



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

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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.

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3 **Discussion:** To our knowledge, few other studies have employed real-time electronic
4 tracking of symptoms to identify AECOPD and potential aetiological triggers. It is
5 anticipated that results of AERIS will increase our understanding of the contribution
6 of bacterial and viral pathogens to AECOPD, potentially leading to research into
7 targeted therapeutic and preventative interventions.
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14 **Registration:** ClinicalTrials.gov NCT01360398
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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

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3 lung function and longer duration of hospitalisation than exacerbations of non-
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5 infectious aetiology.¹⁴
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8 With the introduction of new molecular sequencing techniques, the traditional
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10 belief that healthy lungs are sterile has been refuted. There is increasing evidence that
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12 the lower respiratory tract contains a diverse microbial flora that differs between
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14 health and disease.¹⁶⁻²⁰ The presence of potentially pathogenic microorganisms in the
15
16 inflamed airways of patients with COPD is well-documented, with up to 50% of
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18 patients with stable COPD showing evidence of lower airway bacterial colonisation
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20 using traditional culture techniques.^{15,21,22} In patients with COPD, bacterial detection
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22 in lower airway derived samples is associated with increased airway inflammation,
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24 reduced lung function and more frequent exacerbations.²³⁻²⁵ Acquisition of new
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26 pathogen strains also appears to be associated with an increased risk of
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28 AECOPD.^{15,21,26} Estimates of the relative contribution of different pathogens to
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30 AECOPD vary. However, non-typeable *Haemophilus influenzae* appears to be the
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32 major bacterial pathogen associated with AECOPD, followed by *Streptococcus*
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34 *pneumoniae*, *Moraxella catarrhalis* and *Pseudomonas aeruginosa*.^{14,15} Respiratory
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36 viruses commonly associated with AECOPD include human rhinoviruses, influenza
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38 and parainfluenza viruses, respiratory syncytial virus, coronavirus and adenovirus.¹⁵
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44 Improved understanding of the role of infectious pathogens in AECOPD may
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46 lead to the development of more targeted strategies for treatment and prevention. This
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48 paper describes the objectives and design of Acute Exacerbation and Respiratory
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50 InfectionS in COPD (AERIS), a prospective longitudinal epidemiological study
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52 initiated in the UK to assess the role of respiratory infection in acute exacerbations of
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54 COPD. Molecular diagnostic and typing techniques will be used to describe the
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56 dynamics of airway infection and its potential association with clinical outcome. The
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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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> 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) $\leq 80\%$ 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	<p>Subjects must satisfy ALL of the following criteria at study entry:</p> <ul style="list-style-type: none"> • 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 FEV₁ ≤80% of predicted normal and FEV₁/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	<ul style="list-style-type: none"> • 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 FEV₁), a series of morning questions to identify symptoms of exacerbations²⁹ 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/FEV₁ 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	Study entry and final visit
Body box	Every 6 months and within 72 hours of onset of exacerbation
TLCO ^b	Monthly and within 72 hours of onset of exacerbation
Spirometry	Every 6 months
6-minute walk test	Every 6 months
Questionnaires	
ATS-DLD-78A (Risk factors, disease history and smoking history)	Study entry

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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	Study entry
For routine biochemistry	
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

^aHeight, weight, mid-arm circumference, waist circumference, triceps skin fold measurement, fat free body mass. ^bTLCO: transfer factor. ^cHealthcare 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) ^dmMRC: Medical Research Council Dyspnea Scale score. ^eCAT: COPD Assessment Test. ^fVAS: visual analogue scale. ^gNEADL: Nottingham Extended Daily Activities Scale. ^hCNAQ: Council on Nutrition Appetite Questionnaire. ⁱIn all subjects at study entry and in a subcohort of 30 subjects during the first year. ^jIn a subcohort of approximately 80 subjects. ^kIn 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, FEV₁, FVC, FEV₁/FVC ratio, FEV₁ % 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

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3 Nutritional information (including planned/ unplanned weight loss, and history and
4 changes in food intake patterns) is collected quarterly according to MUST guidelines.
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8 A postero-anterior chest X-ray (and lateral if required) is performed at all
9 exacerbation visits, as per standard clinical practice, in order to exclude pneumonia.
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12 *Questionnaires*

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14 Various outcomes are assessed quarterly and at exacerbation using a series of
15 questionnaires and patient-reported outcomes instruments such as the COPD
16 Assessment Test (CAT),³¹ the Nottingham Extended Daily Activities Scale
17 (NEADL),³² the Council on Nutrition Appetite Questionnaire (CNAQ),³³ and the EQ-
18 5D.³⁴ The BODE index (Body-Mass Index, Degree of Airflow Obstruction, Level of
19 Functional Dyspnea, Exercise Capacity)³⁵ will also be calculated. Healthcare use is
20 recorded at all visits, including medication, vaccination, oxygen therapy, use of
21 mechanical ventilation, pulmonary rehabilitation treatment, surgical intervention,
22 outpatient visits (including GP visits and telephone contacts to COPD team),
23 emergency room visits, hospitalisations, and productivity loss (time missed from work
24 or usual activities due to worsening of COPD symptoms). Potential changes in disease
25 management following an exacerbation (e.g. change in medication use) are also
26 recorded.
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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

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3 spectrometry for identification of volatile organic compounds that may be
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5 characteristic for AECOPD.
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8 Laboratory assays are performed at the Public Health Laboratory of Public Health
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10 England at University Hospital Southampton Foundation NHS Trust, GSK Vaccines
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12 central laboratory, and other GSK Vaccines designated laboratories. The assays use
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14 standardised and validated procedures. Aliquots of all biological samples are
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16 processed (if applicable), frozen and stored for possible further disease-related testing.
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18 Culture isolates are also stored. Any additional laboratory tests will be performed at a
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20 GSK designated laboratory.
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23 **Sample size calculation**

