Title: Associates of, and Time to Recovery from, Eosinopenia in severe COPD exacerbation.

Acronym: A-TREC

Key Study Information

Protocol Version No/Date:	Version 1.1, 8th December 2023
IRAS Number:	333785
Sponsor:	Northumbria Healthcare NHS Foundation Trust
Sponsor Reference No:	NHCT0457
Funder:	Northumbria Healthcare NHS Foundation Trust, Chiesi Limited and GlaxoSmithKline Research & Development Limited
Funding Reference No:	N/A
Chief Investigator:	Professor Stephen Bourke
REC Reference:	23/NW/0346

Protocol Developers

Professor Stephen Bourke	Consultant Respiratory Physician Northumbria Healthcare NHS Foundation Trust Honorary Professor of Respiratory Medicine, Newcastle University
Dr John Steer	Consultant Respiratory Physician Northumbria Healthcare NHS Foundation Trust Associate Clinical Lecturer, Newcastle University
Dr Arun Prasad	Clinical Research Fellow Northumbria Healthcare NHS Foundation Trust
Dr Peter Ireland	Teaching and Research Fellow Northumbria Healthcare NHS Foundation Trust
Dr Eduwin Pakpahan	Assistant Professor in Statistics Northumbria University

Contact Details (Northumbria Healthcare NHS Foundation Trust)

R&D Department:	Tel: 0191 2934087 Email: Jemma.Nelson@northumbria-healthcare.nhs.uk
Chief Investigator:	Tel: 0191 2934026 Email: Stephen.Bourke@nhct.nhs.uk

Signature page

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with GCP guidelines, the Sponsor's (and any other relevant) SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the trial publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the trial will be given; and that any discrepancies and serious breaches of GCP from the trial as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:
Signature: Date: ...../...../.....

.....
Name (please print):

.....

Position: .....

Chief Investigator:
Signature: Date: 08/ 12/ 2023

.....
Name (please print):
Prof Stephen Bourke
.....

Position: Consultant Respiratory Physician.....

Key Study Contacts

Chief Investigator

Professor Stephen Bourke
Consultant Respiratory Physician
Northumbria Healthcare NHS Foundation Trust
Tel: 0191 293 4026
Email: Stephen.Bourke@nhct.nhs.uk

Study Co-ordinator

Jemma Nelson
Research and Development
Northumbria Healthcare NHS Foundation Trust
Tel: 0191 293 4087
Email: Jemma.Nelson@northumbria-healthcare.nhs.uk

Sponsor

Peta Heslop
Head of Research and Development
Northumbria Healthcare NHS Foundation Trust
Tel: 0191 293 4087
Email: Peta.Heslop@northumbria-healthcare.nhs.uk

Funders

Jemma Nelson
Research and Development
Northumbria Healthcare NHS Foundation Trust
Tel: 0191 293 4087
Email: Jemma.Nelson@northumbria-healthcare.nhs.uk

Saarah Niazi-Ali
Chiesi Limited
Email: s.niazi-ali@chiesi.com

Rachel Tse
GlaxoSmithKline Research & Development Limited
Email: Rachel.w.tse@gsk.com

Key Protocol Contributors

Professor Stephen Bourke
Consultant Respiratory Physician
Tel: 0191 293 4026
Email: Stephen.Bourke@northumbria-healthcare.nhs.uk

Dr Peter Ireland
Teaching and Research Fellow
Email: Peter.Ireland@northumbria-healthcare.nhs.uk

Statistician
Dr Eduwin Pakpahan
Assistant Professor in Statistics
Northumbria University
Tel: 0191 349 5317
Email: Eduwin.Pakpahan@northumbria.ac.uk

Trial Steering Committee Chair
Professor Mona Bafadhel
Consultant Respiratory Physician
Email: Mona.Bafadhel@kcl.ac.uk

i. List of Contents

ii. List of Abbreviations

AOT	Ambulatory oxygen therapy
BEC	Blood eosinophil count
BMI	Body mass index
CAT	COPD Assessment Tool
CI	Chief investigator
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CRP	C-reactive protein
DECAF	Dyspnoea, Eosinopenia, Consolidation, Acidosis, Fibrillation
ECG	Electrocardiogram
eCRF	Electronic Case Record Form
eMRCD	Extended Medical Research Council Dyspnoea score
FBC	Full blood count
FeNO	Fractional exhaled Nitric Oxide
FEV1	Forced Expiratory Volume within 1 second
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
HRQoL	Health-related quality of life
ICS	Inhaled corticosteroids
IgE	Immunoglobulin E
IL	Interleukin
LABA	Long-acting beta agonists
LABD	Long-acting bronchodilators
LAMA	Long-acting muscarinic antagonists
LTOT	Long-term oxygen therapy
NEWS2	National early warning score 2
NHCT	Northumbria Healthcare NHS Foundation Trust
NHS	National Health Service
NIV	Non-invasive ventilation
NSECH	Northumbria Specialist Emergency Care Hospital
OCS	Oral corticosteroids
PI	Principal Investigator
PIS	Patient information sheet
PPI	Public and Patient Involvement
REC	Research Ethics Committee
SABD	Short-acting bronchodilators
TMG	Trial Management Group
TNFa	Tumour necrosis factor alpha
TSG	Trial Steering Group

iii. Study Summary

<table>
<tr><td>Study Title</td><td colspan="2">Associates of, and Time to Recovery from, Eosinopenia in severe COPD exacerbation</td></tr>
<tr><td>Acronym</td><td colspan="2">A-TREC</td></tr>
<tr><td>Trial Design</td><td colspan="2">Single centre observational cohort study</td></tr>
<tr><td>Trial Participants</td><td colspan="2">Clinical diagnosis of severe exacerbation of COPD +/- eosinopenia</td></tr>
<tr><td>Planned Sample Size</td><td colspan="2">200 participants</td></tr>
<tr><td>Study Duration</td><td colspan="2">24 months of recruitment</td></tr>
<tr><td>Follow-up Duration</td><td colspan="2">6 weeks follow-up from entry into study</td></tr>
<tr><td>Planned Study Period</td><td colspan="2">FSFV 19/01/2024
LSFV 01/06/2026</td></tr>
<tr><td rowspan="4">Primary</td><td>Objectives</td><td>Outcomes</td></tr>
<tr><td colspan="2">Total Study Population</td></tr>
<tr><td>1. To identify demographic, physiological and clinical factors independently associated with admission eosinopenia in patients with a severe exacerbation of COPD.</td><td>1. Indices independently associated with eosinopenia on admission in patients with a severe exacerbation of COPD.</td></tr>
<tr><td colspan="2">Eosinopenia with uneventful recovery*[1]</td></tr>
<tr><td></td><td>2. To assess the time to recovery from eosinopenia to stable BEC following a severe exacerbation of COPD.</td><td>2. The time taken for BEC to recover to a level equal to or higher than the 42-day assessment.</td></tr>
</table>

