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Acute Exacerbation and Respiratory InfectionS in COPD (AERIS): a prospective, observational cohort study

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ARTICLE SUMMARY

Article focus

- Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) is a prospective longitudinal epidemiological study initiated in the UK to assess how dynamic changes in the COPD airway microbiome contribute to the incidence and severity of acute exacerbations of COPD (AECOPD).

Key messages

- There remains a genuine need to further explore the aetiology and pathogenesis of AECOPD.
- It is anticipated that results of this study will increase our understanding of the contribution of bacterial and viral pathogens to AECOPD, potentially leading to research into targeted therapeutic and preventative interventions.

Strengths and limitations of this study

- Conducted in a specialised hospital that has extensive experience in respiratory research.
- Comprehensive assessment of clinical status, microbiology, functional status, nutritional status, health-related quality-of-life and healthcare resource utilisation in individual patients in a single large cohort during both stable COPD and AECOPD
- AECOPD are proactively identified through patient-completed electronic diaries.
- Cohort retention is a key factor in the successful delivery of such a study and with in-depth sampling protocols, participant engagement, comfort and feedback are key factors in optimising cohort retention and comprehensive data collection.

ABSTRACT

Introduction: The aetiology of acute exacerbations of chronic obstructive pulmonary disease (AECOPD) remains incompletely understood and strategies for the treatment and prevention of AECOPD have not altered significantly for many years. Improved understanding of the role of respiratory pathogens in AECOPD may lead to the development of more targeted therapeutic approaches.

Methods and analyses: Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) is a prospective longitudinal epidemiological study initiated in the UK to assess how dynamic changes in the COPD airway microbiome contribute to the incidence and severity of AECOPD. COPD patients aged 40–85 years are followed monthly for 2 years, and seen in the clinic within 72 hours of onset of symptoms of AECOPD. Exacerbations are detected using electronic diary cards that subjects complete daily. Blood, sputum, nasopharyngeal and urine samples are collected at pre-specified time-points. Molecular diagnostic and typing techniques are used to describe the dynamics of airway infection during AECOPD and stable disease, and its association with clinical outcome. This study also aims at refining the case definition of AECOPD to reflect the possible microbiological aetiology of exacerbations. The study will also assess the impact of AECOPD on health-related quality-of-life and healthcare resource utilisation, as well as the possible interaction between nutritional status, infection and cellular immune responses.

Ethics and dissemination: AERIS is conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and has been approved by the relevant institutional ethics and review board. All subjects must provide written informed consent. The results obtained will be disseminated by presentations at international medical conferences and peer-reviewed publications.

Discussion: To our knowledge, few other studies have employed real-time electronic tracking of symptoms to identify AECOPD and potential aetiological triggers. It is anticipated that results of AERIS will increase our understanding of the contribution of bacterial and viral pathogens to AECOPD, potentially leading to research into targeted therapeutic and preventative interventions.

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an inflammatory disease of the lung, characterised by progressive airflow limitation that is not fully reversible.[1] COPD is the most common chronic respiratory illness in older adults, affecting an estimated 210 million people worldwide.[2] This condition has a substantial impact on quality-of-life.[2] The Global Burden of Disease Study found COPD to be the third leading cause of death globally and the ninth leading cause of years of life lost due to premature mortality in 2010,[3] accounting for 3.7% of years lived with disability and 3.1% of disability-adjusted life years worldwide.[4,5] COPD also imposes a substantial socioeconomic burden. In 2001, the total cost of COPD in Europe was reported to be €38.7 billion.[6]

Considerable progress has been made concerning the epidemiology, pathophysiology and clinical management of COPD in recent years. However, significant challenges remain. Improved understanding of acute exacerbations of COPD (AECOPD) is a key research priority. AECOPD are highly relevant clinically, being a major cause of COPD-related morbidity and mortality,[7–11] as well as accounting for a substantial proportion of the significant social, healthcare and economic burden of COPD.[6] It has been estimated that AECOPD account for approximately 70% of total healthcare costs associated with COPD.[12] Patients with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage II or more disease experience 1 or 2 exacerbations annually. Exacerbation varies from patient to patient with severity of disease.[13] Various triggers for AECOPD have been identified;[1] however, up to 75% of all exacerbations are associated with the detection of bacterial and/or viral respiratory pathogens.[14,15] Exacerbations associated with detectable respiratory pathogens have been shown to have a more marked impact on

lung function and longer duration of hospitalisation than exacerbations of non-infectious aetiology.[14]

With the introduction of new molecular sequencing techniques, the traditional belief that healthy lungs are sterile has been refuted. There is increasing evidence that the lower respiratory tract contains a diverse microbial flora that differs between health and disease.[16–20] The presence of potentially pathogenic microorganisms in the inflamed airways of patients with COPD is well-documented, with up to 50% of patients with stable COPD showing evidence of lower airway bacterial colonisation using traditional culture techniques.[15,21,22] In patients with COPD, bacterial detection in lower airway derived samples is associated with increased airway inflammation, reduced lung function and more frequent exacerbations.[23–25] Acquisition of new pathogen strains also appears to be associated with an increased risk of AECOPD.[15,21,26] Estimates of the relative contribution of different pathogens to AECOPD vary. However, non-typeable *Haemophilus influenzae* appears to be the major bacterial pathogen associated with AECOPD, followed by *Streptococcus pneumoniae, Moraxella catarrhalis and Pseudomonas aeruginosa.*[14,15] Respiratory viruses commonly associated with AECOPD include human rhinoviruses, influenza and parainfluenza viruses, respiratory syncytial virus, coronavirus and adenovirus.[15]

Improved understanding of the role of infectious pathogens in AECOPD may lead to the development of more targeted strategies for treatment and prevention. This paper describes the objectives and design of Acute Exacerbation and Respiratory InfectionS in COPD (AERIS), a prospective longitudinal epidemiological study initiated in the UK to assess the role of respiratory infection in acute exacerbations of COPD. Molecular diagnostic and typing techniques will be used to describe the dynamics of airway infection and its potential association with clinical outcome. The

study will also assess the impact of AECOPD on health-related quality-of-life and healthcare resource utilisation, as well as the possible interaction between disease endotype and exacerbations.

OBJECTIVES

The primary objective of the AERIS study is to estimate the incidence of all-cause AECOPD and of AECOPD with sputum containing bacterial pathogens (overall and by species). Secondary study objectives are summarised in **table 1**.

Table 1 Overview of primary and secondary objectives of the AERIS study

Level	Objective
Primary	• To estimate the incidence rate of all-cause AECOPD • To estimate the incidence rate of AECOPD having sputum containing bacterial pathogens (overall and by species)
Secondary	• To describe the proportion of overall and specific bacterial pathogens detected in sputum by severity of AECOPD • To describe the proportion of overall and specific bacterial pathogens detected in sputum in stable COPD • To estimate the incidence rate of AECOPD having sputum containing viral pathogens (overall and by species) • To describe the proportion of overall and specific viral pathogens detected in sputum by severity of AECOPD • To estimate the time elapsed between consecutive AECOPD episodes • To assess the impact of all-cause AECOPD and stable COPD on health-related quality-of-life • To assess the impact on healthcare use: – Of all-cause AECOPD – Of AECOPD having sputum containing bacterial pathogens – Of AECOPD having sputum containing viral pathogens

AECOPD, acute exacerbations of chronic obstructive pulmonary disease

METHODS

Study design

This is an ongoing, single-centre, prospective, observational cohort study based at University Hospital Southampton, UK.

Study population

Male and female COPD patients between the age of 40 and 85 years are eligible for study participation provided they meet the following inclusion criteria: (i) a confirmed diagnosis of COPD with post-bronchodilator forced expiratory volume in one second (FEV_1) ≤80% of the predicted normal value and FEV_1/forced vital capacity (FVC) ≤0.7, consistent with GOLD stage II–IV disease,[27] (ii) current or ex-smoker with smoking history ≥10 pack years, and (iii) 1 or more documented exacerbations of COPD treated with antibiotics and/or steroids in the 12 months prior to enrolment (**table 2**). Exclusion criteria include other known respiratory conditions, such as asthma, as the only cause of the respiratory obstructive disorder, α-1 antitrypsin deficiency, cystic fibrosis, tuberculosis, lung cancer, previous history of lung surgery and other conditions imposing pneumonia risk. Subjects on long-term antibiotic therapy at the time of enrolment and those who have received antibiotics and/or steroids in the month prior to the enrolment are also excluded.

Table 2 Study inclusion and exclusion criteria

Inclusion criteria	Subjects must satisfy ***ALL*** of the following criteria at study entry: • Subjects who the investigator believes can and will comply with the requirements of the protocol • Written informed consent obtained from the subject • Male or female aged 40–85 years • Confirmed diagnosis of COPD based on post-bronchodilator spirometry (Gold et al. 2009), with FEV_1 ≤80% of predicted normal and FEV_1/FVC <0.7 • Moderate, severe, or very severe COPD, according to GOLD staging (Gold et al. 2009) • Current or prior history of ≥10 pack years of cigarette smoking[a,b] • Documented history of ≥1 exacerbation requiring antibiotics and/or oral corticosteroids or hospitalisation in the previous 12 months[c]
Exclusion criteria	• A confirmed diagnosis of asthma (as only cause of obstructive respiratory disorder), cystic fibrosis, pneumonia risk factors or other respiratory disorders (e.g., tuberculosis, lung cancer) • History of lung surgery • α-1 antitrypsin deficiency as underlying cause of COPD • Moderate or severe COPD exacerbation not resolved at least 1 month prior to enrolment and less than 30 days following the last dose of oral corticosteroids[d] • Use of any antibacterial, antiviral or respiratory investigational drug or vaccine within 30 days of the enrolment visit • Evidence of alcohol or drug abuse • Presence of other conditions that the principal investigator judges may interfere with the study findings • Risk of non-compliance or inability to comply with the study procedures • Women who are pregnant or lactating or are planning on becoming pregnant during the study

[a] Former smokers are defined as those who have stopped smoking for at least 6 months
[b] Number of pack years = (number of cigarettes per day/20) x number of years smoked
[c] Subjects with recent COPD exacerbations, in stable condition, and having stopped antibiotics, can be enrolled one month post-exacerbation
[d] Subjects can be enrolled when their AECOPD or pneumonia has resolved

Clinical data collection

Subjects are seen for an enrolment visit and then monthly for 2 years. Regular review of medications and, when required, changes to medical therapy and active smoking cessation advice are performed according to standard clinical practice at each visit. In addition to these scheduled visits, all subjects are seen in the clinic within 72 hours (3 days) of onset of symptoms of AECOPD. AECOPD is defined as worsening of at least 2 major symptoms (dyspnoea, sputum volume and sputum purulence) or worsening of at least 1 major symptom and 1 minor symptom (wheeze, sore throat, cold [nasal discharge and/or nasal congestion], cough and fever [oral temperature >37.5 °C] without other cause),[28] considered clinically relevant at the site. Exacerbations are identified by means of electronic diary cards that subjects complete daily. The data recorded daily in the electronic diary cards includes self-performed peak flow measurement (peak expiratory flow [PEF] and FEV_1), a series of morning questions to identify symptoms of exacerbations[29] and the EXAcerbations of Chronic Pulmonary Disease Tool Version 1.0 (EXACT-PRO) at bedtime. Subjects are also asked to record any changes to their usual treatment. Data on patient-reported symptoms based on morning questions and on PEF/FEV_1 are transmitted daily to the study clinic. Changes/worsening in these symptoms are monitored by the study staff and subjects are contacted and invited to the clinic when an exacerbation is suspected.

