






Drivers of year-to-year variation in exacerbation frequency of COPD: analysis of the AERIS cohort

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ABSTRACT The association between exacerbation aetiology and exacerbation frequency is poorly understood.

We analysed 2-year follow-up data from a prospective observational study of patients with chronic obstructive pulmonary disease (COPD) (www.clinicaltrials.gov identifier number NCT01360398) to evaluate year-to-year variation in exacerbation frequency and related aetiology. A total of 127 patients underwent blood and sputum sampling monthly and at exacerbation to detect respiratory infections and eosinophilic inflammation; 103 continued into year 2 and 88 completed both years.

The most common bacterial species at stable state and exacerbation was *Haemophilus influenzae*. Among infrequent exacerbators (one exacerbation per year), the incidence of viral infection at exacerbation was high (60.0% (95% CI 35.1–81.7%) in year 1 and 78.6% (53.4–94.2%) in year 2). Those with more frequent exacerbations tended to have higher relative incidence of bacterial than viral infection. Patients with at least two additional exacerbations in year 2 *versus* year 1 had a higher risk of *H. influenzae* colonisation at stable state than those with at least two fewer exacerbations, as detected by culture (OR 1.43 (95% CI 0.71–2.91) *versus* 0.63 (0.40–1.01), $p=0.06$) and PCR (1.76 (95% CI 0.88–3.51) *versus* 0.56 (0.37–0.86), $p<0.01$). This was not seen with other infection types or eosinophilic inflammation.

Analysis of the same cohort over 2 years showed, for the first time, that changes in yearly COPD exacerbation rate may be associated with variations in *H. influenzae* colonisation.

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Analysis of the same cohort of patients over 2 years showed that changes in yearly COPD exacerbation rate may be associated with variations in *Haemophilus influenzae* colonisation
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Introduction

Acute exacerbations of chronic obstructive pulmonary disease (AECOPD) lead to a faster decline in lung function, worsened quality of life and increased risk of hospitalisation [1] and are a significant prognostic factor of reduced survival across all chronic obstructive pulmonary disease (COPD) stages [2].

Exacerbations may be triggered by respiratory bacterial or viral infections and environmental factors, such as pollution [1, 3]. Their frequency can be predicted by patients' exacerbation history [4] and is linked to disease severity, with frequent exacerbators experiencing a greater decline in forced expiratory volume in 1 s over time than infrequent exacerbators [5].

Recent studies have reported an association between exacerbation risk and a shift in the microbiome towards enrichment of Proteobacteria [6–10]. However, little is known about the association between exacerbation aetiology and frequent/infrequent exacerbator phenotypes or the factors that promote a change in exacerbation frequency from one year to the next beyond the effects of prescribed therapies for AECOPD, such as bronchodilator and corticosteroid treatments or macrolide antibiotics [3].

We previously reported first-year results from the prospective longitudinal Acute Exacerbation and Respiratory Infections in COPD (AERIS) study in which COPD patients underwent sputum sampling each month and at exacerbation over 2 years to assess how changes in the airway microbiome contribute to the incidence and severity of AECOPD [9, 11]. During the first year of follow-up, exacerbations were associated with infections with *Moraxella catarrhalis* and nontypeable *Haemophilus influenzae*, and with respiratory viruses, particularly human rhinovirus (HRV) [9]. The composition of the lung microbiome showed unique profiles within subtypes of COPD exacerbations, with nonrandom probabilities of repeating particular subtypes [10].

We now present 2-year follow-up data from the AERIS study with the aim to evaluate year-to-year variation in exacerbation frequency and the aetiology related to these events.

A “focus on the patient” section (figure 1) summarises the clinical relevance and impact of this study on the patient population.

Methods

Study design and procedures

The 2-year prospective, observational cohort AERIS study (www.clinicaltrials.gov registration NCT01360398) was based at University Hospital Southampton, UK. The study protocol has been published [11] and a summary is available at www.gsk-clinicalstudyregister.com (study identifier 114378). AERIS was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice, and was approved by the Southampton and South West Hampshire Research Ethics Committee. All participants provided written informed consent. Results have been reported for subjects followed over 1 year for the primary objective

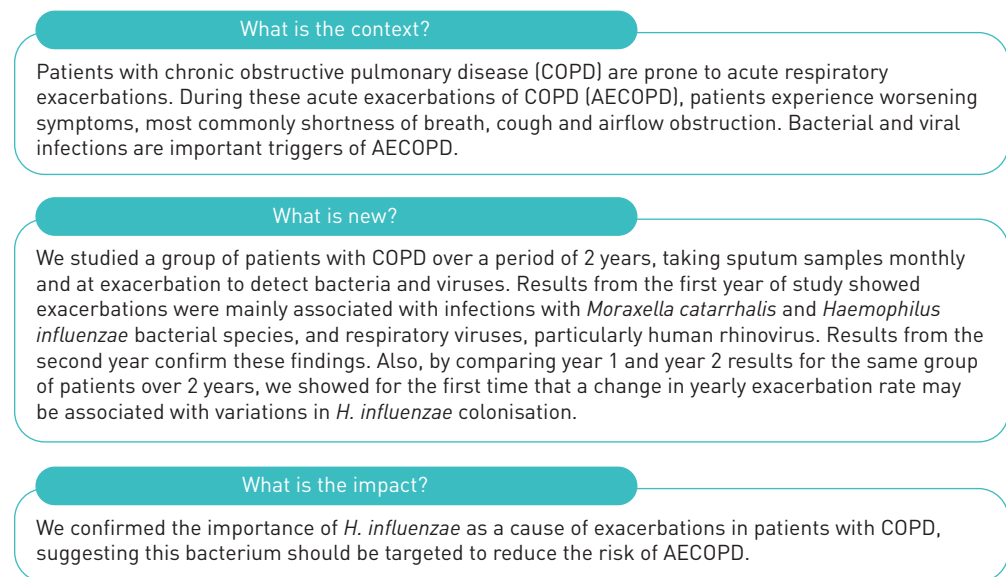


FIGURE 1 Focus on the patient.