Secondary	Objectives	Outcomes
	Total Study Population	
	1. Assess the consistency of eosinophil phenotype during severe exacerbations within individuals. 2. Compare Th1 and Th2 cytokine levels during acute exacerbation of COPD and at the point of recovery.	1. Level of agreement between the index admission eosinophil phenotype and prior admissions for exacerbations of COPD since May 2019[*2]. 2. Change from baseline cytokine levels (Th1 and Th2) at day 28, stratified by intercurrent exacerbation or other acute illness status, including sub-group analysis (eosinopenic cohort vs non-eosinopenic cohort)
	Eosinopenia with uneventful recovery*[1]	
	3. To explore the time to recovery from admission eosinopenia in severe exacerbation of COPD, including the implications for ICS prescribing decisions at the GOLD 2023 threshold.	3. A) The proportion of patients whose BEC reaches 100 cell/uL or higher at each visit 3. B) The time taken to reach peak BEC following a severe exacerbation of COPD. 3. C) Exploratory analysis looking for patient and treatment associations with rate of recovery of BEC.
	Total Eosinopenic Cohort	
	4. Assess the impact of moderate and severe exacerbations on recovery from eosinopenia.	4. Comparison of the recovery of BEC in patients who had further exacerbations of COPD within the study period and patients who have not had further exacerbations.

*[1] Eosinopenia on admission who do not receive a further course of systemic corticosteroids or require emergency hospital admission for an acute illness in the six weeks following admission to hospital with a severe exacerbation of COPD.

*[2] May 2019 is the date that BEC measurements began to be reported to two decimal places at the designated research site – Northumbria Healthcare NHS Foundation Trust.

iv. Funding

Funders	**Financial Support Given**
Northumbria Healthcare NHS Foundation Trust Research and Development North Tyneside General Hospital Rake Lane North Shields NE29 8NH Email: Jemma.Nelson@northumbria-healthcare.nhs.uk	Essential research and laboratory costs to deliver the study.
Northumbria Healthcare NHS Foundation Trust Teaching and Research Fellow Programme North Tyneside General Hospital Rake Lane North Shields NE29 8NH Email: Tracey.Hogg3@northumbria-healthcare.nhs.uk	Complete funding for Principal Investigator's salary for 3-year period.
Chiesi Limited 333 Styal Road Manchester M22 5LG Email: s.niazi-ali@chiesi.com	Desirable research and laboratory costs to deliver the study including statistical analysis
GlaxoSmithKline Research & Development Limited 980 Great West Road Brentford Middlesex TW8 9GS Email: Rachel.w.tse@gsk.com	Desirable research and laboratory costs to deliver the study.

v. Role of Study Sponsor and Funder

Sponsor The Sponsor, Northumbria Healthcare NHS Foundation Trust, assumes overall responsibility for the initiation and management of the trial

Funder The study funders had no role in the study design or protocol development and will have no direct involvement in the study conduct, data analysis and interpretation, manuscript writing or dissemination of results.

vi. Roles and Responsibilities of Trial Management Committees, Groups and Individuals

Trial Steering Committee	A Trial Steering Committee will be formed with an independent Chair, and will meet at least every 12 months and send timely reports to the Sponsor. The independent Chair is: Professor Mona Bafadhel Consultant Respiratory Physician Email: Mona.Bafadhel@kcl.ac.uk
Trial Management Group	The Trial Management Group will meet regularly to ensure all practical details of the study are progressing and working well, and everyone within the study understands them. The Chair will be the Chief Investigator who assumes primary responsibility for the design, conduct and reporting of the study. Chief Investigator: Professor Stephen Bourke Consultant Respiratory Physician Tel: 0191 293 4026 Email: Stephen.Bourke@nhct.nhs.uk

vii. Protocol Contributors

Professor Stephen Bourke	Conceived the study, has led the development of the study design and protocol and will ensure appropriate governance is in place. Professor Bourke will support and supervise Peter Ireland with all other key research activities including mentorship, development and dissemination of study results.
Dr John Steer	Contributed to development of the study design and protocol. Dr Steer will support and supervise Peter Ireland including provision of mentorship and development.
Professor John Simpson	Contributed to development of the study design and protocol. Cytokine analysis will occur in collaboration with Professor Simpson's laboratory at Newcastle University. Professor Simpson will support and supervise Peter Ireland including provision of mentorship and development.
Dr Peter Ireland	Will be involved in all stages of the study, from application to final dissemination of results, including: developing study protocol, developing key study documents, developing the source document worksheet, setting up an electronic database, participant recruitment, performing baseline assessments, data collection, performing data validation, involvement in study management meetings, applying statistical analysis and involvement in preparing publication of results for dissemination.
Dr Arun Prasad	Contributed to development of the study design and protocol. Gathered feedback from patients to help inform study design.
Dr Eduwin Pakpahan	Statistician responsible for development of the initial statistical analysis plan, performing data analysis and assisting with reports and publications.
Patient and Public Involvement	Representative patients from the Northumbria Lung Research PPI group have been involved in the study design.

viii. Key Words

Chronic Obstructive Pulmonary Disease (COPD)	A lung disease causing breathlessness, sputum production and frequent exacerbations
Blood Eosinophil Count (BEC) stable state	Blood eosinophil count during clinical stability
Eosinopenia	Low level of eosinophils in circulating blood defined as $< 0.05 \times 10^9$/L

1. Background

Chronic obstructive pulmonary disease (COPD) is a complex and heterogenous condition which is characterised by persistent airflow limitation and emphysema, often contributing to significant and progressive respiratory symptoms. COPD is extremely prevalent with an estimated 391.9 million people living with the condition worldwide in 2019 (1), more than 1.2 million of whom were living in the UK (2). COPD confers significant mortality and morbidity, with 3.3 million people dying from the condition in 2019 (1), making it the third-leading cause of death worldwide. In the same year it was estimated to be the seventh-leading cause of poor health worldwide accounting for 74.4 million disability-adjusted life years (1).

People living with COPD are often prone to frequent exacerbations. These are characterised by an acute increase in breathlessness and/or cough with a change in sputum production. Exacerbations are often secondary to inflammation caused by infection, pollution or other insults to the airways (3). A severe exacerbation of COPD is commonly defined as the patient requiring admission to hospital for treatment.

Exacerbations of COPD have a significant burden on individuals. Patients who are admitted to hospital with acute exacerbations of their COPD experience a substantial decrease in their health-related quality of life (HRQoL) and lung function (4) and have a significantly increased risk of mortality (5). For patients who survive to be discharged, over 40% will be readmitted within 90 days and over 20% of all patients who are discharged from hospital following an acute exacerbation of COPD will die within 1 year (6).