Study procedures

In addition to the daily monitoring undertaken through the patient-completed electronic diary cards, a wide range of study procedures are performed at study entry, scheduled monthly visits and exacerbation visits (**table 3**).

Table 3 Overview of study assessments performed at the scheduled monthly visits and at exacerbation visits

Description		Frequency of assessment*
Clinical variables		
Physical examination		Monthly and within 72 hours of onset of exacerbation
Anthropometrics and nutritional screening (MUST)[a]		Quarterly
Intercurrent comorbidities		Monthly and within 72 hours of onset of exacerbation
Medical history / medical record review		Study entry and within 72 hours of onset of exacerbation
Vaccination history		Annually
Current medication		Monthly
Smoking status		Monthly
Urine pregnancy test		Study entry, final visit and within 72 hours of onset of exacerbation
Chest CT-scan		Study entry and final visit
Chest X-ray		Within 72 hours of onset of exacerbation
Lung function testing	Body box	Study entry and final visit
	TLCO[b]	Every 6 months and within 72 hours of onset of exacerbation
	Spirometry	Monthly and within 72 hours of onset of exacerbation
6-minute walk test		Every 6 months
Questionnaires		
ATS-DLD-78A (Risk factors, disease history and smoking history)		Study entry

Healthcare use[c]		Monthly and within 72 hours of onset of exacerbation
mMRC[d]		Every 6 months
CAT questionnaire[e]		Quarterly and within 72 hours of onset of exacerbation
EQ-5D index and VAS[f]		Quarterly and within 72 hours of onset of exacerbation
NEADL[g]		Quarterly and within 72 hours of onset of exacerbation
CNAQ[h]		Quarterly and within 72 hours of onset of exacerbation
Biological specimen collection		
Blood sampling	For routine biochemistry	Study entry
	For cell-mediated immune response	Quarterly and within 72 hours of onset of exacerbation
	For biomarkers, blood counts and haematology	Quarterly and within 72 hours of onset of exacerbation
	For RNA transcript profiling	Every 6 months and within 72 hours of onset of exacerbation
	For vitamins, anti-oxidants and nutrients (20 ml)	Every 6 months and within 72 hours of onset of exacerbation
Nasopharyngeal swab sampling[i]		Monthly and within 72 hours of onset of exacerbation
Sputum sampling		Monthly and within 72 hours of onset of exacerbation
Breath sampling[j]		Monthly and within 72 hours of onset of exacerbation
Urine sampling[k]		Monthly and within 72 hours of onset of exacerbation

*In addition to study entry

[a]Height, weight, mid-arm circumference, waist circumference, triceps skin fold measurement, fat free body mass. [b]TLCO: transfer factor. [c]Healthcare use includes medication, vaccination, oxygen therapy, use of mechanical ventilation, pulmonary rehabilitation treatment, surgical intervention, outpatient visits (including GP visit contacts to COPD team), emergency room visits, hospitalisations, and productivity loss (time missed from work or usual activities due to worsening of COPD symptoms) [d]mMRC: Medical Research Council Dyspnea Scale score. [e]CAT: COPD Assessment Test. [f]VAS: visual analogue scale. [g]NEADL: Nottingham Extended Daily Activities Scale. [h]CNAQ: Council on Nutrition Appetite Questionnaire. [i]In all subjects at study entry and in a subcohort of 30 subjects during the first year. jIn a subcohort of approximately 80 subjects. [k]In all subjects at study entry and within 72 hours of every exacerbation and in the subcohort of 30 subjects providing nasopharyngeal swabs during the first year of the study

Clinical assessments

Quantitative high-resolution computed tomography scans are performed at enrolment and study conclusion to describe the degree of bronchiectasis and emphysema resulting from COPD and to exclude other acute or evolving lung pathologies besides COPD and sequelae of COPD. A physical examination is performed at all visits. Medical history, smoking status and details of medication use are updated monthly. Influenza and pneumococcal vaccination status is updated annually.

Lung function testing is performed using spirometry, body plethysmography (lung volumes, body box) and single breath diffusion (gas transfer, TLCO) at specified visits. The following outcomes will be recorded: spirometry, FEV_1, FVC, FEV_1/FVC ratio, FEV_1 % predicted, mid expiratory flow between 25 and 75% of the forced vital capacity (MEF25-75), single breath diffusion (transfer factor [TLCO] and rate of carbon monoxide uptake [KCO], and body plethysmography (total lung capacity [TLC], residual volume [RV], vital capacity [VC] and RV/TLC). At the enrolment visit, subjects are asked to refrain from using short-acting bronchodilators for at least 6 hours and long-acting bronchodilators for at least 12 hours before key procedures. Prior to the subsequent follow-up visits, subjects may use their usual medication normally. Lung function measurements are performed under controlled conditions and in the sitting position as per standard practice.

Anthropometrics (including but not restricted to height, weight, waist and mid-arm circumference and triceps skin-fold circumference) are measured quarterly. Grip strength and fatigability are measured using standard techniques. Anthropometric data are used to compute the Malnutrition Universal Screening Tool (MUST) score.[30]

Nutritional information (including planned/ unplanned weight loss, and history and changes in food intake patterns) is collected quarterly according to MUST guidelines.

A postero-anterior chest X-ray (and lateral if required) is performed at all exacerbation visits, as per standard clinical practice, in order to exclude pneumonia.

Questionnaires

Various outcomes are assessed quarterly and at exacerbation using a series of questionnaires and patient-reported outcomes instruments such as the COPD Assessment Test (CAT),[31] the Nottingham Extended Daily Activities Scale (NEADL),[32] the Council on Nutrition Appetite Questionnaire (CNAQ),[33] and the EQ-5D.[34] The BODE index (Body-Mass Index, Degree of Airflow Obstruction, Level of Functional Dyspnea, Exercise Capacity)[35] will also be calculated. Healthcare use is recorded at all visits, including medication, vaccination, oxygen therapy, use of mechanical ventilation, pulmonary rehabilitation treatment, surgical intervention, outpatient visits (including GP visits and telephone contacts to COPD team), emergency room visits, hospitalisations, and productivity loss (time missed from work or usual activities due to worsening of COPD symptoms). Potential changes in disease management following an exacerbation (e.g. change in medication use) are also recorded.

Biological specimen collection

A wide range of biological specimens are collected from study participants (**table 3**). Blood samples are collected from all patients at study entry, quarterly and at exacerbation. Sputum samples are obtained by spontaneous expectoration or induced by stimulation according to standard methods from all patients at study entry, monthly and at exacerbation. Nasopharyngeal swabs are collected from all patients at study entry and then from a subcohort of 30 patients at monthly follow-up visits and at exacerbation during the first year of follow-up. Urine samples are collected from all patients at study entry and at exacerbation and from the subcohort of 30 patients at monthly follow-up visits during the first year. Breath samples are collected from approximately 80 patients (including the subcohort of 30 patients providing nasopharyngeal swabs) at monthly follow-up visits and at exacerbation visits during the first year.

Blood samples are analysed for disease-related biomarkers, biochemistry, cell-mediated immune response, RNA profile and nutrients. Sputum samples are processed by traditional culture techniques and multiplex PCR analysis for identification of potential respiratory pathogens (including, but not limited to, non-typeable *H. influenzae, M. catarrhalis, S. pneumoniae, P. aeruginosa, Staphylococcus aureus,* respiratory syncytial virus, parainfluenza virus, rhinovirus, human metapneumovirus, influenza virus, adenovirus, and coronavirus). Sputum samples may also be analysed for disease-related biomarkers. Nasopharyngeal swabs are processed by traditional culture techniques and multiplex PCR analysis for potential pathogen identification. Urine samples may be processed for disease-related biomarkers. Breath samples are analysed by the selected ion flow tube mass

spectrometry for identification of volatile organic compounds that may be characteristic for AECOPD.

Laboratory assays are performed at the Public Health Laboratory of Public Health England at University Hospital Southampton Foundation NHS Trust, GSK Vaccines central laboratory, and other GSK Vaccines designated laboratories. The assays use standardised and validated procedures. Aliquots of all biological samples are processed (if applicable), frozen and stored for possible further disease-related testing. Culture isolates are also stored. Any additional laboratory tests will be performed at a GSK designated laboratory.

Sample size calculation

The sample size calculation was based on the primary study endpoint of incidence of all-cause AECOPD. Assuming that, on average, each subject is observed for a period of 18 months and that 2 episodes of AECOPD can be expected per subject per year, if 120 subjects are followed, the number of total person-years would be around 180 and during this time around 360 exacerbation events would be detected. If the distribution of events per subject follows a Poisson distribution with no overdispersion, an overdispersion factor of 1.5, or an overdispersion of 2, the approximate values of the lower and upper bounds of the 95% confidence interval (CI) around the point estimate of 2 events per subject per year would be 1.8-2.2, 1.7-2.3, and 1.7-2.3, respectively. So a sample size of 120 subjects should ensure sufficient precision in the estimation of the incidence rate of all-cause AECOPDs.

Additionally, in order to follow effectively 120 subjects, given the fact that subjects may be eligible but withdraw quite early in the study possibly due to the

deterioration of the subject's health, the decision was taken to replace subjects who withdrew during the first year of follow-up, and recruit additional subjects.

Data analysis

The co-primary study endpoints are the occurrence of all-cause AECOPD and the occurrence of AECOPD having sputum containing bacterial pathogens as detected by culture (overall and by species). The proportion of subjects at each visit for whom a sputum sample was obtained will be computed; overall and by the method the samples were obtained (spontaneous or induced). The proportion of sputum samples obtained at each visit and positive for specific bacterial pathogens (overall and by bacterial species) will also be calculated. The incidence rate of all-cause AECOPD and of AECOPD having sputum containing bacterial pathogens (overall and by bacterial species) will be calculated, with 95% CI. The 95% CI of the incidence rate will be computed using a model which accounts for repeated events, namely 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 as a preliminary approach. Other flexible approaches to statistical analysis may also be used. In addition, the same model with covariates (e.g. smoking status at enrolment, number of moderate/severe exacerbations reported in the 12 months prior to enrolment, presence of respiratory pathogenic bacteria detected at the exacerbation visit and at previous visits) will be applied. Incidence rates will also be calculated for moderate AECOPD and for severe AECOPD.

