Exhaled Breath Metabolomic Biomarkers in the Acutely Breathless Patient
Short Title: EMBER Study

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Confidentiality Statement

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(in cases of Multi-centre studies, this must be replicated for each site)

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1. AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
	3	1/11/17	Dr Neil Greening Prof Salman Siddiqui Dr Wadah Ibrahim	
SA#02	4	1/04/18	Dr Wadah Ibrahim Amisha Singapuri	Clarifications, Typos, Additional Author. - Follow up duration extended to 6 months from 8-16 weeks. - Inclusion Criteria (i) amended to say that all patients need to be able to give consent not just the acute patients - The number of visits the Healthy Volunteers will have is 2 instead of 3. - ACQ questionnaire in the table schedule was supposed to have been the AQLQ questionnaire, however this typo was missed at the last amendment. ACQ amended to AQLQ - Added the NASA Task Load index. - CRP & FBC blood tests have been removed from the pneumonia V1b visit. - Quadriceps Ultrasound removed in Heart failure patients. - 4 Meter Gait in Heart failure subjects removed from Visit V1a and added to. This test has been

				completely removed from Healthy Volunteers. - Physical exam removed from Visit 2 in Healthy Volunteers. - Review of AE & SAE at Visit 1a removed for Paediatric patients. - Lung function clarification given that spirometry is both Pre and Post bronchodilator to match the patient information sheets. - Removed the questionnaire from the appendix of the protocol.

2. SYNOPSIS

Study Title	**Exhaled Breath Metabolomic Biomarkers in the Acutely Breathless Patient.**
Internal ref. no.	Sponsor Reference 0569
Trial Design	**Prospective Acute Care 'Real Life' Observational Study**
Trial Participants	Patients with acute breathlessness in the following (post-acute pathway confirmed) strata: (i) **Acute Heart failure**, characterised by a clinician diagnosis, and a proto-typical response to treatment. (ii) **Community acquired pneumonia**, defined as new radiological consolidation responsive to treatment according to the British Thoracic Society (BTS) community acquired pneumonia guidelines. (iii) **Adult exacerbations of asthma and COPD**, defined as a clinician diagnosis with objective confirmatory evidence of disease based upon historical or prospective measurements, requiring an acute change in treatment (e.g. antibiotics, oral corticosteroids or increased bronchodilator therapy) (iv) **Paediatric exacerbations of acute childhood wheeze and or confirmed asthma** (v) **Age and/or Home environment matched Adult/Sibling/Parent/Spousal healthy volunteers**, who have no prior history of asthma, COPD, heart failure and have not been admitted to hospital with community acquired pneumonia within 6 weeks of the baseline study visit. Additonal healthy volunteers will also be recruited from local databses. (vi) **Stable state aged matched participants** with a confirmed diagnosis of asthma, COPD or heart failure , that have not had and exacerbation of their disease within the preceding 6 weeks.
Planned Sample Size	An interim analysis was conducted in September 2017 to determine the sample size of the ember acute study. Guided by literature search, a panel of 10 pre specified aldehyde biomarkers derived from GCMS analysis were supplied. The biomarkers were normalised to a common standard and were not background subtracted. A closed formula from Hsieh *et al*, Statis med 1998, was utilised to calculate sample sizes from logistic regression models of the 10 aldehydes with acute breathlessness as the outcome measure. The sample size estimates are also relevant to acute class comparisons vs. the sum of other acute classes. Based upon the samples size estimates we would have an 80% power at the 5

	% significance level to detect an odds ratio of association of 1.2 for a given disease class with minimum of 55 patients per disease class. **Discovery Phase (years 1-2):** Acute Heart failure (n=55-100), Community acquired pneumonia (n=55-100), adult exacerbations of asthma and COPD (n=110-200), paediatric exacerbations of acute childhoodwheeze and/or confirmed asthma (n=50-100), Age/Home environment matched Adult/Sibling/Parent/Spousal healthy volunteers (n=55-150 adults/50 children) **Replication Phase (years 3-4):** Acute Heart failure (n=55-100),Community acquired pneumonia (n=55-100), adult exacerbations of asthma and COPD (n=110-200), paediatric exacerbations of acute childhood wheeze and/or confirmed asthma (n=25-50), Age/Home environment matched Adult/Sibling/Parent/Spousal healthy volunteers (n=55-75 adult/25 children) Additional patients with stable COPD/Asthma/Heart failure n=200 **Total Sample Size = 550-1250 patients.**
Follow-up duration	Acute care participants will have up to 2 visits whilst an inpatient followed by a 'Post-Acute Event Recovery' at a minimum of 6 weeks post discharge and up to 6 months. Stable state participants and healthy volunteers will have 2 visits over 16 weeks. All participants will have outcome data 2 years post recruitment.
Planned Trial Period	3 years
Primary Objective	To evaluate the sensitivity, specificity, positive and negative predictive value of metabolomic biomarkers in exhaled breath samples to identify acute breathlessness, defined as one or more of (i) patient defined acute breathlessness and/or a (ii) 1 unit increase in Extended Medical Research Council breathlessness score (eMRC).
Secondary Objectives	1) To replicate selected metabolomic breath biomarker panels of acute breathlessness using a combination of both (i) discovery technologies (including GC-MS) and (ii) point of care auto tuned breath sensing devices (including linear IMS, CMS, PTR-MS). 2) To evaluate the primary endpoint stratified by breathlessness as follows (i) change in eMRC by 1 unit, (ii) patient defined breathlessness and (iii) 100 mm visual analogue score for breathlessness To discover and replicate metabolomic breath biomarkers that differentiate (i) Acute Heart failure, (ii) Community acquired pneumonia, (iii) Adult exacerbations of asthma and COPD

	To quantify the level of clinical uncertainty in the primary diagnosis using a 100 mm visual analogue scale and independent clinical adjudication of case notes blinded to the following blood biomarkers (i) CRP (ii) blood eosinophils (iii) BNP (iv) Troponin-I but not clinical history and chest X-ray. To identify and discover metabolomic breath biomarkers of acute childhood wheeze and or confimed asthma. To clinically validate metabolomic breath biomarkers generated above using a combination of existing pathology markers e.g. BNP, CRP, blood eosinophils and troponin-I and other clinical relevant prognostic scoring tools e.g DECAF, CURB65 and Heart failure risk calculator http://www.heartfailurerisk.org/[19]
Exploratory Objectives	1) To evaluate the dynamic profile of breath biomarkers during (i) the acute state, (ii) in the recovery state post exacerbation. 2) To evaluate the relationship between breath metabolomic biomarkers and health status/ functional measures: - Health status measures - Physical performance 3) To evaluate the relationship between metabolomic breath biomarkers and clinical outcomes including (i) hospital readmission at time points up to 2 years (ii) response to standard clinical therapy and (iii) death. 4) To explore and develop breath metabolomic biomarkers of multi-morbidity 5) To evaluate the relationship between diet, lifestyle and environment upon breath metabolomic biomarkers. 6) To generate a biobank of DNA, RNA, urine, plasma, serum and sputum samples for future breathomic and other 'omic molecular pathology studies (including proteomic, transcriptomic, genomic and metabalomic studies).
Investigational Medicinal Products	**None: Patients will follow their standard acute care pathways**

3. ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
BNP	B-type natiuretic peptide
BRC	Biomedical Research Centre
BRC	Biomedical Research Centre
BTS	British Thoracic Society
CAU	Childrens Assessment Unit
CDU	Clinical Decisions Unit
CI	Chief Investigator
CMS	Compact Mass Spectrometer
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CRP	C-Reactive Protein
CTA	Clinical Trials Authorisation
DDU	Diagnostics Development Unit
DMS	Differential Mobility Spectrometry
DSMC	Data Safety Monitoring Committee
eMRC	Extended Medical Research Council Score
e-CRF	Electronic Case Report Form
ECG	Electrocardiogram
EC	Ethics Committee (see REC)
ECHO	Echocardiography
EPSRC	Engineering and Physical Sciences Research Council
FEV1	Forced Expiratory Volume in 1 second
FeNO	Fraction Exhaled Nitric Oxide
GC-IMS	Gas Chromatography Mobility Spectrometry
GC-FAIMS	Gas Chromatography Field Asymmetric Mobility Spectrometry
GC-MS	Gas Chromatography and Mass Spectroscopy
GCP	Good Clinical Practice
GP	General Practitioner
GTAC	Gene Therapy Advisory Committee
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
ISF	Investigator Site File
IMP	Investigational Medicinal Product
LV	Left Ventricular
MAARA	Midlands Asthma and Allergy Association

MHRA	Medicines and Healthcare products Regulatory Agency
MRC	Medical Research Council
MRC-EMBER	MRC East Midlands Breathomics Molecular Pathway Node
NHS	National Health Service
NIHR	National Institute for Health Reseach
NRES	National Research Ethics Service
PI	Principal Investigator
PIL/S	Participant/ Patient Information Leaflet/Sheet
PTRMS	Proton transfer mass spectroscopy device
PTR-ToF-MS	Real time breath monitoring using protocon transfer reaction time of flight mass spectrometry
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RV	Right Ventricular
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
UHL	University Hospitals of Leicester NHS Trust
VAS	Visual Analogue Score
VOCs	Volatile Organic Compounds

4. BACKGROUND AND RATIONALE

Patients presenting with acute undifferentiated breathlessness are commonly encountered in admissions units & emergency departments across the United Kingdom. The primary mechanisms that underpin acute breathless are driven by diseases within the cardio respiratory systems. Cardiorespiratory disease accounts for approximately 70 % of acute hospital admissions and therefore is a major health priority in the National Health Service (NHS).

The differential diagnosis of undifferentiated breathlessness includes heart failure, community acquired pneumonia, exacerbations of chronic obstructive pulmonary disease (COPD), and asthma. The diagnosis of these common conditions is based on a combination of clinical history and emergency diagnostics. Importantly, acutely ill patients with multi-morbidity can present and accurate diagnosis remains challenging. It is well recognised that when clinicians remain uncertain of the cause of breathlessness, patients have a longer hospital stay as well as increased morbidity and mortality [1].

Currently, blood biomarkers together with clinical, physiological and imaging parameters are used to stratify patients with acute breathlessness. Common examples of blood pathology markers utilised in routine clinical practice include brain derived natriuretic peptide [BNP] in heart failure [2,3], C reactive protein [CRP] and serum procalcitonin in infective bronchitis and pneumonia[4] and blood eosinophils in a proportion of exacerbations of asthma and COPD [5].

These blood biomarkers have clinical utility primarily in patients with single pathologies, but have poor discriminatory power in patients with multi factorial presentations of acute breathlessness. For example the American College of Emergency Physician recommends addition of a single BNP measurement to improve the diagnostic accuracy compared with standard clinical judgment alone in acutely breathless patients. BNP levels of <100 and >500 ng/ml can reliably 'rule out' and 'rule in' acute heart failure respectively [2,3], but there remain a large number of patients with intermediate values between 100-500 where there is often significant diagnostic uncertainty.

It is therefore likely that a variety of clinical, radiological features and biomarkers are required to accurately identify the cause of acute breathlessness. For example the addition of CRP to conventional clinical rule based algorithms modestly improved the positive likelihood ratio of identifying community acquired pneumonia at a low, intermediate or high risk in a large european population study [6].

Therefore better biomarkers are required to identify the cause of breathlessness in acutely unwell patients. Ideally these biomarkers would have the following properties (i) they would originate from the target organ of interest, (ii) they would significantly add value to conventional risk scoring and diagnostic algorithms in acute breathlessness e.g. the DECAF score in COPD[7] exacerbations or the CURB-65 scoring tool in community acquired pneumonia[8], (iii) they would be minimally invasive and suitable for rapid point of care diagnosis in emergency rooms and on acute admissions units and (iv) they would be have diagnostic value in patients with multi factorial acute breathlessness.

Breath Analysis and Disease

Exhaled breath analysis of volatile chemicals offers the potential to develop more effective biomarkers in acutely breathless patients. The use of breath analysis for disease diagnostics dates back to ancient Greeks where physicians used exhaled breath to diagnose different diseases. Breath odours allow correct associations to certain diseases. For example, the sweet smell of diabetic ketoacidosis, the fishy smell of

breath associated to liver illness, the urine-like odour of kidney disease and the smell of the breath of patients with lung abscesses, caused by the proliferation of anaerobic bacteria [9-12].

More recently, exhaled breath analysis has demonstrated early proof of concept in the diagnosis of acute heart failure [13], ventilator associated pneumonia [14] and stable state airways disease [15]. The validity of breath analysis has also been demonstrated in breathless children [16]. This population is likely to prefer breath-based tests, as these are minimally invasive. Importantly, a variety of point of care sensors are now available to evaluate potential exhaled breath biomarkers in emergency care settings.

The proposed program of research will identify and evaluate the diagnostic and prognostic value of exhaled breath metabolomic biomarkers in acute cardio respiratory breathlessness and common indicator diagnoses associated with acute cardio respiratory breathlessness. Specific indicator conditions have been selected according to their (i) relatively high prevalence, (ii) high unmet need, mortality and morbidity and (iii) need to develop better diagnostic and prognostic algorithms in acute care pathways.

The focus of the research program will be to phenotype using a combination of 'discovery' and near-patient care breath sampling technologies in acutely breathless patients attending two acute admissions units within Leicestershire. The indicator diagnoses of interest are (i) acute heart failure, (ii) exacerbations of adult asthma and COPD, (iii) community acquired pneumonia and (iv) paediatric exacerbations of acute childhood wheeze and confirmed asthma. Other disorders that are associated with acute breathlessness including exacerbations and acute presentations of pulmonary fibrosis, acute pulmonary embolism will not be studied within the program due to low prevalence, lack of standardised definitions and likely small sample size (pulmonary fibrosis) and adequate acute companion diagnostics (pulmonary embolism).

The program will be divided into two phases. **Phase 1**, a discovery phase to identify putative breath biomarker panels and **phase 2**, a validation phase to replicate and validate selected biomarkers within phase 1 using point of care breath sensing devices.

Patient level clinic-pathological and outcome data (spanning the entire acute pathway) will be collected in parallel to breath sampling. In addition, breath samples will be acquired during the recovery from the acute state and in the stable state post exacerbation to better understand the kinetics of breath analytes associated with these diseases.

A parallel cohort of age/home environment matched Adult/ parent/sibling/spousal healthy volunteers will also be recruited and evaluated at selected matching time points with breath analysis and a range of questionnaires to evaluate factors that are known to influence the metabolome including diet, lifestyle and the environment. Additonally patients with stable asthma, COPD and heart failure will be recruited from primary and secondary care as disease control populations.

