Asthma: Phenotyping Exacerbations 3

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SYNOPSIS

Title	Asthma: Phenotyping Exacerbations 3
Acronym	APEX 3
Short title	Asthma: Phenotyping Exacerbations 3
Chief Investigator	Dr Matthew Martin
Objectives	Primary Objective • To describe the symptom burden, lung function, inflammatory profile, and infection status in patients admitted to hospital with suspected asthma attacks. Secondary Objectives • Determine the proportion of patients hospitalised with suspected asthma attacks likely due to non-asthma pathology. • Describe and assess the incidence of extra-pulmonary co-morbidities in patients hospitalised with asthma attacks. • Assess incidence of risk factors for attacks based on patients' previous history. • Assess patients' adherence with asthma medications. • Compare the use of Forced Oscillation Technique (FOT) as a non-effort dependent measure of airway obstruction in suspected severe asthma attacks to a standard measure of airway obstruction Peak Expiratory Flow Rate (PEFR). • Compare the use of Fractional Exhaled Nitric Oxide (FeNO) as a measure of airway inflammation against sputum and blood eosinophils. • Describe objective and symptomatic treatment response and recovery from severe asthma attacks following hospital discharge.
Trial Configuration	A Single-Centre, Longitudinal, Observational Study
Setting	Secondary Care.
Sample size estimate	This is an observational, hypothesis-generating study, and due to the lack of previous data on this subject, a meaningful sample size calculation cannot be performed.
Number of participants	100

Eligibility criteria	Inclusion Criteria: • The ability to give fully informed consent • Male or female aged ≥ 18 years of age. • Admission with a suspected acute asthma attack. • Able (in the investigator's opinion) and willing to comply with clinical investigation requirements. Exclusion Criteria • Other clinically significant respiratory diseases including predominant Chronic Obstructive Pulmonary Disease (COPD) and bronchiectasis. • Any other clinically significant medical disease or uncontrolled concomitant disease that is likely, in the investigator's opinion, to impact the ability to participate in the study or the study results. • Pregnant women, lactating women or women who are planning to become pregnant. • Investigator determined apparent other cause for admission. • Acute COVID infection. • Non-English-speaking participants who are unable to comprehend the reasons for the study due to limitations in understanding the English language.
Description of interventions	• The collection of medical history, demographic data, and concomitant medications. • The incidence of upper airway symptoms will be assessed using the Sino-nasal Outcome Test (SNOT). • The assessment of the incidence of Gastroesophageal Reflux Disease using the GERDQ questionnaire. • The assessment of the incidence of vocal cord dysfunction using the Pittsburgh Vocal Cord Dysfunction (VCD) Index. • The assessment of the incidence of dysfunctional breathing using the Nijmegen dysfunctional questionnaire. • The assessment of the incidence of anxiety and depression using the Hospital Anxiety and Depression Scale (HADS). • The assessment of the quality of life using the Acute Asthma Quality of Life Questionnaire. • The assessment of medication adherence using the Medication Adherence Report Scale. • The assessment of lung function by performing Fractional Exhaled Nitric Oxide (FeNO), Forced Oscillation Technique (FOT), Peak Expiratory Flow Rate (PEFR) and Spirometry. • The collection of blood samples for laboratory analysis and storage for future research. • The collection of sputum samples for sputum differential cell count, bacterial culture and sensitivity and storage for future research. • The collection of nasal absorption samples for future cytokine profiling. • The collection of samples via nasal lavage and throat swabs for viral identification. • The collection of optional nasal brushing samples for RNA and DNA extraction.
Duration of study	Study duration: from January 2022 to July 2023.

Randomisation and blinding	Not Applicable
Outcome measures	• Assessment of cytokine levels in nasal lining fluid at attack and recovery visits. • The measurement of vitamin D levels in the blood on admission to the hospital. • Assessment of evidence of infection: Throat swab/nasal lavage for viral PCR, sputum for bacterial culture and sensitivity, CRP. • Assessment of incidence of extra-pulmonary co-morbidities: Questionnaires (SNOT, GERDQ, Pittsburgh VCD index, Nijmegen, HADS) and BMI. • A specific asthma history (polypharmacy, previous asthma attacks, triggers, Personalised Asthma Action Plan) for the assessment of risk factor for attack. • Assessment of adherence to medications (MARS, prescription records, inhaler technique) for the assessment of behavioural attack traits. • Assessment of airway obstruction at attack and recovery visits using peak expiratory flow rate (PEFR), spirometry, physician recorded expiratory wheeze and Forced Oscillation Technique (FOT). • Assessment of airway inflammation at attack and recovery visits using Fractional Exhaled Nitric Oxide (FeNO), sputum inflammatory cell count and blood eosinophil count. • Assessment of symptom burden at attack and recovery visits using the Acute AQLQ questionnaire.
Statistical methods	The main outcomes of the exacerbation are descriptive in nature, and no statistical tests are planned. We will, however, compare key findings at exacerbation visits with those undertaken at the follow-up visits, including: Paired tests (paired t-test or McNemar) will be used to examine changes in fractional exhaled nitric oxide (FeNO), parameters derived from Forced Oscillation Technique (FOT), the Acute Asthma Quality of Life Questionnaire (Acute AQLQ), sputum differential cell count, nasal absorption, haematology, and clinical chemistry values.

