated[a]	2 (3)	2 (5)	0	0	0
EXACT score	n=60	n=40	n=13	n=10	n=20
Mean (SD) slope year	0.007	0.013	−0.011	0.000	−0.004
	(0.057)	(0.066)	(0.027)	(0.034)	(0.032)
State, n (%)					
Improving	24 (38)	12 (28)	9 (69)	6 (60)	12 (60)
Stable	11 (17)	9 (21)	1 (8)	1 (10)	2 (10)
Worsening	25 (40)	19 (44)	3 (23)	3 (30)	6 (30)
No slope calculated[a]	3 (5)	3 (7)	0	0	0

Note: [a]For patients with less than three observations, no mean slope was calculated.

Abbreviations: AECOPD, acute exacerbation of COPD; CAT, COPD Assessment Test; COPD, chronic obstructive pulmonary disease; E-RS, Evaluating Respiratory Symptoms in COPD; EXACT, EXAcerbations of Chronic pulmonary disease Tool; SD, standard deviation; V2, Visit 2.

Clinical Measures

Between baseline and V2, 13 patients had EXACT events, 10 had AECOPD events and 20 had either AECOPD or EXACT events. No differences in either post-BD FEV_1% predicted or FEV_1/FVC ratio were identified between patients with or without EXACT events (Supplementary Materials, Table S3). Changes in CAT, E-RS, and EXACT scores over

Table 5 Change From Baseline Over Time/year in E-RS and EXACT Scores at V2, by PPO Status

	PPO Status		Mean Difference (95% CI)
	Any Positive (n=30)	All Negative (n=32)	
E-RS, mean (SD) slope year	n=28	n=31	
Breathlessness	−0.081 (1.697)	0.495 (2.725)	−0.577 (−1.775, 0.622)
Cough and sputum	−0.188 (1.750)	0.239 (1.529)	−0.427 (−1.282, 0.428)
Chest symptoms	−0.243 (1.739)	0.395 (1.623)	−0.638 (−1.515, 0.238)
EXACT score, mean (SD) slope year	n=27	n=31	−2.985 (−7.134, 1.164)
	−1.445 (7.320)	1.540 (8.314)	

Notes: PPO status, identified by qPCR or bacterial culture, was stratified by all negative or any positive, using baseline sputum samples. Mean difference (95% CI) calculated as any positive minus all negative using ANOVA; for patients with less than three observations, no mean slope was calculated.

Abbreviations: ANOVA, analysis of variance; CI, confidence interval; E-RS, Evaluating Respiratory Symptoms in chronic obstructive respiratory disease; EXACT; EXAcerbations of Chronic pulmonary disease Tool; PPO, potentially pathogenic organism; qPCR, quantitative polymerase chain reaction; SD, standard deviation; V2, Visit 2.

time appeared to be largely unrelated to the occurrence of an EXACT and/or AECOPD event during the study (Table 4). When the sputum samples were stratified by baseline PPO status, the rate of change in E-RS and EXACT scores over time/year all showed trends for improvement in patients who had sputum that tested positive for any PPO, and trends for worsening in patients who had sputum that tested negative for PPOs (Table 5).

Discussion

This study of patients with COPD with moderate airflow limitation found a significant association between airway microbiome diversity, CAT score, and basophil count. This appears to be a new and consistent finding.

Over 1 year, the relative abundance and diversity of the most common major bacterial genera remained consistent. No differences in diversity between mild-to-moderate and severe COPD were observed, which contrasts with the AERIS study in the UK in which microbiome diversity was lower with greater disease severity. AERIS had a greater proportion of patients with severe COPD than in AERIS-J; AERIS-J also had fewer current smokers (19% compared with 40%), patients were older and there was a higher proportion of males than in AERIS.[3] The recruitment of a population with more severe disease in AERIS may explain the differences in results between the two studies and may therefore reflect microbiome differences between milder and more severe disease.