(incidences of AECOPD and AECOPD with sputum containing bacterial pathogens), and on incidences of bacterial and viral pathogens and eosinophilic inflammation in AECOPD and stable state [9, 10, 12].

Study procedures and definitions of AECOPD and COPD severity categories were described previously [9]. Briefly, patients aged 40–85 years with a confirmed diagnosis of COPD, categorised as moderate, severe or very severe [11, 13], were recruited from June 2011 to June 2012. Patients were followed monthly for 24 months in the stable state and reviewed within 72 h of onset of AECOPD symptoms. Exacerbations were detected using daily electronic diary cards.

Sputum samples were obtained by spontaneous expectoration or induced and were processed according to standard methods, as described previously [9]. COPD exacerbation subtypes were classified as bacterial, viral or eosinophilic, using specific criteria [14]: bacterial, if at least one positive potentially pathogenic bacteria culture was isolated (*H. influenzae*, *M. catarrhalis*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* or *Staphylococcus aureus*); viral, if at least one positive viral PCR result; eosinophilic, if eosinophils were >3% of nonsquamous cells from sputum. Bacterial colonisation was indicated by bacterial presence at stable state.

Statistical analysis

The sample size calculation was described previously [11]. Results are presented for the full cohort, defined as all patients considered by the investigator as eligible for study procedures and excluding those who withdrew consent at the first visit. In descriptive analyses that compared follow-up results from the first year of the study to those from the second year (months 13 to 24), patients who completed both years of the study (completer cohort) were considered as well as data for the full cohort.

The percentage of stable-state and exacerbation-state sputum samples containing bacterial or viral pathogens or eosinophils >3% was calculated with 95% confidence intervals computed using the generalised linear model assuming a negative binomial distribution for the response variable with logarithm as link function, and the logarithm of time for follow-up as an offset variable.

To examine the relationship between the number of exacerbations each year and the presence of bacteria, viruses or eosinophils >3%, generalised estimating equation regression models with random intercept were used. These analyses should be considered *post hoc* as they were not included in the AERIS statistical analysis plan.

Statistical analysis was performed using the SAS Drug Development platform version 4.3.2 (SAS Institute, Cary, NC, USA).

Results

Study population

Of the initial 127 subjects, 103 entered year 2 (full cohort, year 2) and 88 completed all follow-up visits (completer cohort) (figure 2). There were no major differences in baseline characteristics, including COPD severity, among the study cohorts (table 1). Rates of sputum collection in year 1 and year 2 were high (figure 2). In both years, most AECOPD samples (67% in year 2 and 71% in year 1) were collected within 2 days of the start of exacerbation symptoms.

Most sputum samples were taken before antibiotic administration. In year 2, no antibiotic had been administered before sputum collection for 91.3% (619 out of 678) stable and 88.7% (173 out of 195) exacerbation samples and, in year 1, for 98.9% (948 out of 959) stable and 91.4% (296 out of 324) exacerbation samples. The percentage of follow-up time during which patients received azithromycin was 3.0% in year 1 and 11.5% in year 2 at stable state. Eosinophilic inflammation (eosinophils >3%) was present in 35.5% of sputum samples at stable state and 24.7% at exacerbation in year 1, and 33.1% and 24.3%, respectively, in year 2.

Incidence and aetiology of AECOPD

In year 1, the mean exacerbation rate was 3.04 (95% CI 2.63–3.50) per patient-year and 2.32 (95% CI 1.99–2.72) per patient-year in the 103 patients who continued into year 2. A total of 223 acute exacerbations were recorded in year 2 and 60.2% of patients had two or more exacerbations (figure 3 and table S1). During the first year of follow-up, 355 acute exacerbations were recorded and 61.4% of patients had two or more exacerbations. The number of exacerbations missed during year 1 and year 2 was 27 and 29, respectively. In both years, most exacerbations were moderate in severity (191 (85.6%) in year 2 *versus* 304 (85.6%) in year 1). Among the 33 patients with five or more exacerbations in year 1 (figure 3), seven (21%) discontinued the study. Among the 88 completers, in year 2 *versus* year 1, 32 (36%) had at least two fewer exacerbations, 13 (15%) had one fewer, 15 (17%) had no change, 13 (15%) had one more and 15 (17%) had at least two more events.