ABBREVIATIONS

Acute AQLQ	Acute Asthma Quality of Life Questionnaire
AE	Adverse Event
CI	Chief Investigator overall
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CRP	C-Reactive Protein
DMC	Data Monitoring Committee
FeNO	Fractional Exhaled Nitric Oxide
FEV1	Forced Expiratory Volume in 1 Second
FOT	Forced Oscillation Technique
GCP	Good Clinical Practice
GERDQ	Gastroesophageal Reflux Disease Questionnaire
HADS	Hospital Anxiety and Depression Scale
ICF	Informed Consent Form
MARS	Medicines Adherence Report Scale
MCS	Microscopy Culture Sensitivities
NHS	National Health Service
PI	Principal Investigator at a local centre
Pittsburgh (VCD) Index	Pittsburgh Vocal Cord Dysfunction (VCD) Index
PEFR	Peak Expiratory Flow Rate
PIS	Participant Information Sheet
PCR	polymerase chain reaction
REC	Research Ethics Committee
R&D	Research and Development department
SAE	Serious Adverse Event

SNOT	Sino-nasal Outcome Test
T2	Type 2
WCC	White Cell Count

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STUDY BACKGROUND INFORMATION AND RATIONALE

Asthma is a common condition affecting 9.8 million people in the United Kingdom[1]. Asthma attacks cause nearly 1500 deaths per year in the UK[2] and account for over 90,000 hospital admissions and 1800 intensive care admissions costing at least 1 billion pounds a year[3].

Non-asthma conditions such as Vocal Cord Dysfunction[4] and Dysfunctional Breathing Pattern[5] can mimic severe asthma and lead to hospital admission and inappropriate asthma treatment with potentially harmful high dose corticosteroids. However, the proportion of patients admitted with these asthma mimics is unclear.

The use of biomarkers to guide treatment in stable asthma is now commonplace, but this prospect has not been fully explored in acute asthma attacks. Determining the type of inflammation underlying an attack can, however be tricky because many patients receive oral corticosteroid prior to, or soon after admission, which promptly and markedly affects the most accessible type 2 asthma (T2) biomarkers[7], the blood eosinophil count. Measurement of another T2 biomarker, fractional exhaled nitric oxide (FENO), during an attack may prove a useful measure of ongoing T2 airway inflammation, but this strategy requires assessment.

Airway obstruction in asthma attacks is assessed and confirmed by measuring peak expiratory flow and/or forced expiratory volumes. These measures are effort-dependent and can, therefore, be unreliable and misleading if improperly performed. We aim to evaluate the potential feasibility and utility of alternative diagnostic methods of assessing airway obstruction and measuring T2 biomarkers to see if these allow the distinction between actual asthma attacks and asthma attack mimics and also if they can distinguish different types of asthma attacks.

The pattern of recovery following asthma attacks is also not fully understood. Studies have reported varying recovery times[8] and inconsistencies in the response of airway obstruction[9] and T2 biomarkers[10] to corticosteroid treatment in acute asthma.

We hypothesise that a significant proportion of patients admitted to hospital with suspected asthma attacks have an alternate cause for their clinical presentation. To further explore this, we propose an observational study to describe and investigate the characteristics of patients admitted with suspected severe asthma. We will assess the utility of point of care, non-invasive biomarker measurements in identifying pulmonary and extrapulmonary traits in patients hospitalised due to presumed asthma attack. A variety of study assessments will be performed at the baseline visit (exacerbation visit), and two follow up visits following hospital discharge. Results of study assessments obtained during each study visit will be compared and analysed. This is the first study to prospectively investigate acute asthma both during and following exacerbation episodes. This will allow a comprehensive understanding and description of pulmonary and extrapulmonary traits as well as the pattern of recovery following asthma attacks.

STUDY OBJECTIVES AND PURPOSE

PURPOSE

The purpose of this study is to describe the biological and physiological changes (pulmonary traits) and physical and psychological comorbidities (extrapulmonary traits) seen in patients requiring hospitalisation for presumed asthma attacks. The recovery and response to standard treatment for asthma attacks following discharge from the hospital will also be described.

The feasibility and utility of measuring non-invasive point of care biomarkers as a means of confirming and quantifying airway obstruction and detecting T2 airway inflammation will be assessed. Blood and sputum samples will be obtained for biomarker profiling.

This will enable the most comprehensive description of asthma attack phenotypes requiring hospitalisation and recovery from these events to date.

PRIMARY OBJECTIVE

- To describe the symptom burden, lung function, inflammatory profile, and infection status in patients admitted to hospital with suspected asthma attacks.

SECONDARY OBJECTIVES

- Determine the proportion of patients hospitalised with suspected asthma attacks likely due to non-asthma pathology.
- Describe and assess the incidence of extra-pulmonary co-morbidities in patients hospitalised with asthma attacks.
- Assess incidence of risk factors for attacks based on patients' previous history.
- Assess patients' adherence with asthma medications.
- Compare the use of Forced Oscillation Technique (FOT) as a non-effort dependent measure of airway obstruction in suspected severe asthma attacks to a standard measure of airway obstruction Peak Expiratory Flow Rate (PEFR).
- Compare the use of Fractional Exhaled Nitric Oxide (FeNO) as a measure of airway inflammation against sputum and blood eosinophils.
- Describe objective and symptomatic treatment response and recovery from severe asthma attacks following hospital discharge.

DETAILS OF PRODUCT(S)

Description of Products

Saline

Sterile saline solution of varying concentrations will be used in the sputum induction and nasal lavage procedures. This will be prepared from a commercially available 7% solution.

Salbutamol

An inhaled short-acting beta$_2$agonist, Salbutamol, will be administered. This will be given prior to the sputum induction. All participants will have salbutamol prescribed to them as part of their routine care.

Packaging and labelling

All products described above are those used in standard NHS practice.