ETHICS AND DISEMMINATION

The AERIS study is conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and has been approved by the relevant institutional

ethics and review board. All subjects must provide written informed consent to participate.

AERIS is being conducted in a specialised hospital that has extensive experience in respiratory research. AECOPD are proactively identified through patient-completed electronic diaries. After confirmation by phone, symptoms of an exacerbation trigger a clinic visit within 72 hours of symptom onset to enable comparisons of samples from same patient in stable COPD and during AECOPD. Although this is an intensive study with prolonged follow up, patients are expected to benefit from the improved access to expert care.

The results obtained will be disseminated by presentations at international medical conferences and peer-reviewed publications.

DISCUSSION

The AERIS study has been initiated to assess the role of infectious pathogens in AECOPD in a well-characterised cohort of patients. The study aims at exploring the dynamics of airway infection and its possible contribution to AECOPD, as well as the potential role of chronic colonization in stable disease. The overall objective of the study will aim at refining the case definition of AECOPD to reflect the possible microbiological aetiology of exacerbations. This is of note, since there is currently no commonly agreed definition of AECOPD and no current case definition includes a microbiological endpoint. The impact of AECOPD on health-related quality-of-life and healthcare use will be assessed in order to provide a complete picture of disease burden. The interaction between airway infection and systemic manifestations of COPD and nutritional status will also be assessed. Biological specimens collected during the study may also be used for further disease-related testing, including

molecular typing to describe and compare selected biomarkers in AECOPD and stable COPD, to explore cell-mediated immune response to specific bacterial antigens, and to develop non-invasive bacterial diagnostic methods.

To our knowledge, few other studies have employed real-time electronic tracking of symptoms to identify AECOPD and potential aetiological triggers. This is important since available data suggest that up to 50% of exacerbations may not be reported to healthcare providers and consequently exacerbation rates are lower in studies employing event-based criteria to define AECOPD.[36] Due to the close daily monitoring of symptoms to identify AECOPD, we anticipate that the exacerbation rate in this study will be higher than previously reported. This close monitoring and early therapeutic intervention at exacerbation may also impact on estimates of the overall burden of disease.

A number of other epidemiological studies have been initiated in recent years to further characterize our understanding of the natural history of AECOPD. However, most of these studies have not included molecular microbiological assessments. Recent large observational studies focusing on biomarker discovery have involved close phenotyping of COPD patients, but have not studied the aetiology of exacerbations in depth.[37–39] In another study, potentially pathogenic bacterial strains were identified using molecular typing techniques, although viruses as potential airway pathogens were not investigated.[21] More recently, the prevalence and load of airway bacteria in stable and exacerbated AECOPD have been assessed in paired samples from 52 patients participating in the London COPD cohort study using modern molecular techniques.[19] Airway bacterial prevalence and load was found to increase significantly during AECOPD, with quantitative molecular techniques proving more discriminatory than culture. However, assessment was limited to only

the three most commonly detected airway bacteria (*H. influenzae, S. pneumoniae,* and *M. catarrhalis).* However, other potential pathogens and the overall respiratory microbiome may also contribute and have not yet been studied in detail.[17,18,20,40,41] In AERIS, samples will be acquired during both AECOPD and stable disease and analysed for a wide range of potentially pathogenic bacteria and viruses using advanced PCR-based techniques as well as traditional culture-based methods.

A major strength of the AERIS study design is the comprehensive assessment of clinical status, microbiology, functional status, nutritional status, health-related quality-of-life and healthcare resource utilisation in individual patients in a single large cohort during both stable COPD and AECOPD. The analyses proposed in this study will generate epidemiological data to complement that derived from existing COPD cohorts and further explore determinants of COPD and the contribution of bacterial and viral pathogens to AECOPD, as well as to provide some understanding of the limitations of existing data. As exacerbation visits are triggered by patient diary data, accurate and timely diary completion is essential. All subjects participating in this study receive diary training at enrolment and support is available from the study team at all times to promote accurate and complete diary keeping. Cohort retention is a key factor in the successful delivery of such a study and with in-depth sampling protocols, participant engagement, comfort and feedback are key factors in optimising cohort retention and comprehensive data collection.

Identification of novel approaches for the prevention of AECOPD is an important research goal. Long-acting beta-agonists (LABA) and long-acting anti-muscarinic bronchodilators remain the cornerstone of treatment for patients with COPD.[42] Combinations of LABA and inhaled corticosteroids are also used in patients with more severe disease and/or frequent exacerbations. Long-term treatment with

macrolide antibiotics and pulsed quinolone therapy may be considered for exacerbation prevention.[7,43,44] However, concerns exist about the potential for development of antimicrobial resistance during long-term antibiotic therapy. Numerous other approaches are under investigation for the prevention of AECOPD, including anti-inflammatory drugs, immunomodulatory agents, immunotherapy, antioxidants and non-pharmacologic strategies. Vaccination is another potential approach meriting investigation for reducing AECOPD risk. However, optimal strategies targeting key respiratory pathogens are not yet available to the clinician.

In conclusion, there have been considerable advances in our understanding of the epidemiology, pathophysiology and clinical management of COPD in recent years. However, there remains a genuine need to further explore the aetiology and pathogenesis of AECOPD. It is anticipated that results of this epidemiological study will increase our understanding of the contribution of bacterial and viral pathogens to AECOPD, potentially leading to research into targeted therapeutic and preventative interventions.

Contributors S. Bourne: principle investigator, closely involved in all steps of the study and specifically wrote substantial parts of the protocol; **C. Cohet**: Epidemiologist responsible for this observational study was closely involved in the design of study; **V. Kim and A. Barton**: co-investigators and closely involved in the conduct of this study; **A. Tuck**: site project manager for microbiology testing and involved in writing of microbiology parts of the protocol; **E. Aris**: project statistician, responsible for writing of statistical analysis plan and definition of statistical outcomes; **S. Mesia Vela**: project manager, closely involved in the discussion on design and follow-up of the study; **J-M. Devaster**: Director clinical research and translational Science, closely involved in all discussions and study accountable

person; **W.R. Ballou:** vice-president of clinical research and translational Science team, closely involved in all discussions of design of this study. **S. Clarke:** co-investigator, closely involved in all steps of the study. **T. Wilkinson**: coordinating lead investigator, involved in all steps of the study.

All authors provided intellectual input into the development of this manuscript. And have critically reviewed and approved the final manuscript.

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Acute Exacerbation and Respiratory InfectionS in COPD (AERIS): protocol for a prospective, observational cohort study

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Acute Exacerbation and Respiratory InfectionS in COPD (AERIS): protocol for a prospective, observational cohort study

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ABSTRACT

Introduction: The aetiology of acute exacerbations of COPD remains incompletely understood and strategies for treatment and prevention have not altered significantly for many years. Improved understanding of the role of respiratory pathogens in AECOPD is required and the use of molecular microbiological techniques may lead to insights into host–pathogen interactions and the development of more targeted therapeutic approaches.

Methods and analyses: Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) is a longitudinal epidemiological study to assess how changes in the COPD airway microbiome contribute to the incidence and severity of AECOPD. COPD patients aged 40–85 are followed monthly for 2 years, and reviewed within 72 hours of onset of symptoms of AECOPD. Exacerbations are detected using daily electronic diary cards . Blood, sputum, nasopharyngeal and urine samples are collected at pre-specified time-points. Molecular diagnostic and typing techniques are used to describe the dynamics of airway infection during AECOPD and stable disease, and associations with clinical outcome. This study aims to refine the case definition of AECOPD to reflect the possible microbiological aetiology. AERIS will assess the impact of AECOPD on health-related quality-of-life and healthcare resource utilisation, and the possible interactions between nutritional status, infection and immune responses.

Ethics and dissemination: AERIS is conducted in accordance with the Declaration of Helsinki and GCP , and has been approved by the institutional ethics and review board. All subjects must provide written informed consent. The results obtained will be disseminated at international medical conferences and in peer-reviewed publications.

Discussion: Few other studies have addressed the complexity of the microbiological and systemic components of COPD or employed real-time electronic tracking of symptoms to identify AECOPD and potential aetiological triggers. Results of AERIS will increase our understanding of the contribution of pathogens to AECOPD, potentially leading to new targeted therapeutic and preventative interventions.

Registration: ClinicalTrials.gov NCT01360398

ARTICLE SUMMARY

Article focus

- Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) is a prospective longitudinal epidemiological study initiated in the UK to assess how dynamic changes in the COPD airway microbiome contribute to the incidence and severity of acute exacerbations of COPD (AECOPD).

Key messages

- There remains a genuine need to further explore the aetiology and pathogenesis of AECOPD.
- It is anticipated that results of this study will increase our understanding of the contribution of bacterial and viral pathogens to AECOPD, potentially leading to research into targeted therapeutic and preventative interventions.

Strengths and limitations of this study

- Conducted in a specialised hospital that has extensive experience in respiratory research.
- Comprehensive assessment of clinical status, microbiology, functional status, nutritional status, health-related quality-of-life and healthcare resource utilisation in individual patients in a single large cohort during both stable COPD and AECOPD
- AECOPD are proactively identified through patient-completed electronic diaries.
- Cohort retention is a key factor in the successful delivery of such a study and with in-depth sampling protocols, participant engagement, comfort and feedback are key factors in optimising cohort retention and comprehensive data collection.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an inflammatory disease of the lung, characterised by progressive airflow limitation that is not fully reversible.[1] COPD is the most common chronic respiratory illness in older adults, affecting an estimated 210 million people worldwide.[2] This condition has a substantial impact on quality-of-life.[2] The Global Burden of Disease Study found COPD to be the third leading cause of death globally and the ninth leading cause of years of life lost due to premature mortality in 2010,[3] accounting for 3.7% of years lived with disability and 3.1% of disability-adjusted life years worldwide.[4,5] COPD also imposes a substantial socioeconomic burden. In 2001, the total cost of COPD in Europe was reported to be €38.7 billion.[6]

Considerable progress has been made concerning the epidemiology, pathophysiology and clinical management of COPD in recent years. However, significant challenges remain. Improved understanding of acute exacerbations of COPD (AECOPD) is a key research priority. AECOPD are highly relevant clinically, being a major cause of COPD-related morbidity and mortality,[7–11] as well as accounting for a substantial proportion of the significant social, healthcare and economic burden of COPD.[6] It has been estimated that AECOPD account for approximately 70% of total healthcare costs associated with COPD.[12] Patients with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage II or more disease experience 1 or 2 exacerbations annually. Exacerbation varies from patient to patient with severity of disease.[13] Various triggers for AECOPD have been identified;[1] however, up to 75% of all exacerbations are associated with the detection

of bacterial and/or viral respiratory pathogens.[14,15] Exacerbations associated with detectable respiratory pathogens have been shown to have a more marked impact on lung function and longer duration of hospitalisation than exacerbations of non-infectious aetiology.[14]