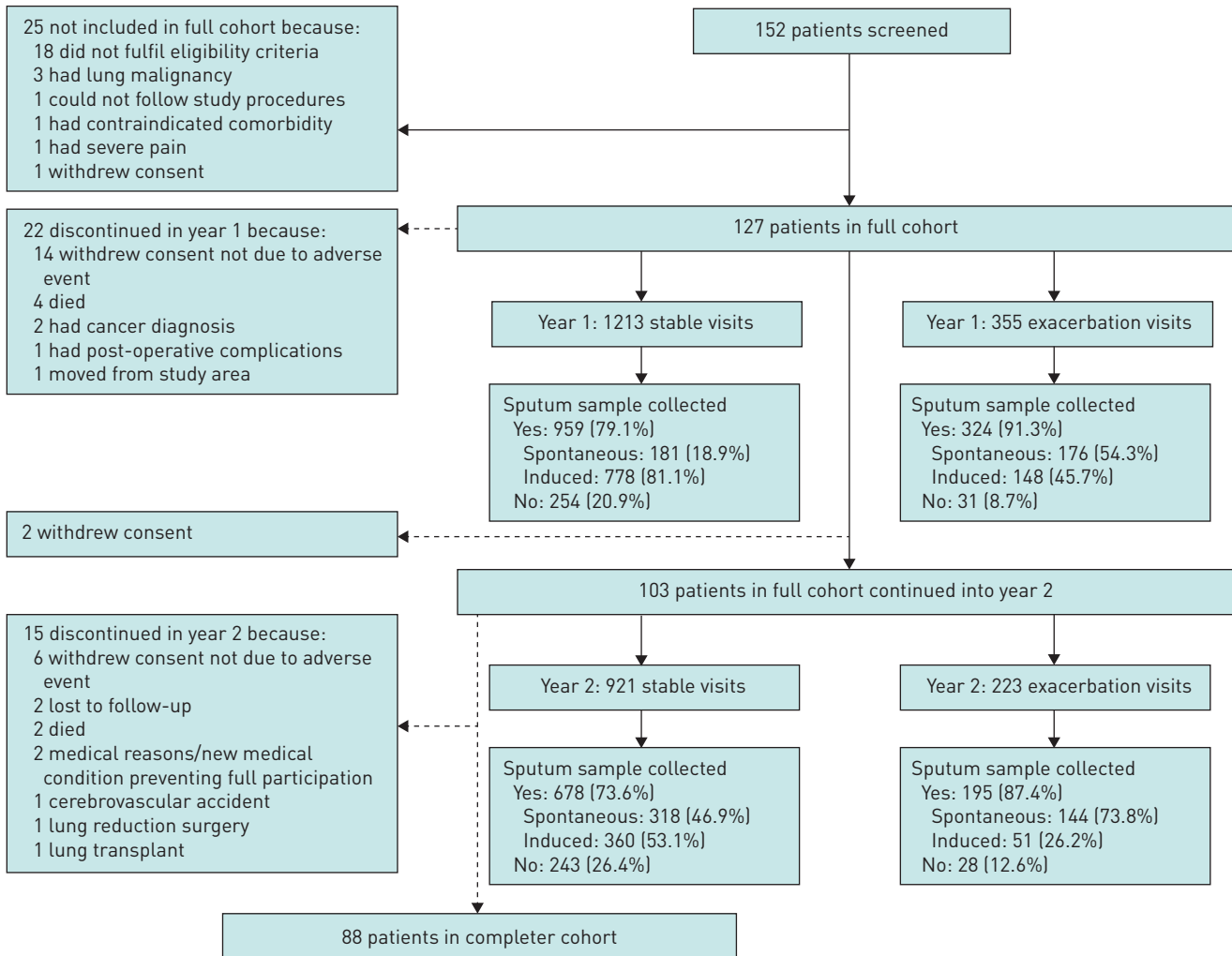


FIGURE 2 Flow chart of patients and sputum sampling in the study.

The most common bacterial species detected by sputum culture and/or PCR in both years was *H. influenzae* (figure 4 and table S2). As in year 1, the percentage of samples in year 2 that were positive by culture for *H. influenzae* or *M. catarrhalis* was higher at exacerbation than at stable state (figure 4). With PCR detection, no increase was found in year 2 in the percentage of samples positive for *H. influenzae* (41.0% (95% CI 36.9–45.2%) at stable state *versus* 39.0% (31.8–46.6%) at exacerbation), while an increase was observed for *M. catarrhalis* (12.5% (95% CI 9.9–15.6%) at stable state *versus* 17.5% (95% CI 12.2–23.9) at exacerbation).

As in year 1, the percentage of sputum samples positive for at least one virus increased from stable state to exacerbation in year 2 (from 11.0% to 33.3%) (figure 4). As expected, the incidence of specific viral pathogens varied from year to year but the most common species detected was HRV, in 6.4% and 18.1% of stable and exacerbation samples, respectively.

Aetiology of AECOPD relative to exacerbator frequency

We examined the presence of bacteria relative to the presence of viral pathogens or eosinophilic sputum samples and number of exacerbations reported each year per patient in the completer cohort. In year 1, among patients with collected sputum, 17 were considered infrequent exacerbators (one exacerbation), 30 were frequent exacerbators (two to four exacerbations) and 24 were very frequent exacerbators (more than four exacerbations). In year 2, 16 patients were infrequent exacerbators, 42 were frequent exacerbators and nine were very frequent exacerbators.

At exacerbation, infrequent exacerbators tended to have a lower incidence of bacterial infection relative to viral infection, while those with more frequent exacerbations tended to have a higher incidence of bacterial

