NIHR | National Institute for Health and Care Research

Health Technology Assessment

Check for updates

Synopsis

Airway-microbiome-driven mechanisms of disease during optimised self-management: a lesson learned from mechanistic study of the Colour-COPD trial

Alice M Turner[1*] Daniella Spittle[2] Karl Staples[3] and David Cleary[4]

[1]Institute of Applied Health Research University of Birmingham, Birmingham, UK
[2]Institute of Inflammation and Aging, University of Birmingham, Birmingham, UK
[3]University of Southampton, Southampton, UK
[4]Institute of Microbiology and Infection, University of Birmingham, Birmingham, UK

*Corresponding author a.m.turner@bham.ac.uk

Published May 2025
DOI: 10.3310/SYTH8546

Abstract

Introduction: Reduced antibiotic consumption due to better self-management could change the profile of bacteria present in the airway, which might benefit the health of chronic obstructive pulmonary disease patients. To test this, we planned to use sputum samples already being collected from Colour-COPD trial patients for mechanistic work. The trial will test whether a sputum colour chart is non-inferior to usual self-management, and has a primary outcome of chronic obstructive pulmonary disease-specific hospital admission. Secondary outcomes include antibiotic consumption and quality of life. Since only half of exacerbations of chronic obstructive pulmonary disease (acute exacerbation of chronic obstructive pulmonary disease) are bacterial, and sputum colour has a good positive predictive value for bacterial presence, it is likely that our intervention will reduce antibiotic consumption. The main route by which our intervention could improve patient outcomes is that it could alter the airway microbiome, and subsequent pathological processes; this add-on study tried to assess that concept.

Methods: We used all sputum samples submitted by Colour-COPD trial patients and processed and stored them for microbiome and cytokine analyses. Sputum plugs were split with one-half being diluted in phosphate-buffered saline, dispersed using glass beads and stored for quantitative polymerase chain reaction/16S analysis. The second portion was dispersed using sequential phosphate-buffered saline and dithiothreitol treatment, generating supernatants and cytospins. Analysis of the microbial patterns, which would have been obtained from the respiratory microbiome, will be compared to antibiotic consumption for acute exacerbation of chronic obstructive pulmonary disease (days/year) steroid load (days/year and mg/year), acute exacerbation of chronic obstructive pulmonary disease rate, forced expiratory volume in 1 second and longitudinally within individuals to determine the impact of frequent courses of antibiotics at group and individual level. This work will now be completed outside the duration of this award. Selected inflammatory markers linked to neutrophilic and eosinophilic inflammation were planned to be measured, but this work was abandoned when the study was terminated early.

Results and study limitations: The trial was stopped prematurely due to low recruitment. This was due to a combination of insufficient trial sites, the impact of COVID-19 on research infrastructure and a reduced rate of acute exacerbation of chronic obstructive pulmonary disease during the COVID-19 pandemic, which affected eligibility in primary care sites, in particular. Since analysis of the microbiome was planned to occur only after trial results, this was abandoned within this award at the termination of the trial. However, since the research questions remained, could be answered in other ways, and patients had consented to the use of their samples for the proposed work, alternative ways to collect samples and fund microbiome analyses were sought. We are now at a point where we expect to have sufficient samples to have adequate power to answer two of our research questions by the time the trial ends, and will conduct their analysis thereafter.

This synopsis should be referenced as follows:
Turner AM, Spittle D, Staples K, Cleary D. Airway-microbiome-driven mechanisms of disease during optimised self-management: a lesson learned from mechanistic study of the Colour-COPD trial. *Health Technol Assess* 2025;**29**(28):45–58. https://doi.org/10.3310/SYTH8546

Conclusion: Although we are not able to address our objectives of describing the airway microbiome in a primary care chronic obstructive pulmonary disease population, and describing the relationship between antibiotic consumption and changes in airway microbiome during the term of the award, we were able to learn lessons about matching mechanistic work to trials.
Future work: We stored samples for a separately funded study to meet our objectives.
Funding: This synopsis presents independent research funded by the National Institute for Health and Care Research (NIHR) Efficacy and Mechanism Evaluation (EME) programme as award number NIHR130938.
A plain language summary of this synopsis is available on the NIHR Journals Library Website https://doi.org/10.3310/SYTH8546.

Introduction

This synopsis paper describes the rationale for add-on work to an ongoing trial (Colour-COPD trial), which compares different self-management (SM) strategies for patients with chronic obstructive pulmonary disease (COPD). The trial design was a pragmatic open-label blinded-end-point study, where patients were to be randomised predominantly from primary care. As the trial has terminated early, with much lower numbers of patients than expected, due primarily to slow recruitment postpandemic, limited conclusions can be drawn. The trial team also felt it was not worthwhile pursuing separate papers for the trial protocol and work packages independent of the synopsis; as such there are no threaded publications to link to at this point. We do, however, anticipate publications of results generated from the samples obtained in the study after submission of the synopsis, since alternative funding sources were obtained for processing of sputum samples, to enable us to answer the research questions independently of this award.

In this report, we also detail some lessons learnt for future trialists who wish to study the mechanism of action of an intervention allied to a NIHR Health Technology Assessment-funded trial. Challenges in overall trial delivery will be reported when the parent study reports in June 2024.

Rationale for mechanistic work pertaining to acute exacerbation of chronic obstructive pulmonary disease

What is the importance of acute exacerbation of chronic obstructive pulmonary disease?