Storage, dispensing and return

All products described above are routinely stored in the Nottingham Respiratory Research Unit (NRRU) Clinical Trials Unit (CTU) at the City Hospital campus.

Placebo
No Placebo is being used.

Known Side Effects

Sputum induction in patients with asthma can cause a small fall in lung function (FEV1). This risk will be minimised with pre-treatment with Salbutamol.

Known side effects of beta2agonists, such as Salbutamol, include fine tremor, headaches, palpitations and tachycardia.

STUDY DESIGN

STUDY CONFIGURATION

This is a single centre observational, longitudinal study. The study will aim to recruit 100 patients hospitalised due to a suspected asthma attack. They will be identified from the Respiratory admission units or inpatient wards at the Nottingham City Hospital or the emergency department at Queens Medical Centre.

The initial assessment will take place at the earliest timepoint possible following hospital admission with a suspected asthma attack. A range of investigations, including lung function tests, blood tests, and questionnaires, will be performed. A follow-up visit will be arranged, which will take place approximately two weeks after hospital discharge at the Nottingham Respiratory Research Unit, at which further lung function tests, blood tests and questionaries will be performed.

A subsequent telephone follow-up visit will be scheduled at around four weeks after hospital discharge, at which additional questionnaires will be performed to determine the degree of symptomatic recovery. A PhD student will perform the majority of study recruitment and study interventions under the fellow's direct supervision.

Primary endpoint

As this is an observational study with a primary objective that is descriptive in nature, there are no endpoints, and all outcome measures are equally essential in describing the primary objective; outcome measures include:

- Assessment of cytokine levels in nasal lining fluid at attack and recovery visits.
- The measurement of vitamin D levels in the blood on admission to the hospital.
- Assessment of evidence of infection: Throat swab/nasal lavage for viral PCR, sputum for bacterial culture and sensitivity, CRP.
- Assessment of incidence of extra-pulmonary co-morbidities: Questionnaires (SNOT, GERDQ, Pittsburgh VCD index, Nijmegen, HADS) and BMI.
- A specific asthma history (polypharmacy, previous asthma attacks, triggers, Personalised Asthma Action Plan) for the assessment of risk factor for attack.
- Assessment of adherence to medications (MARS, prescription records, inhaler technique) for the assessment of behavioural attack traits.
- Assessment of airway obstruction at attack and recovery visits using peak expiratory flow rate (PEFR), spirometry, physician recorded expiratory wheeze and Forced Oscillation Technique (FOT).
- Assessment of airway inflammation at attack and recovery visits using Fractional Exhaled Nitric Oxide (FeNO), sputum inflammatory cell count and blood eosinophil count.
- Assessment of symptom burden at attack and recovery visits using the Acute AQLQ questionnaire.

Safety endpoints

As this is an observational study, there are no defined safety endpoints, although investigations at the time of exacerbation will not be performed if there are safety concerns.

Stopping rules and discontinuation

As this is an observational study, there are no planned stopping criteria other than for individual participants who wish to stop, or who cannot be contacted.

RANDOMIZATION AND BLINDING

There is no randomisation in this cohort study.

STUDY MANAGEMENT

The study will be managed by a Clinical Management Group, who will meet at least monthly to monitor the progress of the study. This will consist of the Chief Investigator, the Co-Investigator (s), the Asthma Research Development Manager, the Database Manager and the Research Nursing Team.

The Chief Investigator has overall responsibility for the study and shall oversee all study management. The data custodian will be the Chief Investigator.

DURATION OF THE STUDY AND PARTICIPANT INVOLVEMENT

Study Duration:

We plan to recruit approximately 100 patients over a period of 18 months. The total duration of the study will be approximately 2.5 years. The study will begin in January 2022 and finish in July 2023.

Participant Duration:

The baseline (Attack) visit will take place at the earliest timepoint possible following hospital admission. A follow up visit will be arranged, approximately 2 weeks after hospital discharge. A third telephone follow up visit will be arranged approximately 4 weeks after hospital discharge.

Study participants will be participating in the study from the time of consent (earliest timepoint possible following hospital admission) up to approximately four weeks following hospital discharge. This could be from 4-12 weeks depending on the length of hospital stay. The maximum length of participation in the study is 12 weeks per participant.

End of the Trial

The end of the study will be the last visit of the last participant.

SELECTION AND WITHDRAWAL OF PARTICIPANTS

Recruitment

Participants will be identified and recruited from admission units or inpatient wards at the Nottingham City Hospital, or the emergency department at Queens Medical Centre. The initial approach will be from a member of the patient's usual care team (which may include the investigator).

The investigator or their nominee, e.g., from the research team or a member of the participant's usual care team, will inform the participant or their nominated representative (other individual or other body with appropriate jurisdiction), of all aspects pertaining to participation in the study.

It will be explained to the potential participant that entry into the trial is entirely voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time, but attempts will be made to avoid this occurrence. In the event of their withdrawal, it will be explained that their data collected so far cannot be erased and we will seek consent to use the data in the final analyses where appropriate.

Eligibility criteria

Inclusion Criteria

- The ability to give fully informed consent
- Male or female aged ≥ 18 years of age.
- Admission with a suspected acute asthma attack.
- Able (in the investigator's opinion) and willing to comply with clinical investigation requirements.

Exclusion Criteria

- Other clinically significant respiratory diseases including predominant Chronic Obstructive Pulmonary Disease (COPD) and bronchiectasis.
- Any other clinically significant medical disease or uncontrolled concomitant disease that is likely, in the investigator's opinion, to impact the ability to participate in the study or the study results.
- Pregnant women, lactating women or women who are planning to become pregnant.
- Investigator determined apparent other cause for admission.
- Acute COVID infection.
- Non-English-speaking participants who are unable to comprehend the reasons for the study due to limitations in understanding the English language.