TABLE 1 Characteristics of the patients at enrolment

Characteristic	Full cohort	Full cohort, year 2	Completer cohort
Patients n	127	103	88
Age at enrolment years	66.8±8.6	66.7±8.7	66.9±8.9
Female sex	59 (46.5%)	45 (43.7%)	37 (42.0%)
Smoking history pack-years	47.0 (33.7–60.0)	50.4 (34.0–60.0)	50.0 (33.5–58.5)
Medication for COPD	127 (100%)	103 (100%)	88 (100%)
Influenza vaccination during previous year	114 (89.8%)	94 (91.3%)	82 (93.2%)
Pneumococcal vaccination during previous year	12 (9.4%)	10 (9.7%)	8 (9.1%)
COPD status by GOLD stage			
Mild	0 (0%)	0 (0%)	0 (0%)
Moderate	57 (44.9%)	46 (44.7%)	42 (47.7%)
Severe	51 (40.2%)	44 (42.7%)	37 (42.0%)
Very severe	19 (15.0%)	13 (12.6%)	9 (10.2%)
BODE index	4 (2–6)	4 (2–5)	4 (2–5)
\dot{V}_{LCO} predicted/actual mmol.kPa⁻¹.min⁻¹	7.9 (7.2–8.8)/4.5 (3.4–5.8)	7.9 (7.4–9.0)/4.5 (3.5–6.1)	8.0 (7.4–8.9)/4.5 (3.6–6.0)
Subjects reporting exacerbations in 12 months before study start			
One exacerbation	28 (22.0%)	21 (20.4%)	20 (22.7%)
Two exacerbations	37 (29.1%)	30 (29.1%)	28 (31.8%)
Three exacerbations	25 (19.7%)	22 (21.4%)	17 (19.3%)
Four or more exacerbations	37 (29.1%)	30 (29.1%)	23 (26.1%)
Exacerbations in preceding 12 months	3.1±2.3/2 (2–4)	3.2±2.4/3 (2–4)	3.1±2.4/2 (2–4)
Exacerbations in preceding 12 months according to severity			
Mild	0.5±1.2	0.4±1.2	0.5±1.3
Moderate	2.3±1.9	2.5±2.0	2.3±2.0
Severe	0.4±0.6	0.3±0.6	0.3±0.5
FEV₁ after bronchodilator use % predicted	46.4±15.2	46.7±14.6	47.7±14.1

Data are presented as mean±sd, n (%) and/or median (interquartile range), unless otherwise stated. COPD: chronic obstructive pulmonary disease; GOLD: Global Initiative for Chronic Obstructive Lung Disease; BODE: body mass index, obstruction, dyspnoea, exercise capacity; \dot{V}_{LCO} : transfer capacity of the lung for carbon monoxide; FEV₁: forced expiratory volume in 1 s.

relative to viral infection (figure 5 and table S3). For example, among frequent exacerbators, in sputum collected during exacerbation, bacterial infection was detected by culture in 60.0% (95% CI 47.9–71.4%) of patients in year 1 and 52.3% (95% CI 41.9–62.5%) in year 2, while viral infection was detected in 43.8% (95% CI 32.0–56.0%) in year 1 and 42.2% (95% CI 31.9–52.9%) in year 2. For infrequent exacerbators, bacterial infection was detected by culture in 76.5% (95% CI 53.5–92.0%) in year 1 and 50.0% (95% CI 26.9–73.1%) in year 2, while viral infection was detected in 60.0% (95% CI 35.1–87.1%) in year 1 and 78.6% (95% CI 53.4–94.2%) in year 2. Evaluation of the presence of infection at stable visits relative to exacerbation frequency did not reveal any clear trends (figure 5 and table S3).

An absence of clear aetiology (bacterial or viral infection or eosinophils >3%) at exacerbation was detected more frequently in very frequent exacerbators (15.6% (95% CI 9.6–23.2%) in year 1 and 16.1% (95% CI 6.1–31.5%) in year 2) than in frequent (6.3% (95% CI 2.0–13.9%) and 12.0% (95% CI 6.2–20.2), respectively) or infrequent exacerbators (0% in both years). Detection percentages were comparable among exacerbator groups at stable state (table S3).

Influence of aetiological factors on change in yearly exacerbation frequency

We examined the influence of bacterial or viral infection or eosinophilic inflammation at stable state on changes in exacerbation frequency from year 1 to year 2 in the completer cohort. Different rates of *H. influenzae* colonisation at stable state appeared to drive the likelihood of frequent exacerbation in year 2, a pattern that was not seen with *M. catarrhalis* colonisation, viral infection or eosinophilic inflammation (figure 6). For example, those with two or more additional exacerbations in year 2 versus year 1 were likely to have a higher rate of *H. influenzae* colonisation detected by culture (7.2% increase in *H. influenzae* presence; OR 1.43, 95% CI 0.71–2.91) than those with at least two fewer exacerbations in year 2 than in year 1 (8.5% decrease; OR 0.63, 95% CI 0.40–1.01) (difference between ORs, $p=0.060$). For *H. influenzae*