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26 The sample size calculation was based on the primary study endpoint of incidence of
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28 all-cause AECOPD. Assuming that, on average, each subject is observed for a period
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30 of 18 months and that 2 episodes of AECOPD can be expected per subject per year, if
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32 120 subjects are followed, the number of total person-years would be around 180 and
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34 during this time around 360 exacerbation events would be detected. If the distribution
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36 of events per subject follows a Poisson distribution with no overdispersion, an
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38 overdispersion factor of 1.5, or an overdispersion of 2, the approximate values of the
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40 lower and upper bounds of the 95% confidence interval (CI) around the point estimate
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42 of 2 events per subject per year would be 1.8-2.2, 1.7-2.3, and 1.7-2.3, respectively.
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44 So a sample size of 120 subjects should ensure sufficient precision in the estimation
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46 of the incidence rate of all-cause AECOPDs.
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52 Additionally, in order to follow effectively 120 subjects, given the fact that
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54 subjects may be eligible but withdraw quite early in the study possibly due to the
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3 deterioration of the subject's health, the decision was taken to replace subjects who
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5 withdrew during the first year of follow-up, and recruit additional subjects.
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8 **Data analysis**

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10 The co-primary study endpoints are the occurrence of all-cause AECOPD and the
11 occurrence of AECOPD having sputum containing bacterial pathogens as detected by
12 culture (overall and by species). The proportion of subjects at each visit for whom a
13 sputum sample was obtained will be computed; overall and by the method the
14 samples were obtained (spontaneous or induced). The proportion of sputum samples
15 obtained at each visit and positive for specific bacterial pathogens (overall and by
16 bacterial species) will also be calculated. The incidence rate of all-cause AECOPD
17 and of AECOPD having sputum containing bacterial pathogens (overall and by
18 bacterial species) will be calculated, with 95% CI. The 95% CI of the incidence rate
19 will be computed using a model which accounts for repeated events, namely the
20 generalised linear model assuming a negative binomial distribution for the response
21 variable with logarithm as link function, and the logarithm of time for follow-up as an
22 offset variable as a preliminary approach. Other flexible approaches to statistical
23 analysis may also be used. In addition, the same model with covariates (e.g. smoking
24 status at enrolment, number of moderate/severe exacerbations reported in the 12
25 months prior to enrolment, presence of respiratory pathogenic bacteria detected at the
26 exacerbation visit and at previous visits) will be applied. Incidence rates will also be
27 calculated for moderate AECOPD and for severe AECOPD.
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50 **ETHICS AND DISEMINATION**

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52 The AERIS study is conducted in accordance with the Declaration of Helsinki and
53 Good Clinical Practice guidelines, and has been approved by the relevant institutional
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3 ethics and review board. All subjects must provide written informed consent to
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5 participate.
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8 AERIS is being conducted in a specialised hospital that has extensive experience
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10 in respiratory research. AECOPD are proactively identified through patient-completed
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12 electronic diaries. After confirmation by phone, symptoms of an exacerbation trigger
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14 a clinic visit within 72 hours of symptom onset to enable comparisons of samples
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16 from same patient in stable COPD and during AECOPD. Although this is an intensive
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18 study with prolonged follow up, patients are expected to benefit from the improved
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20 access to expert care.
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24 The results obtained will be disseminated by presentations at international
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26 medical conferences and peer-reviewed publications.
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29 **DISCUSSION**

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32 The AERIS study has been initiated to assess the role of infectious pathogens in
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34 AECOPD in a well-characterised cohort of patients. The study aims at exploring the
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36 dynamics of airway infection and its possible contribution to AECOPD, as well as the
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38 potential role of chronic colonization in stable disease. The overall objective of the
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40 study will aim at refining the case definition of AECOPD to reflect the possible
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42 microbiological aetiology of exacerbations. This is of note, since there is currently no
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44 commonly agreed definition of AECOPD and no current case definition includes a
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46 microbiological endpoint. The impact of AECOPD on health-related quality-of-life
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48 and healthcare use will be assessed in order to provide a complete picture of disease
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50 burden. The interaction between airway infection and systemic manifestations of
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52 COPD and nutritional status will also be assessed. Biological specimens collected
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54 during the study may also be used for further disease-related testing, including
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3 molecular typing to describe and compare selected biomarkers in AECOPD and stable
4 COPD, to explore cell-mediated immune response to specific bacterial antigens, and
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7 to develop non-invasive bacterial diagnostic methods.
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10 To our knowledge, few other studies have employed real-time electronic tracking
11 of symptoms to identify AECOPD and potential aetiological triggers. This is
12 important since available data suggest that up to 50% of exacerbations may not be
13 reported to healthcare providers and consequently exacerbation rates are lower in
14 studies employing event-based criteria to define AECOPD.³⁶ Due to the close daily
15 monitoring of symptoms to identify AECOPD, we anticipate that the exacerbation
16 rate in this study will be higher than previously reported. This close monitoring and
17 early therapeutic intervention at exacerbation may also impact on estimates of the
18 overall burden of disease.
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30 A number of other epidemiological studies have been initiated in recent years to
31 further characterize our understanding of the natural history of AECOPD. However,
32 most of these studies have not included molecular microbiological assessments.
33 Recent large observational studies focusing on biomarker discovery have involved
34 close phenotyping of COPD patients, but have not studied the aetiology of
35 exacerbations in depth.³⁷⁻³⁹ In another study, potentially pathogenic bacterial strains
36 were identified using molecular typing techniques, although viruses as potential
37 airway pathogens were not investigated.²¹ More recently, the prevalence and load of
38 airway bacteria in stable and exacerbated AECOPD have been assessed in paired
39 samples from 52 patients participating in the London COPD cohort study using
40 modern molecular techniques.¹⁹ Airway bacterial prevalence and load was found to
41 increase significantly during AECOPD, with quantitative molecular techniques
42 proving more discriminatory than culture. However, assessment was limited to only
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3 the three most commonly detected airway bacteria (*H. influenzae*, *S. pneumoniae*, and
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5 *M. catarrhalis*). However, other potential pathogens and the overall respiratory
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7 microbiome may also contribute and have not yet been studied in detail.^{17,18,20,40,41} In
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9 AERIS, samples will be acquired during both AECOPD and stable disease and
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11 analysed for a wide range of potentially pathogenic bacteria and viruses using
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13 advanced PCR-based techniques as well as traditional culture-based methods.
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17 A major strength of the AERIS study design is the comprehensive assessment of
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19 clinical status, microbiology, functional status, nutritional status, health-related
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21 quality-of-life and healthcare resource utilisation in individual patients in a single
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23 large cohort during both stable COPD and AECOPD. The analyses proposed in this
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25 study will generate epidemiological data to complement that derived from existing
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27 COPD cohorts and further explore determinants of COPD and the contribution of
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29 bacterial and viral pathogens to AECOPD, as well as to provide some understanding
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31 of the limitations of existing data. As exacerbation visits are triggered by patient diary
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33 data, accurate and timely diary completion is essential. All subjects participating in
34
35 this study receive diary training at enrolment and support is available from the study
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37 team at all times to promote accurate and complete diary keeping. Cohort retention is
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39 a key factor in the successful delivery of such a study and with in-depth sampling
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41 protocols, participant engagement, comfort and feedback are key factors in optimising
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43 cohort retention and comprehensive data collection.
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49 Identification of novel approaches for the prevention of AECOPD is an important
50
51 research goal. Long-acting beta-agonists (LABA) and long-acting anti-muscarinic
52
53 bronchodilators remain the cornerstone of treatment for patients with COPD.⁴²
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55 Combinations of LABA and inhaled corticosteroids are also used in patients with
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57 more severe disease and/or frequent exacerbations. Long-term treatment with
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3 macrolide antibiotics and pulsed quinolone therapy may be considered for
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5 exacerbation prevention.^{7,43,44} However, concerns exist about the potential for
6
7 development of antimicrobial resistance during long-term antibiotic therapy.
8
9 Numerous other approaches are under investigation for the prevention of AECOPD,
10
11 including anti-inflammatory drugs, immunomodulatory agents, immunotherapy,
12
13 antioxidants and non-pharmacologic strategies. Vaccination is another potential
14
15 approach meriting investigation for reducing AECOPD risk. However, optimal
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17 strategies targeting key respiratory pathogens are not yet available to the clinician.
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21 In conclusion, there have been considerable advances in our understanding of the
22
23 epidemiology, pathophysiology and clinical management of COPD in recent years.
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25 However, there remains a genuine need to further explore the aetiology and
26
27 pathogenesis of AECOPD. It is anticipated that results of this epidemiological study
28
29 will increase our understanding of the contribution of bacterial and viral pathogens to
30
31 AECOPD, potentially leading to research into targeted therapeutic and preventative
32
33 interventions.
34
35