Expected duration of participant participation

Study participants will be participating in the study for 4-12 weeks depending on the length of hospital stay. The maximum length of participation in the study is 12 weeks per participant.

Removal of participants from therapy or assessments/Participant Withdrawal

There are no plans to specifically withdraw participants from the study, unless they withdraw consent or cannot be contacted by the research team, despite several attempts.

Participants may be withdrawn from the trial either at their own request or at the discretion of the Investigator. The participants will be made aware that this will not affect their future care. Participants will be made aware (via the information sheet and consent form) that should they withdraw the data collected to date cannot be erased and may still be used in the final analysis.

Informed consent

All participants will provide written informed consent. The Informed Consent Form will be signed and dated by the participant before they enter the trial. The Investigator will explain the details of the trial and provide a Participant Information Sheet, ensuring that the participant has sufficient time to consider participating or not. The Investigator will answer any questions that the participant has concerning study participation.

Informed consent will be collected from each participant before they undergo any interventions (including physical examination and history taking) related to the study. One copy of this will be kept by the participant, one will be kept by the Investigator, and a third will be retained in the patient's hospital records.

Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the trial, continuing consent will be obtained using an amended Consent form which will be signed by the participant.

Loss of capacity to consent during the course of the study will be managed as detailed on page 27 of this protocol.

STUDY TREATMENT AND REGIMEN

The study will consist of a Baseline (attack) Visit, and two follow up visits. The baseline visit, which will take place at the earliest timepoint possible following hospital admission at the Nottingham City Hospital, will include a range of investigations, including lung function tests, blood tests and questionnaires.

The second visit will take place approximately two weeks after hospital discharge at the Nottingham Respiratory Research Unit, at which further lung function tests, blood tests and questionnaires will be performed.

The third visit (telephone follow up) will be arranged approximately four weeks after hospital discharge and will consist of questionnaires to determine the degree of symptomatic recovery.

Investigations included in the baseline visit, second visit and third visit (telephone follow up) are listed in figure 1 and table 1. Patients admitted on more than one occasion with an attack will undergo attack visit investigation on each occasion if they consent to this.

Study Assessments Algorithm.