5. OBJECTIVES

5.1 Primary Objective

To evaluate the sensitivity, specificity, positive and negative predictive value of metabolomic biomarkers in exhaled breath samples to identify acute breathlessness, defined as one or more of (i) patient defiend acute breathlessness and/or a (ii) 1 unit increase in Extended Medical Research Council breathlessness score (eMRC)

- *Recruitment in to the acute breathlessness group will be according to the following defined primary diagnoses in the discovery phase as defined by the treating physician:*

  (i) Acute Heart failure (n=55-100), (ii) Community acquired pneumonia (n=55-100), (iii) Adult exacerbations of asthma and COPD (n=110-200) and (iv) paediatric exacerbations of acute childhood wheeze and/or confirmed asthma (n=50-100). Additionally age/home environment matched adult/spouse/sibling/parent control subjects (n=55-150 adults/50 children).

5.2 Secondary Objectives

- To replicate selected metabolomic breath biomarker panels of acute breathlessness using a combination of both (i) discovery technologies (including GC-MS) and (ii) point of care auto tuned breath sensing devices (including linear IMS, CMS, PTR-MS).
- To evaluate the primary endpoint stratified by breathlessness as follows (i) change in eMRC by 1 unit, (ii) patient defined breathlessness and (iii) 100 mm visual analogue score for breathlessness
- To discover and replicate metabolomic breath biomarkers that differentiate (i) Acute Heart failure, (ii) Community acquired pneumonia, (iii) Adult exacerbations of asthma and COPD
- To quantify the level of clinical uncertainty in the primary diagnosis using a 100 mm visual analogue scale and independent clinical adjudication of case notes blinded to the following blood biomarkers (i) CRP (ii) blood eosinophils (iii) BNP (iv) Troponin-I but not clinical history and chest X-ray.
- To identify and discover metabolomic breath biomarkers of acute childhood wheeze and or confimed asthma.

To clinically validate metabolomic breath biomarkers generated above using a combination of existing pathology markers e.g. BNP, CRP, blood eosinophils and troponin- I and other clinical relevant prognostic scoring tools e.g DECAF, CURB65 and Heart failure risk calculator http://www.heartfailurerisk.org/[19]

- *Replication of acute breathlessness biomarkers will be according to the following predefined primary diagnoses in the **replication phase**:*

  (i) Acute Heart failure (n=55-100),(ii) Community acquired pneumonia (n=55-100), (iii) adult exacerbations of asthma and COPD (n=110-200),(iv) paediatric exacerbations of acute childhood wheeze and confirmed asthma (n=25-100),(v) age/home environment matched adult/spouse/sibling/parent control subjects (n=55-75 adults/25 children)

5.3 Exploratory End Points (where applicable)

1) To evaluate the dynamic profile of breath biomarkers during (i) the acute state, (ii) in the recovery state post exacerbation.

2) To evaluate the relationship between breath metabolomic biomarkers and health status/ functional measures:

- Health status measures
- Physical performance

3) To evaluate the relationship between metabolomic breath biomarkers and clinical outcomes including (i) hospital readmission at time points up to 2 years (ii) response to standard clinical therapy and (iii) death.

4) To explore and develop breath metabolomic biomarkers of multi-morbidity

5) To evaluate the relationship between diet, lifestyle and environment upon breath metabolomic biomarkers.

To generate a biobank of DNA, RNA, urine, plasma, serum and sputum samples for future breathomic and other 'omic molecular pathology studies (including proteomic, transcriptomic, genomic and metabalomic studies).

6 STUDY DESIGN

6.1 Summary of Trial Design

The clinical study will be a prospective observational study across two acute clinical sites that routinely assess and treat cardio-respiratory admissions due to breathlessness within Leicester.

(i) Glenfield Hospital (GGH): Adult cardio-respiratory presentations to Glenfield Hospital (including Clinical Decisions Unit (CDU) & inpatient wards), outpatient clinics and NIHR Leicester Biomedical Research Centre (BRC)
(ii) Leicester Royal Infirmary: Adult and paediatric cardio-respiratory presentations to A&E, Children's Assessment Unit (CAU), Diagnostics Development Unit (DDU), inpatient wards, outpatient clinics and Research Space

Follow up and stable state controls will be assessed in the outpatient setting within University Hospitals of Leicester including the BRC. The study flow is summarised in Figure 1 below.

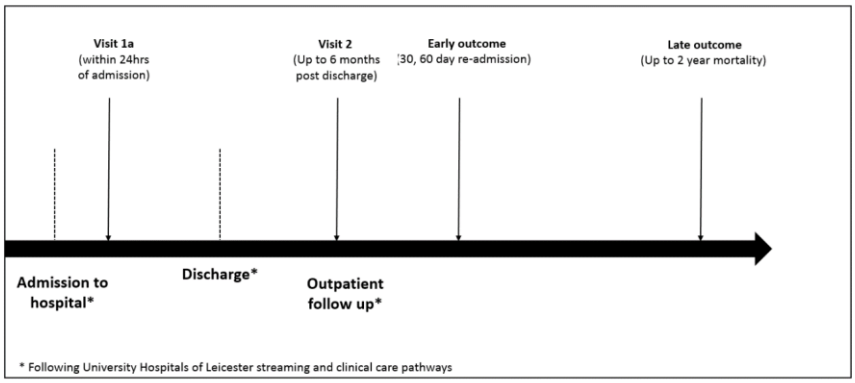


Fig 1: Study flow chart. Figure demonstrates the patient journey from admission through to outcome. Assessments carried out at each visit are outlined in table 1.

Figure 1: Study Flow chart

Prospective participants with self reported acute breathlessness, either requiring admission or a change in baseline treatment, presenting within UHL. The streaming and care pathways at UHL allocate ambulance admissions to the two units described above with appropriate clinical care being provided at each site according to national guidelines and local protocols / policies.
Patients with confirmed acute airwary exacerbation or heart failure decompensation as part of other research studies at the NIHR Leicester BRC may also be included.

Age- and/or home environment matched adult/spousal/parent and sibling healthy volunteers will be recruited where possible on the admissions unit and when not possible in the research units across both sites at a separate visit. All attempts will be made to sample breath on *at least two of* the same time points as patients within these disease groups, however sampling may occur in a different location and at different serial time points. All healthy volunteers will complete a range of questionnaires adapted from generic cohort studies to characterise their diet, lifestyle and environmental exposures. A stable state control population will also be recruited to compare with the unstable state. This will consist of participants with a confirmed diagnosis matched with the acute care population but not in the exacerbation state.

Specifically after triage and senior clinical assessment at each of the acute EMBER sites (CDU, DDU, CAU) if a primary clinical diagnosis of (i) exacerbation of heart failure, ii) exacerbation of asthma/COPD, (iii) adult community acquired pneumonia or (iv) paediatric exacerbations of acute childhood wheeze and confirmed asthma has been made, members of the EMBER research team will evaluate patients using breath diagnosis and sensing technologies and additional tools to characterise patients that are acutely unwell.

The following steps will be employed at all sites:

1) Identify patients with acute breathlessness defined as one or more of (i) patient defined acute breathlessness and/or a (ii) 1 unit increase in (eMRC)
2) Completeion of study diagnosis at the point of senior clinical decision maker review
3) Informed consent

4) In selected patients that have not been captured during their acute admission and have been discharged prior to EMBER assessments (for example patients that may have been discharged rapidly due to the use of an ambulatory care pathway), ***recall within 48 hours of discharge*** will be perfomed for EMBER assessments

5) Breath sampling using a standardised CE marked multi-platform breath sampling face mask as well as real time breath sampling.