36
37 **Contributors S. Bourne:** principle investigator, closely involved in all steps of the
38
39 study and specifically wrote substantial parts of the protocol; **C. Cohet:**
40
41 Epidemiologist responsible for this observational study was closely involved in the
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43 design of study; **V. Kim and A. Barton:** co-investigators and closely involved in the
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45 conduct of this study; **A. Tuck:** site project manager for microbiology testing and
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49 responsible for writing of statistical analysis plan and definition of statistical
50
51 outcomes; **S. Mesia Vela:** project manager, closely involved in the discussion on
52
53 design and follow-up of the study; **J-M. Devaster:** Director clinical research and
54
55 translational Science, closely involved in all discussions and study accountable
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3 person; **W.R. Ballou:** vice-president of clinical research and translational Science
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5 team, closely involved in all discussions of design of this study. **S. Clarke:** co-
6
7 investigator, closely involved in all steps of the study. **T. Wilkinson:** coordinating
8
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10

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12 All authors provided intellectual input into the development of this manuscript. And
13
14 have critically reviewed and approved the final manuscript.
15

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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.

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3 **Discussion:** Few other studies have addressed the complexity of the microbiological
4 and systemic components of COPD or employed real-time electronic tracking of
5 symptoms to identify AECOPD and potential aetiological triggers. Results of AERIS
6 will increase our understanding of the contribution of pathogens to AECOPD,
7 potentially leading to new targeted therapeutic and preventative interventions.
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14 **Registration:** ClinicalTrials.gov NCT01360398
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20 **ARTICLE SUMMARY**

21 **Article focus**

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26 • Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) is a
27 prospective longitudinal epidemiological study initiated in the UK to assess how
28 dynamic changes in the COPD airway microbiome contribute to the incidence
29 and severity of acute exacerbations of COPD (AECOPD).
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35 **Key messages**

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

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3 of bacterial and/or viral respiratory pathogens.^{14,15} Exacerbations associated with
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5 detectable respiratory pathogens have been shown to have a more marked impact on
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7 lung function and longer duration of hospitalisation than exacerbations of non-
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9 infectious aetiology.¹⁴
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12 With the introduction of new molecular sequencing techniques, the traditional
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14 belief that healthy lungs are sterile has been refuted. There is increasing evidence that
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16 the lower respiratory tract contains a diverse microbial flora that differs between
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18 health and disease.¹⁶⁻²⁰ The presence of potentially pathogenic microorganisms in the
19
20 inflamed airways of patients with COPD is well-documented, with up to 50% of
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22 patients with stable COPD showing evidence of lower airway bacterial colonisation
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24 using traditional culture techniques.^{15,21,22} In patients with COPD, bacterial detection
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26 in lower airway derived samples is associated with increased airway inflammation,
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28 reduced lung function and more frequent exacerbations.²³⁻²⁵ Acquisition of new
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30 pathogen strains also appears to be associated with an increased risk of
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32 AECOPD.^{15,21,26} Estimates of the relative contribution of different pathogens to
33
34 AECOPD vary. However, non-typeable *Haemophilus influenzae* appears to be the
35
36 major bacterial pathogen associated with AECOPD, followed by *Streptococcus*
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38 *pneumoniae*, *Moraxella catarrhalis* and *Pseudomonas aeruginosa*.^{14,15} Respiratory
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40 viruses commonly associated with AECOPD are diverse and include human
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42 rhinoviruses, influenza and parainfluenza viruses, respiratory syncytial virus,
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44 coronavirus and adenovirus.¹⁵
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51 Improved understanding of the role of infectious pathogens in AECOPD is
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53 required to better understand the pathophysiology of the disease and may lead to the
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55 development of more targeted strategies for treatment and prevention. This paper
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57 describes the objectives and design of Acute Exacerbation and Respiratory InfectionS
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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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> Of all-cause AECOPD

- Of AECOPD having sputum containing bacterial pathogens
 - Of AECOPD having sputum containing viral pathogens
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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) $\leq 80\%$ 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	<p>Subjects must satisfy <i>ALL</i> of the following criteria at study entry:</p> <ul style="list-style-type: none"> • 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 FEV₁ ≤80% of predicted normal and FEV₁/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	<ul style="list-style-type: none"> • 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 FEV₁), a series of morning questions to identify symptoms of exacerbations²⁹ 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/FEV₁ 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	Study entry and final visit
Body box	Every 6 months and within 72 hours of onset of exacerbation
TLCO ^b	Monthly and within 72 hours of onset of exacerbation
Spirometry	Every 6 months
6-minute walk test	
Questionnaires and patient-reported outcome instruments	
ATS-DLD-78A (Risk factors, disease history and smoking history)	Study entry