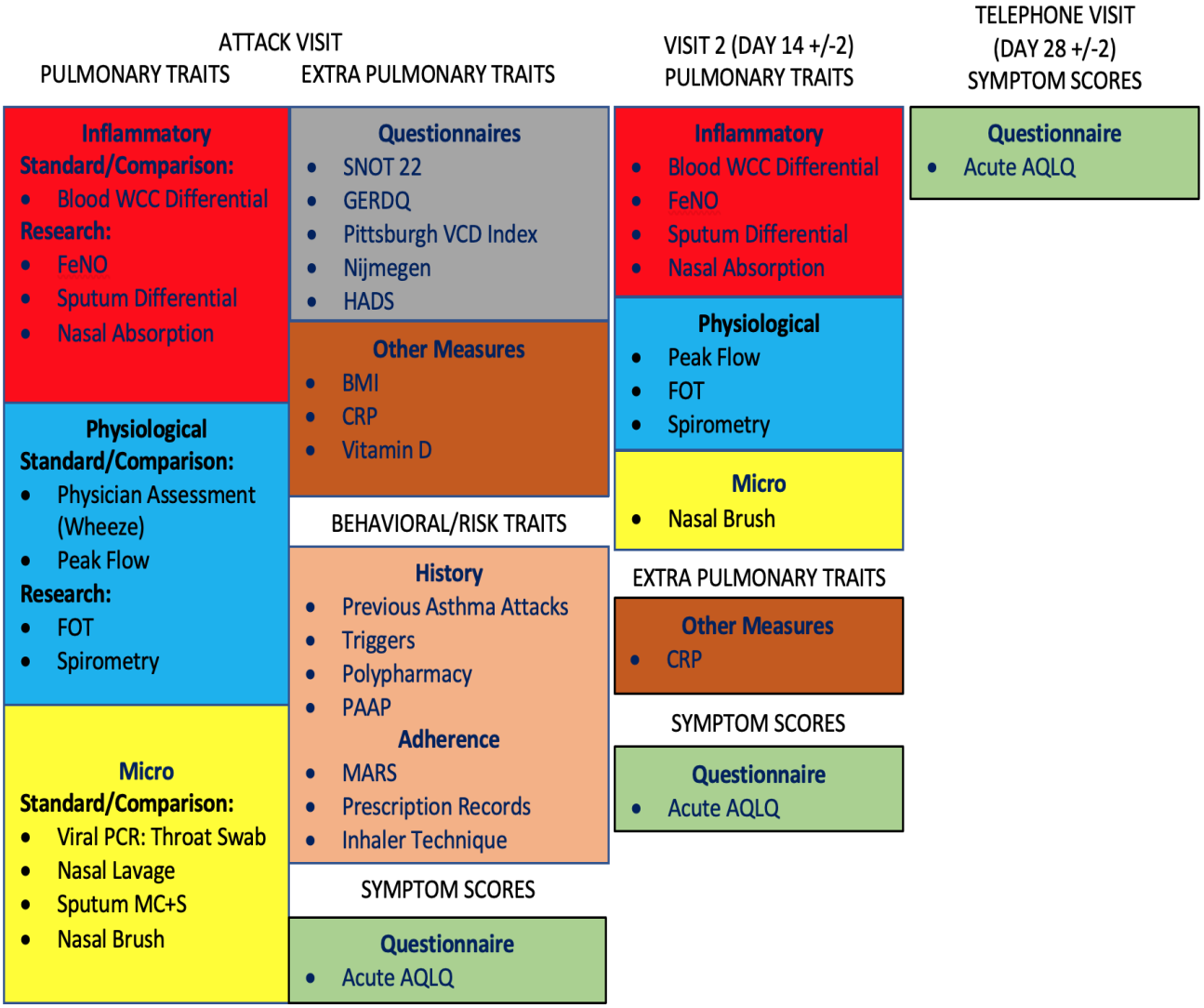


Figure 1: Study assessments algorithm.
Definition of abbreviations: WCC = white cell count; FeNO = fractional exhaled nitric oxide; FOT = forced oscillation technique; PCR = polymerase chain reaction; MC+S = microscopy culture + sensitivity; SNOT = Sino-nasal outcome test; GERDQ = gastroesophageal reflux disease questionnaire; VCD = vocal cord dysfunction; HADS = hospital anxiety and depression scale; BMI = body mass index; CRP = c-reactive protein; PAAP = Personalised Asthma Action Plan; MARS = medication adherence report scale; AQLQ = asthma quality of life questionnaire.

Schedule of study assessments.

Visit	Baseline visit	Second visit	Telephone visit
Time	Attack	Day 14 +/-2	Day 28 +/-2
Investigations Performed			
Informed Consent	X		
Medical History	X		
Concomitant Medications	X		
Demographic Data	X		
Sino-nasal Outcome Test	X		
Gastroesophageal Reflux Disease Questionnaire	X		
Pittsburgh Vocal Cord Dysfunction Index	X		
Nijmegen	X		
Hospital Anxiety and Depression Scale	X		
Medication Adherence Report Scale	X		
Inhaler Technique check	X		
Acute Asthma Quality of Life Questionnaire	X	X	X
Spirometry and PEFR	X	X	
Fractional Exhaled Nitric Oxide (FeNO)	X	X	
Forced Oscillation Technique (FOT)	X	X	
Clinical Chemistry (C- Reactive Protein)	X	X	
Sputum (induced or spontaneous) for differential cell count, supernatant storage and microbiological assessment	X	X	
Blood White Cell Count Differential	X	X	
Vitamin D	X		
Viral PCR: Throat swab.	X		
Nasal lavage	X		
Nasosorption Sample	X	X	
Nasal Epithelium Samples for Genetic Analysis (optional)	X	X	

Table 1: Schedule of study assessments.

Study Assessments

Medical History, Demographics and Concomitant Medications

Medical history will include clinically significant diseases and prior surgeries and smoking history. A specific respiratory history will also be recorded. Demographic data will include age, sex and self- reported race/ethnicity. Medication use will also be collected.

Height, Weight and Blood Pressure

Height, weight and blood pressure measurements will be obtained from medical record.

Sino-nasal Outcome Test

The incidence of upper airway symptoms will be assessed using Sino-nasal Outcome Test (SNOT).

Gastroesophageal Reflux Disease questionnaire.

The incidence of Gastroesophageal Reflux Disease will be assessed using GERDQ questionnaire.

Pittsburgh Vocal Cord Dysfunction Index

The incidence of vocal cord dysfunction will be assessed using Pittsburgh Vocal Cord Dysfunction (VCD) Index

Nijmegen Questionnaire.

The incidence of dysfunctional breathing will be assessed using Nijmegen dysfunctional questionnaire.

Hospital Anxiety and Depression Scale

The incidence of anxiety and depression will be assessed using Hospital Anxiety and Depression Scale (HADS).

Medication Adherence Report Scale

Participants' adherence to medication will be assessed using the Medication Adherence Report Scale (MARS).

Acute Asthma Quality of Life Questionnaire

The impact of acute asthma on quality of life will be assessed using Acute Asthma Quality of Life Questionnaire at baseline and follow up.

Fractional Exhaled Nitric Oxide (FeNO)

FeNO will be measured using a standard device (NIOXX Vero®) in accordance with the manufacturer's guidelines. The same type of device will be used at each visit. FeNO should be performed prior to any other respiratory assessment if possible.

Forced Oscillation Technique (FOT)

FOT is a non-invasive method that evaluates resistance and reactance in the respiratory system during tidal breathing. It should be performed after FeNO, but prior to Spirometry and will be conducted according to a study SOP.

Spirometry

Spirometry will be conducted according to a study SOP. The Global Lung Function (GLI) 2012 equations will be used. Reversibility testing will not be routinely performed. Thus, asthma medications will not normally be withheld for study visits and patients should take their inhalers as usual. The same type of device will be used at each visit.

Induced and Spontaneous Sputum

Sputum induction will be performed according to an SOP, which includes measures to protect the safety of participants. These consist of pre-treatment with Salbutamol, a short acting bronchodilator, stipulated levels of lung function in order to perform the test and stopping criteria. If the participant is unable to undergo the procedure, due to safety reasons, they will be asked to produce a spontaneous sputum sample. Sputum samples will be collected for a differential cell count, bacterial culture and sensitivity and storage for future research. Procedures for sampling and processing will be detailed in a study SOP.

Laboratory Assessments

Blood will be collected for laboratory analysis, including Vitamin D, Haematology, WCC differential, C-Reactive protein. Sputum will be collected for microbiological assessment. These samples will be analysed at the local NHS laboratory.

Blood Samples for Biomarkers

Serum blood samples for biobanking will be collected at baseline (attack) and at second visits and stored for future research.