COPD is a chronic condition affecting 2 million people in the UK,[1] causing over 140,000 hospital admissions and 1.7% of UK hospital bed-days[1] annually. Common day-to-day symptoms include breathlessness, which is typically worse on exertion, and cough productive of sputum. COPD is defined by airflow obstruction on spirometry, this being a ratio < 0.7 and lower than the lower limit of normal for age in the forced expiratory volume in 1 second (FEV1) and forced vital capacity after administration of a bronchodilator.[2] COPD severity is often determined by the degree of FEV1 impairment relative to a normal individual of the same age, sex and height, expressed as the % predicted for age.[2] However, it is also possible to define severity by symptoms and exacerbation frequency, as described by the Global Initiative for Obstructive Lung Disease (GOLD) outcomes strategy,[3] which are independent of spirometry and determined by the presence of symptoms and hospital admissions – thus GOLD C and D patients have ≥ 2 exacerbations/year or ≥ 1, which results in hospital admission, and are distinguished by their degree of breathlessness [Medical Research Council (MRC) score] or quality of life [COPD assessment test (CAT) score]. This grading system was proposed in part as it may allow greater personalisation of daily therapy[3] by taking exacerbations into account. National data suggests 46% of primary care COPD patients fall into GOLD C and D,[4] although some debate over this exists, as a more recent clinical practice research datalink study has shown that 29% of patients experience ≥ 2 exacerbations/year (albeit without GOLD grades known).[5] Irrespective of this debate, exacerbations are clearly an important problem.

Which mechanisms are relevant for the Colour-COPD trial?

A key effect of optimising acute exacerbation of chronic obstructive pulmonary disease (AECOPD) recognition and subtyping to bacterial or non-bacterial events, as with the Colour-COPD intervention (a standardised COPD SM plan + sputum colour chart in the intervention arm), will be reduced antibiotic ingestion. Previous work has demonstrated alterations in the lung microbiome (defined as the microorganisms present in respiratory secretions, comprising an ecological community of commensal, symbiotic and pathogenic organisms) in both beneficial and harmful ways after antibiotic usage, and relationships between microbial patterns and inflammation, which is linked to poor outcomes.[6] Whether the microbiota of the lung can be manipulated therapeutically to improve AECOPD rate and disease progression remains unknown,

but there is growing recognition of a need to facilitate the design of intervention studies that aim to conserve the lung microbial flora.[7] If Colour-COPD reduces antibiotic use as expected then we would expect it to meet this aim, but the parent trial does not fund assessment of the microbiome. However, irrespective of the outcome of the trial, the data being collected will allow us to assess the relationship between antibiotic use, microbiota and inflammation, which in turn could influence disease progression and risk of future AECOPD.

Antibiotic usage is known to affect the microbiome, both in the gut[8] and in the respiratory tract. In a study of medium-term (8 weeks) azithromycin use, the number of microorganisms seen on sequencing was no different, but the degree of within-host diversity reduced and anti-inflammatory changes were observed.[9] It appeared that benefits of treatment were mediated by altering the lung microbiome interaction with the host immune system, which highlights the relevance of understanding resident microbes as potential targets for immunomodulation. Although this study was small (n = 20 patients), results were supported by ex vivo cell-based assays, and a more recent systematic review of prophylactic antibiotics of various types in COPD [n = 12 randomised controlled trials (RCTs), n = 3683 patients in primary review][10] demonstrated that QoL improved, and supported the fact that bacterial numbers do not change, albeit with only one of three studies looking at bacterial load assessing this via 16S quantitative polymerase chain reaction (qPCR).[11] Other techniques, such as quantitative culture (measures bacteria in colony forming units/ml), are able to identify far fewer microorganisms than 16S qPCR, hence two/three of the included studies were not using the most sensitive technique. In addition, the *balance* of flora was not assessed in this review and the available data did not concur regarding inflammation, which was static in two studies that reported it, and may be very relevant. In animal models of microbiome depletion secondary to antibiotic use, alterations in downstream inflammation are seen, and animals with high levels of *selected* genera (generally after depletion) were likely to exhibit progression of inflammatory lung disease.[12] However, the degree of inflammation in such animal models was far in excess of that seen in COPD, and it is unclear whether such effects would be recapitulated in COPD. Thus the evidence is inconsistent on whether antibiotic usage is beneficial (e.g. long-term macrolides) or harmful (e.g. depletion regimes) to the lung microbiome. Further work is therefore required, and in particular this should focus on real-life regimes so that we can understand the effects likely to be present in patients in UK practice. Colour-COPD enrols sufficient patients that we could do subgroup analyses assessing long-term macrolides, and will give information on repeated short courses of antibiotics as well, which effectively represent the potentially beneficial and harmful types of antibiotic use with respect to the microbiome and downstream inflammation.

Haemophilus sp. colonisation as identified by 16S ribonucleic acid (rRNA) sequencing has also been reported to lead to more frequent exacerbation.[13] Previous research has shown that *Haemophilus*, and the immune response to it, is particularly important in frequent exacerbators,[13–15] and hence it should be sought specifically in our trial, which focuses on this group. Colonisation might decrease efficacy of the intervention in Colour-COPD if widely present, due to baseline alterations in sputum colour – treatment targeting bacterial exacerbations could end up being given to a person whose sputum may appear green due to colonisation instead of active infection. Whether routine NHS culture is capable of picking up all colonising organisms is unclear, and the study may help in this regard because of the number of samples, and parallel use of the current NHS standard alongside sequencing. Similarly, it is possible that microbiome data could explain hitherto inexplicable AECOPD events, where routine NHS processes do not pick up a precipitating organism, but it is present. If this is the case then our intervention may not have the expected effect, and patients might require antibiotics more often than sputum colour changes suggest.

The relationship between the respiratory bacterial microbiome, antibiotics and viruses may be bidirectional: the pattern of dominant organism present may influence the response to treatment with antibiotics,[16] as well as being influenced by treatment. Equally, it may influence susceptibility to viral infection,[14,17] and viral infection may then alter susceptibility to subsequent bacterial overgrowth. Viruses are an important driver of seasonal variation in AECOPD rate, and coinfection with both bacteria and viruses is a strong independent risk factor for frequent AECOPD,[14] as well as prolonged hospital stay.[18] Furthermore, some viruses (e.g. adenovirus) stimulate greater pro-inflammatory effects in airway disease, such as COPD, than in healthy people,[19] and immunological effects, such as the response to influenza vaccine,[20] are also enhanced in COPD. Understanding the effect of changes in the viral population in the airway, and markers of downstream inflammation will therefore also be important during Colour-COPD, if antibiotic prescription rates change as expected. The key inflammatory mechanisms for study are the innate immune cytokines, such as IL-1β, which

we have shown to be elevated in bacterially colonised COPD patients.[15] With regard to the interaction with viral infection, the antiviral type I interferon (IFN) pathways are also a target of study as previous data suggest a defect in the production of IFN-β in response to rhinoviral infection in COPD patients that may be predisposed to viral infections.[21] In addition, there are data to support a rhinovirus infection-induced degradation of antimicrobial peptides, such as elafin and secretory leukocyte protease inhibitor (SLPI) that may underlie the relationship between viral and bacterial infection of the airway.[21]

Aims and objectives

Our main aim was to answer the research question

Do alterations in the airway microbiome and downstream inflammation occur if patients optimise SM of exacerbations of COPD?