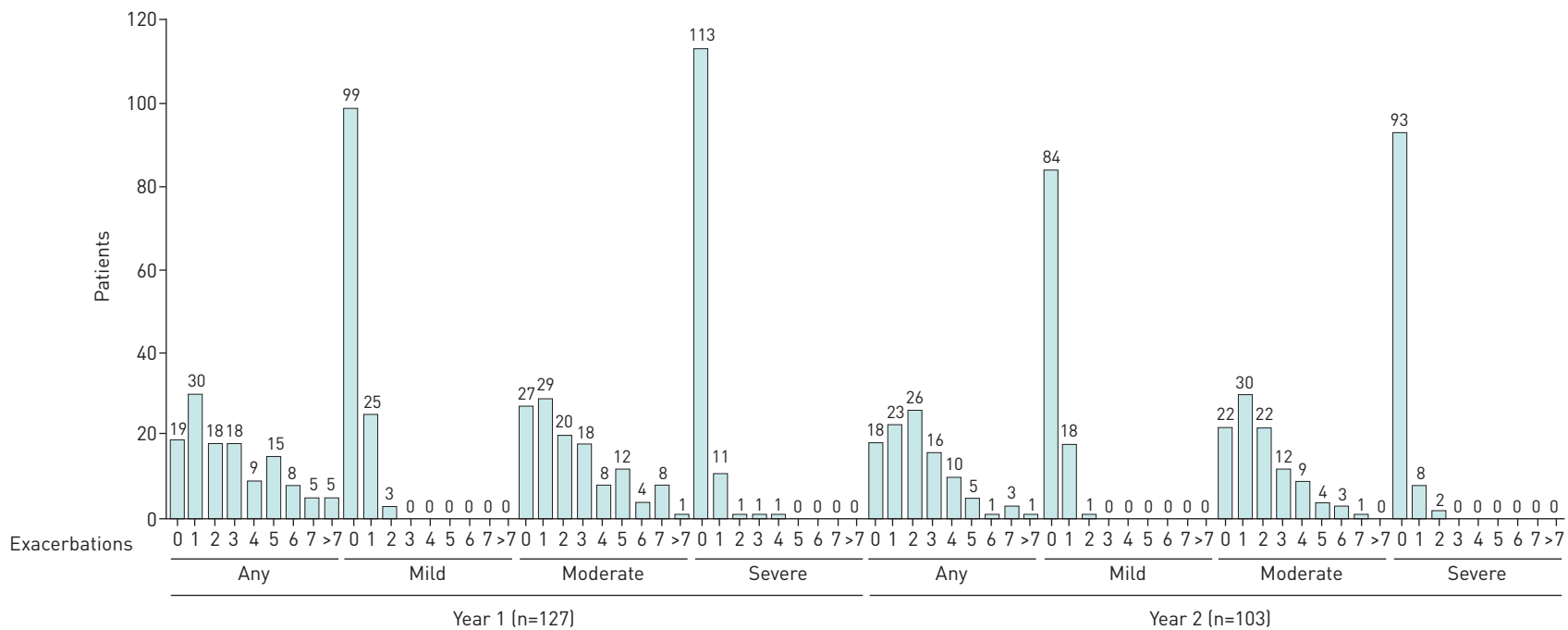


FIGURE 3 Distribution of patients in the first- or second-year follow-up according to the number of acute exacerbations of chronic obstructive pulmonary disease presented and their severity [full cohort, year 1 or year 2]. The “any” category indicates overall classification; patients could experience exacerbations in one or more severity category.