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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

^aHeight, weight, mid-arm circumference, waist circumference, triceps skin fold measurement, fat free body mass. ^bTLCO: transfer factor. ^cHealthcare 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) ^dmMRC: Medical Research Council Dyspnea Scale score. ^eCAT: COPD Assessment Test. ^fVAS: visual analogue scale. ^gNEADL: Nottingham Extended Daily Activities Scale. ^hCNAQ: Council on Nutrition Appetite Questionnaire. ⁱIn all subjects at study entry and in a subcohort of 30 subjects during the first year. ^jIn a subcohort of approximately 80 subjects. ^kIn 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, FEV₁, FVC, FEV₁/FVC ratio, FEV₁ % 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

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3 Nutritional information (including planned/ unplanned weight loss, and history and
4 changes in food intake patterns) is collected quarterly according to MUST guidelines.
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8 A postero-anterior chest X-ray (and lateral if required) is performed at all
9 exacerbation visits, as per standard clinical practice, in order to exclude pneumonia.
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12 *Questionnaires and patient-reported outcome instruments*

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14 Various outcomes are assessed quarterly and at exacerbation using a series of
15 questionnaires and patient-reported outcome instruments such as the COPD
16 Assessment Test (CAT),³¹ the Nottingham Extended Daily Activities Scale
17 (NEADL),³² the Council on Nutrition Appetite Questionnaire (CNAQ),³³ and the EQ-
18 5D.³⁴ The five items included in the EQ-5D index are mobility, self-care, usual
19 activities, pain/discomfort and anxiety/depression. The BODE index (Body-Mass
20 Index, Degree of Airflow Obstruction, Level of Functional Dyspnea, Exercise
21 Capacity)³⁵ will also be calculated.
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34 Healthcare use is recorded at all visits, including medication, vaccination,
35 oxygen therapy, use of mechanical ventilation, pulmonary rehabilitation treatment,
36 surgical intervention, outpatient visits (including GP visits and telephone contacts to
37 COPD team), emergency room visits, hospitalisations, and productivity loss (time
38 missed from work or usual activities due to worsening of COPD symptoms). Potential
39 changes in disease management following an exacerbation (e.g. change in medication
40 use) are also recorded.
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49 *Biological specimen collection*

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51 A wide range of biological specimens are collected from study participants (**table 3**).
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53 Blood samples are collected from all patients at study entry, quarterly and at
54 exacerbation. Sputum samples are obtained by spontaneous expectoration or induced
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3 by stimulation according to standard methods from all patients at study entry, monthly
4 and at exacerbation. Nasopharyngeal swabs are collected from all patients at study
5 entry and then from a subcohort of 30 patients at monthly follow-up visits and at
6 exacerbation during the first year of follow-up. Urine samples are collected from all
7 patients at study entry and at exacerbation and from the subcohort of 30 patients at
8 monthly follow-up visits during the first year. Breath samples are collected from
9 approximately 80 patients (including the subcohort of 30 patients providing
10 nasopharyngeal swabs) at monthly follow-up visits and at exacerbation visits during
11 the first year.
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23 Blood samples are analysed for disease-related biomarkers, biochemistry, cell-
24 mediated immune response, RNA profile and nutrients. Sputum samples are
25 processed by traditional culture techniques and multiplex PCR analysis for
26 identification of potential respiratory pathogens (including, but not limited to, non-
27 typeable *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, *P. aeruginosa*, *Staphylococcus*
28 *aureus*, respiratory syncytial virus, parainfluenza virus, rhinovirus, human
29 metapneumovirus, influenza virus, adenovirus, and coronavirus). Sputum samples
30 may also be analysed for disease-related biomarkers. Nasopharyngeal swabs are
31 processed by traditional culture techniques and multiplex PCR analysis for potential
32 pathogen identification. Urine samples may be processed for disease-related
33 biomarkers. Breath samples are analysed by the selected ion flow tube mass
34 spectrometry for identification of volatile organic compounds that may be
35 characteristic for AECOPD.
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52 Laboratory assays are performed at the Public Health Laboratory of Public Health
53 England at University Hospital Southampton Foundation NHS Trust, GSK Vaccines
54 central laboratory, and other GSK Vaccines designated laboratories. The assays use
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3 standardised and validated procedures. Aliquots of all biological samples are
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5 processed (if applicable), frozen and stored for possible further disease-related testing.
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7 Culture isolates are also stored. Any additional laboratory tests will be performed at a
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9 GSK designated laboratory.
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11 **Sample size calculation**

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15 The sample size calculation was based on the primary study endpoint of incidence of
16
17 all-cause AECOPD. Assuming that, on average, each subject is observed for a period
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19 of 18 months and that 2 episodes of AECOPD can be expected per subject per year, if
20
21 120 subjects are followed, the number of total person-years would be around 180 and
22
23 during this time around 360 exacerbation events would be detected. If the distribution
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25 of events per subject follows a Poisson distribution with no overdispersion, an
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27 overdispersion factor of 1.5, or an overdispersion of 2, the approximate values of the
28
29 lower and upper bounds of the 95% confidence interval (CI) around the point estimate
30
31 of 2 events per subject per year would be 1.8-2.2, 1.7-2.3, and 1.7-2.3, respectively.
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33 So a sample size of 120 subjects should ensure sufficient precision in the estimation
34
35 of the incidence rate of all-cause AECOPDs.
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40 Additionally, in order to follow effectively 120 subjects, given the fact that
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42 subjects may be eligible but withdraw quite early in the study possibly due to the
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44 deterioration of the subject's health, the decision was taken to replace subjects who
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46 withdrew during the first year of follow-up, and recruit additional subjects.
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49 We will construct a CONSORT diagram and capture where possible reasons for
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51 screen failure, drop-outs and loss to follow-up.
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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.