With the introduction of new molecular sequencing techniques, the traditional belief that healthy lungs are sterile has been refuted. There is increasing evidence that the lower respiratory tract contains a diverse microbial flora that differs between health and disease.[16–20] The presence of potentially pathogenic microorganisms in the inflamed airways of patients with COPD is well-documented, with up to 50% of patients with stable COPD showing evidence of lower airway bacterial colonisation using traditional culture techniques.[15,21,22] In patients with COPD, bacterial detection in lower airway derived samples is associated with increased airway inflammation, reduced lung function and more frequent exacerbations.[23–25] Acquisition of new pathogen strains also appears to be associated with an increased risk of AECOPD.[15,21,26] Estimates of the relative contribution of different pathogens to AECOPD vary. However, non-typeable *Haemophilus influenzae* appears to be the major bacterial pathogen associated with AECOPD, followed by *Streptococcus pneumoniae, Moraxella catarrhalis and Pseudomonas aeruginosa.*[14,15] Respiratory viruses commonly associated with AECOPD are diverse and include human rhinoviruses, influenza and parainfluenza viruses, respiratory syncytial virus, coronavirus and adenovirus.[15]

Improved understanding of the role of infectious pathogens in AECOPD is required to better understand the pathophysiology of the disease and may lead to the development of more targeted strategies for treatment and prevention. This paper describes the objectives and design of Acute Exacerbation and Respiratory InfectionS

in COPD (AERIS), a prospective longitudinal epidemiological study initiated in the UK to assess the role of respiratory infection in acute exacerbations of COPD. Molecular diagnostic and typing techniques will be used to describe the dynamics of airway infection and its potential association with clinical outcome. The study will also assess the impact of AECOPD on health-related quality-of-life and healthcare resource utilisation, as well as the possible interaction between disease endotype and exacerbations.

OBJECTIVES

The primary objective of the AERIS study is to estimate the incidence of all-cause AECOPD and of AECOPD with sputum containing bacterial pathogens (overall and by species). Secondary study objectives are summarised in **table 1**.

Table 1 Overview of primary and secondary objectives of the AERIS study

Level	Objective
Primary	• To estimate the incidence rate of all-cause AECOPD • To estimate the incidence rate of AECOPD having sputum containing bacterial pathogens (overall and by species)
Secondary	• To describe the proportion of overall and specific bacterial pathogens detected in sputum by severity of AECOPD • To describe the proportion of overall and specific bacterial pathogens detected in sputum in stable COPD • To estimate the incidence rate of AECOPD having sputum containing viral pathogens (overall and by species) • To describe the proportion of overall and specific viral pathogens detected in sputum by severity of AECOPD • To estimate the time elapsed between consecutive AECOPD episodes • To assess the impact of all-cause AECOPD and stable COPD on health-related quality-of-life • To assess the impact on healthcare use: – Of all-cause AECOPD

- Of AECOPD having sputum containing bacterial pathogens
- Of AECOPD having sputum containing viral pathogens

AECOPD, acute exacerbations of chronic obstructive pulmonary disease

METHODS

Study design

This is an ongoing, single-centre, prospective, observational cohort study based at University Hospital Southampton, UK.

Study population

Male and female COPD patients between the age of 40 and 85 years are eligible for study participation provided they meet the following inclusion criteria: (i) a confirmed diagnosis of COPD with post-bronchodilator forced expiratory volume in one second (FEV_1) ≤80% of the predicted normal value and FEV_1/forced vital capacity (FVC) ≤0.7, consistent with GOLD stage II–IV disease,[27] (ii) current or ex-smoker with smoking history ≥10 pack years, and (iii) 1 or more documented exacerbations of COPD treated with antibiotics and/or steroids in the 12 months prior to enrolment (**table 2**). Subjects with recent COPD exacerbations, in stable condition, and having stopped antibiotics, can be enrolled one month post-exacerbation. Exclusion criteria include other known respiratory conditions, such as asthma, as the only cause of the respiratory obstructive disorder, α-1 antitrypsin deficiency, cystic fibrosis, tuberculosis, lung cancer, previous history of lung surgery and other conditions imposing pneumonia risk. Subjects on long-term corticosteroid or antibiotic therapy at the time of enrolment and those who have received antibiotics and/or steroids in the month prior to the enrolment are also excluded.

Table 2 Study inclusion and exclusion criteria

Inclusion criteria	Subjects must satisfy ***ALL*** of the following criteria at study entry: • Subjects who the investigator believes can and will comply with the requirements of the protocol • Written informed consent obtained from the subject • Male or female aged 40–85 years • Confirmed diagnosis of COPD based on post-bronchodilator spirometry (Gold et al. 2009), with FEV_1 ≤80% of predicted normal and FEV_1/FVC <0.7 • Moderate, severe, or very severe COPD, according to GOLD staging (Gold et al. 2009) • Current or prior history of ≥10 pack years of cigarette smoking[a,b] • Documented history of ≥1 exacerbation requiring antibiotics and/or oral corticosteroids or hospitalisation in the previous 12 months[c]
Exclusion criteria	• A confirmed diagnosis of asthma (as only cause of obstructive respiratory disorder), cystic fibrosis, pneumonia risk factors or other respiratory disorders (e.g., tuberculosis, lung cancer) • History of lung surgery • α-1 antitrypsin deficiency as underlying cause of COPD • Moderate or severe COPD exacerbation not resolved at least 1 month prior to enrolment and less than 30 days following the last dose of oral corticosteroids[d] • Long-term corticosteroid or antibiotic therapy • Use of any antibacterial, antiviral or respiratory investigational drug or vaccine within 30 days of the enrolment visit • Evidence of alcohol or drug abuse • Presence of other conditions that the principal investigator judges may interfere with the study findings • Risk of non-compliance or inability to comply with the study procedures • Women who are pregnant or lactating or are planning on becoming pregnant during the study

[a] Former smokers are defined as those who have stopped smoking for at least 6 months
[b] Number of pack years = (number of cigarettes per day/20) x number of years smoked
[c] Subjects with recent COPD exacerbations, in stable condition, and having stopped antibiotics, can be enrolled one month post-exacerbation
[d] Subjects can be enrolled when their AECOPD or pneumonia has resolved

Clinical data collection

Subjects are seen for an enrolment visit and then monthly for 2 years. Regular review of medications and, when required, changes to medical therapy and active smoking cessation advice are performed according to standard clinical practice at each visit. In addition to these scheduled visits, all subjects are seen in the clinic within 72 hours (3 days) of onset of symptoms of AECOPD. AECOPD is defined as worsening of at least 2 major symptoms (dyspnoea, sputum volume and sputum purulence) or worsening of at least 1 major symptom and 1 minor symptom (wheeze, sore throat, cold [nasal discharge and/or nasal congestion], cough and fever [oral temperature >37.5 °C] without other cause),[28] considered clinically relevant at the site. Exacerbations are identified by means of electronic diary cards that subjects complete daily. The data recorded daily in the electronic diary cards includes self-performed peak flow measurement (peak expiratory flow [PEF] and FEV_1), a series of morning questions to identify symptoms of exacerbations[29] and the EXAcerbations of Chronic Pulmonary Disease Tool Version 1.0 (EXACT-PRO) at bedtime. Subjects are also asked to record any changes to their usual treatment. Data on patient-reported symptoms based on morning questions and on PEF/FEV_1 are transmitted daily to the study clinic. Changes/worsening in these symptoms are monitored by the study staff and subjects are contacted and invited to the clinic when an exacerbation is suspected.

Study procedures

In addition to the daily monitoring undertaken through the patient-completed electronic diary cards, a wide range of study procedures are performed at study entry, scheduled monthly visits and exacerbation visits (**table 3**).

Table 3 Overview of study assessments performed at the scheduled monthly visits and at exacerbation visits

Description		Frequency of assessment*
Clinical variables		
Physical examination		Monthly and within 72 hours of onset of exacerbation
Anthropometrics and nutritional screening (MUST)[a]		Quarterly
Intercurrent comorbidities		Monthly and within 72 hours of onset of exacerbation
Medical history / medical record review		Study entry and within 72 hours of onset of exacerbation
Vaccination history		Annually
Current medication		Monthly
Smoking status		Monthly
Urine pregnancy test		Study entry, final visit and within 72 hours of onset of exacerbation
Chest CT-scan		Study entry and final visit
Chest X-ray		Within 72 hours of onset of exacerbation
Lung function testing	Body box	Study entry and final visit
	TLCO[b]	Every 6 months and within 72 hours of onset of exacerbation
	Spirometry	Monthly and within 72 hours of onset of exacerbation
6-minute walk test		Every 6 months
Questionnaires and patient-reported outcome instruments		
ATS-DLD-78A (Risk factors, disease history and smoking history)		Study entry

Healthcare use[c]		Monthly and within 72 hours of onset of exacerbation
mMRC[d]		Every 6 months
CAT questionnaire[e]		Quarterly and within 72 hours of onset of exacerbation
EQ-5D index and VAS[f]		Quarterly and within 72 hours of onset of exacerbation
NEADL[g]		Quarterly and within 72 hours of onset of exacerbation
CNAQ[h]		Quarterly and within 72 hours of onset of exacerbation
Biological specimen collection		
Blood sampling	For routine biochemistry	Study entry
	For cell-mediated immune response	Quarterly and within 72 hours of onset of exacerbation
	For biomarkers, blood counts and haematology	Quarterly and within 72 hours of onset of exacerbation
	For RNA transcript profiling	Every 6 months and within 72 hours of onset of exacerbation
	For vitamins, anti-oxidants and nutrients (20 ml)	Every 6 months and within 72 hours of onset of exacerbation
Nasopharyngeal swab sampling[i]		Monthly and within 72 hours of onset of exacerbation
Sputum sampling		Monthly and within 72 hours of onset of exacerbation
Breath sampling[j]		Monthly and within 72 hours of onset of exacerbation
Urine sampling[k]		Monthly and within 72 hours of onset of exacerbation

*In addition to study entry

[a]Height, weight, mid-arm circumference, waist circumference, triceps skin fold measurement, fat free body mass. [b]TLCO: transfer factor. [c]Healthcare use includes medication, vaccination, oxygen therapy, use of mechanical ventilation, pulmonary rehabilitation treatment, surgical intervention, outpatient visits (including GP visit contacts to COPD team), emergency room visits, hospitalisations, and productivity loss (time missed from work or usual activities due to worsening of COPD symptoms) [d]mMRC: Medical Research Council Dyspnea Scale score. [e]CAT: COPD Assessment Test. [f]VAS: visual analogue scale. [g]NEADL: Nottingham Extended Daily Activities Scale. [h]CNAQ: Council on Nutrition Appetite Questionnaire. [i]In all subjects at study entry and in a subcohort of 30 subjects during the first year. jIn a subcohort of approximately 80 subjects. [k]In all subjects at study entry and within 72 hours of every exacerbation and in the subcohort of 30 subjects providing nasopharyngeal swabs during the first year of the study

Clinical assessments

Quantitative high-resolution computed tomography scans are performed at enrolment and study conclusion to describe the degree of bronchiectasis and emphysema noted and to exclude other acute or evolving lung pathologies besides COPD and sequelae of COPD. A physical examination is performed at all visits. Medical history, smoking status and details of medication use are updated monthly. Influenza and pneumococcal vaccination status is updated annually.