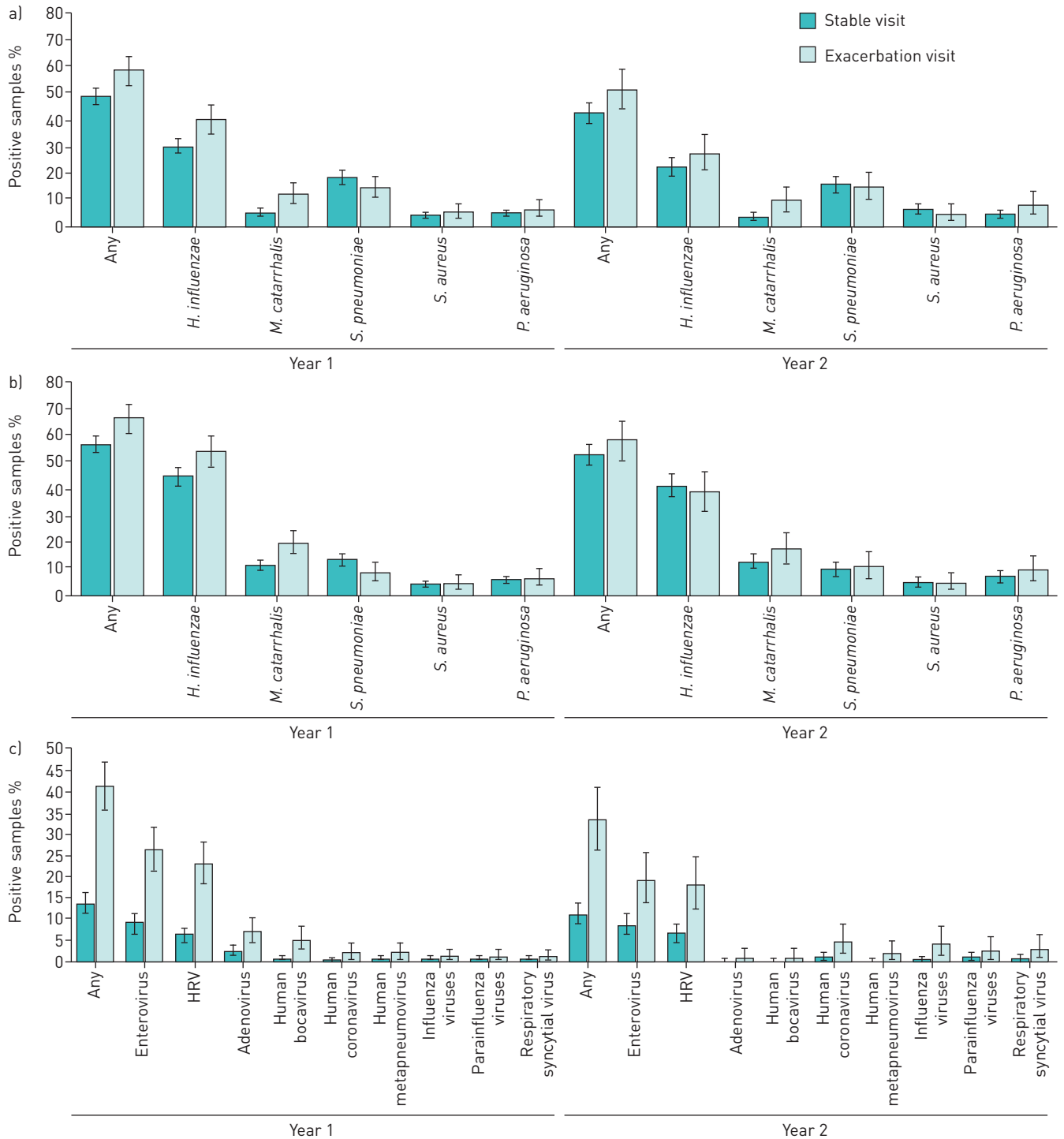


FIGURE 4 Percentage of culture-positive or PCR-positive sputum samples at stable state and exacerbation in year 1 and year 2 [full cohort, year 1 or year 2]. a) Percentage culture-positive for bacteria. b) Percentage PCR-positive for bacteria. c) Percentage PCR-positive for virus. Error bars represent 95% confidence intervals. HRV: human rhinovirus.

colonisation detected by PCR, there was a 13.1% increased detection (OR 1.76, 95% CI 0.88–3.51) for those with two or more additional exacerbations in year 2 *versus* year 1 and a 13.7% decrease (OR 0.56, 95% CI 0.37–0.86) for those with at least two fewer exacerbations ($p=0.006$). Repetition of this analysis for any study visit showed similar trends, with a slightly stronger effect for *H. influenzae* ($p<0.01$ for both detection methods) (figure S1), possibly because of increased detection at exacerbation *versus* stable state (figure 4).

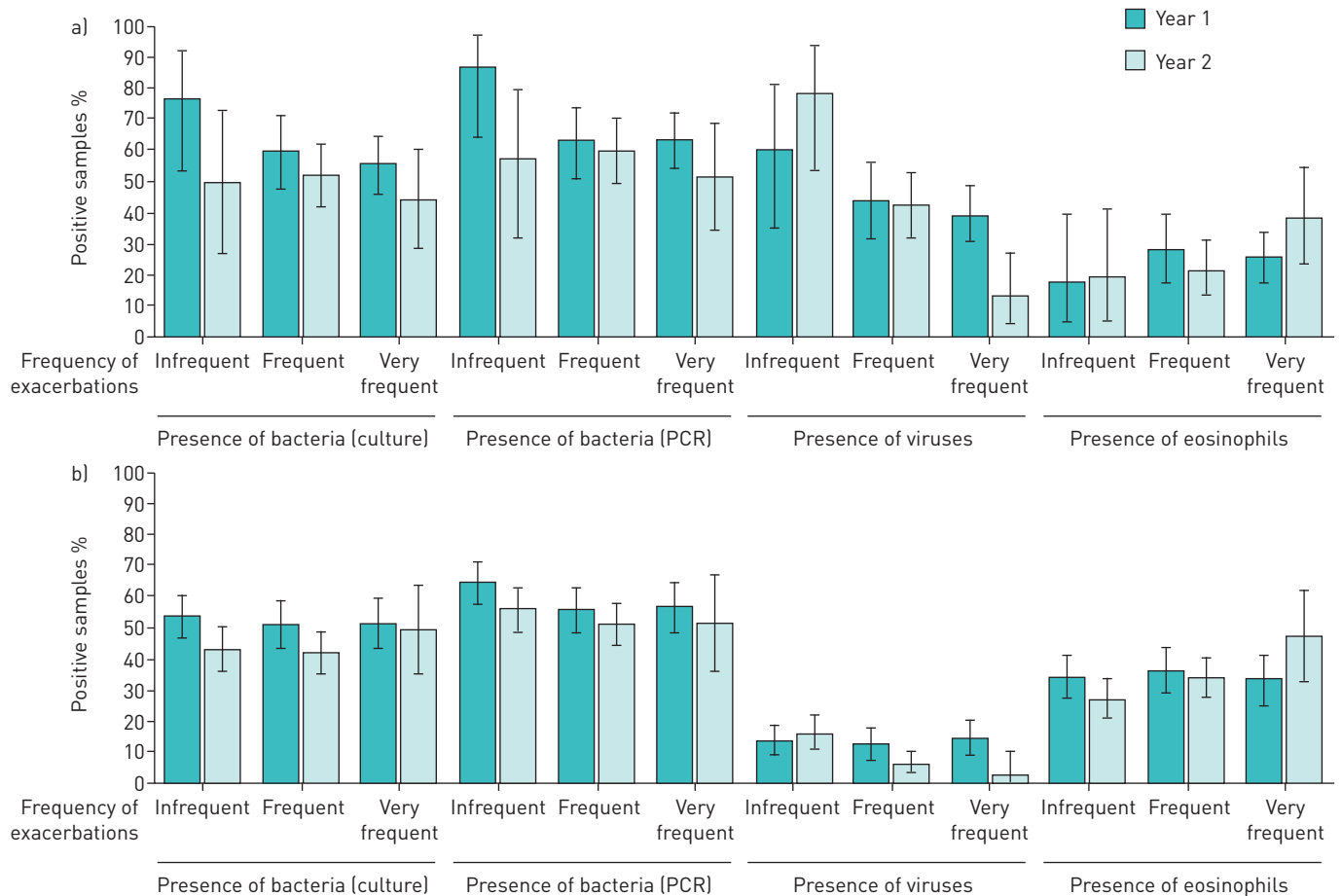


FIGURE 5 Percentage of chronic obstructive pulmonary disease patients with culture- or PCR-positive sputum samples for bacterial or viral pathogens or eosinophilic sputum samples [presence of eosinophils >3%] at a) exacerbation and b) stable visits, by frequency of exacerbations reported during year 1 or year 2 (completer cohort). Exacerbation frequency in specified year: infrequent, one or (for stable visits) none; frequent, two to four; very frequent, more than four. Error bars represent 95% confidence intervals.