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3 AERIS is being conducted in a specialised hospital that has extensive experience
4 in respiratory research. AECOPD are proactively identified through patient-completed
5 electronic diaries. After confirmation by phone, symptoms of an exacerbation trigger
6 a clinic visit within 72 hours of symptom onset to enable comparisons of samples
7 from same patient in stable COPD and during AECOPD. Although this is an intensive
8 study with prolonged follow up, patients are expected to benefit from the improved
9 access to expert care.
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19 The results obtained will be disseminated by presentations at international
20 medical conferences and peer-reviewed publications. Reporting will be in accordance
21 with STROBE guidance.
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25 26 **DISCUSSION**

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29 The AERIS study has been initiated to comprehensively assess the role of infectious
30 pathogens in AECOPD in a well-characterised cohort of patients. The study aims to
31 explore the dynamics of airway infection and its possible contribution to AECOPD, as
32 well as the potential role of chronic colonization in stable disease. The overall
33 objective of the study will aim at refining the case definition of AECOPD to reflect
34 the possible microbiological aetiology of exacerbations. This is of note, since there is
35 currently no commonly agreed definition of AECOPD and no current case definition
36 includes a microbiological endpoint. The impact of AECOPD on health-related
37 quality-of-life and healthcare use will be assessed in order to provide a complete
38 picture of disease burden. The interaction between airway infection and systemic
39 manifestations of COPD and nutritional status will also be assessed in detail for the
40 first time. Biological specimens collected during the study may also be used for
41 further disease-related testing, including molecular typing to describe and compare
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3 selected biomarkers in AECOPD and stable COPD, to explore cell-mediated immune
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5 response to specific bacterial antigens, and to develop non-invasive bacterial
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7 diagnostic methods.
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10 To our knowledge, few other studies have employed real-time electronic tracking
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12 of symptoms to identify AECOPD and potential aetiological triggers. This is
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14 important since available data suggest that up to 50% of exacerbations may not be
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16 reported to healthcare providers and consequently exacerbation rates are lower in
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18 studies employing event-based criteria to define AECOPD.³⁶ Due to the close daily
19
20 monitoring of symptoms to identify AECOPD, we anticipate that the exacerbation
21
22 rate in this study will be higher than previously reported. This close monitoring and
23
24 early therapeutic intervention at exacerbation may also impact on estimates of the
25
26 overall burden of disease.
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30 A number of other epidemiological studies have been initiated in recent years to
31
32 further characterize our understanding of the natural history of AECOPD. However, it
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34 is important to recognise that most of these studies have not included molecular
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36 microbiological assessments. Recent large observational studies focusing on
37
38 biomarker discovery have involved close phenotyping of COPD patients, but have not
39
40 studied the aetiology of exacerbations in depth.³⁷⁻³⁹ In another study, potentially
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42 pathogenic bacterial strains were identified using molecular typing techniques,
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44 although viruses as potential airway pathogens were not investigated.²¹ More recently,
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46 the prevalence and load of airway bacteria in stable and exacerbated AECOPD have
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48 been assessed in paired samples from 52 patients participating in the London COPD
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50 cohort study using modern molecular techniques.¹⁹ Airway bacterial prevalence and
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52 load was found to increase significantly during AECOPD, with quantitative molecular
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54 techniques proving more discriminatory than culture. However, assessment was
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3 limited to only the three most commonly detected airway bacteria (*H. influenzae*, *S.*
4 *pneumoniae*, and *M. catarrhalis*). However, other potential pathogens and the overall
5 respiratory microbiome may also contribute and have not yet been studied in
6 detail.^{17,18,20,40,41} In AERIS, samples will be acquired during both AECOPD and stable
7 disease and analysed for a wide range of potentially pathogenic bacteria and viruses
8 using advanced PCR-based techniques as well as traditional culture-based methods.
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17 A major strength of the AERIS study design is the comprehensive assessment of
18 clinical status, microbiology, functional status, nutritional status, health-related
19 quality-of-life and healthcare resource utilisation in individual patients in a single
20 large cohort during both stable COPD and AECOPD. The selection of subjects with a
21 history of at least a single exacerbation enriches the cohort to some degree and
22 ensures an adequate number of exacerbations are sampled. It is accepted that some
23 aspects of the analysis may not be generalizable to the subgroup of patients who never
24 exacerbate. The analyses proposed in this study will generate epidemiological data to
25 complement that derived from existing COPD cohorts and further explore
26 determinants of COPD and the contribution of bacterial and viral pathogens to
27 AECOPD, as well as to provide some understanding of the limitations of existing
28 data. As exacerbation visits are triggered by patient diary data, accurate and timely
29 diary completion is essential. All subjects participating in this study receive diary
30 training at enrolment and support is available from the study team at all times to
31 promote accurate and complete diary keeping. Cohort retention is a key factor in the
32 successful delivery of such a study and with in-depth sampling protocols, participant
33 engagement, comfort and feedback are key factors in optimising cohort retention and
34 comprehensive data collection.
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3 Identification of novel approaches for the prevention of AECOPD is an important
4 research goal. Long-acting beta-agonists (LABA) and long-acting anti-muscarinic
5 bronchodilators remain the cornerstone of treatment for patients with COPD.⁴²
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7 Combinations of LABA and inhaled corticosteroids are also used in patients with
8 more severe disease and/or frequent exacerbations. Long-term treatment with
9 macrolide antibiotics and pulsed quinolone therapy may be considered for
10 exacerbation prevention.^{7,43,44} However, concerns exist about the potential for
11 development of antimicrobial resistance during long-term antibiotic therapy.
12
13 Numerous other approaches are under investigation for the prevention of AECOPD,
14 including anti-inflammatory drugs, immunomodulatory agents, immunotherapy,
15 antioxidants and non-pharmacologic strategies. Vaccination is another potential
16 approach meriting investigation for reducing AECOPD risk. However, optimal
17 strategies targeting key respiratory pathogens are not yet available to the clinician.
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32 In conclusion, there have been considerable advances in our understanding of the
33 epidemiology, pathophysiology and clinical management of COPD in recent years.
34 However, there remains a genuine need to further explore the aetiology and
35 pathogenesis of AECOPD. It is anticipated that results of this epidemiological study
36 will increase our understanding of the contribution of bacterial and viral pathogens to
37 AECOPD, the natural history of these events in association with the timing of
38 symptoms and physiological changes, and will offer new direction for research into
39 targeted therapeutic and preventative interventions.
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4 participating in this study. We also wish to acknowledge the support of the
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12 involved in the design of study;
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36 involved in all discussions and study accountable person;
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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~~ refine 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 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