COPD is also associated with a high burden on healthcare and the economy. There are over 140,000 emergency hospital admissions for acute exacerbations of COPD each year in the UK accounting for almost 2% of all hospital admissions (2). COPD costs the NHS approximately £1.9 billion per year, almost 20% of the cost of all lung conditions (7). In the European Union respiratory disease accounts for 6% of the annual healthcare budget and COPD accounts for more than 50% of this (8).

Inhaled corticosteroids (ICS) are of benefit in some patients with COPD. In isolation, ICS have not been proven to reduce the long-term decline in FEV1 or improve mortality (9) and their use may increase the risk of pneumonia (10). When ICS is combined with long-acting bronchodilators (LABD) as a triple-therapy inhaler (LABA+LAMA+ICS) there is a reduction in exacerbation rates plus improvement in lung function, HRQoL and survival when compared to mono- and dual-therapy (11, 12, 13).

Blood eosinophil count (BEC) can be used as a biomarker to predict whether the addition of ICS to LABD will be beneficial in reducing future COPD exacerbations (14). Similarly, BEC informs selection for emerging biologic therapy in COPD, including Mepolizumab (15) and Dupilumab (16). The association between BEC and reduction in exacerbation frequency is based on BEC measured when the patient is clinically stable. The relationship between BEC and effect of ICS is continuous, with increasing effects seen as BEC rises (17). ICS have very little effect when the BEC is < 100 cells/uL and a post hoc risk-benefit analysis suggested that at lower levels of BEC the harm of ICS due to pneumonia is greater than the benefit of

severe exacerbation reduction (18). Patients with BEC >= 300 cells/uL are most likely to gain treatment benefit from the addition of ICS. Current national and international COPD guidelines suggest the use of BEC as a biomarker to help inform the decision of whether to commence or withdraw ICS (19), but do not specify measuring BEC at a time of clinical stability.

BEC fall transiently and often profoundly during severe ECOPD (20). In the Dyspnoea, Eosinopenia, Consolidation, Acidaemia and atrial Fibrillation (DECAF) score developmental cohorts, admission eosinopenia (BEC< 50 cells/µL) was present in 1,340 of 2,645 severe ECOPD (21). In the recent BEC COPD study (IRAS 285200) eosinopenia was less common on discharge compared to admission, despite in-hospital prednisolone therapy (22), suggesting that it is primarily driven by factors other than corticosteroid use. BEC is also transiently suppressed during other acute illnesses such as sepsis (23) and in response to increases in adrenaline (24), further suggesting that there may be other independent factors behind transient eosinopenia during exacerbations of COPD.

It is unclear at what point eosinopenia following an exacerbation of COPD recovers to stable state BEC. The timing of measuring BEC as a biomarker to inform the use of ICS as a maintenance therapy in COPD is integral, as measurements during an exacerbation will not accurately capture all patients who may benefit from this treatment. This is not recognised in current national and international guidelines related to COPD.

2. Rationale

Decisions regarding long-term management of COPD are often made in the acute setting. Given that transient eosinopenia occurs in approximately 50% of patients with severe exacerbations of COPD (21), measuring BEC during an acute admission or illness risks incorrectly identifying all patients who may benefit from the introduction of ICS. In the current BEC COPD study (IRAS 285200), reliance on admission and discharge BEC inappropriately denied ICS to 47% and 33% of patients respectively compared to a confirmed stable-state measure.

A co-primary aim of this study is to determine when BEC will recover to stable state following a severe exacerbation of COPD. This will inform the optimum timing to measure BEC for use as a biomarker in decisions related to management escalation. Based on the results of BEC COPD, it is likely that an increased number of patients will be identified as being above the BEC threshold and therefore likely to benefit from ICS when time is allowed for BEC to recover to stable state. Increasing the number of patients with COPD who are appropriately escalated to ICS maintenance therapy should reduce the burden of COPD on patients and the NHS by reducing exacerbation and hospitalisation rate (11) and may also improve mortality (25).

Eosinopenia during exacerbation of COPD is associated with increased short-term mortality (21). Treatment with oral corticosteroids does not fully explain the mechanisms behind the development of eosinopenia, with the phenomenon being less common on discharge compared to admission despite inpatient oral corticosteroid treatment, as seen during BEC

COPD. Another co-primary aim of this study is to identify demographic, physiological and clinical factors independently associated with admission eosinopenia in patients with a severe exacerbation of COPD, providing useful mechanistic information regarding the relationship between BEC and short-term mortality.

The hypothesis for this study is that 90% of participants who are admitted to hospital with a severe exacerbation of COPD and eosinopenia will have recovery of their BEC to baseline stable state within 4 weeks. We also hypothesize that there are demographic, physiological and clinical factors independently associated with admission eosinopenia other than prior systemic corticosteroid use.

3. Objectives and Outcome Measure/Endpoint

3.1 Co-primary aims

1. To identify demographic, physiological and clinical factors independently associated with admission eosinopenia in patients with a severe exacerbation of COPD.

2. To assess the time to recovery from eosinopenia to stable BEC following a severe exacerbation of COPD with uneventful recovery. *[1]

3.2 Secondary aims

1. To assess the consistency of eosinophil phenotype during severe exacerbations within individuals.

2. To compare Th1 and Th2 cytokine levels during acute exacerbation of COPD and at the point of recovery.

3. To further explore the duration of recovery from admission eosinopenia in severe exacerbation of COPD, including the implications for ICS prescribing decisions at the GOLD 2023 threshold. *[1]

4. To assess the impact of moderate and severe exacerbations on recovery from eosinopenia. *[2]

3.3 Co-primary outcomes

1. Indices independently associated with eosinopenia on admission in patients with a severe exacerbation of COPD.

2. The time taken for BEC to recover to a level equal to or higher than the 42-day assessment. *[1]

3.4 Secondary outcomes

1. Level of agreement between the index admission eosinophil phenotype and prior admissions for exacerbations of COPD since May 2019[*3].

2. Change from baseline cytokine levels (Th1 and Th2) at day 28, stratified by intercurrent exacerbation or other acute illness status, including sub-group analysis (eosinopenic cohort vs non-eosinopenic cohort)

3.
    a. The time taken to reach peak BEC following a severe exacerbation of COPD. [*1]
    b. The proportion of patients whose BEC reaches 100 cells/uL or higher at each visit. [*1]
    c. Exploratory analysis looking for patient and treatment associations with rate of recovery of BEC. [*1]

4. Comparison of the recovery of BEC in patients who had further exacerbations of COPD within the study period and patients who have not had further exacerbations. [*2]

[*1] Eosinopenia on admission who do not receive a further course of systemic corticosteroids or require emergency hospital admission for an acute illness in the six weeks following admission to hospital with a severe exacerbation of COPD

[*2] Total eosinopenic cohort

[*3] May 2019 is the date that BEC measurements began to be reported to two decimal places at the designated research site – Northumbria Healthcare NHS Foundation Trust

4. Study Design

A single-centre observational cohort study.