Nasal Absorption

Nasal absorption will be used to collect mucosal lining fluid from the respiratory tract for cytokines profiling at baseline (attack) and at second visits.

Nasal Lavage

Nasal lavage samples will be taken at the baseline (attack) visit. Samples will be obtained by injecting 5ml of sterile normal saline into the participant's nostrils via a nasal adaptor and then

immediately aspirated. These will be placed in a sterile container and transported to the laboratory for viral identification. This procedure will be detailed in a study SOP.

Nasal Epithelium Sample

Nasal brushings samples may be taken at baseline (attack) and at second visits for RNA and DNA extraction. This will be optional and specific consent from participants will be sought. Procedures for sampling and processing will be detailed in a study SOP.

Throat Swab

A throat swab will be taken at the baseline (attack) visit for viral identification.

Compliance and adherence

In order to assess adherence as a factor in exacerbations, a participant's beliefs and attitudes towards medicines will be explored using the Medicines Adherence Report Scale (MARS) questionnaire. Non-intentional non-compliance with usual treatment will be assessed by checking inhaler technique; poor inhaler technique will be reported to the participant's clinical team. Also, GP records will be reviewed for information on medications prescribed in the last 12 months.

Participants who do not attend study visits and then unable to be contacted despite several attempts may be withdrawn from the study.

Criteria for terminating trial

There are no planned criteria for terminating the trial, as we do not anticipate obtaining data or identifying circumstances that would necessitate closing the study early. For individual participants, discontinuation will be considered on a case-by-case basis.

TRANSPORT AND STORAGE OF THE TISSUES

Samples will be labelled in the same format, using study reference, unique patient identifier, time and date taken, and participant visit number.

All biological (blood, sputum, nasal absorption samples and nasal lavage, nasal brushes and throat swabs) samples will be stored in a linked, anonymised format and labelled using a combination of study reference, unique study identifier and cross referenced with location code numbers to permit accurate linkage to study data and the consent form. The master database will be held by the NRRU Database Manager or delegate, in a password encrypted file.

The analysis of samples will either take place in Nottingham, including University of Nottingham premises at Queens Medical Centre and also at the University of Nottingham itself, or in the laboratories of third party national and international partners, following appropriate Tissue Transfer Agreements.

Samples for NHS laboratory analysis (blood samples for haematology, clinical chemistry and sputum for microbiological assessment) will be labelled and analysed in accordance with local NHS procedures.

Once analysis has taken place, blood and sputum samples will be stored at University of Nottingham premises at Respiratory Medicine, The Clinical Sciences Building, Nottingham City Hospital, Hucknall Road, Nottingham, NG5 1PB for future research (DI William Dunn – Licence Number 12265), if participants are agreeable and sign the optional clause on the consent form.

Where participants do not agree to the future use of the samples, they will be destroyed in accordance with the Human Tissue Act, 2004.

LABORATORY ANALYSES

Sputum samples will be analyzed at NUH pathology labs for microscopy, culture and sensitivity, following a structured protocol.

Sputum supernatant will be stored in aliquots at -80 degrees centrigrade, in monitored and alarmed freezers for future cytokine analysis. Cytospin slides for the differential cell count, will be fixed, stained and counted, before being stored.

Nasal absorption samples will be analyzed in the laboratories of the University of Nottingham.

Samples from nasal lavage and the throat swabs will be analysed in Nottingham, including University of Nottingham premises at Queens Medical Centre and also at the University of Nottingham itself, or in the laboratories of third-party partners, following appropriate Tissue Transfer Agreements.

Samples from nasal brushings will be collected for RNA and DNA extraction and genetic analysis. This will be optional and specific consent from participants will be sought. Sample analysis will take place on University of Nottingham premises. Results of genetic testing will not be shared with participants, however, participants' GP/Consultants will be informed of any incidental findings.

Laboratory analyses will be detailed in a study laboratory manual.

STATISTICS

Methods

Data analysis will be performed by a PhD student, Mouaid Aljehani, under the supervision of Professor Tricia McKeever, Professor Tim Harrison and Dr Matthew Martin.

The main outcomes of the exacerbation are descriptive in nature, and no statistical tests are planned. We will, however, compare key findings at exacerbation visits with those undertaken at the follow-up visits, including: Paired tests (paired t-test or McNemar) will be used to examine changes in fractional exhaled nitric oxide (FeNO), parameters derived from Forced Oscillation Technique (FOT), the Acute Asthma Quality of Life Questionnaire (Acute AQLQ), sputum differential cell count, nasal absorption, haematology, and clinical chemistry values.

Sample size and justification

This is an observational, hypothesis-generating study, and due to the lack of previous data on this subject, a meaningful sample size calculation cannot be performed.

Assessment of safety

An assessment of safety to conduct study procedures will be carried out for each patient prior to recruitment in study and again prior to potential sputum induction. Participants will specifically be asked about the need for further urgent or emergency assessments or treatment for acute asthma at their follow up visit.

Procedures for missing, unused and spurious data

Values outside the accepted range will be investigated for accuracy. As the focus of this study is descriptive, complete cases analyses will be the primary method for data analysis.

ADVERSE EVENTS

As this is an observational study, only unexpected AE's and all SAE's related directly to the trial investigations will be collected and reported.

Definitions

Adverse Events

An adverse event is any unfavourable and unintended sign, symptom, syndrome or illness that develops or worsens during the period of observation in the study.

An AE does include a / an:

1. An exacerbation of a pre-existing illness.

2. An increase in frequency or intensity of a pre-existing episodic event or condition.

3. A condition detected or diagnosed after medicinal product administration even though it may have been present prior to the start of the study.