In this study, no correlation between microbiome diversity and sputum or blood eosinophils was observed, which is in contrast to other reports that specific microbial species are associated with neutrophilic and eosinophilic inflammation.[12,21] However, such associations appear to be complex, since another study reported that blood eosinophil counts showed a positive relationship with the percentage of *Firmicutes* and *Streptococcus* and a negative association with the percentage of *Proteobacteria* and *Haemophilus*.[22] We also found no correlation between sputum neutrophils and relative abundance of organisms, particularly *H. influenzae*, which contrasts with a previous report,[23] although in that study there was no association between sputum neutrophils and colonization with *S. pneumoniae* or the presence of >1 PPO. The strength of these associations may, therefore, depend on the relative abundance of different organisms in different study populations and on the level of Type 2 inflammation in the airways. For example, in AERIS-J, despite the inclusion of a proportion of patients with physician-diagnosed ACO, the median sputum eosinophil count was 1.8%, and 75% of patients had a count of less than 3.8%.

In contrast to studies that have examined the relationship between sputum cytology and specific organisms, fewer studies have examined the relationship between overall microbiome diversity and sputum cell counts. We found a significant correlation between lower blood basophil count and reduced microbiome diversity and a positive correlation between higher basophils and a greater relative abundance of benign organisms, consistent with studies conducted in European populations.[9,12] The association between basophil count and microbiome diversity suggests that it was not

a chance finding, and it was significant after correction for a false discovery rate. The clinical and inflammatory roles of basophils in COPD pathogenesis are poorly understood, but data published in 2021 from a cross-sectional study identified increased basophil and mast cell gene signatures in patients with stable-state COPD versus healthy controls.[24] These gene signatures were positively correlated with genes that regulate eosinophilic airway inflammation and with blood eosinophils, and negatively correlated with lung function.[24] Circulating blood basophils are scarce and are therefore thought to perform a regulatory rather than effector role, which is associated with innate immunity.[25] A plausible hypothesis is that the observed association between lower basophil count and reduced microbiome diversity seen in AERIS-J relates to lower innate immunity in this patient population.[25] In support of this hypothesis, it has been suggested that the absence of a diverse microbiome may reduce bone marrow production of granulocytes.[26] Despite the paucity of data on the role of basophils in COPD, the observed association between blood basophils and microbiome diversity warrants further research.

There was a consistent negative correlation between CAT score and microbial diversity. This is consistent with findings from SPIROMICS, a multicenter, observational study, in which a significant association was reported between airway microbiome and spirometry and symptoms, including CAT score, in patients with mild COPD.[27] The most plausible mechanism is an association between lower diversity and more chronic airway inflammation,[1] which may, in turn, worsen symptoms.[8] In this study, similar alpha and beta diversity was observed between PPO-positive and -negative sputum, suggesting that the presence of PPOs may not be directly associated with the airway microbiome. In this context it is important to remember that these patients generally had mild-to-moderate disease, which may account for the absence of *Pseudomonas aeruginosa*, although this organism had low abundance even in patients with very severe disease in AERIS.[3]

In this study, we carried out an exploratory analysis of the change in COPD symptoms recorded using an eDiary over 1 year. We found that symptoms did not correlate with the degree of airflow limitation or occurrence of exacerbations over the study period. However, patients who were positive for PPOs at baseline tended to have improved breathlessness, cough and sputum and chest symptoms over 1 year, and conversely, patients who were negative for PPOs had worsening scores, as expected given the progressive nature of COPD. A possible explanation is that patients who were positive for PPOs at baseline experienced prolonged improvement from a PPO-associated infection that occurred before recruitment to the study. A history of exacerbations during the previous 2 years was an inclusion criterion, but the timing of exacerbations prior to study recruitment was not collected.

There are several strengths to this study, including the insight it provides on the microbiome for patients in the Asia–Pacific region, for which data are limited. Furthermore, the microbiome was investigated using a combination of techniques, which allowed for a more in-depth characterization and resulted in both species and genus level data. A limitation of this study was the impact of the COVID-19 pandemic on data collection, therefore, analyses of V2 data should be considered exploratory. Only a modest number of sputum samples were available for analysis, and these had to be obtained from patients with stable-state disease, albeit with a history of respiratory infection or exacerbations in the prior 24 months, limiting the generalizability of these results. Moreover, it cannot be excluded that the airway microbiome changed due to patients having fewer exacerbations and fewer viral infections as a result of social isolation and mask wearing during the COVID-19 pandemic. A further limitation was due to the exclusion of patients with no exacerbations in the previous 24 months. This meant the influence of exacerbations on comparisons between airway microbiome and clinical features could not be assessed, although the aim of this study was to evaluate the etiology of AECOPDs in Japanese patients. Future work may benefit from including a subgroup of patients without previous exacerbations. In addition, an array of culture-based methods was utilized by local laboratories for the analysis of bacterial pathogens. The lack of a harmonized culture-based method across laboratories may have influenced the results. Similarly, there may have been differences between bacteria identified by culture and qPCR due to differences in sensitivity of the two methods used.[28] Lastly, the absence of sputum cytology data prevented the assessment of any potential associations between the microbiome and sputum immune cell counts, which may have yielded important comparisons with those from European studies.[9]