5. Study Setting

Patients will be recruited from a single secondary care centre within the UK. The confirmed recruiting site is Northumbria Healthcare NHS Foundation Trust (NHCT). Patients will be recruited from Northumbria Specialist Emergency Care Hospital (NSECH) and will be identified by daily screening of acute admissions.

6. Participant Eligibility Criteria

6.1 Inclusion Criteria

1. Admitted to hospital with primary clinical diagnosis of exacerbation of COPD*
2. Age at least 35 years
3. Smoking history of at least 10 pack years
4. Airflow obstruction: FEV1/FVC ratio < 0.7 confirmed on historic or inpatient spirometry
5. Capacity to give informed consent to participate
6. Recruitment within 36 hours of initial full blood count following presentation to hospital

*Primary diagnosis to be ratified by study investigator at time of eligibility review

6.2 Exclusion Criteria

1. Parasitic infection, systemic fungal infection (excluding infection limited to nails or skin), eosinophilic granulomatosis with polyangiitis, hypereosinophilic syndrome or other conditions associated with a high eosinophil count [#]
2. Active malignancy
3. Maintenance oral prednisolone or other systemic steroids, anti-interleukin 5 therapy or other medication known to influence BEC
4. Patients with poor venous access
5. Investigator confirmed history of asthma in adulthood
6. Non-COPD related health problems which in the view of the primary investigator may compromise the conduct and completion of the study

[#] Patients with atopic conditions such as allergic rhinitis, allergic conjunctivitis and eczema will be eligible.

6.3 Additional criteria for the time to recovery from eosinopenia analysis only

Inclusion criteria:

a. Eosinopenia on admission
b. Uneventful recovery[*1]

[*1] Eosinopenia on admission who do not receive a further course of systemic corticosteroids or require emergency hospital admission for an acute illness in the six weeks following admission to hospital with a severe exacerbation of COPD

7. Study Procedures

A schedule of activities is included in the appendix of this protocol (appendix 1).

The initial assessments and investigations will be performed within 36 hours of the participant's initial full blood count on presentation to hospital with a severe exacerbation of COPD. Length of stay as an inpatient can vary considerably for exacerbations of COPD and therefore the settings for the follow up assessments and investigations will vary considerably. These will include inpatient setting, outpatient setting or in the patient's home. There will be a 1-day window for the day 3 assessment, and a 3-day window either side of patient follow-up assessments and investigations from day 7 of the scheduled activities. All visits are timed from the date and time of participant's initial full blood count on presentation to hospital.

Given current evidence which suggests that BEC may express a circadian rhythm in patients with COPD (27), blood samples will be taken between 10:00 – 18:00 as BEC are stable between these time points, which are also consistent with the timing of most stable state blood tests in clinical practice (e.g. during COPD reviews and clinic visits). The time from sampling to analysis will be within a 24-hour window to reduce the reduction in BEC which is associated with a longer time from sample collection to analysis (28). These parameters should reduce variability in BEC measurements which could affect the validity of our results.

Reason for attrition from the time to recovery from eosinopenia analysis will be recorded (e.g. acute illness, steroid use) and rates of attrition will be closely monitored. We will introduce a procedure for participants and/or their community clinical team to contact the research team to provide advice on when treatment with steroids in the community is clinically appropriate. This may help to reduce inappropriate use of steroids and therefore reduce attrition rates, as well as reducing risk to participants.

7.1 Recruitment

Patients who may be eligible for the study (severe exacerbations of COPD) will be provided with a participant information sheet (PIS) on admission. This will provide basic information about the study to help facilitate discussions about participation in the study when approached by a member of the research team.

Any recruitment successes or concerns will be identified by the research team and subsequently discussed in regular meetings with supervisors and during monthly NHCT respiratory research meetings. Feedback will also be obtained from participants in the study and shared with the Northumbria Lung Research PPI Group, the study Trial Management Group and the Trial Steering Group. Facilitators and barriers to recruitment identified by the research team and feedback obtained from study participants will be used to inform ongoing recruitment to improve recruitment success.

We will record the details of patients with exacerbations of COPD who are not eligible or who decline to take part in a screening log.

7.1.1 Participant Identification

Participants will be identified by the usual care team based on the eligibility criteria and approached to discuss participation in the study.

Eligibility will be confirmed by the research team and recorded on the source document worksheet and eCRF using information obtained from historic and current electronic and paper clinical records.

7.1.2 Screening

Acute medical admissions to NSECH will be screened daily by the usual care team. Informed consent will need to be obtained and initial assessments and additional study specific baseline investigations performed within 36 hours of the participant's initial full blood count on presentation to hospital with a severe exacerbation of COPD (for overnight urinary metanephrines, collection needs to commence within the 36-hour window). We will aim to discuss participation in the study with eligible patients at the earliest opportunity from time of initial full blood count.

7.2 Consent

Once identified as eligible, patients will be approached by a member of the research team. If not already provided, information about the study will be given in the form of the PIS and any initial questions will be answered. The PIS will be available in large font to aid those with visual impairment. We will support recruitment of patients from diverse backgrounds (e.g. translators and language aids to support patients with language barriers).

The initial information about the study will be offered to patients in 2 formats:

- Read the PIS themselves; or
- Listen to the PIS being read to them

Given the high incidence of exacerbations of COPD requiring hospital admission, it is possible that multiple eligible patients will be identified daily. All eligible patients will be given time to receive and understand the initial information regarding the study. The consent, assessment and investigation process will then be performed for all patients who wish to be involved in the study. Informed consent will only be taken by individuals who have the appropriate GCP training and prior to any study assessments or investigations being performed.

It is likely that the consent and collection of baseline data will take approximately 90 minutes per patient (15 minutes for initial information followed by 30 minutes for consent and 45 minutes for assessments and investigations).

All participants in the study will be 35 years or older.

7.3 Baseline Data

A source document worksheet will be used to collect baseline data. Key data to be collected is shown below.