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3 informed consent. The results obtained will be disseminated ~~by presentations~~ at
4 international medical conferences and in peer-reviewed publications.
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8 **Discussion:** ~~To our knowledge, f~~ew other studies have addressed the complexity of
9 the microbiological and systemic components of COPD or employed real-time
10 electronic tracking of symptoms to identify AECOPD and potential aetiological
11 triggers. ~~It is anticipated that R~~esults of AERIS will significantly increase our
12 understanding of the contribution of ~~bacterial and viral pathogens~~ pathogens to
13 AECOPD, potentially leading to ~~research into~~ new targeted therapeutic and
14 preventative interventions.
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24 **Registration:** ClinicalTrials.gov NCT01360398
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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

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2
3 lung function and longer duration of hospitalisation than exacerbations of non-
4
5 infectious aetiology.¹⁴
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8 With the introduction of new molecular sequencing techniques, the traditional
9
10 belief that healthy lungs are sterile has been refuted. There is increasing evidence that
11
12 the lower respiratory tract contains a diverse microbial flora that differs between
13
14 health and disease.¹⁶⁻²⁰ The presence of potentially pathogenic microorganisms in the
15
16 inflamed airways of patients with COPD is well-documented, with up to 50% of
17
18 patients with stable COPD showing evidence of lower airway bacterial colonisation
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20 using traditional culture techniques.^{15,21,22} In patients with COPD, bacterial detection
21
22 in lower airway derived samples is associated with increased airway inflammation,
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24 reduced lung function and more frequent exacerbations.²³⁻²⁵ Acquisition of new
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26 pathogen strains also appears to be associated with an increased risk of
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28 AECOPD.^{15,21,26} Estimates of the relative contribution of different pathogens to
29
30 AECOPD vary. However, non-typeable *Haemophilus influenzae* appears to be the
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32 major bacterial pathogen associated with AECOPD, followed by *Streptococcus*
33
34 *pneumoniae*, *Moraxella catarrhalis* and *Pseudomonas aeruginosa*.^{14,15} Respiratory
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36 viruses commonly associated with AECOPD are diverse and include human
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38 rhinoviruses, influenza and parainfluenza viruses, respiratory syncytial virus,
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40 coronavirus and adenovirus.¹⁵
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46 Improved understanding of the role of infectious pathogens in AECOPD is
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48 required to better understand the pathophysiology of the disease and may lead to the
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50 development of more targeted strategies for treatment and prevention. This paper
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52 describes the objectives and design of Acute Exacerbation and Respiratory InfectionS
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54 in COPD (AERIS), a prospective longitudinal epidemiological study initiated in the
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56 UK to assess the role of respiratory infection in acute exacerbations of COPD.
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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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> 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) $\leq 80\%$ 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 <i>ALL</i> of the following criteria at study entry: <ul style="list-style-type: none"> • 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 FEV₁ ≤80% of predicted normal and FEV₁/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	<ul style="list-style-type: none"> • 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 • <u>Long-term corticosteroid or antibiotic therapy</u> • 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 FEV₁), a series of morning questions to identify symptoms of exacerbations²⁹ 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/FEV₁ 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	Study entry and final visit
Body box	
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

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7	Healthcare use ^c	Monthly and within 72 hours of onset of exacerbation
8	mMRC ^d	Every 6 months
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10	CAT questionnaire ^e	Quarterly and within 72 hours of onset of exacerbation
11	EQ-5D index and VAS ^f	Quarterly and within 72 hours of onset of exacerbation
12		
13	NEADL ^g	Quarterly and within 72 hours of onset of exacerbation
14		
15	CNAQ ^h	Quarterly and within 72 hours of onset of exacerbation
16		
17	Biological specimen collection	
18	Blood sampling	Study entry
19	For routine biochemistry	
20	For cell-mediated immune response	Quarterly and within 72 hours of onset of exacerbation
21	For biomarkers, blood counts and haematology	Quarterly and within 72 hours of onset of exacerbation
22	For RNA transcript profiling	Every 6 months and within 72 hours of onset of exacerbation
23	For vitamins, anti-oxidants and nutrients (20 ml)	Every 6 months and within 72 hours of onset of exacerbation
24		
25		
26	Nasopharyngeal swab sampling ⁱ	Monthly and within 72 hours of onset of exacerbation
27		
28	Sputum sampling	Monthly and within 72 hours of onset of exacerbation
29	Breath sampling ^j	Monthly and within 72 hours of onset of exacerbation
30		
31	Urine sampling ^k	Monthly and within 72 hours of onset of exacerbation
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*In addition to study entry

^aHeight, weight, mid-arm circumference, waist circumference, triceps skin fold measurement, fat free body mass. ^bTLCO: transfer factor. ^cHealthcare 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) ^dmMRC: Medical Research Council Dyspnea Scale score. ^eCAT: COPD Assessment Test. ^fVAS: visual analogue scale. ^gNEADL: Nottingham Extended Daily Activities Scale. ^hCNAQ: Council on Nutrition Appetite Questionnaire. ⁱIn all subjects at study entry and in a subcohort of 30 subjects during the first year. ^jIn a subcohort of approximately 80 subjects. ^kIn 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, FEV₁, FVC, FEV₁/FVC ratio, FEV₁ % 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