Lung function testing is performed using spirometry, body plethysmography (lung volumes, body box) and single breath diffusion (gas transfer, TLCO) at specified visits. The following outcomes are recorded: spirometry, FEV_1, FVC, FEV_1/FVC ratio, FEV_1 % predicted, mid expiratory flow between 25 and 75% of the forced vital capacity (MEF25-75), single breath diffusion (transfer factor [TLCO] and rate of carbon monoxide uptake [KCO], and body plethysmography (total lung capacity [TLC], residual volume [RV], vital capacity [VC] and RV/TLC). At the enrolment visit, subjects are asked to refrain from using short-acting bronchodilators for at least 6 hours and long-acting bronchodilators for at least 12 hours before key procedures. Prior to the subsequent follow-up visits, subjects may use their usual medication normally. Lung function measurements are performed under controlled conditions and in the sitting position as per standard practice.

Anthropometrics (including but not restricted to height, weight, waist and mid-arm circumference and triceps skin-fold circumference) are measured quarterly. Grip strength and fatigability are measured using standard techniques. Anthropometric data are used to compute the Malnutrition Universal Screening Tool (MUST) score.[30]

Nutritional information (including planned/ unplanned weight loss, and history and changes in food intake patterns) is collected quarterly according to MUST guidelines.

A postero-anterior chest X-ray (and lateral if required) is performed at all exacerbation visits, as per standard clinical practice, in order to exclude pneumonia.

Questionnaires and patient-reported outcome instruments

Various outcomes are assessed quarterly and at exacerbation using a series of questionnaires and patient-reported outcome instruments such as the COPD Assessment Test (CAT),[31] the Nottingham Extended Daily Activities Scale (NEADL),[32] the Council on Nutrition Appetite Questionnaire (CNAQ),[33] and the EQ-5D.[34] The five items included in the EQ-5D index are mobility, self-care, usual activities, pain/discomfort and anxiety/depression. The BODE index (Body-Mass Index, Degree of Airflow Obstruction, Level of Functional Dyspnea, Exercise Capacity)[35] will also be calculated.

Healthcare use is recorded at all visits, including medication, vaccination, oxygen therapy, use of mechanical ventilation, pulmonary rehabilitation treatment, surgical intervention, outpatient visits (including GP visits and telephone contacts to COPD team), emergency room visits, hospitalisations, and productivity loss (time missed from work or usual activities due to worsening of COPD symptoms). Potential changes in disease management following an exacerbation (e.g. change in medication use) are also recorded.

Biological specimen collection

A wide range of biological specimens are collected from study participants (**table 3**). Blood samples are collected from all patients at study entry, quarterly and at exacerbation. Sputum samples are obtained by spontaneous expectoration or induced

by stimulation according to standard methods from all patients at study entry, monthly and at exacerbation. Nasopharyngeal swabs are collected from all patients at study entry and then from a subcohort of 30 patients at monthly follow-up visits and at exacerbation during the first year of follow-up. Urine samples are collected from all patients at study entry and at exacerbation and from the subcohort of 30 patients at monthly follow-up visits during the first year. Breath samples are collected from approximately 80 patients (including the subcohort of 30 patients providing nasopharyngeal swabs) at monthly follow-up visits and at exacerbation visits during the first year.

Blood samples are analysed for disease-related biomarkers, biochemistry, cell-mediated immune response, RNA profile and nutrients. Sputum samples are processed by traditional culture techniques and multiplex PCR analysis for identification of potential respiratory pathogens (including, but not limited to, non-typeable *H. influenzae, M. catarrhalis, S. pneumoniae, P. aeruginosa, Staphylococcus aureus,* respiratory syncytial virus, parainfluenza virus, rhinovirus, human metapneumovirus, influenza virus, adenovirus, and coronavirus). Sputum samples may also be analysed for disease-related biomarkers. Nasopharyngeal swabs are processed by traditional culture techniques and multiplex PCR analysis for potential pathogen identification. Urine samples may be processed for disease-related biomarkers. Breath samples are analysed by the selected ion flow tube mass spectrometry for identification of volatile organic compounds that may be characteristic for AECOPD.

Laboratory assays are performed at the Public Health Laboratory of Public Health England at University Hospital Southampton Foundation NHS Trust, GSK Vaccines central laboratory, and other GSK Vaccines designated laboratories. The assays use

standardised and validated procedures. Aliquots of all biological samples are processed (if applicable), frozen and stored for possible further disease-related testing. Culture isolates are also stored. Any additional laboratory tests will be performed at a GSK designated laboratory.

Sample size calculation

The sample size calculation was based on the primary study endpoint of incidence of all-cause AECOPD. Assuming that, on average, each subject is observed for a period of 18 months and that 2 episodes of AECOPD can be expected per subject per year, if 120 subjects are followed, the number of total person-years would be around 180 and during this time around 360 exacerbation events would be detected. If the distribution of events per subject follows a Poisson distribution with no overdispersion, an overdispersion factor of 1.5, or an overdispersion of 2, the approximate values of the lower and upper bounds of the 95% confidence interval (CI) around the point estimate of 2 events per subject per year would be 1.8-2.2, 1.7-2.3, and 1.7-2.3, respectively. So a sample size of 120 subjects should ensure sufficient precision in the estimation of the incidence rate of all-cause AECOPDs.

Additionally, in order to follow effectively 120 subjects, given the fact that subjects may be eligible but withdraw quite early in the study possibly due to the deterioration of the subject's health, the decision was taken to replace subjects who withdrew during the first year of follow-up, and recruit additional subjects.

We will construct a CONSORT diagram and capture where possible reasons for screen failure, drop-outs and loss to follow-up.

Data analysis

The co-primary study endpoints are the occurrence of all-cause AECOPD and the occurrence of AECOPD having sputum containing bacterial pathogens as detected by culture (overall and by species). The proportion of subjects at each visit for whom a sputum sample was obtained will be computed; overall and by the method the samples were obtained (spontaneous or induced). The proportion of sputum samples obtained at each visit and positive for specific bacterial pathogens (overall and by bacterial species) will also be calculated. The incidence rate of all-cause AECOPD and of AECOPD having sputum containing bacterial pathogens (overall and by bacterial species) will be calculated, with 95% CI. The 95% CI of the incidence rate will be computed using a model which accounts for repeated events, namely 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 as a preliminary approach. Other flexible approaches to statistical analysis may also be used. In addition, the same model with covariates (e.g. smoking status at enrolment, number of moderate/severe exacerbations reported in the 12 months prior to enrolment, presence of respiratory pathogenic bacteria detected at the exacerbation visit and at previous visits) will be applied. Incidence rates will also be calculated for moderate AECOPD and for severe AECOPD.

ETHICS AND DISSEMINATION

The AERIS study is conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and has been approved by the relevant institutional ethics and review board. All subjects must provide written informed consent to participate.

AERIS is being conducted in a specialised hospital that has extensive experience in respiratory research. AECOPD are proactively identified through patient-completed electronic diaries. After confirmation by phone, symptoms of an exacerbation trigger a clinic visit within 72 hours of symptom onset to enable comparisons of samples from same patient in stable COPD and during AECOPD. Although this is an intensive study with prolonged follow up, patients are expected to benefit from the improved access to expert care.

The results obtained will be disseminated by presentations at international medical conferences and peer-reviewed publications. Reporting will be in accordance with STROBE guidance.

DISCUSSION

The AERIS study has been initiated to comprehensively assess the role of infectious pathogens in AECOPD in a well-characterised cohort of patients. The study aims to explore the dynamics of airway infection and its possible contribution to AECOPD, as well as the potential role of chronic colonization in stable disease. The overall objective of the study will aim at refining the case definition of AECOPD to reflect the possible microbiological aetiology of exacerbations. This is of note, since there is currently no commonly agreed definition of AECOPD and no current case definition includes a microbiological endpoint. The impact of AECOPD on health-related quality-of-life and healthcare use will be assessed in order to provide a complete picture of disease burden. The interaction between airway infection and systemic manifestations of COPD and nutritional status will also be assessed in detail for the first time. Biological specimens collected during the study may also be used for further disease-related testing, including molecular typing to describe and compare

selected biomarkers in AECOPD and stable COPD, to explore cell-mediated immune response to specific bacterial antigens, and to develop non-invasive bacterial diagnostic methods.

To our knowledge, few other studies have employed real-time electronic tracking of symptoms to identify AECOPD and potential aetiological triggers. This is important since available data suggest that up to 50% of exacerbations may not be reported to healthcare providers and consequently exacerbation rates are lower in studies employing event-based criteria to define AECOPD.[36] Due to the close daily monitoring of symptoms to identify AECOPD, we anticipate that the exacerbation rate in this study will be higher than previously reported. This close monitoring and early therapeutic intervention at exacerbation may also impact on estimates of the overall burden of disease.

A number of other epidemiological studies have been initiated in recent years to further characterize our understanding of the natural history of AECOPD. However, it is important to recognise that most of these studies have not included molecular microbiological assessments. Recent large observational studies focusing on biomarker discovery have involved close phenotyping of COPD patients, but have not studied the aetiology of exacerbations in depth.[37–39] In another study, potentially pathogenic bacterial strains were identified using molecular typing techniques, although viruses as potential airway pathogens were not investigated.[21] More recently, the prevalence and load of airway bacteria in stable and exacerbated AECOPD have been assessed in paired samples from 52 patients participating in the London COPD cohort study using modern molecular techniques.[19] Airway bacterial prevalence and load was found to increase significantly during AECOPD, with quantitative molecular techniques proving more discriminatory than culture. However, assessment was

limited to only the three most commonly detected airway bacteria (*H. influenzae, S. pneumoniae,* and *M. catarrhalis).* However, other potential pathogens and the overall respiratory microbiome may also contribute and have not yet been studied in detail.[17,18,20,40,41] In AERIS, samples will be acquired during both AECOPD and stable disease and analysed for a wide range of potentially pathogenic bacteria and viruses using advanced PCR-based techniques as well as traditional culture-based methods.