6) Collection of additional biomarkers for future biomarker discovery campaigns Including a (i) urine sample, (ii) blood sample (up to 85 mls) (DNA, RNA ,plasma and serum), peripheral blood cell flow cytometry and (iii) spontaneous sputum samples (plugs and supernatants). These samples will be collected at time point 1a/1b and at 2

1) Stable state follow up of all patients will occur following recovery from the acute event. Assessment will occur at time of clinical follow up, or as a separate research visit.

The expected duration of participants within the study will therefore be equivalent to their length of hospital stay and up to the stable state follow up visit, with a similar duration for healthy volunteers and stable state patients.

Readmission to hospital within the protocol defined recovery period. Patients that re admit to hospital between visits 1 and 2, can have additional 1a ±1b assessments. Visit 2 will be taken as recovery following the subsequent admission. If a patient is admitted to hospital after visit 2 then they will be eligible to be recruited as a new study participant.

Definition of Acute Breatlessness

A standard definition for acute breathlessness does not exist. We will use the presence of patient reported acute breathlessness, above their usual baseline breathlessness (i.e. in stable state) as a binary variable. We will also use the extended MRC score, and 100mm visual analogue score (VAS), as a continuous variable.

The extended MRC score has been validated in chronic disease populations, including in the acute state, and may be applied to diseases other than COPD where originaly validated. We will define a change in breathlessness of the eMRC as at least a unit change from stable state. In addition a VAS score will be used to measure breathlessness.

Definition of Post Exacerbation Recovery

Patient recovery will be defined as

(i) Patient reported recovery from the acute exacerbation spell and back to their baseline extended MRC score or clinician defined recovery from the acute exacerbation spell

and

(ii) At least 6 weeks post exacerbation event (up to 6 months).

6.2 Summary of Additional Assessment and breath sampling procedures/ devices

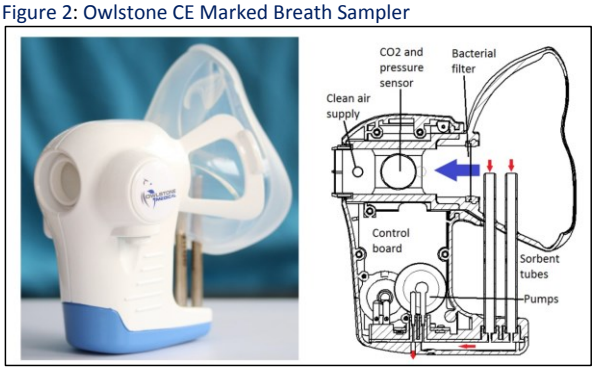
A number of analytical devices will be utilised to sample breath in acutely ill patients and based upon the (i) clinical site, (ii) operational needs and likely patient grouping (severity of underlying condition at the point of admission) within each clinical site, (iii) balance of discovery and point of care sensing technologies: breath sensors will be co-located to the two EMBER acute admissions units. All devices will be maintained to a high level of operational readiness by the technicians and device specialists within the clinical operations group. All devices will be checked for safety by Medical Physics at University Hospitals of Leicester NHS Trust.

The following devices will be utilised to evaluate breath volatile organic compounds

- A portable mass spectroscopy device (Advion Ltd) for discovery based metabolomic breath sampling. This device is also capable of real time sampling.
- A proton transfer mass spectroscopy device (PTRMS) for discovery based metabolomic breath sampling.
- A point of care GC-FAIMS Lonestar device (Owlstone Nanotech Ltd)
- A point of care linear IMS Bioscout device (Owlstone Ltd: GC-FAIMS Lonestar analyser)

A CE marked breath sampling device developed by Owlstone Nanotech Ltd (Appendix A) will be used to sample breath in the majority of patients onto adsorbent Tenax tubes as well as acquiring online sampling. This effectively allows de coupling of the breath sampling from the breath sensor and analysis platforms in selected patients and allows patients that are not able to be mobilised in a chair to a real time breath sampling device to be sampled at their bedside. The Owlstone RECIVA sampler (Figure 3) is capable of entraining oxygen and is therefore suitable for patients in respiratory failure requiring low to moderate flow rates of oxygen to maintain target oxygen saturations. CE marking approvals are appended to this protocol in (Appendix B).

Figure 2: Owlstone CE Marked Breath Sampler



7 TRIAL PARTICIPANTS

7.1 Overall Description of Trial Participants

Each site will recruit patients with acute breathlessness. Final confirmation of the primary indicator diagnosis is usually available at discharge (following visit 1b) and after further diagnostics at the stable state follow up clinic (at visit 2). The final primary indicator diagnosis and additional comorbidities will be recorded at initial senior clinical review, discharge and at follow up. The indicator diagnosis will be used to define the number of patients recruited within each disease stratum.

The study will have two distinct phases:
Phase 1: Discovery of novel breath metabolomic biomarkers.
Phase 2: Replication and validation of selected biomarkers in Phase 1.

Planned recruitment to meet the primary, secondary and exploratory study endpoints is outlined below.

(i) Acute Heart failure (n=55-100 Phase 1, n=55-100 phase 2)
(ii) Adult community acquired pneumonia (n=55-100 Phase 1, n=55-10 Phase 2). This condition will be defined as new radiological consolidation responsive to treatment according to the British Thoracic Society (BTS) community acquired pneumonia guidelines.
(iii) Adult exacerbations of airway diseases asthma and COPD (n=110-200 Phase 1, n=110-200 Phase 2). This condition will be defined as a clinician diagnosis with subsequent or historical evidence of objective diagnostic criteria according to national and international guidelines.
(iv) Paediatric exacerbations of acute childhood wheeze and/or confirmed asthma (n=55-100 Phase 1, n=25-50 Phase 2).
This condition will be defined as a clinician diagnosis and for childhood wheeze/asthma subsequent or historical evidence of objective diagnostic criteria according to national and international guidelines.
(v) Age/environment matched Adult/spousal/parental/ sibling volunteers (n=55-150 adults/50 children) discovery phase, (n=55-75 adult/25 children)
replication phase) with no evidence or history of the indicator disease being evaluated in their spouse/sibling/child.
(vi) Stable state participants with a confirmed diagnosis of asthma, COPD or heart failure that have not had and exacerbation of their disease within the preceding 6 weeks (n=200 (100 in each phase)).

7.2 Inclusion Criteria

(i) Able to give informed consent for participation in the study.
(ii) Male or Female, aged 16 years or above (adult cohort) and 5-15 years for paediatric patients attending the acute care paediatric pathway.
(iii) Capable (in the opinion of the EMBER clinical research investigator(s) of providing serial breath samples.
(iv) Diagnosed with acute breathlessness as one of the primary indicator reasons by the clinical acute care team. This is not a requirement for healthy subjects or matched controls.
(v) One of the indicator provisional diagnoses identified in section 7.1 following senior review by the clinical acute care team. This is not a requirement for healthy subjects or matched controls
(vi) Able (in the Investigators opinion) and willing to comply with all study requirements.
(vii) Willing to allow his or her General Practitioner and consultant, if appropriate, to be notified of participation in the study.
(viii) Ability to understand English.

7.3 Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

(i) Female participants who are known to be pregnant, lactating or planning pregnancy during the course of the study.
(ii) Current participation in a clinical trial of an investigative medicinal product or within 3 months or 5.5 half-lives of the IMP whichever is longer.
(iii) Active or clinically suspected pulmonary tuberculosis
(iv) In the opinion of the treating physician, breath sampling during the acute admission would be clinically unsafe or inappropriate due to the patient's condition or poor prognosis. Examples include malignancy or autoimmune disease with anticipated survival of under 1 year, and chronic renal replacement therapy.
(v) Unable or unwilling to give informed consent by visit 1b.
(vi) Any other significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the study, or may influence the result of the study, or the participant's ability to participate in the study.