Our objectives were to

1. Describe the airway microbiome in a primary care COPD population.

We planned to test the hypothesis that the microbiological drivers of disease in primary care do not differ from a secondary care population by comparison to samples from our prior work[14] using samples taken at baseline.

2. Describe the relationship between antibiotic consumption and changes in airway microbiome.

This would have tested the hypothesis that reducing antibiotic consumption leads to changes in the airway microbiome at 12 months, specifically preserved bacterial diversity. We also planned to explore the impact of chronic antibiotic use, and of specific drug classes used acutely or chronically (e.g. macrolides).

3. Explore the relationship between microbiome profile, inflammation and prognosis.

Key prognostic factors in COPD include FEV1 decline and AECOPD rate. This will test the hypothesis that the degree of bacterial diversity present determines the degree of downstream neutrophilic inflammation, which in turn determines an individual's future exacerbation risk, as well as risk of decline. This was planned using samples from baseline and 12 months. We would have examined specifically for the effect of *Haemophilus* sp. on these features.

Deliverables from this project included

1. A large biobank of sputum, and of 16S rRNA sequencing data.
2. A greater understanding of the common bacteria present in UK primary care, whether these differ from secondary care COPD patients, and the impact of repeated antibiotic courses for AECOPD.
3. Understanding of the value of the microbiome over standard culture for prediction of prognosis and other relevant longitudinal features, such as AECOPD rate.

Progress towards these objectives and deliverables is reported in the results section.

Methods

Research design: lessons learnt

This study planned to examine samples from a RCT, but it can be seen both as a test of the mechanism by which enhanced SM could influence pathogenesis in the lung, and a cohort study exploring how our innate microbial profile relates to important clinical aspects of COPD, such as QoL and disease progression. Unfortunately, allying the work to a trial, which then struggled to recruit, meant that the work could no longer be funded by a grant stream, which required the trial to continue.

Some of our objectives could be achieved irrespective of the results of the trial, namely objectives 1 and 3 – these involve use of baseline microbiome samples alongside clinical data. To assess objective 2 and limit costs to the NIHR we proposed a stop/go point where the analyses for Colour-COPD are performed; if antibiotic use *or* QoL *or* AECOPD rate differed between arms then we would proceed to mechanistic work on follow-up samples. When the study terminated early such that none of these points were met then the remaining money allocated to microbiome sequencing and bioinformatics was returned to the NIHR, and other research strategies and funding sources considered. Since a trial was not the only way to collect samples for our research questions, we are now pursuing our objectives using a cohort instead, which is in part industry funded, partly locally funded. While we will not achieve our original objectives in quite the same way as a result, we learnt that the Efficacy and Mechanism Evaluation (EME) programme as a funder is able to accept cohort studies for mechanistic work separate from a trial, and now recognise that submission without linking to a trial would have been a better choice. We would encourage future investigators to consider the full range

of study designs that could address their questions and consider cohorts where this may be more cost-effective and lower risk in comparison to a trial.

Sample size considerations

Based on targeted trial recruitment, if 70% of our sputum producers complied we would have had 2481 sputum samples, half in each of the intervention and control groups, assuming that 60% of patients[22] remain frequent exacerbators (≥ 2/year) and the remainder have one or zero events. From these we estimated that 50% of samples would be of sufficient size to progress to the additional mechanistic work proposed herein (n ~ 1240 samples, with two samples per patient at 0 and 12 months).

Determining accurate power and sample size calculations for microbiome studies is an unresolved problem. In addition, there is limited data for effect sizes, in particular those based on compositional changes, hence these are difficult to model. Nevertheless, we used https://fedematt.shinyapps.io/shinyMB to model sample size calculations using available data on microbiome composition at COPD exacerbation with the limited data available on the impact of antibiotics in COPD airways. This application provides simulated power calculations, which make use of the Dirichlet–multinomial model for OTU abundances implementing both Wald and Wilcoxon–Mann–Whitney tests. The abundance distribution model we used is based on saliva microbiome data from the Human Microbiome Project. We considered a model that examines the 50 most abundant OTUs and determined power for a range of sample sizes.

Assumptions for effect size of antibiotic therapy on microbiome (objective 2) are as follows:

A. Based on previous data we assume a reduction of *Haemophilus* OTUs in response to antibiotic therapy of ~27% (−6.8-fold).
B. At the time of exacerbation we assume that *Haemophilus* is the most abundant genus.
C. We also assume a 10% reduction in the next four most abundant genera, which includes both *Streptococcus* and *Moraxella*.

One thousand Monte Carlo replications (simulated datasets) were done, with an alpha of 0.05. The results can be submitted as figures if desired. This shows that at a sample size of 200, power surpasses 80%.

Assumptions for the effect size of the microbiome on exacerbation risk (part of objective 3):

A. We assume a reduction of *Veillonella*, being the most abundant OTU, of 25%.
B. We assume an increase of the next four most abundant OTUs of ~10% – this represents a conservative estimate based on the increase of *Moraxella*, the fifth most abundant OTU in our previous research, from ~0% to 10% to 14.3% and *Haemophilus* increases of ~12%.

Again, 1000 Monte Carlo replications (simulated datasets) were done, with an alpha of 0.05, with a sample size of 250, power is 90.8% (Wald), and approaches 80% with n = 200.