A major strength of the AERIS study design is the comprehensive assessment of clinical status, microbiology, functional status, nutritional status, health-related quality-of-life and healthcare resource utilisation in individual patients in a single large cohort during both stable COPD and AECOPD. The selection of subjects with a history of at least a single exacerbation enriches the cohort to some degree and ensures an adequate number of exacerbations are sampled. It is accepted that some aspects of the analysis may not be generalizable to the subgroup of patients who never exacerbate. The analyses proposed in this study will generate epidemiological data to complement that derived from existing COPD cohorts and further explore determinants of COPD and the contribution of bacterial and viral pathogens to AECOPD, as well as to provide some understanding of the limitations of existing data. As exacerbation visits are triggered by patient diary data, accurate and timely diary completion is essential. All subjects participating in this study receive diary training at enrolment and support is available from the study team at all times to promote accurate and complete diary keeping. Cohort retention is a key factor in the successful delivery of such a study and with in-depth sampling protocols, participant engagement, comfort and feedback are key factors in optimising cohort retention and comprehensive data collection.

Identification of novel approaches for the prevention of AECOPD is an important research goal. Long-acting beta-agonists (LABA) and long-acting anti-muscarinic bronchodilators remain the cornerstone of treatment for patients with COPD.[42] Combinations of LABA and inhaled corticosteroids are also used in patients with more severe disease and/or frequent exacerbations. Long-term treatment with macrolide antibiotics and pulsed quinolone therapy may be considered for exacerbation prevention.[7,43,44] However, concerns exist about the potential for development of antimicrobial resistance during long-term antibiotic therapy. Numerous other approaches are under investigation for the prevention of AECOPD, including anti-inflammatory drugs, immunomodulatory agents, immunotherapy, antioxidants and non-pharmacologic strategies. Vaccination is another potential approach meriting investigation for reducing AECOPD risk. However, optimal strategies targeting key respiratory pathogens are not yet available to the clinician.

In conclusion, there have been considerable advances in our understanding of the epidemiology, pathophysiology and clinical management of COPD in recent years. However, there remains a genuine need to further explore the aetiology and pathogenesis of AECOPD. It is anticipated that results of this epidemiological study will increase our understanding of the contribution of bacterial and viral pathogens to AECOPD, the natural history of these events in association with the timing of symptoms and physiological changes, and will offer new direction for research into targeted therapeutic and preventative interventions.

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Contributors S. Bourne: principle investigator, closely involved in all steps of the study and specifically wrote substantial parts of the protocol;

C. Cohet: epidemiologist responsible for this observational study was closely involved in the design of study;

V. Kim and A. Barton: co-investigators and closely involved in the conduct of this study;

A. Tuck: site project manager for microbiology testing and involved in writing of microbiology parts of the protocol;

E. Aris: project statistician, responsible for writing of statistical analysis plan and definition of statistical outcomes;

S. Mesia Vela: project manager, closely involved in the discussion on design and follow-up of the study;

J-M. Devaster: director of clinical research and translational science, closely involved in all discussions and study accountable person;

W.R. Ballou: vice-president of clinical research and translational science team, closely involved in all discussions of design of this study.

S. Clarke: co-investigator, closely involved in all steps of the study.

T. Wilkinson: coordinating lead investigator, involved in all steps of the study.

All authors provided intellectual input into the development of this manuscript. And have critically reviewed and approved the final manuscript.

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Acute Exacerbation and Respiratory InfectionS in COPD (AERIS): protocol for a prospective, observational cohort study

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ARTICLE SUMMARY

Article focus

- Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) is a prospective longitudinal epidemiological study initiated in the UK to assess how dynamic changes in the COPD airway microbiome contribute to the incidence and severity of acute exacerbations of COPD (AECOPD).

Key messages

- There remains a genuine need to further explore the aetiology and pathogenesis of AECOPD.
- It is anticipated that results of this study will increase our understanding of the contribution of bacterial and viral pathogens to AECOPD, potentially leading to research into targeted therapeutic and preventative interventions.

Strengths and limitations of this study

- Conducted in a specialised hospital that has extensive experience in respiratory research.
- Comprehensive assessment of clinical status, microbiology, functional status, nutritional status, health-related quality-of-life and healthcare resource utilisation in individual patients in a single large cohort during both stable COPD and AECOPD
- AECOPD are proactively identified through patient-completed electronic diaries.
- Cohort retention is a key factor in the successful delivery of such a study and with in-depth sampling protocols, participant engagement, comfort and feedback are key factors in optimising cohort retention and comprehensive data collection.

ABSTRACT

Introduction: The aetiology of acute exacerbations of COPD remains incompletely understood and strategies for ~~the~~ treatment and prevention ~~of AECOPD~~ have not altered significantly for many years. Improved understanding of the role of respiratory pathogens in AECOPD is required and the use of ~~modern~~ molecular microbiological techniques may lead to insights into host–pathogen interactions and the development of more targeted therapeutic approaches.

Methods and analyses: Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) is a ~~prospective~~ longitudinal epidemiological study ~~initiated in the UK~~ to assess how ~~dynamic~~ changes in the COPD airway microbiome contribute to the incidence and severity of AECOPD. COPD patients aged 40–85 ~~years~~ are followed monthly for 2 years, and ~~seen in the clinic~~reviewed within 72 hours of onset of symptoms of AECOPD. Exacerbations are detected using daily electronic diary cards ~~that subjects complete daily~~. Blood, sputum, nasopharyngeal and urine samples are collected at pre-specified time-points. Molecular diagnostic and typing techniques are used to describe the dynamics of airway infection during AECOPD and stable disease, and ~~its~~ associations with clinical outcome. This study ~~also~~ aims to~~at~~ refin~~c~~~~ing~~ the case definition of AECOPD to reflect the possible microbiological aetiology. ~~of exacerbations. The study~~AERIS will ~~also~~ assess the impact of AECOPD on health-related quality-of-life and healthcare resource utilisation, ~~as well as~~and the possible interactions between nutritional status, infection and ~~cellular~~ immune responses.

Ethics and dissemination: AERIS is conducted in accordance with the Declaration of Helsinki and ~~Good Clinical Practice~~ GCP ~~guidelines~~, and has been approved by the ~~relevant~~ institutional ethics and review board. All subjects must provide written

informed consent. The results obtained will be disseminated ~~by presentations~~ at international medical conferences and in peer-reviewed publications.

Discussion: ~~To our knowledge, f~~Few other studies have addressed the complexity of the microbiological and systemic components of COPD or employed real-time electronic tracking of symptoms to identify AECOPD and potential aetiological triggers. ~~It is anticipated that R~~results of AERIS will ~~significantly~~ increase our understanding of the contribution of ~~bacterial and viral pathogens~~pathogens to AECOPD, potentially leading to ~~research into~~new targeted therapeutic and preventative interventions.

Registration: ClinicalTrials.gov NCT01360398

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an inflammatory disease of the lung, characterised by progressive airflow limitation that is not fully reversible.[1] COPD is the most common chronic respiratory illness in older adults, affecting an estimated 210 million people worldwide.[2] This condition has a substantial impact on quality-of-life.[2] The Global Burden of Disease Study found COPD to be the third leading cause of death globally and the ninth leading cause of years of life lost due to premature mortality in 2010,[3] accounting for 3.7% of years lived with disability and 3.1% of disability-adjusted life years worldwide.[4,5] COPD also imposes a substantial socioeconomic burden. In 2001, the total cost of COPD in Europe was reported to be €38.7 billion.[6]

Considerable progress has been made concerning the epidemiology, pathophysiology and clinical management of COPD in recent years. However, significant challenges remain. Improved understanding of acute exacerbations of COPD (AECOPD) is a key research priority. AECOPD are highly relevant clinically, being a major cause of COPD-related morbidity and mortality,[7–11] as well as accounting for a substantial proportion of the significant social, healthcare and economic burden of COPD.[6] It has been estimated that AECOPD account for approximately 70% of total healthcare costs associated with COPD.[12] Patients with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage II or more disease experience 1 or 2 exacerbations annually. Exacerbation varies from patient to patient with severity of disease.[13] Various triggers for AECOPD have been identified;[1] however, up to 75% of all exacerbations are associated with the detection of bacterial and/or viral respiratory pathogens.[14,15] Exacerbations associated with detectable respiratory pathogens have been shown to have a more marked impact on

lung function and longer duration of hospitalisation than exacerbations of non-infectious aetiology.[14]

With the introduction of new molecular sequencing techniques, the traditional belief that healthy lungs are sterile has been refuted. There is increasing evidence that the lower respiratory tract contains a diverse microbial flora that differs between health and disease.[16–20] The presence of potentially pathogenic microorganisms in the inflamed airways of patients with COPD is well-documented, with up to 50% of patients with stable COPD showing evidence of lower airway bacterial colonisation using traditional culture techniques.[15,21,22] In patients with COPD, bacterial detection in lower airway derived samples is associated with increased airway inflammation, reduced lung function and more frequent exacerbations.[23–25] Acquisition of new pathogen strains also appears to be associated with an increased risk of AECOPD.[15,21,26] Estimates of the relative contribution of different pathogens to AECOPD vary. However, non-typeable *Haemophilus influenzae* appears to be the major bacterial pathogen associated with AECOPD, followed by *Streptococcus pneumoniae, Moraxella catarrhalis and Pseudomonas aeruginosa.*[14,15] Respiratory viruses commonly associated with AECOPD are diverse and include human rhinoviruses, influenza and parainfluenza viruses, respiratory syncytial virus, coronavirus and adenovirus.[15]

Improved understanding of the role of infectious pathogens in AECOPD is required to better understand the pathophysiology of the disease and may lead to the development of more targeted strategies for treatment and prevention. This paper describes the objectives and design of Acute Exacerbation and Respiratory InfectionS in COPD (AERIS), a prospective longitudinal epidemiological study initiated in the UK to assess the role of respiratory infection in acute exacerbations of COPD.

Molecular diagnostic and typing techniques will be used to describe the dynamics of airway infection and its potential association with clinical outcome. The study will also assess the impact of AECOPD on health-related quality-of-life and healthcare resource utilisation, as well as the possible interaction between disease endotype and exacerbations.

OBJECTIVES

The primary objective of the AERIS study is to estimate the incidence of all-cause AECOPD and of AECOPD with sputum containing bacterial pathogens (overall and by species). Secondary study objectives are summarised in **table 1**.

Table 1 Overview of primary and secondary objectives of the AERIS study

Level	Objective
Primary	• To estimate the incidence rate of all-cause AECOPD • To estimate the incidence rate of AECOPD having sputum containing bacterial pathogens (overall and by species)
Secondary	• To describe the proportion of overall and specific bacterial pathogens detected in sputum by severity of AECOPD • To describe the proportion of overall and specific bacterial pathogens detected in sputum in stable COPD • To estimate the incidence rate of AECOPD having sputum containing viral pathogens (overall and by species) • To describe the proportion of overall and specific viral pathogens detected in sputum by severity of AECOPD • To estimate the time elapsed between consecutive AECOPD episodes • To assess the impact of all-cause AECOPD and stable COPD on health-related quality-of-life • To assess the impact on healthcare use: – Of all-cause AECOPD – Of AECOPD having sputum containing bacterial pathogens – Of AECOPD having sputum containing viral pathogens

AECOPD, acute exacerbations of chronic obstructive pulmonary disease

METHODS

Study design

This is an ongoing, single-centre, prospective, observational cohort study based at University Hospital Southampton, UK.