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3 Nutritional information (including planned/ unplanned weight loss, and history and
4 changes in food intake patterns) is collected quarterly according to MUST guidelines.
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8 A postero-anterior chest X-ray (and lateral if required) is performed at all
9 exacerbation visits, as per standard clinical practice, in order to exclude pneumonia.
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13 *Questionnaires [and patient-reported outcome instruments](#)*
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15 Various outcomes are assessed quarterly and at exacerbation using a series of
16 questionnaires and patient-reported outcomes instruments such as the COPD
17 Assessment Test (CAT),³¹ the Nottingham Extended Daily Activities Scale
18 (NEADL),³² the Council on Nutrition Appetite Questionnaire (CNAQ),³³ and the EQ-
19 5D.³⁴ The five items included in the EQ-5D index are mobility, self-care, usual
20 activities, pain/discomfort and anxiety/depression. The BODE index (Body-Mass
21 Index, Degree of Airflow Obstruction, Level of Functional Dyspnea, Exercise
22 Capacity)³⁵ will also be calculated.
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34 Healthcare use is recorded at all visits, including medication, vaccination,
35 oxygen therapy, use of mechanical ventilation, pulmonary rehabilitation treatment,
36 surgical intervention, outpatient visits (including GP visits and telephone contacts to
37 COPD team), emergency room visits, hospitalisations, and productivity loss (time
38 missed from work or usual activities due to worsening of COPD symptoms). Potential
39 changes in disease management following an exacerbation (e.g. change in medication
40 use) are also recorded.
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49 *Biological specimen collection*
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51 A wide range of biological specimens are collected from study participants (**table 3**).
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53 Blood samples are collected from all patients at study entry, quarterly and at
54 exacerbation. Sputum samples are obtained by spontaneous expectoration or induced
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3 by stimulation according to standard methods from all patients at study entry, monthly
4 and at exacerbation. Nasopharyngeal swabs are collected from all patients at study
5 entry and then from a subcohort of 30 patients at monthly follow-up visits and at
6 exacerbation during the first year of follow-up. Urine samples are collected from all
7 patients at study entry and at exacerbation and from the subcohort of 30 patients at
8 monthly follow-up visits during the first year. Breath samples are collected from
9 approximately 80 patients (including the subcohort of 30 patients providing
10 nasopharyngeal swabs) at monthly follow-up visits and at exacerbation visits during
11 the first year.
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23 Blood samples are analysed for disease-related biomarkers, biochemistry, cell-
24 mediated immune response, RNA profile and nutrients. Sputum samples are
25 processed by traditional culture techniques and multiplex PCR analysis for
26 identification of potential respiratory pathogens (including, but not limited to, non-
27 typeable *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, *P. aeruginosa*, *Staphylococcus*
28 *aureus*, respiratory syncytial virus, parainfluenza virus, rhinovirus, human
29 metapneumovirus, influenza virus, adenovirus, and coronavirus). Sputum samples
30 may also be analysed for disease-related biomarkers. Nasopharyngeal swabs are
31 processed by traditional culture techniques and multiplex PCR analysis for potential
32 pathogen identification. Urine samples may be processed for disease-related
33 biomarkers. Breath samples are analysed by the selected ion flow tube mass
34 spectrometry for identification of volatile organic compounds that may be
35 characteristic for AECOPD.
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52 Laboratory assays are performed at the Public Health Laboratory of Public Health
53 England at University Hospital Southampton Foundation NHS Trust, GSK Vaccines
54 central laboratory, and other GSK Vaccines designated laboratories. The assays use
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3 standardised and validated procedures. Aliquots of all biological samples are
4 processed (if applicable), frozen and stored for possible further disease-related testing.
5
6 Culture isolates are also stored. Any additional laboratory tests will be performed at a
7
8 GSK designated laboratory.
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11 **Sample size calculation**

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13 The sample size calculation was based on the primary study endpoint of incidence of
14
15 all-cause AECOPD. Assuming that, on average, each subject is observed for a period
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17 of 18 months and that 2 episodes of AECOPD can be expected per subject per year, if
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19 120 subjects are followed, the number of total person-years would be around 180 and
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21 during this time around 360 exacerbation events would be detected. If the distribution
22
23 of events per subject follows a Poisson distribution with no overdispersion, an
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25 overdispersion factor of 1.5, or an overdispersion of 2, the approximate values of the
26
27 lower and upper bounds of the 95% confidence interval (CI) around the point estimate
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29 of 2 events per subject per year would be 1.8-2.2, 1.7-2.3, and 1.7-2.3, respectively.
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31 So a sample size of 120 subjects should ensure sufficient precision in the estimation
32
33 of the incidence rate of all-cause AECOPDs.
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40 Additionally, in order to follow effectively 120 subjects, given the fact that
41
42 subjects may be eligible but withdraw quite early in the study possibly due to the
43
44 deterioration of the subject's health, the decision was taken to replace subjects who
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46 withdrew during the first year of follow-up, and recruit additional subjects.
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49 [We will construct a CONSORT diagram and capture where possible reasons for](#)
50 [screen failure, drop-outs and loss to follow-up.](#)
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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.

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3 AERIS is being conducted in a specialised hospital that has extensive experience
4 in respiratory research. AECOPD are proactively identified through patient-completed
5 electronic diaries. After confirmation by phone, symptoms of an exacerbation trigger
6 a clinic visit within 72 hours of symptom onset to enable comparisons of samples
7 from same patient in stable COPD and during AECOPD. Although this is an intensive
8 study with prolonged follow up, patients are expected to benefit from the improved
9 access to expert care.
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19 The results obtained will be disseminated by presentations at international
20 medical conferences and peer-reviewed publications. [Reporting will be in accordance](#)
21 [with STROBE guidance.](#)
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26 **DISCUSSION**