As such, we are now aiming for a sample size of 200 obtained from Colour-COPD and the other cohorts.

Sample receipt and processing

Processes being followed in Colour-COPD will be described in the trial report in due course (report due June 2024) in which the Consolidated Standards of Reporting Trials' diagram will be included. Most patients submitted samples to the central laboratory by post, while Birmingham samples were received fresh. This allowed us to plan work to assess whether the microbiome can be adequately assessed on posted samples. The additional sputum processing required for the mechanistic work involved (1) assessing sample quality visually, as well as looking at the time taken for transit to the central laboratory. This impacted upon whether samples can be stored for subsequent work or not, (2) selection of sputum plugs to reduce the possibility of oral contamination in downstream analysis, (3) splitting of samples, with one-half being dispersed using glass beads and stored for qPCR/16S analysis of both viruses and bacteria. The second portion was dispersed using sequential PBS and DTT treatment generating supernatants for analysis of inflammatory markers. Where the sample was small (< 0.2 g), it was prioritised for qPCR/16S analysis.

Analysis of samples to infer if postage is appropriate for microbiome studies

There are no data on whether posting affects the sputum microbiome, and only limited work on other sample types. In large studies of the faecal microbiome differences were seen after posting dry swabs; a consequence of growth 'blooms' of some bacterial taxa.[23] However, the fraction of the sequencing reads belonging to each sample that were identified as belonging to a bloom was low (~7%)[23] and given that faecal samples are inherently more nutrient rich compared to sputum, this is likely to be lower in the latter case. In order to explore whether changes could occur during the pilot phase of the parent trial we split samples

given face to face to the study team at baseline, with one-half being processed immediately (i.e. usual practice) and the other half being left at ambient temperature in the laboratory for processing after 24 hours, this being equivalent to a postal route. At the end of the pilot phase, all samples processed in this way were planned to be compared to one another using methods previously developed to address this issue;[24] importantly this could provide methodological data for future trials that focus on the sputum microbiome. We collected sufficient samples for this work and will report results after batch processing with the samples from the parallel (non-EME funded) cohort.

Microbiome analyses

This work is no longer being funded by this award, but we plan to complete it for samples obtained from the trial and from the parallel (separately funded) cohort to answer our research questions, such that methods are provided for completeness. The V4 hypervariable region of the 16S rRNA gene will be amplified using barcoded primers 515F and 806R[25] from samples in triplicate. Additional samples will include sampling, deoxyribonucleic acid (DNA) extraction, and PCR amplification controls, which will also be processed in order to identify and remove potential contaminating sequences. Triplicate PCR products will be pooled and sequenced using a HiSeq 2500. Analysis will be conducted in R v3.60 (The R Foundation for Statistical Computing, Vienna, Austria).[11] Sequence reads will be filtered (chimera removal), trimmed to remove low-quality positions and amplicon sequence variants (ASVs) inferred using dada2.[26] Taxonomic classifications will be made using the RDP Naive Bayesian Classifier and the Greengenes database v13_8.[27] Alpha diversity (using measures of richness and evenness) and clustering analysis with both Unifrac and Bray–Curtis will be done within phyloseq.[28] Using principle co-ordinates analysis and permutational multivariate analysis of variance these distance matrices will be used to describe both phylogenetic and compositional differences between groups. This analysis is naïve to trial arm and metadata variables and therefore will be initially applied to the complete cohort. The subsequent overlaying of metadata variables, including arm of study, allows for specific comparisons to be made (e.g. use and duration of antibiotics). Similarly, between-group comparisons using all available metadata will identify whether group differences are explained by confounding variables, which can then be accounted for. The identification of bacterial taxa that are responsible for any compositional differences observed will be done using a combination of approaches, including differential expression analysis for sequence count data (DESeq2),[29] linear discriminant analysis effect size (LEfSe)[30] and analysis and composition of microbiomes.[31]

Inflammation analyses

Innate immune cytokines such as IL-1β, TNF, IL-6 and IFNβ were to be measured in the sputum PBS supernatants using the MesoScale Discovery multiplex ELISA system. This work was abandoned when the study terminated early and is not yet funded elsewhere.

Study population

The patient population were those in the sputum substudy of Colour-COPD, drawn from a mixture of UK primary and secondary care, being defined by a clinical diagnosis of COPD, and two or more AECOPD (or one hospital admission for AECOPD) in the preceding 12 months. The study was approved by the South Yorkshire Research Ethics Committee (20/YH/0273), and all patients gave informed consent. The study expanded to recruit from secondary care when recruitment proved difficult in primary care alone; greater numbers of secondary care patients chose to participate in the sputum substudy; nevertheless we will be able to make comparisons between primary and secondary care patients, according to our objectives. The additional cohort studies from which relevant COPD samples are now being obtained are funded by Vertex Pharmaceuticals and the Alpha 1 Foundation, among others. These studies are separately ethically approved and funded, and all patients have given informed consent. Inclusion criteria are similar to Colour-COPD, but not identical – for example, all frequencies of AECOPD are allowed.

Statistical analysis

No interim analyses are planned, and all analyses will use both 0- and 12-month stable state samples unless otherwise stated.

Describe the impact of postage/ room temperature storage on airway-microbiome samples

Specific comparisons will be done on paired samples to examine compositional changes in microbiome profiles arising from room-temperature growth blooms. Here we will follow methods previously developed for large, population-based microbiome studies to identify and remove specific ASVs from samples prior to further analysis.[24]

Describe the airway microbiome: using baseline samples from all patients (Colour-COPD trial and cohort patients) patterns of microbiota will initially be identified through comparisons of transformed abundance at both the phylum and genus taxonomic levels. Significant differences in alpha diversity using both species richness (observed and Shannon Index) and evenness (Simpson's diversity

and Peilou evenness) estimators will be determined using either Mann–Whitney U or Kruskal–Wallis H tests depending on the number of groups being compared. Beta diversity (between-group comparisons of composition) will be calculated using presence/absence measures, which leverage phylogenetic information (Unifrac) and those that also include counts (Bray–Curtis). Significant differences in these distance matrices will be computed using PERMANOVA. These measures will also be applied to comparisons based on other metadata and will enable the identification of potentially confounding variables among subgroups, for example, those based on smoking status, inhaled corticosteroid etc. Comparison between primary and secondary care patients will be conducted.