8 STUDY PROCEDURES

A personal legal representative (PeLR) is defined as : A person not connected with the conduct of the trial who is suitable to act as a legal representative by virtue of their relationship with the child and available and willing to do so.

8.3 Informed Consent

Informed consent in acutely unwell patients (both adults and children) poses a number of potential barriers to the conduct of research. However the clinical study team have extensive experience (See Coats TJ *et al* [23]) in consenting both adults and children during acute care settings. During the current study we will recruit participants during the acute phase, with full informed consent from the participant or personal legal representative (PeLR), in the case of paediatrics.

The patient/PeLR must personally sign and date the latest approved version of the informed consent form before any study specific procedures are performed. In the case of paediatric participants they will be asked to complete an assent form, in addition to the PeLR consent form.

Written and verbal versions of the participant information and Informed consent will be presented to the patient/PeLR detailing no less than: the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the patient is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal. In the event that the participant cannot read the information sheet it will be presented verbally only.

All Informed Consent will be obtained by means of participant dated signature and dated signature of the person who presented and obtained the informed consent. The person who obtains the consent will be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator as detailed on the Delegation of Authority and Signature log for the study. The original signed form will be retained at the study site within the Trial Master File (TMF) or Investigator Site File (ISF). A copy of the signed Informed Consent will be given to participants/parent/legal guardians and a copy retained in the participant medical notes.

8.2 Screening and Eligibility Assessment

Patients (trial participant groups (i), (ii), (iii), (iv) – see synopsis) will be identified by dedicated member of the research team working across both hospital sites following confirmation of the suspected acute diagnosis once the patient has been clinically reviewed by a senior clinical decision maker.

The research team will be fully ICH-GCP competent and trained in obtaining informed consent and assent according to the hierarchy of consent process outlined in section 8 of this protocol. The research team will use available clinical information systems to confirm the nature of previous admissions and indicator diagnoses such as the UHL-ICE system, iLAB and the radiology systems for pathology and image viewing respectively. Clinical IT systems used to capture clinical meta data are outlined in (Figure 5) below.

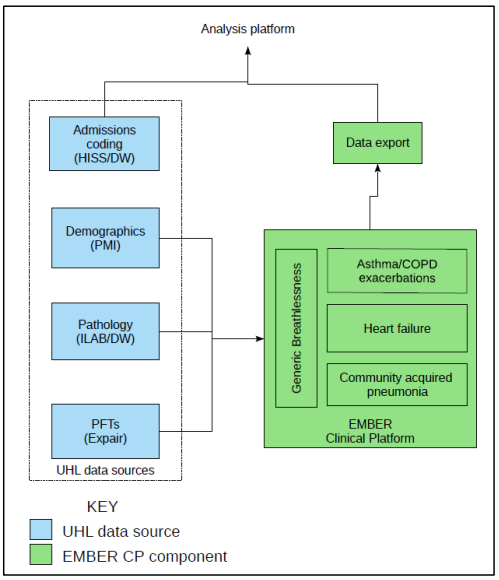
Potential participants to the study will be identified shortly after presentation to each of the sites at Glenfield Hospital, Diagnostics Development Unit (DDU)/Children's Assessment Units (CAU) at the Leicester Royal Infirmary, by members of the healthcare team using the inclusi on criteria of the study. Once the decision has been made to admit a patient onto the acute medical unit, potential participants will be approached by a member of the healthcare team and asked if they would like to consider taking part in the study. If the patient is willing to consider participating in the trial, they will be approached by a member of the research team for further information and consent. In many occasions, the healthcare team will also be members of the research team. Due to the nature of acute research the research team will identify themselves with both the clinical nurse responsible for the patient in question on the admissions unit and the clinician(s) responsible so that the consent and screening process can take place in a seamlessness manner and with minimal disruption alongside the patients usual care pathway.

Environment matched adult/spouses/siblings/parents volunteers (trial participants group (v) – see synopsis) will be identified and recruited from a number of sources:

- For acute admission the study team will approach the spouse, parent or sibling of the index case and seek informed consent for study assessments. All healthy subjects will undergo at last two assessments seperated by a duration of up to 6 months to match the acute (visit 1a) and stable state (visit 2) time period elapsed in their index case partner/spouse/sibling/child.
- Additional healthy volunteers will be identified from local recruitment databases and via advertising

Additional patients with chronic asthma, COPD and heart failure (trial participants group (vi) – see synopsis) will be identified via cardiology and respiratory outpatient clinics at Glenfield Hospital, paediatric respiratory outpatient clinics at the Leicester Royal Infirmary, from NIHR Leicester Biomedical Research Centre (BRC) patient research databases and will undergo up to 2 visits over a 6 months period. In all cases permission for recruitment from outpatients will be sought from the responsible consultant for the patient. The rationale for inclusion of this population is to have a stabe state disease control population that have been characterised over the same overall reporting period (stable state for measurements and 2 years for outcome data collection) as the acute population.

Figure 5: Clinical informatics organogram



8.3 Study Assessments

A summary of both baseline and follow up assessments is outlined in Table 1 (adults) and Table 2 (children) below. If appropriate consent is in place the subjects may undertake any combination of any of the investigations listed in the investigation table at any of these visits.

Table 1: Summary of adult assessments

Assessments	COPD			Asthma			Pneumonia			Heart Failure			Healthy	
Time point	1a	1b	2	1a	1b	2	1a	1b	2	1a	1b	2	1a	2
Written Informed Consent	X			X			X			X			X	
Collection of demographic data (age, gender, height, weight, ethnicity, smoking status)*	X^		X^	X^		X^	X^		X^	X^		X^	X	X
Time of date of admission/discharge#	X^	X^		X^	X^		X^	X^		X^	X^			
Breathless Scores*														
Patient defined breathlessness	X^		X	X^		X	X^		X	X^		X	X	X
Extended MRC dyspnoea score	X^		X^	X		X	X		X	X		X	X	X
New York Heart Association Dyspnoea Score (NYHA)										X^		X^		
100 mm visual analogue breathlessness score	X		X	X		X	X		X	X		X	X	X
Exacerbation history (previous health care utilisation and acute use of medication)*	X^		X^	X^		X^	X^		X^	X^		X^	X	X
Quality of breath VOCs questionnaire	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Structured Clinical History of presenting complaint and past medical history*	X^		X^	X^		X^	X^		X^	X^		X^	X	X
Assessment of acute disease severity#*														
DECAF Score	X^													
CURB 65 Score							X^							
BTS Asthma Severity Score				X^										
Heart failure risk calculator										X^				
Concurrent medication usage	X^	X^	X^	X^	X^	X^	X^	X^	X^	X^	X^	X^	X	X
12 lead ECG*	X^			X^			X^			X^				
Chest X-ray*	X^		X^	X^		X^	X^		X^	X^		X^		
Health Status Questionnaires*														
Asthma Quality of Life questionnaire (AQLQ)				X		X^								
COPD assessment tool (CAT)	X		X^											