Conclusions

Our data build on the global literature surrounding microbial diversity by focusing on an Asian population with COPD. AERIS-J found consistent evidence to suggest that low blood basophil counts were associated with lower sputum microbial diversity, and it is conceivable that there is a self-reinforcing cycle in which lower diversity reduces blood basophils, which in turn reduces innate immunity. Additionally, though analyses regarding correlations between clinical outcomes and patients' microbiome were exploratory, the significant association between lower CAT score and reduced microbiome diversity aligns with previous research identifying an association between microbial diversity and chronic airway inflammation.

Abbreviations

ACO, asthma-COPD overlap; AECOPD, acute exacerbations of COPD; AERIS, Acute Exacerbation and Respiratory InfectionS in COPD; AERIS-J, Acute Exacerbation and Respiratory InfectionS in COPD Japan; BD, bronchodilator; CAT, COPD Assessment Test; CI, confidence interval; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; E-RS, Evaluating Respiratory Symptoms in COPD; EXACT, EXAcerbations of Chronic pulmonary disease Tool; FEV_1, forced expiratory volume in 1 second; FVC, forced volume capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; OCS, oral corticosteroid; PPO, potentially pathogenic organism; qPCR, quantitative polymerase chain reaction; SCS, systemic corticosteroid; SD, standard deviation; V2, visit 2.

Data Sharing Statement

GSK makes available anonymized individual participant data and associated documents from interventional clinical studies that evaluate medicines, upon approval of proposals submitted to https://www.gsk-studyregister.com/en/. To access data for other types of GSK sponsored research, for study documents without patient-level data and for clinical studies not listed, please submit an enquiry via the website.

Ethics Approval and Informed Consent

The study was conducted in accordance with consensus ethical principles derived from international guidelines, including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines, ethical guidelines for Medical and Health Research Involving Human Subjects, and other applicable laws and regulations. All study documents were reviewed and approved by institutional review boards and/or independent ethics committee(s) at all investigational sites (Table S4). Patients provided informed consent.

Acknowledgments

The authors would like to thank the participating patients and their families, clinicians, and study investigators.

Editorial support in the form of preparation of the first draft based on input from all authors, and collation and incorporation of author feedback to develop subsequent drafts, was provided by Katie Crossland, PhD, and Alexandra Berry, PhD, of Fishawack Indicia Ltd, UK, part of Avalere Health, and was funded by GSK.

The institutions presented for Hiroki Kawabata, Takeo Ishii and Tamami Kobayashi are their affiliations at the time of the study.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Funding

The AERIS-J study was funded by GSK (GSK study identifier: 208636).

Disclosure

KY has received lecture fees and honoraria from GSK.

ZW and TI are former GSK employees and TI holds financial equities in GSK.

YS has received lecture fees from GSK.

NI has received lecture fees and honoraria from GSK.

TH has received lecture fees from GSK.

KF declares no conflict of interest.

OH has received lecture fees from GSK, AstraZeneca, Boehringer Ingelheim, Eli Lilly, and Novartis.

YI reports grants from Japanese Ministry of Health, Labour, and Welfare, grants from Japan Agency for Medical Research and Development, advisory board participation and lecture fees from Boehringer Ingelheim, Inc., lecture fees from Shionogi & Co. Ltd, Kyorin Pharmaceutical Co. Ltd, GSK, and Novartis, and advisory board participation for Roche/Promedior, Galapagos, Taiho Pharmaceutical, CSL Behring, and Vicore Pharma, outside the submitted work.

HK declares no conflict of interest.

KM declares no conflict of interest.