Category	Data
Patient demographics	Name, NHS number, hospital number, Gender, Date of birth, Ethnicity, Postcode, Indices of Multiple Deprivation
Admission indices	Date and time of arrival at hospital, date and time of admission to ward, highest NEWS2 score and associated observations within 24 hours of presentation at hospital including respiratory rate, oxygen saturations, inspired oxygen, heart rate, blood pressure, temperature, DECAF score, requirement for invasive or non-invasive ventilation within 24 hours of presentation, worst arterial blood gas measurement within 24 hours of presentation, admission ECG (rate and rhythm), chest x-ray findings (including diaphragm height), height, weight, BMI
Admission history	Requirement for invasive or non-invasive ventilation beyond 24 hours of presentation, development of new chest x-ray consolidation during admission, evidence of development of respiratory infection during admission, non-respiratory medical deterioration during admission, date of discharge
Previous admission history	Initial FBC for any previous admission with a severe exacerbation of COPD since May 2019
COPD History	Smoking status, pack year history, e-cigarette use, other inhaled recreational drugs, other relevant exposures notably occupational, exacerbation history, admission history, acute NIV history, previous spirometry, LTOT/AOT use, home NIV use
Past medical history	Various conditions
Medication history	Relevant long-term and acute medications
Social history	Home residence, Rockwood clinical frailty score
Blood results	FBC indices, CRP, albumin, procalcitonin, fibrinogen, lactate, copeptin, cortisol, IgE, pneumococcal antigen
Urinary investigations	Overnight urinary metanephrines
Biofire Respiratory 2.1 Panel	Common respiratory viruses and bacteria

Sputum	Microbiological culture results and purulence (patient reported and lab/research team reported)
Cytokine panel	Th1 and Th2
Spirometry and FeNO	FEV1, FVC, VC, FeNO
Assessments	eMRCD, CAT Score

7.4 Follow-up Data

The source document worksheet will be used to collect data throughout the 6-week follow-up period. Key data to be collected at each assessment is outlined below.

Follow-up assessment	**Data**
Day 3	Acute prednisolone and antibiotic therapy use, FBC indices, CRP
Day 7	Acute prednisolone and antibiotic therapy, FBC indices, CRP
Day 14	Clinical history, full medication history including acute prednisolone and antibiotic therapy, FBC indices, CRP, FeNO, CAT Score, eMRCD Score, Rockwood Clinical Frailty Score
Day 21	Acute prednisolone and antibiotic therapy, FBC indices, CRP
Day 28	Clinical history, full medication history including acute prednisolone and antibiotic therapy, FBC indices, CRP, copeptin, overnight urinary metanephrines, cytokine panel, Spirometry, FeNO, CAT Score, eMRCD Score, Rockwood Clinical Frailty Score
Day 42	Clinical history, full medication history including acute prednisolone and antibiotic therapy, FBC indices, CRP

7.5 Study Assessments

The following data will be collected during day 1 of the study once patients have met the inclusion/exclusion criteria and given fully informed consent:

1) Socioeconomic details
2) Full medical history
3) COPD history (confirmation of diagnosis, exacerbation history, admission history, NIV history, smoking history, LTOT/AOT use and home NIV use)
4) Spirometry and FeNO
5) Extended Medical Research Council Dyspnoea Score (eMRCD)
6) COPD Assessment Tool Score (CAT)

7) Rockwood Clinical Frailty Score

During the 6-week follow-up period many of the above assessments will be repeated at certain follow-up appointments including:

1) Clinical history
2) Medication history
3) Spirometry and FeNO
4) Extended Medical Research Council Dyspnoea Score (eMRCD)
5) COPD Assessment Tool Score (CAT)
6) Rockwood Clinical Frailty Score

7.6 Withdrawal criteria

Participants are free to withdraw from the study at any time without giving an explanation. Participants will be offered an interview at the point of withdrawal to ascertain their reasons for withdrawing. Participants will be given the option to make requests that, if met, will retain them in the study. If these requests are reasonable (e.g. changing the timing or location of visits) then this will be sought as a solution to retain participants and reduce attrition. Date and time of withdrawal will be recorded on the source document worksheet by the research team.

We will seek consent to continue to collect electronic data from any participants in the study who withdraw, but no further study specific assessments will occur from the point of participant withdrawal. Any blood sample that has been taken and stored for cytokine analysis, prior to participant withdrawal, will continue to be stored for subsequent analysis unless participants specifically withdraw consent for this.

7.7 Storage and analysis of clinical samples

Clinical samples collected throughout the study will include blood (plasma and serum), urine, sputum and nasopharyngeal swabs. Blood, urine, sputum and nasopharyngeal samples will be labelled with patient identifiable data on collection before being securely transferred to Northumbria Specialist Emergency Care Hospital, North Tyneside General Hospital or Wansbeck General Hospital laboratories at the earliest possible time from collection. This may vary slightly depending on follow-up visits as occasionally they may be collected in the community. Samples will be securely stored for the standard duration of time that each sample type are stored in the hospital laboratory. During this time only laboratory staff will have physical access to the samples and their security will be the responsibility of the laboratory manager. Following analysis, samples will be disposed of as per local Trust guidelines.

Blood plasma samples for cytokine panel testing will be collected, labelled with an individual participant identification number which will be linked to personal identifiers, and transferred to North Tyneside General Hospital. At North Tyneside General Hospital, they will be centrifuged into aliquots which will be securely stored in a specialist freezer at -80C +/- 10C. Length of time in storage will vary depending on when the sample was taken during

the recruitment period but will be between 1 – 24 months. During storage the samples will remain labelled with individual participant identification numbers.

Study samples will be transferred in batches to Newcastle University where they will continue to be securely stored in a specialist freezer at -80C +/- 10C to await analysis. During this time only laboratory staff will have physical access to the samples and their security will be the responsibility of the laboratory manager. Following analysis, samples will be disposed of as per local Newcastle University guidelines.

7.8 End of the study

The recruitment phase of the study will aim to end once 200 patients have been recruited, however the study will follow an adaptive design. We will map the number of participants with uneventful recovery from eosinopenia and closely monitor rates of attrition. We will extend recruitment if necessary to ensure that we have the required sample size for the time to recovery from eosinopenia analysis as per our power calculation (n = 70). The follow up phase of the study will end after the 6-week follow-up appointment of the last patient recruited.

We will ensure that all participants are on optimal therapy on completion of the trial.

8. Statistics and Data Analysis

8.1 Sample size calculation

There is a lack of published data on longitudinal blood eosinophils during an admission for exacerbation of COPD and within 6 weeks following an exacerbation. From observations during clinical practice, blood eosinophils often recover within 2-3 weeks after admission for an exacerbation of COPD.

We predict that 90% of our population will have recovery of BEC to baseline within 4 weeks. Using a one-proportion test based on the above hypothesis, for an 80% statistical power, we believe a sample size of at least 69 is needed. Based on the estimated prevalence of admission eosinopenia (approximately 50%) and attrition including due to intercurrent illness or prednisolone use, we will recruit 200 patients.

This will also ensure that we are adequately powered for the co-primary outcome of identifying independent associates of eosinopenia in the total population. We expect 100 participants will have eosinopenia, and expect less than 10 parameters to be included in the regression equation, meeting the standard criterion of 10 outcomes per parameter.

8.2 Planned Recruitment Rate

Recently, recruitment to COPD studies has been challenging due to the impact of the Covid-19 pandemic. A-TREC requires 200 patients to be recruited from a single centre. During the Covid-19 pandemic 2 studies recruited 115 and 214 COPD patients respectively from our site. Thus, anticipated rate of recruitment will be 10-15 patients per month. Anticipated duration of recruitment will be 30 months.