	COPD			Asthma			Pneumonia			Heart Failure			Healthy	
	1a	1b	2	1a	1b	2	1a	1b	2	1a	1b	2	1a	2
NASA Task Load Index	X		X	X		X	X		X	X		X	X	X
Blood tests*														
BNP	X^		X	X			X			X^		X^	X	
Troponin-I	X			X			X			X^			X	
CRP	X^		X^	X^		X^	X^		X^	X^		X^	X	X
Full blood count + differential count	X^		X^	X^		X^	X^		X^	X^		X^	X	X
DNA and RNA (PAXGENE)~	X		X	X		X	X		X	X		X	X	X
Plasma and serum	X		X	X		X	X		X	X		X	X	X
Arterial or capillary blood gas*#	X^			X^			X^			X^				
Peripheral blood flow cytometry	X		X	X		X	X		X	X		X	X	X
Lung function test														
Spirometry*	X^		X^	X^		X^							X	X
Oscillometry*	X		X	X		X	X		X	X		X	X	X
Exhaled nitric oxide (FeNO)				X		X^								
Sputum sample (spontaneous^ or induced)	X^		X	X^		X	X^		X	X^		X	X	X
Volatile organic compounds (VOCs)	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine sample*	X		X	X		X	X		X	X		X	X	X
Echocardiography*	X									X^				
Quadriceps ultrasound	X	X	X											
4 meter gait speed		X	X								X	X		
Review of Adverse Events & Serious Adverse Events		X	X		X	X		X	X		X	X		X
Physical Exam*	X^		X^	X^		X^	X^		X^	X^		X^	X	

*Can be performed at time points 1a or 1b. for healthy volunteers this can be completed at visit 1 or 2. For informed consent see protocol, ^ may be part of routine clinical care according to UHL Trust Guidelines. Arterial, capillary blood gases and blood cultures only done in selected clinical cases, # Acute patients only ~If DNA taken at time point 1a will not be repeated at timepoint 2.

Table 2: Summary of children assessments

Assessments	Childhood Wheeze /Confirmed Asthma			Healthy	
Time point	1a	1b	2	1a	2
Written Informed Consent	X			X	
Collection of demographic data (age, gender, height, weight, ethnicity, smoking status)*	X^		X^	X	X
Time of date of admission/discharge#	X^	X^			
100 mm visual analogue breathlessness score*	X		X	X	X
Exacerbation history (previous health care utilisation and acute use of medication)*	X^		X^	X	X
Quality of breath VOCs questionnaire	X	X	X	X	X
Structured Clinical History of presenting complaint and past medical history*	X^		X^	X	X
Concurrent medication usage	X^	X^	X^	X	X
Chest X-ray*	X^		X^		
Health Status Questionnaires*					
Asthma control questionnaire (ACQ)	X^		X^		
Asthma quality of life questionnaire (AQLQ)	X^		X^		
NASA Task Load Index	X	X	X	X	X
Blood tests*+$					
CRP	X^		X^	X	X
Full blood count + differential count	X^		X^	X	X
DNA and RNA (PAXGENE)	X		X	X	X
Plasma and serum	X		X	X	X
Arterial or capillary blood gas*	X^				
Blood culture*	X^				
Lung function test					
Spirometry*	X^		X^	X	X
Exhaled nitric oxide (FeNO)	X		X	X	X
Sputum sample (spontaneous^ or induced)$	X^		X^	X	X
Volatile organic compounds (VOCs)	X	X	X	X	X
Urine sample*	X		X	X	X
Review of Adverse Events & Serious Adverse Events		X	X		X
Physical Exam*	X^		X^	X	X

*Can be performed at time points 1a or 1b. For informed consent see protocol, ^ Part of routine clinical care as part of the University Hospitals of Leicester, acute care clinical pathway. Will be performed in selected clinical cases based on clinical need, # Acute patients only, + up to 35 mls maximum per visit, $ optional

A description of the research assessments is outlined below

- Demographics

All standard clinical demographics routinely recorded in acutely unwell patients will be captured using study specific case report forms (CRFs). These will include the age, sex, height, weight, smoking status and ethnicity of the patient.

- Medical History

All standard clinical data routinely recorded in acutely unwell patients will be captured using study specific case report forms (CRFs). This will include co morbidities usually supplied with the GP referral letter and the use of hospital clinical information systems such as ICE to cross check co morbidities where necessary using discharge summaries as well as case notes review.

- Concomitant Medication

Current medication will be collected. This will comprise of usual medication, medication in the two weeks prior to visit 1a .In the case of admission, additional confirmation will be performed after standard pharmacy review by the admissions pharmacist allocated to the admissions unit in question.

- Physical Examination

All standard clinical data routinely recorded in acutely unwell patients will be captured using study specific electronic case report forms (CRFs). Standard clinical examination findings relevant to the indicator diagnosis in question will be recording and will include pulse, blood pressure, temperature, respiratory rate, oxygen saturations and impairment of cognition.

- Collection and recording of standard clinical care pathway diagnostic

The majority of acute care admissions with undifferentiated breathlessness will undergo various combinations of the following assessments dependent upon their admission diagnosis.
- Chest x ray
- 12 lead ECG
- Laboratory blood tests may include: Urea and electrolytes, liver function tests, albumin, corrected calcium,phosphate, D-Dimer, BNP, Troponin-I, full blood count, haematocrit and differential white cell count.
- Additional investigation such echocardiography (will be done during acute admission between time points 1a and 1b). Echo will measure LV and RV function, LA size, vavular function, LV wall thickness and e/e' on tissue Doppler.

***The responsibility to act upon and record abnormal investigations within the patient's clinical notes will remain with the admitting clinical team who will have ordered and requested the investigations.*

- Breath sampling procedures (PLEASE REFER TO APPENDIX D: ASSET ALLOCATION APPENDIX FOR A DETAILED SUMMARY OF THE CORE BREATH DETECTION TECHNOLOGIES)

Breath sampling will be performed using various combinations of the devices below dependent upon patient location, level of illness (acuity) and phase of the program (discovery/replication). Breath sampling will be limited to a duration acceptable to a patient (typically 30 mins) at any particular study visit to minimise disruption to the patient care pathway and flow.

Advion CMS: Discovery Phase (Phase 1) Breath Analysis:

The Advion Expression compact mass spectrometer (CMS) is a single quadrupole mass spectrometer that enables the study of volatiles in breath by adding a simple modification at the front end of the instrument. The volatiles are drawn into the mass spectrometer ion source directly from the patient's breath using a Venturi pump. This pump creates a Venturi effect sucking the volatiles into the mass spectrometer at a rate of approximately 300 mL/min. When sampling, patients wear a facemask which is fed with approximately 35 l/min of purified air to which the Venturi pump inlet is connected. The extracted breath samples pass through a transfer line heated to an optimal temperature to avoid condensation of the volatiles. The minimum time required to perform a breath sample is 2 min. After this sampling time the data can be extracted immediately for analysis.

GC-IMS (B&S Analytiks): Replication Phase (Phases 2) Breath Analysis:

Gas chromatography ion mobility spectrometry (GC-IMS) enables the detection of VOCs in exhaled breath of patients in trace gas concentrations (ng/L-pg/L range). Sampling takes place using a Spiroscout spirometer. The patients exhale through a disposable mouth piece connected to a Teflon tube. A piezoelectric pressure sensor is used to monitor the breathing profile, this opens the sampling valve at the appropriate point in the breath profile to collect end-tidal breath in a sample loop of 10 mL volume. After filling this loop, the collected sample air is then transferred to a multicapillary column for a chromatographic separation, which is achieved in 12 min. The separated molecules are then transferred into the IMS, ionised and then separated according to their mobility in a weak electric field.