Describe the relationship between antibiotic consumption and changes in airway microbiome

A key secondary outcome of Colour-COPD is the number of antibiotic courses, and the number of treatment failure episodes, which require prolonged antibiotic use. These quantitative outcomes will be compared to patterns of microbiota, and to follow-up stable state 12-month sample by modelling the transition probabilities between states (i.e. stable vs. treatment failure) using Markov chains.[13] We will also describe differences by tertiles in terms of antibiotic course amount (high → low), and days of antibiotic use, specifically comparing the top tertile of high antibiotic users to the lowest using group. Two specific subgroup analyses will be done: patients taking prophylactic macrolides versus. not, and those on inhaled steroids versus not. The cohort studies from which additional samples are now being obtained also collect data on AECOPD rate and days of antibiotic use, as well as the named concomitant medications, hence analysis is unaffected by the new recruitment methods.

Describe the relationship between QoL and changes in airway microbiome

A key secondary outcome of Colour-COPD is QoL; for the purpose of this analysis, we will use CAT score. This integer-based outcome will be compared in the context of changes from baseline to follow-up samples by using a generalised linear mixed model that incorporates phylogenetic information (glmmTree[32]) to determine if microbiome composition is predictive of CAT score. Once more, our cohorts collect CAT, so the analysis is unaffected.

Explore the relationship between microbiome profile, inflammation and exacerbation risk

Machine-learning methods, such as neural-network models, will be used to examine the interaction of variables. As these data are longitudinal, and therefore include dynamic microbial abundances, dynamic Bayesian networks (DBN) will also be used. Topological data analysis (TDA) will permit the integration of this multidimensional data to build a visual network.[33] This analysis will be more limited with the lower sample numbers, nevertheless we will assess if it is possible upon completion of Colour-COPD follow-up.

Determine whether microbial profile has an influence on progression of COPD

Key classes of microbiota, in particular *Haemophilus* sp., will be compared with respect to decline in FEV1, CAT and MRC score. Where serial values are available from the routine care record progression will be determined by slope analysis (as used in our previous work[34,35]) across those who have ≥ 3 measures, and between those who progress, are stable, or improve from the first to last measure gained in the trial and routine care data from the cohorts. We will define colonisation by any positive culture for a PPM in the stable state (NHS standard) and by the lower limit of detection for microbiome analyses, which was 2000 copies/ml for NTHI in the AERIS study (recognising that this threshold requires clinical validation such as that in the proposed analyses to validate it as an acceptable definition of colonisation). We will compare colonisation as defined by standard NHS culture to that defined by microbiome analysis with respect to average progression values, using standard statistical techniques for comparison of means (e.g. Mann–Whitney), and regression where necessary to adjust for potential confounders.

Determine whether good SM of AECOPD has an effect on the microbiome

We had planned to compare well-delivered versus poorly delivered SM training (defined in Colour-COPD as ≥ 70% of the key items involved in giving a plan done when seeing the patient, of which we have 10 for colour chart + SM, and 7 for SM alone) to microbiome pattern at 12 months, dividing into those categories or patterns that have beneficial outcomes, such as improved QoL or reduced progression of disease. In the patients in whom we have e-diary data we were going to divide patients into those who managed their symptom-defined AECOPD promptly within 24 hours, with appropriate treatment (steroids alone for non-purulent AECOPD, antibiotics ± steroids in purulent AECOPD) to those who did not, and align this in a similar way to microbiome patterns. However, the low number of participants means this analysis will no longer be possible as these data are not collected in the cohorts from which recruitment is now coming.

Results

Recruitment from Colour-COPD trial

One hundred and twenty-eight patients were screened for the trial, of whom 115 were randomised. Twenty-six primary care sites and two secondary care sites were opened to achieve this level of recruitment, with 50 patients being recruited by the secondary care sites. Average recruits/practice in primary care was therefore two to three, though there were some sites who recruited no patients; reasons for this will be covered in the main trial termination report. During follow-up to date, two have died and eight withdrew for various reasons. This level of loss to follow-up was within the range expected at the outset. Among the remaining patients, 42 consented to the sputum substudy and at least one sample has been received from 38/42 people. Characteristics of the included patients are shown in *Table 1*; notably the prevalence of bronchiectasis is higher than in a typical primary care population due to the necessity of expanding recruitment into secondary care when delivery in primary care proved problematic. There was also a high number of non-smokers. Further results from the trial will be available in the trial synopsis in 2024.

Of the 51 samples received to date, 23 were of sufficient size and quality to be stored for microbiome analysis.

TABLE 1 Patient characteristics

Age (years), mean (SD, range)	63.4 (9.3, 42–77)
Sex, %	
Male	55
Female	45
Ethnicity, %	
White British	91
Mixed – White and Black Caribbean	4.5
Asian and Asian British – Indian	4.5
Smoking status, %	
Never	27
Ex	59
Current	14
No. of exacerbations in past year, median [IQR]	4 [2.75–5]
Bronchiectasis, %	
Yes	27
No	73

In order to ensure that all samples from the eventual combined study (Colour-COPD patients + cohort patients) are processed together, and that the maximum amount of funds could be returned to EME at the time of closedown, no microbiome processing has occurred to date. This is anticipated at the end of the Colour-COPD study in March 2024, when we should have reached $n = 200$ samples overall based on sample receipt levels in the trial and recruitment rates to the cohorts at present. The merged cohort formed, and all analysis of samples within it, is funded by other sources and thus not reported here.

Discussion

Since the parent trial terminated earlier than planned, and none of the original analyses of sputum are now funded within the grant, there are limited points for discussion. However, there are some lessons that can be learnt regarding the practicality of future mechanistic studies.