We will monitor recruitment closely and consider opening to external sites if necessary.

8.3 Statistical Analysis Plan

Our initial statistical analysis plan has been designed with support from Newcastle University and the trial statistician (Eduwin Pakpahan, Northumbria University). A full statistical analysis plan will be confirmed with the trial statistician prior to data hard-lock, and uploaded to the trial registration entry on clinicaltrials.gov.

8.3.1 Baseline characteristics of population

For all outcomes (both primary and secondary) we will describe baseline characteristics of the population and follow-up measurements using suitable measures of tendencies. For continuous variables, mean with the associated standard deviation and 95% confidence interval, or median and interquartile range, will be provided based on their distribution. For categorical variables (including binary variables), frequency and proportions will be given. To test for significance between the arms, Chi-Squared test (or Fisher's Exact test in case of small number of observations), t-test, or Mann-Whitney U test will be used depending on whether the variable is categorical, parametric, or non-parametric.

8.3.2 Primary outcome analysis

Time to recovery from eosinopenia
A 1 proportion test will be used to assess the proportion of patients who do not recover within 4 weeks as compared to the hypothesised proportion.

A Chi-squared test will examine whether patients recover within 2, 3 and 4 weeks after hospital admission.

Independent associates of eosinopenia
Candidate indices will be identified by:

- Univariate analysis: include indices related to eosinopenia at the 0.1 threshold.
- Domain knowledge: include additional indices thought likely to be related to eosinopenia even if no association on univariate analysis.
- Create summary indices to reduce the number of variables – e.g. evidence bacterial infection will include a positive blood culture, positive sputum culture or positive antigens.
- Address collinearity.

- Independent associates of eosinopenia will be identified by logistic regression (backwards stepwise elimination).

8.3.3 Secondary outcomes analysis

To assess recovery from eosinopenia (as a binary outcome), Cochran Q test will examine which timepoints are significantly different from each other and compare the proportion that recover by a certain time period in a pairwise model. A Life table analysis will be used to determine probability of no recovery within specific time periods.

Linear regression models will be used to identify factors that may influence recovery time, and a logistic regression model will assess the association between index admission eosinophil phenotype and separate exacerbation eosinophil phenotypes, with adjustment for demographic and disease characteristics. This will be summarised with odds ratios and 95% confidence intervals.

8.4 Subgroup analyses

In the subgroup who attain a stable state BEC of 100 cell/uL or higher, a 1 proportion test will be repeated at each timepoint.

8.5 Participant population

Participants will be aged 35 years and over. They will have been admitted to hospital with an exacerbation of COPD +/- eosinopenia. We will support recruitment of patients from diverse backgrounds (e.g. language aids to support patients with language barriers, home visits for follow-up to support those patients unable to travel due to socioeconomic or health disability).

8.6 Procedure to account for loss to follow-up or missing data

We consider the tolerable level of loss to follow-up or missing data to be less than 10%. Every effort will be made to minimise missing baseline and outcome data. 1 in 10 participants will undergo data verification and validation. We will allow a 2% error rate for objective measures (such as BEC) and a 10% error rate for subjective measures. Standard approaches will be used to detect patterns in missing data. The level and pattern of the missing data in the baseline variables and outcomes will be established by forming appropriate tables and the likely causes of any missing data will be investigated. This information will be used to determine whether the level and type of missing data has the potential to introduce bias into the analysis results for the proposed statistical methods.

9. Data Management

9.1 Data collection

Data for each participant will be collected on a paper source document worksheet that has been designed specifically for the study.

9.2 Data handling and record keeping

Paper research material will be stored within a locked filing cabinet in a key-pad secured office at North Tyneside General Hospital. Only the research team will have access to this location. The paper research material will not contain personal data but be identified by a unique individual participant identification number. The file linking this to personal data will be held securely, separately and electronically.

Data from the paper source document worksheet will be transcribed onto an electronic Case Report Form (eCRF). This will be stored on a secure online database (REDCap) which will be password protected. The REDCap database will have a security system to protect against unauthorised access. There will be regular data back up to prevent any loss of data.

Upon completion of the study (including data analysis and publication), personal data will be securely destroyed including the linking files for the individual participant identification numbers. Anonymised, electronic research data generated by the study will be stored for 25 years.

Data will be handled and stored in accordance with the 2018 General Data Protection Regulation (GDPR).

9.3 Access to Data

Direct access to the data will be granted to the CI and PI at Northumbria Healthcare NHS Foundation Trust.

9.4 Archiving

Study data will be archived for 25 years following completion of the study.

10. Monitoring, Audit and Inspection

A Trial Steering Group (TSG) will be formed with an independent Chair and will include a patient representative. They will meet at least every 12 months and send timely reports to the Sponsor and any funders.

A Trial Management Group (TMG) will also be formed which will be chaired by the CI. They will meet at least monthly to ensure effective day to day running of the study.

The PI will assist the CI in monitoring the study. On-site data verification and validation will be performed for 1 in 10 participants by an independent investigator. This will include participant eligibility and primary and secondary outcome data.

11. Ethical and Regulatory Considerations

11.1 Research Ethics Committee (REC) review and reports

Prior to commencement of the study, approval will be sought from an NHS REC for the study protocol and other relevant documents e.g. participant information sheet and consent forms.

Substantial amendments will not be implemented until the REC grants a favourable opinion for the study.

All correspondence with the REC will be retained in the Trial Master File/Investigator Site File.

An annual progress report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended.

It is the Chief Investigator's responsibility to produce the annual reports as required.

The Chief Investigator will notify the REC of the end of the study.

If the study is ended prematurely, the Chief Investigator will notify the REC, including the reasons for the premature termination.

Within one year after the end of the study, the Chief Investigator will submit a final report with the results, including any publications/abstracts, to the REC.

The study will be registered on ClinicalTrials.gov prior to commencement.

11.2 Peer review

The study has been reviewed by 2 independent experts.

11.3 Public and Patient Involvement

We have sought the views of patients with COPD on the planned research. Participants from the Northumbria Lung Research Patient and Public Involvement (PPI) group (expert patients with COPD and experience taking part in and reviewing research proposals) and from an existing clinical COPD study were contacted via the telephone to obtain their feedback on the proposed study.

Questions which were asked of these patients included:

1. Is the study of an interest to you?
2. Do you think it is important for the NHS?
3. Is the study design clear?
4. Are the number of study visits appropriate for you?
5. Is there anything that would put you off taking part?

All participants thought the study was interesting and useful. They felt it would be important to the NHS and potentially answer important clinical questions. They thought the study design was clear and not too complicated, with none of the participants recommending any changes to the study design. 75% of participants thought the number of study visits were appropriate and the number of tests were not too high.