GC-FAIMS (Owlstone Nanotech Ltd): Replication Phase (Phase 2) Breath analysis:

Gas chromatography field asymmetric ion mobility spectrometry (GC-FAIMS) is a gas detection technology that separates and identifies chemical ions based on their mobility under a varying electric fields at atmospheric pressure. Samples are collected on adsorbent tubes (Tenax/Carbograph) using a CE marked handheld sampling device. Samples are then extracted from tubes using thermal desorption and then analysed by GC-FAIMS. The combination of the high selectivity of GC with the high sensitivity of FAIMS enables the detection of volatiles in a wide range of matrices such as aqueous, solid and gaseous. FAIMS is also known as differential mobility spectrometry (DMS).

Real time breath monitoring using proton transfer reaction time of flight mass spectrometry (PTR-ToF-MS):

In order to sample a patient's breath using this technique, two breath sampling devices will be used. The first device is a Loccioni SOFIA GSI-S; the subject is required to exhale a single breath, five times (three if providing five samples proves too difficult) into a sterile mouthpiece connected to an electrostatic bacterial/viral filter whilst wearing a nose clip (all CE marked). Flow from the mouthpiece passes into a gas sampling interface capnograph (Loccioni GSI-S – CE marked) and real-time user feedback of flow is provided on screen, allowing the regulation of the breath sampling rate. The gas sampling interface acts to simultaneously trigger the acquisition of the PTR-ToF-MS data and the exhaled breath travels through the capnograph down a heated sample line into the ion source of the PTR-ToF-MS

The second breath sampling device is a ReCIVA breath sampler (Owlstone), as described in Section 6.2, with one of the adsorbent Tenax tubes replaced with an outlet tube adapted for online sampling. The exhaled breath is transferred to the PTR-ToF-MS via a heated transfer line connected to the outlet tube, continuously drawn at a constant flow rate by the PTR-ToF-MS. The online adaptation of the consumable adsorbent tube does not affect the CE mark of the ReCIVA sampling device.

Once the breath sample reaches the PTR-ToF-MS, via either breath sampler, the breath mixes with protonated water (H_3O^+) inducing proton transfer to the target volatile organic compounds (VOCs) present, resulting in their ionisation. Sample ions are then guided into the time of flight mass spectrometer and mass spectra, showing the abundance and mass of the VOCs present, are collected throughout the exhalation. Following sampling, mouthpieces, filters and nose clips are disposed of and all patient contacted surfaces wiped down with antiseptic cleaning wipes in preparation for the next patient.

- Sputum collection

Spontaneous or induced sputum samples can be collected. In the case of induced sputum sample collection all subjects will have a baseline FEV_1 measured. Sputum collection will be collected using the principles as defined by the European Respiratory Society Guidelines. All equipment used for performing the following procedure will be de-contaminated according to local working instructions. The procedure may be stopped prematurely if: (1) the subject's FEV_1 drops by greater than 20% of the post-salbutamol FEV_1 baseline value, (2) the subject feels any discomfort and does not want to continue with saline inhalation procedure or (3) the investigator feels that it is unsafe to continue with the saline inhalation. Sputum supernatants will be stored at the investigative site in dedicated -80 C freezers.

- Urine Collection

Urine will be collected from subjects. It will be collected into a suitable container and stored at the investigative site in dedicated -80 C freezers.

- Lung function

Lung function testing will include spirometry (pre and post bronchodilator), FeNO (Fraction exhaled Nitric Oxide), hand held oscillometry (adults asthma patients only). Peak flow measurements (to be recorded as part of routine care in asthmatics), and volatile breath analysis. All of these are non-invasive clinical tests.

- Research Echocardiography (ECHO)

For patients (excluding healthy volunteers) where there is no clinical indication for ECHO a research ECHO will be performed by a certified technician following written informed consent from the patient any time point between visit 1a and visit 1b.

- Blood sampling

In addition to the routine clinical blood samples will be taken for RNA and DNA, serum and plasma, peripheral blood cell flow cytometry analysis per visit. This may total up to 85 mls for an adult and 35mls for a child. Samples will be barcoded and stored at the investigative site in dedicated -80 C freezers. Samples may be transferred to the NIHR national bio sample centre subject to available funding. Blood samples will be taken at the same time point as the clinical samples to minimise inconvenience to the patient.

- Health Questionnaires

Questionnaires regarding the subjects' health and disease status will be completed. These will include measures of health status.

- NASA Task Load Index

It is a widely used, subjective, multidimensional assessment tool that rates perceived workload in order to assess a task, system, or team's effectiveness or other aspects of performance. It assesses work load on five 7-point scales including mental demand, physical demand, temporal demand, performance, effort and

frustration. Increments of high, medium and low estimates for each point result in 21 gradations on the scales. This tool will be incorporated into EMBER to assess the overall performance and feasibility of using ReCIVA mask in breath collection.

- Physical performance

4 meter gait speed (the time it takes to walk 4 meters) and quadriceps size measured using ultrasound will be performed to assess physical performance in selected patients. Both are measures of frailty, non-invasive, and validated in the acute care setting in chronic respiratory disease. If ultrasound measure is missed at timepoint 1 then it will not be taken at timepoint 2.

8.6 Definition of End of Study

The end of trial is the date of the last patient last visit (Visit 2).

Each participant will have the right to withdraw from the study at any time. In addition, the investigator may discontinue a participant from the study at any time if the investigator considers it necessary for any reason including:

- Ineligibility (either arising during the study or retrospective having been overlooked at screening)
- Pregnancy
- Significant protocol deviation
- A serious adverse event which requires results in inability to continue to comply with study procedures
- Disease progression which results in inability to continue to comply with study procedures
- Consent withdrawn
- Lost to follow up

The reason for withdrawal will be recorded in the CRF.

8.7 Source Data

In this study a paper CRF will be collected for all ' research only' measurements defined in tables 1 and 2. The electronic CRFs will be used as the source document for the captured data fields described in Table 1 and 2 that are part of routine clinical care. The rationale for this is that data collection for routine clinical care measures within MRC EMBER is populated in to the eCRF from existing UHL IM&T systems (Figure 4 e.g. IMPAX, ilab, CODE BREAKER) as well as prospectively confirmed by the research team for relevant study clinical meta data of interest e.g. smoking status, medication.

9. SAFETY REPORTING

9.1 Definitions

9.1.1 Adverse Event (AE)

An AE or adverse experience is:
Any untoward medical occurrence in a patient or clinical investigation participants, which does not necessarily have to have a causal relationship with this treatment.
An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the the study, whether or not considered related to the study.

9.1.2 Adverse Reaction (AR)

All untoward and unintended responses related to the study.
All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the study qualify as adverse reactions.

9.1.3 Severe Adverse Events

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following note of clarification is provided:
The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a participant's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

9.1.4 Serious Adverse Event or Serious Adverse Reaction

A serious adverse event or reaction is any untoward medical occurrence that:

- Results in death,
- Is life-threatening,

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

- Requires inpatient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

Other important medical events*
*Other events that may not result in death, are not life threatening, or do not require hospitalisation, may be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

9.1.5 Expected Serious Adverse Events/Reactions

The patient cohort being studied are frail and have a high hospital readmission rate and mortality rate. For example in those with a diagnosis of COPD both national and local data suggest readmission to hospital will be 20% at one month, 33% at 3 months and 60% at one year. A number of these patients will be readmitted on more than one occasion (our study Greening N *et* al, BMJ 2014) demonstrated 599 hospitalisations in one year in a similar COPD population of 389. In addition mortality is expected to be 14% at 3 months and 20% at one year.