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29 The AERIS study has been initiated to [comprehensively](#) assess the role of infectious
30 pathogens in AECOPD in a well-characterised cohort of patients. The study aims [at](#)
31 [exploring](#) the dynamics of airway infection and its possible contribution to
32 AECOPD, as well as the potential role of chronic colonization in stable disease. The
33 overall objective of the study will aim at refining the case definition of AECOPD to
34 reflect the possible microbiological aetiology of exacerbations. This is of note, since
35 there is currently no commonly agreed definition of AECOPD and no current case
36 definition includes a microbiological endpoint. The impact of AECOPD on health-
37 related quality-of-life and healthcare use will be assessed in order to provide a
38 complete picture of disease burden. The interaction between airway infection and
39 systemic manifestations of COPD and nutritional status will also be assessed [in detail](#)
40 [for the first time.](#) Biological specimens collected during the study may also be used
41 for further disease-related testing, including molecular typing to describe and compare
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3 selected biomarkers in AECOPD and stable COPD, to explore cell-mediated immune
4 response to specific bacterial antigens, and to develop non-invasive bacterial
5 diagnostic methods.
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10 To our knowledge, few other studies have employed real-time electronic tracking
11 of symptoms to identify AECOPD and potential aetiological triggers. This is
12 important since available data suggest that up to 50% of exacerbations may not be
13 reported to healthcare providers and consequently exacerbation rates are lower in
14 studies employing event-based criteria to define AECOPD.³⁶ Due to the close daily
15 monitoring of symptoms to identify AECOPD, we anticipate that the exacerbation
16 rate in this study will be higher than previously reported. This close monitoring and
17 early therapeutic intervention at exacerbation may also impact on estimates of the
18 overall burden of disease.
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30 A number of other epidemiological studies have been initiated in recent years to
31 further characterize our understanding of the natural history of AECOPD. However, it
32 is important to recognise that most of these studies have not included molecular
33 microbiological assessments. Recent large observational studies focusing on
34 biomarker discovery have involved close phenotyping of COPD patients, but have not
35 studied the aetiology of exacerbations in depth.³⁷⁻³⁹ In another study, potentially
36 pathogenic bacterial strains were identified using molecular typing techniques,
37 although viruses as potential airway pathogens were not investigated.²¹ More recently,
38 the prevalence and load of airway bacteria in stable and exacerbated AECOPD have
39 been assessed in paired samples from 52 patients participating in the London COPD
40 cohort study using modern molecular techniques.¹⁹ Airway bacterial prevalence and
41 load was found to increase significantly during AECOPD, with quantitative molecular
42 techniques proving more discriminatory than culture. However, assessment was
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3 limited to only the three most commonly detected airway bacteria (*H. influenzae*, *S.*
4 *pneumoniae*, and *M. catarrhalis*). However, other potential pathogens and the overall
5 respiratory microbiome may also contribute and have not yet been studied in
6 detail.^{17,18,20,40,41} In AERIS, samples will be acquired during both AECOPD and stable
7 disease and analysed for a wide range of potentially pathogenic bacteria and viruses
8 using advanced PCR-based techniques as well as traditional culture-based methods.
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17 A major strength of the AERIS study design is the comprehensive assessment of
18 clinical status, microbiology, functional status, nutritional status, health-related
19 quality-of-life and healthcare resource utilisation in individual patients in a single
20 large cohort during both stable COPD and AECOPD. The selection of subjects with a
21 history of at least a single exacerbation enriches the cohort to some degree and
22 ensures an adequate number of exacerbations are sampled. It is accepted that some
23 aspects of the analysis may not be generalizable to the subgroup of patients who never
24 exacerbate. The analyses proposed in this study will generate epidemiological data to
25 complement that derived from existing COPD cohorts and further explore
26 determinants of COPD and the contribution of bacterial and viral pathogens to
27 AECOPD, as well as to provide some understanding of the limitations of existing
28 data. As exacerbation visits are triggered by patient diary data, accurate and timely
29 diary completion is essential. All subjects participating in this study receive diary
30 training at enrolment and support is available from the study team at all times to
31 promote accurate and complete diary keeping. Cohort retention is a key factor in the
32 successful delivery of such a study and with in-depth sampling protocols, participant
33 engagement, comfort and feedback are key factors in optimising cohort retention and
34 comprehensive data collection.
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3 Identification of novel approaches for the prevention of AECOPD is an important
4 research goal. Long-acting beta-agonists (LABA) and long-acting anti-muscarinic
5 bronchodilators remain the cornerstone of treatment for patients with COPD.⁴²
6
7 Combinations of LABA and inhaled corticosteroids are also used in patients with
8 more severe disease and/or frequent exacerbations. Long-term treatment with
9 macrolide antibiotics and pulsed quinolone therapy may be considered for
10 exacerbation prevention.^{7,43,44} However, concerns exist about the potential for
11 development of antimicrobial resistance during long-term antibiotic therapy.
12
13 Numerous other approaches are under investigation for the prevention of AECOPD,
14 including anti-inflammatory drugs, immunomodulatory agents, immunotherapy,
15 antioxidants and non-pharmacologic strategies. Vaccination is another potential
16 approach meriting investigation for reducing AECOPD risk. However, optimal
17 strategies targeting key respiratory pathogens are not yet available to the clinician.
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32 In conclusion, there have been considerable advances in our understanding of the
33 epidemiology, pathophysiology and clinical management of COPD in recent years.
34 However, there remains a genuine need to further explore the aetiology and
35 pathogenesis of AECOPD. It is anticipated that results of this epidemiological study
36 will increase our understanding of the contribution of bacterial and viral pathogens to
37 AECOPD, the natural history of these events in association with the timing of
38 symptoms and physiological changes, and will offer new direction potentially leading
39 to for research into targeted therapeutic and preventative interventions.
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50 **Contributors S. Bourne:** principle investigator, closely involved in all steps of the
51 study and specifically wrote substantial parts of the protocol; **C. Cohet:**
52 **e**pidemiologist responsible for this observational study was closely involved in the
53 design of study; **V. Kim and A. Barton:** co-investigators and closely involved in the
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3 conduct of this study; **A. Tuck**: site project manager for microbiology testing and
4 involved in writing of microbiology parts of the protocol; **E. Aris**: project statistician,
5 responsible for writing of statistical analysis plan and definition of statistical
6 outcomes; **S. Mesia Vela**: project manager, closely involved in the discussion on
7 design and follow-up of the study; **J-M. Devaster**: ~~d~~Director of clinical research and
8 translational sScience, closely involved in all discussions and study accountable
9 person; **W.R. Ballou**: vice-president of clinical research and translational sScience
10 team, closely involved in all discussions of design of this study. **S. Clarke**: co-
11 investigator, closely involved in all steps of the study. **T. Wilkinson**: coordinating
12 lead investigator, involved in all steps of the study.
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25 All authors provided intellectual input into the development of this manuscript. And
26 have critically reviewed and approved the final manuscript.
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29

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32
33
34

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36 Wilkinson received an institutional grant from GlaxoSmithKline (GSK) group of
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39 lecturing, advisory boards and teaching from Novartis, Astra Zeneca and Boehringer
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41 attendance and advisory board payments from Pfizer, GSK and Novartis (all
42 payments were made to employing institution). T. Wilkinson has also received travel
43 expenses, fees for advisory boards GSK related to this study, fees for advisory boards
44 from Pfizer and AstraZeneca, reimbursement for travel and conference attendance
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3 from Boehringer Ingelheim, consultancy fees from Almirall, and financial support
4
5 from Novartis and Retroscreen (travel expenses, consultancy and project support
6
7 costs). C. Cohet, E. Aris, S. Mesia Vela, J-M. Devaster and W.R. Ballou are
8
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10
11 stock options.
12
13

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