Feedback on the A-TREC PIS was obtained from two members of the Northumbria Lung Research PPI Group. The PIS was sent out via post and then a focussed telephone interview was performed with each member.

Both members felt that the language used in the PIS was understandable and appropriate for patients with COPD. They also felt that there was an appropriate amount of information within the PIS, and neither member felt that there should be additional information added. One member thought that additional information would likely lead to information overload for patients who were considering taking part in the study.

Both members felt that the information relayed in the PIS was clear and that there were no areas which required further clarity. One member stated that they found the PIS more understandable than previous information sheets that they have read related to clinical research. They described the A-TREC PIS as ‘inclusive’ for participants, as it was easy to understand and did not contain any jargon. The other member described the A-TREC PIS as ‘impressive’ and thought that it was straightforward to read and understand.

One member stated that they thought patients would find it reassuring to be followed up in the study after an admission to hospital with an exacerbation of COPD, as often patients are discharged without future follow up plans. They also thought that some patients would welcome additional blood tests for further reassurance and did not see these as an extra burden.

Overall, both members thought that the PIS was suitable for the study. They also thought that the study was worthwhile and important. One member specifically stated that they would want to be involved in the study if they were admitted to hospital with an exacerbation of their COPD. They were both supportive of research that sought to improve care for patients with COPD.

11.4 Regulatory Compliance

The study will not commence until a favourable REC opinion is obtained. The anticipated start date for recruitment is 19th January 2024.

For any amendment to the study, the CI, in agreement with the sponsor will submit information to the appropriate body for them to issue approval for the amendment.

11.5 Protocol Compliance

A protocol non-compliance is a departure from the ethically approved study protocol, study process (e.g. consent) or from Good Clinical Practice or other applicable regulatory requirements. Any protocol deviations will be documented on a protocol deviation form, filed in the study master file, and reported to the CI and sponsor immediately.

11.6 Notification of Serious Breaches to GCP and/or the protocol

A serious breach of the GCP and/or protocol will be immediately notified to the CI and sponsor.

11.7 Data protection and patient confidentiality

All members of the research team will comply with the requirements of the Data Protection Act 1998 with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

Participant's personal identifiable information will be replaced with an unrelated sequence of characters, with the data and the linking code secured in separate locations. Access to this data will be limited a to a need only basis. Personal data, including the linking files, will be destroyed upon completion of the study.

All electronic data will be transferred via the secure database (REDCap). Anonymised, electronic research data generated by the study will be stored for 25 years.

11.8 Financial and other competing interests for the chief investigator, PIs and committee members for the overall study management

Funding for the Teaching and Research Fellowship has been secured which will cover the PI expenses for the entirety of the study.

Essential laboratory costs will be covered by available funds from the Respiratory Medicine research budget within the Research and Development department at Northumbria Healthcare NHS Foundation Trust.

External funding applications have been made to Chiesi Limited and GlaxoSmithKline for additional desirable research costs.

None of the funders have had, or will have, any influence on the design or conduct of the study, and none of the participant data will be shared with funders.

11.9 Indemnity

Northumbria Healthcare NHS Foundation Trust (NHCT) has liability for clinical negligence. NHS Indemnity covers NHS staff and medical academic staff with honorary contracts for potential liability in respect of negligent harm arising from the conduct of the study.

As Sponsor, NHCT will provide indemnity in respect of potential liability and negligent harm arising from study management.

Indemnity in respect of potential liability arising from negligent harm related to study design is provided by NHCT.

11.10 Amendments

All amendments will be discussed with the Chief Investigator who will be responsible for the final decision to make an amendment and whether the amendment is substantial or non-substantial.

When necessary, approval for amendments to the study protocol and/or original supporting documents will be obtained from an appropriate Research Ethics Committee.

An amendment history will be recorded and the most up to date versions of protocols and supporting documents, and previous versions, will be stored and available for audit purposes.

11.11 Post study care

This study will be conducted in accordance with relevant regulations, Good Clinical Practice and the principles of the Declaration of Helsinki.

11.12 Access to the final study dataset

The CI, PI, host organisation and statistician will have access to the full dataset, for the duration of the trial.

12. Dissemination Policy

12.1 Dissemination Policy

Upon completion of the study, and with the PPI representatives, we will facilitate dissemination of the results to people with COPD. Results will be fully anonymised and shared with study participants, presented at conferences, and published in medical journals. We will engage the relevant British Thoracic Society Specialist Advisory Groups, British Lung Foundation and the National Institute for Health and Care Excellence to share the impact of the study.

12.2 Authorship Eligibility Guidelines

The CI and NHCT will be writing manuscripts, abstracts, press releases and any other publications arising from the study. Authorship will be determined in accordance with the International Committee of Medical Journal Editors guidelines and other contributors will be acknowledged.

13. References

1. The top 10 causes of death. World Health Organization; 2020. Available from: The top 10 causes of death (who.int)
2. British Lung Foundation. Lung disease in the UK – big picture statistics. Available from: https://statistics.blf.org.uk/lung-disease-uk-big-picture.
3. Celli BR, Fabbri LM, Aaron SD, et al. An Updated Definition and Severity Classification of Chronic Obstructive Pulmonary Disease Exacerbations: The Rome Proposal. *Am J Respir Crit Care Med* 2021; **204**(11): 1251-8.
4. Zarrintan S. An Invited Commentary on: Moderate and severe exacerbations have a significant impact on health-related quality of life, utility, and lung function in patients with chronic obstructive pulmonary disease: A meta-analysis. Int J Surg. 2020 Jun;78:166-167.
5. Owusuaa C, Dijkland SA, Nieboer D, van der Rijt CCD, van der Heide A. Predictors of mortality in chronic obstructive pulmonary disease: a systematic review and meta-analysis. BMC Pulm Med. 2022 Apr 4;22(1):125.
6. Echevarria C, Steer J, Heslop-Marshall K, Stenton SC, Hickey PM, Hughes R, et al. The PEARL score predicts 90-day readmission or death after hospitalisation for acute exacerbation of COPD. Thorax. 2017;72:686-93.
7. Respiratory disease. NHS England; 2023. Available from: NHS England » Respiratory disease
8. Forum of International Respiratory Societies (FIRS). The global impact of respiratory disease. Third Edition. European Respiratory Society, 2021 available at: https://www.who.int/gard/publications/The_Global_Impact_of_Respiratory_Disease.pdf
9. Yang IA, Clarke MS, Sim EH, Fong KM. Inhaled corticosteroids for stable chronic obstructive pulmonary disease. *Cochrane Database Syst Rev* 2012; **7**(7): CD002991.
10. Dransfield MT, Bourbeau J, Jones PW, et al. Once-daily inhaled fluticasone furoate and vilanterol versus vilanterol only for prevention of exacerbations of COPD: two replicate double-blind, parallel-group, randomised controlled trials. *Lancet Respir Med* 2013; **1**(3): 210-23.
11. Lipson DA, Barnhart F, Brealey N, Brooks J, Criner GJ, Day NC, Dransfield MT, Halpin DMG, Han MK, Jones CE, Kilbride S, Lange P, Lomas DA, Martinez FJ, Singh D, Tabberer M, Wise RA, Pascoe SJ; IMPACT Investigators. Once-Daily Single-Inhaler Triple versus Dual Therapy in Patients with COPD. N Engl J Med. 2018 May 3;378(18):1671-1680.
12. Rabe KF, Martinez FJ, Ferguson GT, Wang C, Singh D, Wedzicha JA, Trivedi R, St Rose E, Ballal S, McLaren J, Darken P, Aurivillius M, Reisner C, Dorinsky P; ETHOS Investigators. Triple Inhaled Therapy at Two Glucocorticoid Doses in Moderate-to-Very-Severe COPD. N Engl J Med. 2020 Jul 2;383(1):35-48.
13. Vestbo J, Papi A, Corradi M, et al. Single inhaler extrafine triple therapy versus long-acting muscarinic antagonist therapy for chronic obstructive pulmonary disease (TRINITY): a double-blind, parallel group, randomised controlled trial. *Lancet* 2017; 389(10082): 1919-29
14. Bafadhel M, Peterson S, De Blas MA, Calverley PM, Rennard SI, Richter K, Fagerås M. Predictors of exacerbation risk and response to budesonide in patients with chronic