Achievement of objectives

Early termination of the parent trial means that we will not be able to answer our primary research question, and there are few conclusions that can be drawn against our original objectives at this stage, however we will eventually be able to answer objectives 1 and 2 using the methods described. This will be achieved by parallel and extended recruitment for sputum and data collection from COPD patients by separately funded studies. We will also achieve all three deliverables, albeit with a smaller biobank than expected. Shared funding of the complete dataset means that governance is more complex, involving multiple funders and two sponsors. Nevertheless, we will be able to grant access to data collected in the project to future researchers once it is complete, in a deidentified form, after proper request.

Lessons learnt for future researchers

The parent study was set up at the start of the pandemic and it proved very difficult to open primary care sites, and for them to recruit once opened, this was mainly due to the effects of the pandemic on research infrastructure and capacity in primary care. Opening sites often took much longer than normal, presumably due to managerial staff being diverted onto restructuring primary care multiple times to comply with pandemic-related restrictions. While we had enabled remote delivery of the intervention (so that it could continue during the pandemic, and reassure patients they were safe), practices largely proved unable to do this, perhaps because staff were prioritised to important public health-related tasks, such as vaccine delivery. Further detail on these issues will be included

in the main trial closedown report. Despite epidemiology suggesting high numbers of eligible patients prior to the pandemic, eligible numbers fell in the immediate period of opening up as the initial waves of the pandemic subsided, since social restrictions had led to less exposure to infection, and consequently fewer AECOPD; this probably also influenced the slow recruitment rate. In our original application, we had planned to open secondary care sites where frequent exacerbators might constitute a higher proportion of the overall COPD population if recruitment was low, hence some secondary care sites were added. However, we were only able to open two hospitals before a decision on closedown had to be made. At the time there were extremely long timelines for R&D to open non-COVID studies, due to the public health priority of COVID studies, and many sites felt unable to take the study on due to lack of research capacity with COVID studies. Recruitment rose rapidly in secondary care by the end of 2022 into early 2023, but by then it was too late in our pilot period to make a meaningful difference to the outcome – namely closedown. Again, this will be covered further in our main trial report.

The number of sputum producers willing to ship samples to a central laboratory was marginally higher than anticipated, possibly because much of the recruitment came from secondary care patients in the end, where chronic sputum production and frequent AECOPD are more common. Postal receipt was generally possible within 48 hours, which was sufficient to obtain good-quality samples suitable for culture, and where initial extraction appeared to yield good-quality DNA for subsequent microbiome work. The positive experience we had of this part of the study has led us to replicate it within a subsequent trial protocol in COPD (STABILISE study, under set up).

Conclusion

Since this study has closed earlier than planned with the NIHR, but funding has been obtained from other sources to continue sample and data collection it is not possible to draw conclusions yet. We believe the samples obtained will secure generalisable results for a UK COPD population, on the basis of the patient characteristics and the pragmatic nature of recruitment methods. The results may enable broader understanding of the limitations of current sputum culture techniques with respect to overall bacterial drivers of disease in COPD, by identifying the pattern of organisms present within the microbiome. They could also help understand the degree to which bacteria influence and promote harmful pulmonary inflammation, and thus the decline of lung function. Revealing this could enable future studies to optimise patient selection for prophylactic antibiotics, or other anti-inflammatory agents, and could inform public health initiatives to reduce the development of antibiotic resistance in vulnerable COPD patients. Our main conclusion is that the objectives of the study as planned remain important to achieve, hence why we pursued alternative strategies to completion, independent of NIHR EME.

Patient and public involvement

Patients were involved prior to the parent study, where they were advised about postage of sputum, and sputum collection, at inception, where they advised on patient-facing materials, such as the patient information sheet. During the study, we produced some materials to improve the quality of submitted samples, in the form of a video and an information sheet, which patients codeveloped with us. This contact was mainly through a respiratory patient advisory group (PAG) whom we contact roughly quarterly in Birmingham, to update them on the range of respiratory studies run and led locally, and inform them of progress. This has been achieved mainly through Zoom meetings (Zoom Video Communications, San Jose, CA, USA) and a digital newsletter, because this was patients' preference, avoiding travel and potential exposure to viruses; many respiratory patients still have some fear of COVID-19 (and other common respiratory infections) such that avoiding hospital contact and large groups is their wish. Patient involvement was also embedded in the governance processes in numerous ways. Firstly, our patient co-applicant was invited to trial management meetings, and kept informed via their minutes. Unfortunately, during the study, his health deteriorated and he died during 2022. We also have patient representation on our trial steering group, and they have been helpful in guiding our plans for study closedown, and how to retain impact and relevance to patients despite early termination. For example, they suggested that we engage with charities such as Asthma + Lung UK to highlight the issues that have particularly affected closure of respiratory studies during the pandemic. Finally, the parent trial involves coanalysis by patients of the qualitative work assessing acceptability of the intervention, and its practical deployment.

We found it challenging to retain patient enthusiasm from the PAG in particular, who found it disheartening to hear about studies they had advised on discontinuing; the need for repeated engagement as we attempted amendments to the study proposal to try and retain funding led to

This synopsis should be referenced as follows:
Turner AM, Spittle D, Staples K, Cleary D. Airway-microbiome-driven mechanisms of disease during optimised self-management: a lesson learned from mechanistic study of the Colour-COPD trial. *Health Technol Assess* 2025;**29**(28):45–58. https://doi.org/10.3310/SYTH8546

some participants feeling it was all a bit too much and dropping out. We are widening our participation group to avoid such challenges in the future, recognising that a larger group may help retain involvement, even if it is not always the same individuals. In parallel, we are working with our departmental PPI leads to learn lessons from management of groups allied to NIHR infrastructure, such as the ARC, to help us maintain the PAG long-term, even if there are individual study setbacks. While people were initially keen to be involved in coanalysis, enthusiasm for this also waned during the study, and it is not yet clear from the parent trial whether this will be meaningfully achieved.

Equality, diversity and inclusion

Our PPI group, and the patients enrolled to the study were predominantly but not exclusively white British and of a middle-older age group. Women were slightly over-represented in our PPI groups. We worked with local NHS trusts to access a diverse range of patients through clinical services, and the recruitment to the trial probably represents the typical demographic of a COPD patient in England.