KS has received lecture fees from GSK, AstraZeneca, Boehringer Ingelheim, Ono Pharmaceutical, and Sanofi.

KT has received lecture fees from GSK, AstraZeneca, and Boehringer Ingelheim.

MY declares no conflict of interest.

RI and SK are employees of GSK.

TK and CHC were employees of GSK at the time of the study and CHC holds financial equities in GSK.

PWJ is an Emeritus Professor of Respiratory Medicine at St George's, University of London, and a former full-time employee of GSK at the time of protocol development and contributed to study design and protocol on behalf of GSK. He is a part-time consultant at GSK and holds financial equities in GSK.

References

1. Ramsheh MY, Haldar K, Esteve-Codina A, et al. Lung microbiome composition and bronchial epithelial gene expression in patients with COPD versus healthy individuals: a bacterial 16S rRNA gene sequencing and host transcriptomic analysis. *Lancet Microbe*. 2021;2(7):e300–e310. doi:10.1016/s2666-5247(21)00035-5
2. Yagi K, Huffnagle GB, Lukacs NW, Asai N. The Lung Microbiome During Health and Disease. *Int J Mol Sci*. 2021;22(19):10872. doi:10.3390/ijms221910872
3. Mayhew D, Devos N, Lambert C, et al. Longitudinal profiling of the lung microbiome in the AERIS study demonstrates repeatability of bacterial and eosinophilic COPD exacerbations. *Thorax*. 2018;73(5):422–430. doi:10.1136/thoraxjnl-2017-210408
4. Yan Z, Chen B, Yang Y, et al. Multi-omics analyses of airway host-microbe interactions in chronic obstructive pulmonary disease identify potential therapeutic interventions. *Nat Microbiol*. 2022;7(9):1361–1375. doi:10.1038/s41564-022-01196-8
5. D'Alessandro-Gabazza CN, Yasuma T, Kobayashi T, et al. Inhibition of lung microbiota-derived proapoptotic peptides ameliorates acute exacerbation of pulmonary fibrosis. *Nat Commun*. 2022;13(1):1558. doi:10.1038/s41467-022-29064-3
6. D'Alessandro-Gabazza CN, Kobayashi T, Yasuma T, et al. A Staphylococcus pro-apoptotic peptide induces acute exacerbation of pulmonary fibrosis. *Nat Commun*. 2020;11(1):1539. doi:10.1038/s41467-020-15344-3
7. Shimizu K, Yoshii Y, Morozumi M, et al. Pathogens in COPD exacerbations identified by comprehensive real-time PCR plus older methods. *Int J Chron Obstruct Pulmon Dis*. 2015;10:2009–2016. doi:10.2147/copd.s82752
8. Global Initiative for Chronic Obstructive Lung Disease. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease: 2024 Report. Available from: https://goldcopd.org/2024-gold-report/. Accessed 11, October, 2024.
9. Wang Z, Locantore N, Haldar K, et al. Inflammatory Endotype-associated Airway Microbiome in Chronic Obstructive Pulmonary Disease Clinical Stability and Exacerbations: A Multicohort Longitudinal Analysis. *Am J Respir Crit Care Med*. 2021;203(12):1488–1502. doi:10.1164/rccm.202009-3448OC
10. Wang Z, Bafadhel M, Haldar K, et al. Lung microbiome dynamics in COPD exacerbations. *Eur Respir J*. 2016;47(4):1082–1092. doi:10.1183/13993003.01406-2015
11. Bafadhel M, McKenna S, Terry S, et al. Acute exacerbations of chronic obstructive pulmonary disease: identification of biologic clusters and their biomarkers. *Am J Respir Crit Care Med*. 2011;184(6):662–671. doi:10.1164/rccm.201104-0597OC
12. Wang Z, Liu H, Wang F, et al. A Refined View of Airway Microbiome in Chronic Obstructive Pulmonary Disease at Species and Strain-Levels. *Front Microbiol*. 2020;11:1758. doi:10.3389/fmicb.2020.01758
13. Morimoto C, Matsumoto H, Nomura N, et al. Sputum microbiota and inflammatory subtypes in asthma, COPD, and its overlap. *J Allergy Clin Immunol Glob*. 2024;3(1):100194. doi:10.1016/j.jacig.2023.100194
14. Kato M, Tomii K, Hashimoto K, et al. The IMPACT Study - Single Inhaler Triple Therapy (FF/UMEC/VI) Versus FF/VI And UMEC/VI In Patients With COPD: Efficacy And Safety In A Japanese Population. *Int J Chron Obstruct Pulmon Dis*. 2019;14:2849–2861. doi:10.2147/copd.s226601