obstructive pulmonary disease: a post-hoc analysis of three randomised trials. Lancet Respir Med. 2018 Feb;6(2):117-126.
15. Pavord ID, Chanez P, Criner GJ, Kerstjens HAM, Korn S, Lugogo N, Martinot JB, Sagara H, Albers FC, Bradford ES, Harris SS, Mayer B, Rubin DB, Yancey SW, Sciurba FC. Mepolizumab for Eosinophilic Chronic Obstructive Pulmonary Disease. N Engl J Med. 2017 Oct 26;377(17):1613-1629.
16. Bhatt SP, Rabe KF, Hanania NA, Vogelmeier CF, Cole J, Bafadhel M, Christenson SA, Papi A, Singh D, Laws E, Mannent LP, Patel N, Staudinger HW, Yancopoulos GD, Mortensen ER, Akinlade B, Maloney J, Lu X, Bauer D, Bansal A, Robinson LB, Abdulai RM; BOREAS Investigators. Dupilumab for COPD with Type 2 Inflammation Indicated by Eosinophil Counts. N Engl J Med. 2023 Jul 20;389(3):205-214.
17. Singh D, Agusti A, Martinez FJ, Papi A, Pavord ID, Wedzicha JA, Vogelmeier CF, Halpin DMG. Blood Eosinophils and Chronic Obstructive Pulmonary Disease: A Global Initiative for Chronic Obstructive Lung Disease Science Committee 2022 Review. Am J Respir Crit Care Med. 2022 Jul 1;206(1):17-24.
18. Suissa S, Ernst P. Precision Medicine Urgency: The Case of Inhaled Corticosteroids in COPD. Chest. 2017;152(2):227-31.
19. Global Strategy for Prevention, Diagnosis and Management of COPD: 2023 Report. Global Initiative for Chronic Obstructive Lung Disease, 2023.
20. Schumann DM, Tamm M, Kostikas K, Stolz D. Stability of the Blood Eosinophilic Phenotype in Stable and Exacerbated COPD. Chest. 2019 Sep;156(3):456-465.
21. Echevarria C, Steer J, Prasad A, Quint JK, Bourke SC. Admission blood eosinophil count, inpatient death and death at 1 year in exacerbating patients with COPD. Thorax. 2023 Jul 24:thorax-2022-219463.
22. Prasad A, Echevarria C, Steer J, Bourke SC. Blood eosinophil counts during severe exacerbations do not reflect stable state inflammatory phenotype in COPD. Eur Respi J 2022; 60: Supple. 66, 1870.
23. Bass DA, Gonwa TA, Szejda P, Cousart MS, DeChatelet LR, McCall CE. Eosinopenia of acute infection: Production of eosinopenia by chemotactic factors of acute inflammation. Journal of Clinical Investigation. 1980;65(6):1265-71
24. Godlowski ZZ. Eosinopenia caused by adrenaline infusion and by insulin hypoglycaemia. British medical journal 1948;1(4540):46-8
25. Lipson DA, Crim C, Criner GJ, et al. Reduction in All-Cause Mortality with Fluticasone Furoate/Umeclidinium/Vilanterol in Patients with Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 2020; **201**(12): 1508-16.
26. Van Rossem I, Hanon S, Verbanck S, Vanderhelst E. Blood Eosinophil Counts in Chronic Obstructive Pulmonary Disease: Adding Within-Day Variability to the Equation. Am J Respir Crit Care Med. 2022 Mar 15;205(6):727-729.
27. Chipps BE, Jarjour N, Calhoun WJ, Iqbal A, Haselkorn T, Yang M, Brumm J, Corren J, Holweg CTJ, Bafadhel M. A Comprehensive Analysis of the Stability of Blood Eosinophil Levels. Ann Am Thorac Soc. 2021 Dec;18(12):1978-1987.

14. Appendices

1. Schedule of Activities

	Admission Day 1 (EC & NEC)	Day 3 (EC)	Day 7 (EC)	Day 14 (EC)	Day 21 (EC)	Day 28 (EC)	Day 28 (NEC)	Day 42 (EC)
Screening	X							
Consent	X							
Eligibility review	X							
Demographics	X							
Co-Morbidities	X							
Admission indices	X							
COPD History	X							
Clinical History	X			X		X	X	X
Full medication history	X			X		X	X	X
Prednisolone and antibiotic therapy	X	X	X	X	X	X	X	X
FBC within 2 years during severe ECOPD	X							
FBC	X	X	X	X	X	X	X	X
CRP	X	X	X	X	X	X	X	X
Procalcitonin	X							
Fibrinogen	X							
Lactate	X							
Copeptin	X					X	X	
Cortisol	X							
IgE	X							
Overnight urinary metanephrines	X					X	X	
Biofire Respiratory 2.1 Panel	X							
Streptococcal antigen	X							
Sputum microbiology and purulence	X							
Cytokine panel	X					X	X	
Spirometry	X					X		

FeNO	**X**			**X**		**X**		
CAT Score	**X**			**X**		**X**	**X**	
eMRCD Score	**X**			**X**		**X**	**X**	
Rockwood clinical frailty scale	**X**			**X**		**X**	**X**	

EC = Eosinopenic cohort (n = 100) NEC = Non-eosinopenic cohort (n = 100)