Study population

Male and female COPD patients between the age of 40 and 85 years are eligible for study participation provided they meet the following inclusion criteria: (i) a confirmed diagnosis of COPD with post-bronchodilator forced expiratory volume in one second (FEV_1) ≤80% of the predicted normal value and FEV_1/forced vital capacity (FVC) ≤0.7, consistent with GOLD stage II–IV disease,[27] (ii) current or ex-smoker with smoking history ≥10 pack years, and (iii) 1 or more documented exacerbations of COPD treated with antibiotics and/or steroids in the 12 months prior to enrolment (**table 2**). Subjects with recent COPD exacerbations, in stable condition, and having stopped antibiotics, can be enrolled one month post-exacerbation. Exclusion criteria include other known respiratory conditions, such as asthma, as the only cause of the respiratory obstructive disorder, α-1 antitrypsin deficiency, cystic fibrosis, tuberculosis, lung cancer, previous history of lung surgery and other conditions imposing pneumonia risk. Subjects on long-term corticosteroid or antibiotic therapy at the time of enrolment and those who have received antibiotics and/or steroids in the month prior to the enrolment are also excluded.

Table 2 Study inclusion and exclusion criteria

Inclusion criteria	Subjects must satisfy ***ALL*** of the following criteria at study entry: • Subjects who the investigator believes can and will comply with the requirements of the protocol • Written informed consent obtained from the subject • Male or female aged 40–85 years • Confirmed diagnosis of COPD based on post-bronchodilator spirometry (Gold et al. 2009), with FEV_1 ≤80% of predicted normal and FEV_1/FVC <0.7 • Moderate, severe, or very severe COPD, according to GOLD staging (Gold et al. 2009) • Current or prior history of ≥10 pack years of cigarette smoking[a,b] • Documented history of ≥1 exacerbation requiring antibiotics and/or oral corticosteroids or hospitalisation in the previous 12 months[c]
Exclusion criteria	• A confirmed diagnosis of asthma (as only cause of obstructive respiratory disorder), cystic fibrosis, pneumonia risk factors or other respiratory disorders (e.g., tuberculosis, lung cancer) • History of lung surgery • α-1 antitrypsin deficiency as underlying cause of COPD • Moderate or severe COPD exacerbation not resolved at least 1 month prior to enrolment and less than 30 days following the last dose of oral corticosteroids[d] • Long-term corticosteroid or antibiotic therapy • Use of any antibacterial, antiviral or respiratory investigational drug or vaccine within 30 days of the enrolment visit • Evidence of alcohol or drug abuse • Presence of other conditions that the principal investigator judges may interfere with the study findings • Risk of non-compliance or inability to comply with the study procedures • Women who are pregnant or lactating or are planning on becoming pregnant during the study

[a] Former smokers are defined as those who have stopped smoking for at least 6 months
[b] Number of pack years = (number of cigarettes per day/20) x number of years smoked
[c] Subjects with recent COPD exacerbations, in stable condition, and having stopped antibiotics, can be enrolled one month post-exacerbation
[d] Subjects can be enrolled when their AECOPD or pneumonia has resolved

Clinical data collection

Subjects are seen for an enrolment visit and then monthly for 2 years. Regular review of medications and, when required, changes to medical therapy and active smoking cessation advice are performed according to standard clinical practice at each visit. In addition to these scheduled visits, all subjects are seen in the clinic within 72 hours (3 days) of onset of symptoms of AECOPD. AECOPD is defined as worsening of at least 2 major symptoms (dyspnoea, sputum volume and sputum purulence) or worsening of at least 1 major symptom and 1 minor symptom (wheeze, sore throat, cold [nasal discharge and/or nasal congestion], cough and fever [oral temperature >37.5 °C] without other cause),[28] considered clinically relevant at the site. Exacerbations are identified by means of electronic diary cards that subjects complete daily. The data recorded daily in the electronic diary cards includes self-performed peak flow measurement (peak expiratory flow [PEF] and FEV_1), a series of morning questions to identify symptoms of exacerbations[29] and the EXAcerbations of Chronic Pulmonary Disease Tool Version 1.0 (EXACT-PRO) at bedtime. Subjects are also asked to record any changes to their usual treatment. Data on patient-reported symptoms based on morning questions and on PEF/FEV_1 are transmitted daily to the study clinic. Changes/worsening in these symptoms are monitored by the study staff and subjects are contacted and invited to the clinic when an exacerbation is suspected.

Study procedures

In addition to the daily monitoring undertaken through the patient-completed electronic diary cards, a wide range of study procedures are performed at study entry, scheduled monthly visits and exacerbation visits (**table 3**).

Table 3 Overview of study assessments performed at the scheduled monthly visits and at exacerbation visits

Description		Frequency of assessment*
Clinical variables		
Physical examination		Monthly and within 72 hours of onset of exacerbation
Anthropometrics and nutritional screening (MUST)[a]		Quarterly
Intercurrent comorbidities		Monthly and within 72 hours of onset of exacerbation
Medical history / medical record review		Study entry and within 72 hours of onset of exacerbation
Vaccination history		Annually
Current medication		Monthly
Smoking status		Monthly
Urine pregnancy test		Study entry, final visit and within 72 hours of onset of exacerbation
Chest CT-scan		Study entry and final visit
Chest X-ray		Within 72 hours of onset of exacerbation
Lung function testing	Body box	Study entry and final visit
	TLCO[b]	Every 6 months and within 72 hours of onset of exacerbation
	Spirometry	Monthly and within 72 hours of onset of exacerbation
6-minute walk test		Every 6 months
Questionnaires and patient-reported outcome instruments		
ATS-DLD-78A (Risk factors, disease history and smoking history)		Study entry

Healthcare use[c]		Monthly and within 72 hours of onset of exacerbation
mMRC[d]		Every 6 months
CAT questionnaire[e]		Quarterly and within 72 hours of onset of exacerbation
EQ-5D index and VAS[f]		Quarterly and within 72 hours of onset of exacerbation
NEADL[g]		Quarterly and within 72 hours of onset of exacerbation
CNAQ[h]		Quarterly and within 72 hours of onset of exacerbation
Biological specimen collection		
Blood sampling	For routine biochemistry	Study entry
	For cell-mediated immune response	Quarterly and within 72 hours of onset of exacerbation
	For biomarkers, blood counts and haematology	Quarterly and within 72 hours of onset of exacerbation
	For RNA transcript profiling	Every 6 months and within 72 hours of onset of exacerbation
	For vitamins, anti-oxidants and nutrients (20 ml)	Every 6 months and within 72 hours of onset of exacerbation
Nasopharyngeal swab sampling[i]		Monthly and within 72 hours of onset of exacerbation
Sputum sampling		Monthly and within 72 hours of onset of exacerbation
Breath sampling[j]		Monthly and within 72 hours of onset of exacerbation
Urine sampling[k]		Monthly and within 72 hours of onset of exacerbation

*In addition to study entry

[a]Height, weight, mid-arm circumference, waist circumference, triceps skin fold measurement, fat free body mass. [b]TLCO: transfer factor. [c]Healthcare use includes medication, vaccination, oxygen therapy, use of mechanical ventilation, pulmonary rehabilitation treatment, surgical intervention, outpatient visits (including GP visit contacts to COPD team), emergency room visits, hospitalisations, and productivity loss (time missed from work or usual activities due to worsening of COPD symptoms) [d]mMRC: Medical Research Council Dyspnea Scale score. [e]CAT: COPD Assessment Test. [f]VAS: visual analogue scale. [g]NEADL: Nottingham Extended Daily Activities Scale. [h]CNAQ: Council on Nutrition Appetite Questionnaire. [i]In all subjects at study entry and in a subcohort of 30 subjects during the first year. jIn a subcohort of approximately 80 subjects. [k]In all subjects at study entry and within 72 hours of every exacerbation and in the subcohort of 30 subjects providing nasopharyngeal swabs during the first year of the study

Clinical assessments

Quantitative high-resolution computed tomography scans are performed at enrolment and study conclusion to describe the degree of bronchiectasis and emphysema ~~resulting from COPD~~noted and to exclude other acute or evolving lung pathologies besides COPD and sequelae of COPD. A physical examination is performed at all visits. Medical history, smoking status and details of medication use are updated monthly. Influenza and pneumococcal vaccination status is updated annually.

Lung function testing is performed using spirometry, body plethysmography (lung volumes, body box) and single breath diffusion (gas transfer, TLCO) at specified visits. The following outcomes ~~will be~~are recorded: spirometry, FEV_1, FVC, FEV_1/FVC ratio, FEV_1 % predicted, mid expiratory flow between 25 and 75% of the forced vital capacity (MEF25-75), single breath diffusion (transfer factor [TLCO] and rate of carbon monoxide uptake [KCO], and body plethysmography (total lung capacity [TLC], residual volume [RV], vital capacity [VC] and RV/TLC). At the enrolment visit, subjects are asked to refrain from using short-acting bronchodilators for at least 6 hours and long-acting bronchodilators for at least 12 hours before key procedures. Prior to the subsequent follow-up visits, subjects may use their usual medication normally. Lung function measurements are performed under controlled conditions and in the sitting position as per standard practice.

Anthropometrics (including but not restricted to height, weight, waist and mid-arm circumference and triceps skin-fold circumference) are measured quarterly. Grip strength and fatigability are measured using standard techniques. Anthropometric data are used to compute the Malnutrition Universal Screening Tool (MUST) score.[30]

Nutritional information (including planned/ unplanned weight loss, and history and changes in food intake patterns) is collected quarterly according to MUST guidelines.

A postero-anterior chest X-ray (and lateral if required) is performed at all exacerbation visits, as per standard clinical practice, in order to exclude pneumonia.

Questionnaires and patient-reported outcome instruments

Various outcomes are assessed quarterly and at exacerbation using a series of questionnaires and patient-reported outcomes instruments such as the COPD Assessment Test (CAT),[31] the Nottingham Extended Daily Activities Scale (NEADL),[32] the Council on Nutrition Appetite Questionnaire (CNAQ),[33] and the EQ-5D.[34] The five items included in the EQ-5D index are mobility, self-care, usual activities, pain/discomfort and anxiety/depression. The BODE index (Body-Mass Index, Degree of Airflow Obstruction, Level of Functional Dyspnea, Exercise Capacity)[35] will also be calculated.

Healthcare use is recorded at all visits, including medication, vaccination, oxygen therapy, use of mechanical ventilation, pulmonary rehabilitation treatment, surgical intervention, outpatient visits (including GP visits and telephone contacts to COPD team), emergency room visits, hospitalisations, and productivity loss (time missed from work or usual activities due to worsening of COPD symptoms). Potential changes in disease management following an exacerbation (e.g. change in medication use) are also recorded.