Additional information

CRediT contribution statement

Alice M Turner (https://orcid.org/0000-0002-5947-3254): Conceptualisation (lead), Funding acquisition (lead), Project administration (lead), Supervision (lead), Writing – original draft (lead).

Daniella Spittle (https://orcid.org/0000-0002-4054-0314): Data curation (lead), Investigation (lead), Formal analysis (lead), Writing – reviewing and editing (equal).

Karl Staples (https://orcid.org/0000-0003-3844-6457): Conceptualisation (equal), Funding acquisition (equal), Methodology (lead), Writing – reviewing and editing (equal).

David Cleary (https://orcid.org/0000-0003-4533-0700): Funding acquisition (equal), Methodology (equal), Writing – reviewing and editing (equal).

Disclosure of interests

Full disclosure of interests: Completed ICMJE forms for all authors, including all related interests, are available in the toolkit on the NIHR Journals Library report publication page https://doi.org/10.3310/SYTH8546.

Primary conflicts of interest: Alice M Turner is funded by the NIHR Patient Safety Research Collaboration (Midlands PSRC), NIHR HTA and EME programmes. She has also been funded by NIHR i4i during the term of the reported study. She reports institutional funding from Vertex Pharmaceuticals, Takeda, Grifols Biotherapeutics, Chiesi and CSL Behring for research projects outside this study.

Daniella Spittle is funded by the NIHR Efficacy and Mechanism Evaluation programme and Vertex Pharmaceuticals.

Karl Staples is funded by the NIHR EME programme for this work and by EpiEndo Pharmaceuticals for research outside this study.

David Cleary is funded by the NIHR EME programme for this work.

Data-sharing statement

All data requests should be submitted to the corresponding author for consideration. Access to anonymised data may be granted following review.

Ethics statement

The study was approved by the South Yorkshire Research Ethics Committee (20/YH/0273), and all patients gave informed consent.

Information governance statement

The University of Birmingham is committed to handling all personal information in line with the UK Data Protection Act (2018) and the General Data Protection Regulation (EU GDPR) 2016/679. Under the Data Protection legislation, the University of Birmingham is the Data Controller, and you can find out more about how we handle personal data, including how to exercise your individual rights and the contact details for our Data Protection Officer here: www.birmingham.ac.uk/university/leadership/governance/policies-regs/data-protection.

Department of Health and Social Care disclaimer

This publication presents independent research commissioned by the National Institute for Health and Care Research (NIHR). The views and opinions expressed by authors in this publication are those of the authors and do not necessarily reflect those of the NHS, the NIHR, MRC, NIHR Coordinating Centre, the EME programme or the Department of Health and Social Care.

This synopsis was published based on current knowledge at the time and date of publication. NIHR is committed to being inclusive and will continually monitor best practice and guidance in relation to terminology and language to ensure that we remain relevant to our stakeholders.

Trial registration

This trial is registered as ISRCTN14955629.

Funding

This synopsis presents independent research funded by the National Institute for Health and Care Research (NIHR) Efficacy and Mechanism Evaluation (EME) programme as award number NIHR130938.

This synopsis provided an overview of the research award Airway microbiome driven mechanisms of disease after optimised self management: Colour-COPD trial. For more information about this research please view the award page (https://www.fundingawards.nihr.ac.uk/award/NIHR130938).

About this synopsis

The contractual start date for this research was in January 2021. This article began editorial review in November 2023 and was accepted for publication in August 2024. The authors have been wholly responsible for all data collection, analysis and interpretation, and for writing up their work. The EME editors and publisher have tried to ensure the accuracy of the authors' article and would like to thank the reviewers for their constructive comments on the draft document. However, they do not accept liability for damages or losses arising from material published in this article.

List of abbreviations

AECOPD	acute exacerbation of COPD
CAT	COPD assessment test
COPD	chronic obstructive pulmonary disease
DNA	deoxyribonucleic acid
EME	Efficacy Mechanism and Evaluation
FEV1	forced expiratory volume in 1 second
GOLD	Global Initiative for Obstructive Lung Disease
IFN	interferon
MRC	Medical Research Council
NIHR	National Institute for Health and Care Research
PAG	patient advisory group
QOL	quality of life
QPCR	quantitative polymerase chain reaction
RCT	randomised controlled trial
RRNA	ribonucleic acid
SM	self-management

References

1. British Lung Foundation. *The Battle for Breath – the Impact of Lung Disease in the UK*. London: British Lung Foundation; 2016.
2. National Institute for Health and Care Excellence. *Chronic Obstructive Pulmonary Disease*: *NICE Guideline*. London: National Institute for Health and Care Excellence; 2018.
3. Global Initiative for Obstructive Lung Disease. URL: https://goldcopd.org/2024-gold-report/
4. Merinopoulou E, Raluy-Callado M, Ramagopalan S, MacLachlan S, Khalid JM. COPD exacerbations by disease severity in England. *Int J Chron Obstruct Pulmon Dis* 2016;**11**:697–709.
5. Pilsczek FH, Salina D, Poon KK, Fahey C, Yipp BG, Sibley CD, *et al*. A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to *Staphylococcus aureus*. *J Immunol* 2010;**185**:7413–25.
6. Agusti A, Edwards LD, Rennard SI, Macnee W, Tal-Singer R, Miller BE, *et al*.; Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) Investigators. Persistent systemic inflammation is associated with poor clinical outcomes in COPD: a novel phenotype. *PLOS ONE* 2012;**7**:e37483.
7. Toraldo DM, Conte L. Influence of the lung microbiota dysbiosis in chronic obstructive pulmonary disease exacerbations: the controversial use of corticosteroid and antibiotic treatments and the role of eosinophils as a disease marker. *J Clin Med Res* 2019;**11**:667–75.