Chronic macrolide use tended to be associated with fewer exacerbations in year 2. Among the 88 completers, 16 patients were administered chronic azithromycin treatment (six and 10 starting in year 1 and year 2, respectively). For those who started the antibiotic in year 1, the exacerbation rate decreased by at least two. In year 2, rates decreased or increased *versus* year 1, possibly because antibiotic treatments were initiated at different times, with variable patient follow-up. Ranking patients by difference in number of exacerbations in year 2 *versus* year 1 (table S4) showed those with at least two fewer exacerbations had the highest proportion of visits with chronic azithromycin treatment during the first (5.8%) and second year (18.6%). For those with one fewer exacerbation, the proportion of visits under azithromycin were, respectively, 0% and 15.6%. No patients with the same or an increased exacerbation rate in year 2 received chronic azithromycin treatment in the first year.

Discussion

We used 2-year follow-up data from the AERIS study to evaluate, for the first time, how year-to-year variation in exacerbation frequency is influenced by the aetiology of AECOPD, thus gaining novel insights into the drivers of exacerbation frequency in an individual patient. Our analysis shows that infrequent exacerbators are likely to have high incidence of viral infection at exacerbation, while those with more frequent exacerbations tend to have a higher relative incidence of bacterially associated events. This suggests that bacterial infection at exacerbation may be a key driver of the frequent exacerbator phenotype rather than viruses and that, as exacerbation frequency increases, a higher proportion of events in an individual is likely to be bacterially related. This effect was not seen at stable state, which is possibly indicative of a relatively constant microbiome, as reported previously in clinically stable COPD patients [10, 15]. In terms of viral infections, these are deemed to be stochastic events that occur following transmission and are identified less frequently than bacterial infection during an exacerbation [16].