4. A continuous persistent disease or symptoms present at baseline that worsen following the start of the study.

An AE does not include a / an:

1. A medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); but the condition that lead to the procedure is an AE.

2. A pre-existing disease or conditions present or detected at the start of the study that did not worsen.

3. Situations where an untoward medical occurrence has not occurred (e.g., hospitalisations for cosmetic elective surgery, social and / or convenience admissions).

4. A disease or disorder being studied or sign or symptom associated with the disease or disorder unless more severe than expected for the participant's condition.

5. An overdose of concurrent medication without any signs or symptoms.

Serious Adverse Events

A Serious Adverse Event (SAE) is any adverse event occurring following study mandated procedures, having received the intervention that results in any of the following outcomes:

1. Death

2. A life-threatening adverse event

3. Inpatient hospitalisation or prolongation of existing hospitalisation

4. A disability / incapacity

5. A congenital anomaly in the offspring of a participant

Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

All adverse events will be assessed for seriousness, expectedness and causality.

A distinction is drawn between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined using the criteria above. Hence, a severe AE need not necessarily be serious.

Causality

Not related or improbable: a clinical event including laboratory test abnormality with temporal relationship to trial intervention administration which makes a causal relationship incompatible or for which other treatments, chemicals or disease provide a plausible explanation**.** This will be counted as “unrelated” for notification purposes.

Possible: a clinical event, including laboratory test abnormality, with temporal relationship to trial intervention administration which makes a causal relationship a reasonable possibility, but which could also be explained by other interventions, chemicals or concurrent disease. This will be counted as “related” for notification purposes.

Probable: a clinical event, including laboratory test abnormality, with temporal relationship to trial intervention administration which makes a causal relationship a reasonable possibility, and is unlikely to be due to other interventions, chemicals or concurrent disease. This will be counted as “related” for notification purposes.

Definite: a clinical event, including laboratory test abnormality, with temporal relationship to trial intervention administration which makes a causal relationship a reasonable possibility, and which can definitely not be attributed to other causes. This will be counted as “related” for notification purposes.

With regard to the criteria above, medical and scientific judgment shall be used in deciding whether prompt reporting is appropriate in that situation.

Reporting of adverse events

Participants will be asked to contact the study site immediately in the event of any serious adverse event. All adverse events will be recorded and closely monitored until resolution, stabilisation, or until it has been shown that the study intervention is not the cause. The Chief Investigator shall be informed immediately of any serious adverse events and shall determine seriousness and causality in conjunction with any treating medical practitioners.

All trial investigation related serious adverse events will be recorded and reported to the REC as part of the annual reports. Unexpected serious adverse events will be reported within the timeframes to the REC as stated below. The Chief Investigator shall be responsible for all adverse event reporting.

Trial Investigation Related SAEs

A serious adverse event that is unexpected in its severity and seriousness *and* deemed directly related to or suspected to be related to the trial investigation shall be reported to the ethics committee that gave a favourable opinion as stated below.

The event shall be reported immediately of knowledge of its occurrence to the Chief Investigator.

The Chief Investigator will:

- Assess the event for seriousness, expectedness and relatedness to the trial investigation.
- Take appropriate medical action, which may include halting the trial and inform the Sponsor of such action.
- If the event is deemed related to the trial investigation or device shall inform the REC using the reporting form found on the NRES web page within 7 days of knowledge of the event.
- Shall, within a further eight days send any follow-up information and reports to the REC.
- Make any amendments as required to the study protocol and inform the REC as required

Participant removal from the study due to adverse events

Any participant who experiences an adverse event may be withdrawn from the study at the discretion of the Investigator.

ETHICAL AND REGULATORY ASPECTS

ETHICS COMMITTEE AND REGULATORY APPROVALS

The trial will not be initiated before the protocol, informed consent forms and participant and GP information sheets have received approval / favourable opinion from the Research Ethics Committee (REC), the respective National Health Service (NHS) or other healthcare provider's Research & Development (R&D) department, and the Health Research Authority (HRA) if required. Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed consent forms and participant and GP information sheets (if appropriate) have been reviewed and received approval / favourable opinion from the REC and R&D departments. A protocol amendment

intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the REC are notified as soon as possible and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of Good Clinical Practice, and the UK Department of Health Policy Framework for Health and Social Care, 2017.

INFORMED CONSENT AND PARTICIPANT INFORMATION

The process for obtaining participant informed consent will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. The investigator or their nominee and the participant shall both sign and date the Informed Consent Form before the person can participate in the study.

The participant will receive a copy of the signed and dated forms and the original will be retained in the Trial Master File. A second copy will be filed in the participant's medical notes and a signed and dated note made in the notes that informed consent was obtained for the trial.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to potential participant, that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No trial-specific interventions will be done before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the course of the study, and will discuss with them, whether they wish to continue with the study. If applicable they will be asked to sign revised consent forms.

If the Informed Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

There will be no official form of capacity assessment during the study. Where the research team perceive, that there are changes to a participant's well-being, likely to impact on capacity to consent, the participant will be withdrawn from the study.

RECORDS

Case Report Forms

Each participant will be assigned a trial identity code number, for use on CRFs other trial documents and the electronic database. The documents and database will also use their initials (of first and last names separated by a hyphen or a middle name initial when available) and date of birth (dd/mm/yy) and the visit.

CRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number, and Participant Trial Number (the Trial

Recruitment Log), to permit identification of all participants enrolled in the trial, in accordance with regulatory requirements and for follow-up as required
CRFs shall be restricted to those personnel approved by the Chief or local Principal Investigator and recorded on the 'Trial Delegation Log.'