15. Ishii T, Nishimura M, Akimoto A, James MH, Jones P. Understanding low COPD exacerbation rates in Japan: a review and comparison with other countries. *Int J Chron Obstruct Pulmon Dis*. 2018;13:3459–3471. doi:10.2147/copd.s165187
16. Cheng DO, Hurst JR. COVID-19 and 'basal' exacerbation frequency in COPD. *Thorax*. 2021;76(5):432–433. doi:10.1136/thoraxjnl-2020-216637
17. Alqahtani JS, Oyelade T, Aldhahir AM, et al. Reduction in hospitalised COPD exacerbations during COVID-19: a systematic review and meta-analysis. *PLoS One*. 2021;16(8):e0255659. doi:10.1371/journal.pone.0255659
18. Leidy NK, Murray LT, Monz BU, et al. Measuring respiratory symptoms of COPD: performance of the EXACT- Respiratory Symptoms Tool (E-RS) in three clinical trials. *Respir Res*. 2014;15(1):124. doi:10.1186/s12931-014-0124-z
19. Leidy NK, Wilcox TK, Jones PW, Roberts L, Powers JH, Sethi S. Standardizing measurement of chronic obstructive pulmonary disease exacerbations. Reliability and validity of a patient-reported diary. *Am J Respir Crit Care Med*. 2011;183(3):323–329. doi:10.1164/rccm.201005-0762OC
20. Leidy NK, Murray LT, Jones P, Sethi S. Performance of the EXAcerbations of chronic pulmonary disease tool patient-reported outcome measure in three clinical trials of chronic obstructive pulmonary disease. *Annals of the American Thoracic Society*. 2014;11(3):316–325. doi:10.1513/AnnalsATS.201309-305OC
21. Sze MA, Dimitriu PA, Suzuki M, et al. Host Response to the Lung Microbiome in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. 2015;192(4):438–445. doi:10.1164/rccm.201502-0223OC
22. Dicker AJ, Huang JTJ, Lonergan M, et al. The sputum microbiome, airway inflammation, and mortality in chronic obstructive pulmonary disease. *J Allergy Clin Immunol*. 2021;147(1):158–167. doi:10.1016/j.jaci.2020.02.040
23. Beech AS, Lea S, Kolsum U, et al. Bacteria and sputum inflammatory cell counts; a COPD cohort analysis. *Respir Res*. 2020;21(1):289. doi:10.1186/s12931-020-01552-4
24. Winter NA, Gibson PG, McDonald VM, Fricker M. Sputum Gene Expression Reveals Dysregulation of Mast Cells and Basophils in Eosinophilic COPD. *Int J Chron Obstruct Pulmon Dis*. 2021;16:2165–2179. doi:10.2147/copd.s305380
25. Chirumbolo S, Bjørklund G, Sboarina A, Vella A. The role of basophils as innate immune regulatory cells in allergy and immunotherapy. *Hum Vaccin Immunother*. 2018;14(4):815–831. doi:10.1080/21645515.2017.1417711
26. Balmer ML, Schürch CM, Saito Y, et al. Microbiota-Derived Compounds Drive Steady-State Granulopoiesis via MyD88/TICAM Signaling. *The Journal of Immunology*. 2014;193(10):5273–5283. doi:10.4049/jimmunol.1400762
27. Opron K, Begley LA, Erb-Downward JR, et al. Lung microbiota associations with clinical features of COPD in the SPIROMICS cohort. *NPJ Biofilms Microbiomes*. 2021;7(1):14. doi:10.1038/s41522-021-00185-9
28. Schoonbroodt S, Ichanté JL, Boffé S, et al. Real-time PCR has advantages over culture-based methods in identifying major airway bacterial pathogens in chronic obstructive pulmonary disease: Results from three clinical studies in Europe and North America. *Front Microbiol*. 2022;13:1098133. doi:10.3389/fmicb.2022.1098133