8. Kim S, Covington A, Pamer EG. The intestinal microbiota: antibiotics, colonization resistance, and enteric pathogens. *Immunol Rev* 2017;**279**:90–105.
9. Segal LN, Clemente JC, Wu BG, Wikoff WR, Gao Z, Li Y, *et al*. Randomised, double-blind, placebo-controlled trial with azithromycin selects for anti-inflammatory microbial metabolites in the emphysematous lung. *Thorax* 2017;**72**:13–22.
10. Wang Y, Zijp TR, Bahar MA, Kocks JWH, Wilffert B, Hak E. Effects of prophylactic antibiotics on patients with stable COPD: a systematic review and meta-analysis of randomized controlled trials. *J Antimicrob Chemother* 2018;**73**:3231–43.
11. Brill SE, Law M, El-Emir E, Allinson JP, James P, Maddox V, *et al*. Effects of different antibiotic classes on airway bacteria in stable COPD using culture and molecular techniques: a randomised controlled trial. *Thorax* 2015;**70**:930–8.
12. Yadava K, Pattaroni C, Sichelstiel AK, Trompette A, Gollwitzer ES, Salami O, *et al*. Microbiota promotes chronic pulmonary inflammation by enhancing IL-17A and autoantibodies. *Am J Respir Crit Care Med* 2016;**193**:975–87.
13. Mayhew D, Devos N, Lambert C, Brown JR, Clarke SC, Kim VL, *et al*.; AERIS Study Group. Longitudinal profiling of the lung microbiome in the AERIS study demonstrates repeatability of bacterial and eosinophilic COPD exacerbations. *Thorax* 2018;**73**:422–30.
14. Wilkinson TMA, Aris E, Bourne S, Clarke SC, Peeters M, Pascal TG, *et al*.; AERIS Study Group. A prospective, observational cohort study of the seasonal dynamics of airway pathogens in the aetiology of exacerbations in COPD. *Thorax* 2017;**72**:919–27.
15. Staples KJ, Taylor S, Thomas S, Leung S, Cox K, Pascal TG, *et al*. Relationships between mucosal antibodies, non-typeable *Haemophilus influenzae* (NTHi) Infection and airway inflammation in COPD. *PLOS ONE* 2016;**11**:e0167250.
16. Haldar K, Bafadhel M, Lau K, Berg A, Kwambana B, Kebadze T, *et al*. Microbiome balance in sputum determined by PCR stratifies COPD exacerbations and shows potential for selective use of antibiotics. *PLOS ONE* 2017;**12**:e0182833.
17. Edouard S, Million M, Bachar D, Dubourg G, Michelle C, Ninove L, *et al*. The nasopharyngeal microbiota in patients with viral respiratory tract infections is enriched in bacterial pathogens. *Eur J Clin Microbiol Infectious Dis* 2018;**37**:1725–33.
18. MacDonald M, Korman T, King P, Hamza K, Bardin P. Exacerbation phenotyping in chronic obstructive pulmonary disease. *Respirology* 2013;**18**:1280–1.
19. Kushwah R, Cao H, Hu J. Potential of helper-dependent adenoviral vectors in modulating airway innate immunity. *Cell Mol Immunol* 2007;**4**:81–9.
20. Chuaychoo B, Wongsurakiat P, Nana A, Kositanont U, Maranetra KN. The immunogenicity of intradermal influenza vaccination in COPD patients. *Vaccine* 2010;**28**:4045–51.
21. Mallia P, Message SD, Gielen V, Contoli M, Gray K, Kebadze T, *et al*. Experimental rhinovirus infection as a human model of chronic obstructive pulmonary disease exacerbation. *Am J Respir Crit Care Med* 2011;**183**:734–42.
22. Hurst JR, Vestbo J, Anzueto A, Locantore N, Mullerova H, Tal-Singer R, *et al*.; Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) Investigators. Susceptibility to exacerbation in chronic obstructive pulmonary disease. *N Engl J Med* 2010;**363**:1128–38.
23. McDonald D, Hyde E, Debelius JW, Morton JT, Gonzalez A, Ackermann G, *et al*. American gut: an open platform for citizen science microbiome research. *mSystems* 2018;**3**:10–128.
24. Amir A, McDonald D, Navas-Molina JA, Debelius J, Morton JT, Hyde E, *et al*. Correcting for microbial blooms in fecal samples during room-temperature shipping. *mSystems* 2017;**2**:10–128.
25. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, *et al*. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci U S A* 2011;**108**:4516–22.
26. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;**13**:581–3.
27. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, *et al*. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* 2006;**72**:5069–72.
28. McMurdie PJ, Holmes S. An R package for reproducible interactive analysis and graphics of microbiome census data. *PLOS ONE* 2013;**8**:e61217.
29. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;**15**:550.

30. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;**12**:R60.

31. Mandal S, Van Treuren W, White RA, Eggesbo M, Knight R, Peddada SD. Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microb Ecol Health Dis* 2015;**26**:27663.

32. Xiao J, Chen L, Johnson S, Yu Y, Zhang X, Chen J. Predictive modeling of microbiome data using a phylogeny-regularized generalized linear mixed model. *Front Microbiol* 2018;**9**:1391.

33. Hinks T, Zhou X, Staples K, Dimitrov B, Manta A, Petrossian T, *et al*. Multidimensional endotypes of asthma: topological data analysis of cross-sectional clinical, pathological, and immunological data. *Lancet* 2015;**385**:S42.

34. Stockley RA, Edgar RG, Pillai A, Turner AM. Individualized lung function trends in alpha-1-antitrypsin deficiency: a need for patience in order to provide patient centered management? *Int J Chron Obstruct Pulmon Dis* 2016;**11**:1745–56.

35. Wood AM, Harrison RM, Semple S, Ayres JG, Stockley RA. Outdoor air pollution is associated with rapid decline of lung function in alpha-1-antitrypsin deficiency. *Occup Environ Med* 2010;**67**:556–61.

This synopsis should be referenced as follows:
Turner AM, Spittle D, Staples K, Cleary D. Airway-microbiome-driven mechanisms of disease during optimised self-management: a lesson learned from mechanistic study of the Colour-COPD trial. *Health Technol Assess* 2025;**29**(28):45–58. https://doi.org/10.3310/SYTH8546