Biological specimen collection

A wide range of biological specimens are collected from study participants (**table 3**). Blood samples are collected from all patients at study entry, quarterly and at exacerbation. Sputum samples are obtained by spontaneous expectoration or induced

by stimulation according to standard methods from all patients at study entry, monthly and at exacerbation. Nasopharyngeal swabs are collected from all patients at study entry and then from a subcohort of 30 patients at monthly follow-up visits and at exacerbation during the first year of follow-up. Urine samples are collected from all patients at study entry and at exacerbation and from the subcohort of 30 patients at monthly follow-up visits during the first year. Breath samples are collected from approximately 80 patients (including the subcohort of 30 patients providing nasopharyngeal swabs) at monthly follow-up visits and at exacerbation visits during the first year.

Blood samples are analysed for disease-related biomarkers, biochemistry, cell-mediated immune response, RNA profile and nutrients. Sputum samples are processed by traditional culture techniques and multiplex PCR analysis for identification of potential respiratory pathogens (including, but not limited to, non-typeable *H. influenzae, M. catarrhalis, S. pneumoniae, P. aeruginosa, Staphylococcus aureus,* respiratory syncytial virus, parainfluenza virus, rhinovirus, human metapneumovirus, influenza virus, adenovirus, and coronavirus). Sputum samples may also be analysed for disease-related biomarkers. Nasopharyngeal swabs are processed by traditional culture techniques and multiplex PCR analysis for potential pathogen identification. Urine samples may be processed for disease-related biomarkers. Breath samples are analysed by the selected ion flow tube mass spectrometry for identification of volatile organic compounds that may be characteristic for AECOPD.

Laboratory assays are performed at the Public Health Laboratory of Public Health England at University Hospital Southampton Foundation NHS Trust, GSK Vaccines central laboratory, and other GSK Vaccines designated laboratories. The assays use

standardised and validated procedures. Aliquots of all biological samples are processed (if applicable), frozen and stored for possible further disease-related testing. Culture isolates are also stored. Any additional laboratory tests will be performed at a GSK designated laboratory.

Sample size calculation

The sample size calculation was based on the primary study endpoint of incidence of all-cause AECOPD. Assuming that, on average, each subject is observed for a period of 18 months and that 2 episodes of AECOPD can be expected per subject per year, if 120 subjects are followed, the number of total person-years would be around 180 and during this time around 360 exacerbation events would be detected. If the distribution of events per subject follows a Poisson distribution with no overdispersion, an overdispersion factor of 1.5, or an overdispersion of 2, the approximate values of the lower and upper bounds of the 95% confidence interval (CI) around the point estimate of 2 events per subject per year would be 1.8-2.2, 1.7-2.3, and 1.7-2.3, respectively. So a sample size of 120 subjects should ensure sufficient precision in the estimation of the incidence rate of all-cause AECOPDs.

Additionally, in order to follow effectively 120 subjects, given the fact that subjects may be eligible but withdraw quite early in the study possibly due to the deterioration of the subject's health, the decision was taken to replace subjects who withdrew during the first year of follow-up, and recruit additional subjects.

We will construct a CONSORT diagram and capture where possible reasons for screen failure, drop-outs and loss to follow-up.

Data analysis

The co-primary study endpoints are the occurrence of all-cause AECOPD and the occurrence of AECOPD having sputum containing bacterial pathogens as detected by culture (overall and by species). The proportion of subjects at each visit for whom a sputum sample was obtained will be computed; overall and by the method the samples were obtained (spontaneous or induced). The proportion of sputum samples obtained at each visit and positive for specific bacterial pathogens (overall and by bacterial species) will also be calculated. The incidence rate of all-cause AECOPD and of AECOPD having sputum containing bacterial pathogens (overall and by bacterial species) will be calculated, with 95% CI. The 95% CI of the incidence rate will be computed using a model which accounts for repeated events, namely 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 as a preliminary approach. Other flexible approaches to statistical analysis may also be used. In addition, the same model with covariates (e.g. smoking status at enrolment, number of moderate/severe exacerbations reported in the 12 months prior to enrolment, presence of respiratory pathogenic bacteria detected at the exacerbation visit and at previous visits) will be applied. Incidence rates will also be calculated for moderate AECOPD and for severe AECOPD.

ETHICS AND DISSEMINATION

The AERIS study is conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and has been approved by the relevant institutional ethics and review board. All subjects must provide written informed consent to participate.

AERIS is being conducted in a specialised hospital that has extensive experience in respiratory research. AECOPD are proactively identified through patient-completed electronic diaries. After confirmation by phone, symptoms of an exacerbation trigger a clinic visit within 72 hours of symptom onset to enable comparisons of samples from same patient in stable COPD and during AECOPD. Although this is an intensive study with prolonged follow up, patients are expected to benefit from the improved access to expert care.

The results obtained will be disseminated by presentations at international medical conferences and peer-reviewed publications. Reporting will be in accordance with STROBE guidance.

DISCUSSION

The AERIS study has been initiated to comprehensively assess the role of infectious pathogens in AECOPD in a well-characterised cohort of patients. The study aims ~~a~~to~~t~~ explor~~e~~ing the dynamics of airway infection and its possible contribution to AECOPD, as well as the potential role of chronic colonization in stable disease. The overall objective of the study will aim at refining the case definition of AECOPD to reflect the possible microbiological aetiology of exacerbations. This is of note, since there is currently no commonly agreed definition of AECOPD and no current case definition includes a microbiological endpoint. The impact of AECOPD on health-related quality-of-life and healthcare use will be assessed in order to provide a complete picture of disease burden. The interaction between airway infection and systemic manifestations of COPD and nutritional status will also be assessed in detail for the first time. Biological specimens collected during the study may also be used for further disease-related testing, including molecular typing to describe and compare

selected biomarkers in AECOPD and stable COPD, to explore cell-mediated immune response to specific bacterial antigens, and to develop non-invasive bacterial diagnostic methods.

To our knowledge, few other studies have employed real-time electronic tracking of symptoms to identify AECOPD and potential aetiological triggers. This is important since available data suggest that up to 50% of exacerbations may not be reported to healthcare providers and consequently exacerbation rates are lower in studies employing event-based criteria to define AECOPD.[36] Due to the close daily monitoring of symptoms to identify AECOPD, we anticipate that the exacerbation rate in this study will be higher than previously reported. This close monitoring and early therapeutic intervention at exacerbation may also impact on estimates of the overall burden of disease.

A number of other epidemiological studies have been initiated in recent years to further characterize our understanding of the natural history of AECOPD. However, it is important to recognise that most of these studies have not included molecular microbiological assessments. Recent large observational studies focusing on biomarker discovery have involved close phenotyping of COPD patients, but have not studied the aetiology of exacerbations in depth.[37–39] In another study, potentially pathogenic bacterial strains were identified using molecular typing techniques, although viruses as potential airway pathogens were not investigated.[21] More recently, the prevalence and load of airway bacteria in stable and exacerbated AECOPD have been assessed in paired samples from 52 patients participating in the London COPD cohort study using modern molecular techniques.[19] Airway bacterial prevalence and load was found to increase significantly during AECOPD, with quantitative molecular techniques proving more discriminatory than culture. However, assessment was

limited to only the three most commonly detected airway bacteria (*H. influenzae, S. pneumoniae,* and *M. catarrhalis).* However, other potential pathogens and the overall respiratory microbiome may also contribute and have not yet been studied in detail.[17,18,20,40,41] In AERIS, samples will be acquired during both AECOPD and stable disease and analysed for a wide range of potentially pathogenic bacteria and viruses using advanced PCR-based techniques as well as traditional culture-based methods.

A major strength of the AERIS study design is the comprehensive assessment of clinical status, microbiology, functional status, nutritional status, health-related quality-of-life and healthcare resource utilisation in individual patients in a single large cohort during both stable COPD and AECOPD. The selection of subjects with a history of at least a single exacerbation enriches the cohort to some degree and ensures an adequate number of exacerbations are sampled. It is accepted that some aspects of the analysis may not be generalizable to the subgroup of patients who never exacerbate. The analyses proposed in this study will generate epidemiological data to complement that derived from existing COPD cohorts and further explore determinants of COPD and the contribution of bacterial and viral pathogens to AECOPD, as well as to provide some understanding of the limitations of existing data. As exacerbation visits are triggered by patient diary data, accurate and timely diary completion is essential. All subjects participating in this study receive diary training at enrolment and support is available from the study team at all times to promote accurate and complete diary keeping. Cohort retention is a key factor in the successful delivery of such a study and with in-depth sampling protocols, participant engagement, comfort and feedback are key factors in optimising cohort retention and comprehensive data collection.

Identification of novel approaches for the prevention of AECOPD is an important research goal. Long-acting beta-agonists (LABA) and long-acting anti-muscarinic bronchodilators remain the cornerstone of treatment for patients with COPD.[42] Combinations of LABA and inhaled corticosteroids are also used in patients with more severe disease and/or frequent exacerbations. Long-term treatment with macrolide antibiotics and pulsed quinolone therapy may be considered for exacerbation prevention.[7,43,44] However, concerns exist about the potential for development of antimicrobial resistance during long-term antibiotic therapy. Numerous other approaches are under investigation for the prevention of AECOPD, including anti-inflammatory drugs, immunomodulatory agents, immunotherapy, antioxidants and non-pharmacologic strategies. Vaccination is another potential approach meriting investigation for reducing AECOPD risk. However, optimal strategies targeting key respiratory pathogens are not yet available to the clinician.

In conclusion, there have been considerable advances in our understanding of the epidemiology, pathophysiology and clinical management of COPD in recent years. However, there remains a genuine need to further explore the aetiology and pathogenesis of AECOPD. It is anticipated that results of this epidemiological study will increase our understanding of the contribution of bacterial and viral pathogens to AECOPD, the natural history of these events in association with the timing of symptoms and physiological changes, and will offer new direction ~~potentially leading to~~ for research into targeted therapeutic and preventative interventions.

Contributors S. Bourne: principle investigator, closely involved in all steps of the study and specifically wrote substantial parts of the protocol; **C. Cohet**: ~~e~~Epidemiologist responsible for this observational study was closely involved in the design of study; **V. Kim and A. Barton**: co-investigators and closely involved in the

conduct of this study; **A. Tuck**: site project manager for microbiology testing and involved in writing of microbiology parts of the protocol; **E. Aris**: project statistician, responsible for writing of statistical analysis plan and definition of statistical outcomes; **S. Mesia Vela**: project manager, closely involved in the discussion on design and follow-up of the study; **J-M. Devaster**: dDirector of clinical research and translational sScience, closely involved in all discussions and study accountable person; **W.R. Ballou:** vice-president of clinical research and translational sScience team, closely involved in all discussions of design of this study. **S. Clarke:** co-investigator, closely involved in all steps of the study. **T. Wilkinson**: coordinating lead investigator, involved in all steps of the study.

All authors provided intellectual input into the development of this manuscript. And have critically reviewed and approved the final manuscript.

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