All paper forms shall be filled in using black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated.
The Chief or local Principal Investigator shall sign a declaration ensuring accuracy of data recorded in the CRF.

Sample Labelling

Each participant will be assigned a trial identity code number for use on the samples, consent forms and other study documents and the electronic database. The documents and database will also use their initials (of first and last names separated by a hyphen or a middle name initial when available) and date of birth (dd/mm/yy) and visit.

Samples for NHS pathology analysis will be labelled in accordance with local NHS procedures.

Source documents

Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms, current medical records, laboratory results and records. A CRF may also completely serve as its own source data. Only trial staff as listed on the Delegation Log shall have access to trial documentation other than the regulatory requirements listed below.

Direct access to source data / documents

The CRF and all source documents, including progress notes and copies of laboratory and medical test results shall made be available at all times for review by the Chief Investigator, Sponsor's designee and inspection by relevant regulatory authorities (e.g. DH, Human Tissue Authority).

DATA PROTECTION

All trial staff and investigators will endeavour to protect the rights of the trial's participants to privacy and informed consent, and will adhere to the Data Protection Act, 2018. The CRF will only collect the minimum required information for the purposes of the trial. CRFs will be held securely, in a locked room, or locked cupboard or cabinet. Access to the information will be limited to the trial staff and investigators and relevant regulatory authorities (see above). Computer held data including the trial database will be held securely and password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one way encryption method).

Information about the trial in the participant's medical records / hospital notes will be treated confidentially in the same way as all other confidential medical information.

Electronic data will be backed up every 24 hours to both local and remote media in encrypted format.

QUALITY ASSURANCE & AUDIT

INSURANCE AND INDEMNITY

Insurance and indemnity for trial participants and trial staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but trial participants may have recourse through the NHS complaints procedures.

The University of Nottingham as research Sponsor indemnifies its staff, research participants and research protocols with both public liability insurance and clinical trials insurance. These policies include provision for indemnity in the event of a successful litigious claim for proven non-negligent harm.

TRIAL CONDUCT

Trial conduct may be subject to systems audit of the Trial Master File for inclusion of essential documents; permissions to conduct the trial; Trial Delegation Log; CVs of trial staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, correct randomisation, timeliness of visits); adverse event recording and reporting; accountability of trial materials and equipment calibration logs.

The Trial Coordinator, or where required, a nominated designee of the Sponsor, shall carry out a site systems audit at least yearly and an audit report shall be made to the Clinical Management Group.

TRIAL DATA

Monitoring of trial data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. The Trial Coordinator or where required, a nominated designee of the Sponsor, shall carry out monitoring of trial data as an ongoing activity.

Entries on CRFs will be verified by inspection against the source data. A sample of CRFs (10% or as per the study risk assessment) will be checked on a regular basis for verification of all entries made. In addition the subsequent capture of the data on the trial database will be checked. Where corrections are required these will carry a full audit trail and justification.

Trial data and evidence of monitoring and systems audits will be made available for inspection by REC as required.

RECORD RETENTION AND ARCHIVING

In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Nottingham Research Code of Conduct and Research Ethics, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The Trial Master File and trial documents held by the Chief Investigator on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Nottingham. This archive shall include all trial databases and associated meta-data encryption codes.

DISCONTINUATION OF THE TRIAL BY THE SPONSOR

The Sponsor reserves the right to discontinue this trial at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons. The Sponsor shall take advice from the Trial Steering Committee and Data Monitoring Committee as appropriate in making this decision.

STATEMENT OF CONFIDENTIALITY

Individual participant medical information obtained as a result of this study are considered confidential and disclosure to third parties is prohibited with the exceptions noted above. Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in the computer files.

Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare.

If information is disclosed during the study that could pose a risk of harm to the participant or others, the researcher will discuss this with the CI and where appropriate report accordingly.

Data generated as a result of this trial will be available for inspection on request by the participating physicians, the University of Nottingham representatives, the REC, local R&D Departments and the regulatory authorities.

PUBLICATION AND DISSEMINATION POLICY

Throughout the study, the NRRU and BRC websites will carry stories and information will be provided to participants via newsletters, telephone, and social media at regular intervals, if they give permission to be contacted. Our results will initially be shared with our PPI members and those who participated in this study. We anticipate many outputs from this study, much of which will be included in a PhD thesis, presented at scientific meetings, and prepared for publication in peer-reviewed journals and through relevant organisations, such as Asthma UK.

USER AND PUBLIC INVOLVEMENT

A specific Patient and Public Involvement (PPI) group has been formed which consists of patients with asthma. Initial meetings with the research team have been held and it is planned that these will take place at least 3 times per year, throughout the study. This group were consulted on the priority and importance of this research and have advised on the study design and the participant recruitment and retention strategy.

They have also been involved in the development of participant information and will advise on engagement activities, the dissemination of results and ongoing study conduct and management.

STUDY FINANCES

Funding source

Internal Funds

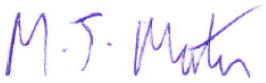
Participant stipends and payments

Participants will not be paid to participate in the trial. Travel expenses will be offered for any hospital visits in excess of usual care.

SIGNATURE PAGES

Signatories to Protocol:

Chief Investigator: (name) Matthew Martin

Signature:____  ____

Date: 22/2/22

Co- investigator: (name) _____

Signature:_____

Date: _____

Co- investigator: (name) Mouaid Aljehani

Signature: 

Date: 22/2/22

Trial Statistician: (name)__Tricia McKeever_____

Signature:__  _____

Date: __23/02/22_____

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