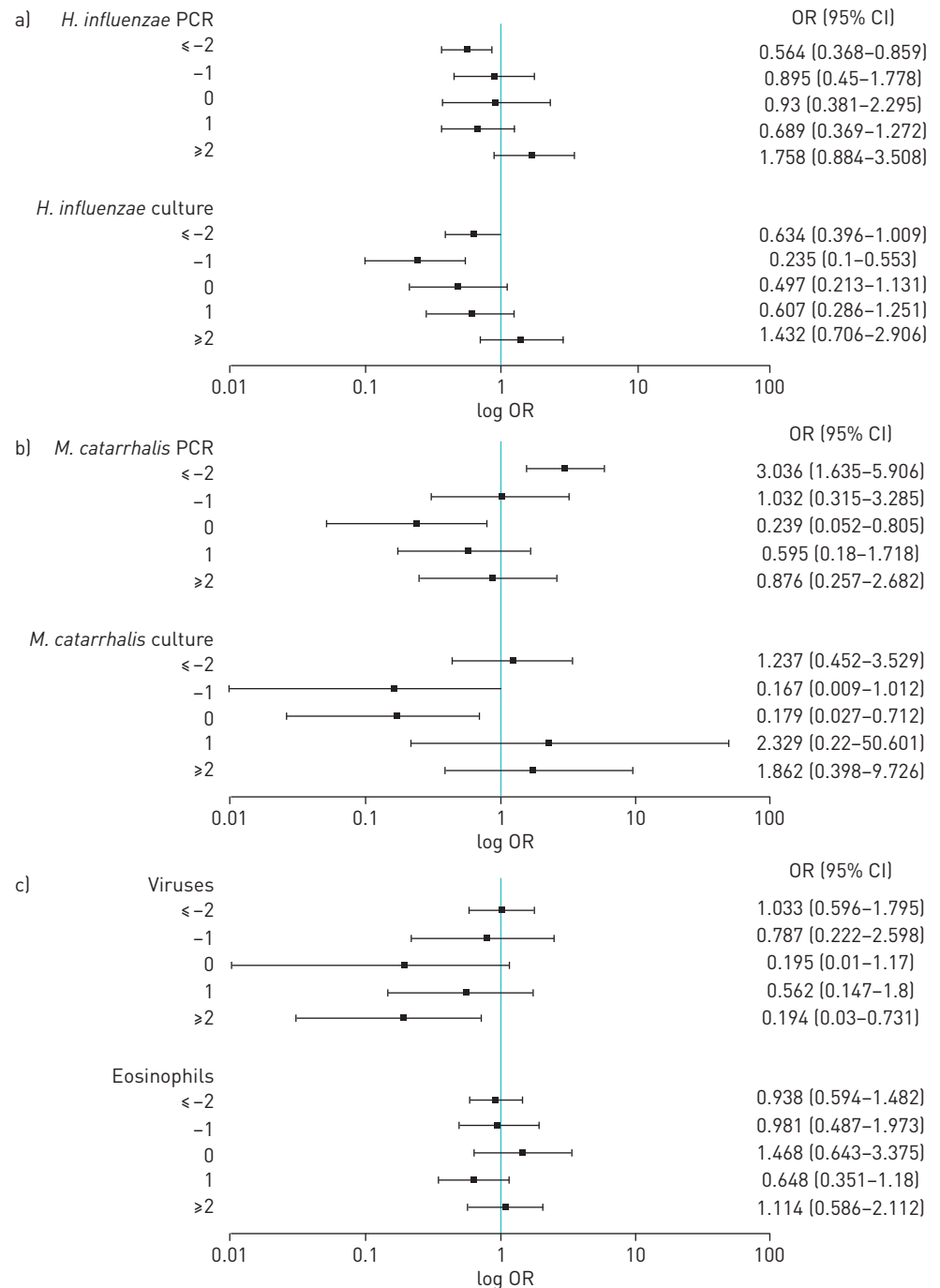


FIGURE 6 Effect of the presence of aetiological factors (bacterial or viral infection, or eosinophilic inflammation) at stable visits on the odds of experiencing a change in number of exacerbations in year 2 relative to year 1 (completer cohort). a) *Haemophilus influenzae* detected by PCR or culture. Comparison of ≤-2 and ≥2 groups, $p=0.006$ (PCR) and $p=0.060$ (culture). b) *Moraxella catarrhalis* detected by PCR or culture. Comparison of ≤-2 and ≥2 groups, $p=0.068$ (PCR) and 0.673 (culture). c) Viruses and eosinophils. Comparison of ≤-2 and ≥2 groups, $p=0.052$ (viruses) and $p=0.669$ (eosinophils). ≤-2: at least two fewer exacerbations in year 2 than in year 1; -1: one fewer exacerbation; 0: no change; 1: one more exacerbation; ≥2: at least two more exacerbations. Error bars represent 95% confidence intervals. p -values were estimated using the Wald test.

Bacterial and viral co-infection is associated with more impaired lung function and longer hospital stays than only bacterial or viral infection [17–19]. Therefore, viral infections alone are unlikely to drive the more frequent exacerbation phenotype. Among the very frequent exacerbation phenotype, exacerbations not related to infection or associated with eosinophilic inflammation were detected more often than in the

frequent or infrequent exacerbation groups, possibly due to the frailty of these COPD patients and the impact of comorbidities in driving symptomatic deteriorations.

We also examined the effect of bacterial or viral infection or eosinophilic inflammation on a change in exacerbation frequency from one year to the next. *H. influenzae* colonisation at stable state appears to drive the likelihood of exacerbation since those with additional exacerbations in year 2 *versus* year 1 had a higher rate of *H. influenzae* colonisation at stable state than those who experienced no increase or fewer exacerbations in year 2 than in year 1. Consistent results were obtained for the full cohort, including patients with incomplete follow-up (data not shown). Persistent infection of the COPD airways with *H. influenzae* has been shown to contribute to chronic airway inflammation that increases symptoms and accelerates pulmonary disease progression [20]. Our results support chronic colonisation with *H. influenzae* as an important, potentially modifiable risk factor for exacerbations. The mechanism for this susceptibility to exacerbation in *H. influenzae*-colonised individuals may be partly related to an interaction with viral infection, as noted from previous AERIS study results [9], as well as independent effects. Moreover, the inflammatory milieu has been shown to be altered in the *H. influenzae*-colonised airway of COPD patients, with significantly greater levels of interleukin (IL)-1 β in bronchoalveolar lavage samples than in COPD patients negative for *H. influenzae* [21]. This may be a direct result of infection and stimulation of inflammasomes and IL-1 β secretion [22].

There were similarities year-to-year in relative incidence of the most common bacterial and viral pathogenic species in AECOPD. As expected, some variation in incidence of viruses was observed but HRV remained the most common virus detected in year 2. *H. influenzae* was the most common bacterial infection in both years, in line with previous study results [23–25]. However, the rate of exacerbations that were positive for *H. influenzae* was lower in the second year than in the first year for the full cohort and the same trend was observed for *M. catarrhalis*. This may be related to dissimilarities between the year 1 and year 2 populations, although no clear differences in cohort demographics were seen. The intensive nature of the study could have been a contributing factor since patients received a high level of clinical attention. Fewer patients with a very frequent exacerbator phenotype in year 2 suggests patients in the completer cohort may have been less frail in the second year than in year 1. However, among patients with at least five exacerbations in the first year, only 21% did not complete two years of follow-up. Also, importantly, there is an indication of changes in clinical practice since stable state patients in the full cohort received azithromycin during 3.0% of their follow up in year 1 compared with 11.5% in year 2, which is likely to have altered airway microbial patterns and exacerbation frequency. Azithromycin use is clinically indicated by UK national guidelines [26] and our observation of a lower exacerbation rate in year 2 adds further support for the use of azithromycin in AECOPD treatment at least in the short term.

The strengths, including the early capture of exacerbations, and limitations of the AERIS study design were described in detail previously [9] and limitations specific to the year-to-year analyses have been discussed regarding possible clinical differences between the year 1 and year 2 study populations. With the stratification of patients according to exacerbator phenotype, the number of patients per exacerbation rate category was small. The results of these *post hoc* analyses should therefore be regarded as trends observed in the context of complex effects contributed by various aetiological factors, which need to be investigated further in larger prospective studies.

In conclusion, this year-to-year analysis of patients with COPD found that, whilst overall patterns of infection were similar across both years of the study, there were important differences within certain patients, which give valuable insights into the aetiology of exacerbations. By analysing the same cohort of patients over two years, we show for the first time that a change in yearly exacerbation rate may be associated with variations in *H. influenzae* colonisation. This confirms the importance of *H. influenzae* as a driver of exacerbation risk and identifies this bacterium as a modifiable factor and hence therapeutic target for future clinical trials of interventions to reduce the risk of exacerbation with this common, global disease.

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