Given this is an observational study and the rates that are expected we would reasonably expect that hospitalisation or death are not routinely reported as SAEs (we would anticipated an excess of 600 SAEs if not). Therefore expected serious adverse events include (i) clinical deterioration and death of acutely breathless patients, (ii) adverse drug reactions to pharmacotherapy administered during the patients clinical care pathway, (iii) readmission to hospital for acute breathlessness due to acute exacerbations of co morbid conditions during the study period.

However, if any adverse events are experienced during any of the measures performed specifically related to research procedures (Table 1 and 2) e.g. breath sampling, administration of questionnaires, then these will be reported using the approved reporting procedure.

9.1.6 Suspected Unexpected Serious Adverse Reactions

A serious adverse reaction, the nature or severity of which is not consistent with the applicable product information

9.2 Reporting Procedures for All Adverse Events

All AEs occurring during the study observed by the investigator or reported by the participant, whether or not attributed to study, will be recorded on the CRF.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to study, other suspect device and action taken. Follow-up information should be provided as necessary.

AEs considered related to the study as judged by a medically qualified investigator or the sponsor will be followed until resolution or the event is considered stable. All related AEs that result in a participant's withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs.

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the participant's removal from the study. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must undergo an end of study assessment and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable.

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

The relationship of AEs to the study will be assessed by a medically qualified investigator.

9.3 Reporting Procedures for Serious Adverse Events

All SAEs, except those expected ones defined in section 9.1.5 that do not require immediate reporting (see 9.1.5), must be reported to the Sponsor within 24 hours day of discovery or notification of the event. The Sponsor will perform an initial check of the information and ensure that it is reviewed at the next R&D Management meeting. All SAE information must be recorded on an SAE form and sent to the Sponsor using the appropriate reporting form and the contact details on there. Additional information received for a case (follow-up or corrections to the original case) needs to be detailed on a new SAE form which must be sent to the Sponsor using the appropriate reporting form and the contact details on there.

The Sponsor will report all SUSARs to the Research Ethics Committee concerned. Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. The CI will inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

In addition to the expedited reporting above, the CI shall submit once a year throughout the study or on request an Annual Report to the Ethics Committee which lists all SAEs / SUSARs that have occurred during the preceding 12 months.

10. STATISTICS

10.1 Description of Statistical Methods (Detailed statistical anad analytical methods are outlined in the MRC-EMBER data analysis plan).

Primary Analysis

For the primary analysis the outcome is a nominal variable with levels (1) Acute breathlessness (2) Recovery form acute breathlessness. The relationship between the primary outcome and the metabolomics biomarkers in exhaled breath sample will be modelled using multinomial logistic regression. Acute

breathlessness will be defined as one or more of (i) patient defined acute breathlessness and/or a (ii) 1 unit increase in Extended Medical Research Council breathlessness score (eMRC). In addition to metabolomics markers the following independent variables will be included in the model: age, sex, standard acute care pathology markers blood eosinophils, BNP, CRP, Troponin-I, canonical VOCs extracted from breath analysis and where relevant validated co morbidity scores (e.g. Charlson comorbidity score).

Adjudication

All acute/healthy adult patients' notes will be sequentially divided. These will then be reviewed and independently adjudicated by two independent respiratory consultants, blinded to admission bloods and diagnosis. The panel will independantly decide the primary diagnosis as well as mark their level of certainty on a 100 mm VAS scale. The panel member will then be able to review imaging, ECGs, and ABGs but not blood results. Results will be both -validated by separate panel member to ensure agreement using Bland-Altman and Correlations and within rater agreement will be assessed by replicate analysis of at least 20 cases 4 weeks apart.

Binary logistic regressions will be utilised to assess associations between VOCs and acute disease. ROC curves will be produced for individual predictors in the primary analysis. Additional techniques for multi variate analysis including but not limited to cluster analysis, structural equation modelling and topological data analyses will be applied where relevant. Tables of descriptive statistics will be compiled for all key variables. All analysis will be performed using SAS 9.4 http://www.sas.com and R 3.2.0 https://www.r-project.org/ .

Secondary Analysis
For the secondary analysis pertaining to other definitions of acute breathlessness, visual analogue breathlessness scores (100mm) and a 1 unit increase in the extended medical research council analysis outlined above for the primary endpoint will be performed based upon stratification of breathlessness type.
.
For the secondary analyses pertaining to discovery of breath metabolomics markers the outcome is a nominal variable with levels (1) Acute Heart failure (2) Community acquired pneumonia 3) Adult exacerbations of asthma/COPD (both in combination and considered individually) and (4) Acute exacerbations childhood wheeze and confirmed asthma in children. The relationship between the primary outcome and the metabolomics biomarkers in exhaled breath sample will be modelled using multinomial logistic regression and ROC curves. In addition to metabolomics markers the following independent variables will be included in the models where necessary (improve model fit): age, sex, validated co morbidity scores such as the Charlson score, indicator disease risk scores such as the CURB-65 pneumonia score, DECAF COPD exacerbation scores and treatments commonly used acutely for the indicator diagnoses.

Subsidiary Analysis

For subsidiary analyses modelling risk of re admission, death and length of stay we will utilise the following approaches

- Cox proportional hazards model will be fitted and competing risk models will be fitted for readmission. Logistic regression analysis will be used when looking at risk at particular time points for readmission.
- The number of exacerbation events will be modelled using Poisson or Negative Binomial regression.
- Relationship between death and breath biomarkers will be evaluated using a logistic regression model.

For subsidiary analyses pertaining to dynamic profile of breath biomarkers during (i) the acute states (time points 1a), (ii) transition to the stable state (time point 1b) and (iii) in the chronic state 8-12 weeks post exacerbation (time point 2), a repeated measures model with a random intercept and random effect for time will be fitted, the random effects will be fitted for each patient. For the repeated measures mixed model an unstructured covariance will be assumed.

Other outcomes are exploratory and based around feasibility of an acute care platform for airways disease. Statistical analyses for these outcomes will be performed as appropriate for hypotheses derived in this exploration.

10.2 The Number of Participants

The sample size for the study is based on the requirements of the multinomial logistic regression model for the primary analysis. We estimate that if the number of events to number of variables in the model is in the ratio 20:1 a sufficiently large sample size will be obtained[24]. We regard this study as being exploratory it will serve as a basis for determining the sample size required for a larger study in the future. (Appendix D)

10.3 The Level of Statistical Significance

All tests will be performed at the 5% significance level.

10.4 Criteria for the Termination of the Trial.

Last participant, last visit

10.5 Procedure for Accounting for Missing, Unused, and Spurious Data.
Describe

A table listing all key variables with percentage of missing data will be reported and where possible the reason for missing data will be reported. Missing data will be imputed using PROC MI in SAS. Spurious data will be reported and where possible correct values will be inserted, if it is not possible to supply correct values value will be deleted.

10.6 Procedures for Reporting any Deviation(s) from the Original Statistical Plan

An interim sample size calculation will be performed at the end of the discovery phase (Quarter 2, year 3) of the project; this calculation will identify the optimal number of patients in each replication phase disease indicator group based upon the optimal number of metabolomic biomarkers associated with the disease and the key outcome data from the discovery phase e.g. risk prediction, 30 / 60 day re admissions, discrimination of disease state from healthy state.

10.7 Inclusion in Analysis

All eligible participants, that have provided informed consent and participated in either discovery or replication phase studies.

11. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access will be granted to authorised representatives from the sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

12. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures.

Internal monitoring by the research team within the BRC will be performed according to ICH GCP. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, we will verify that the clinical study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

The EMBER clinical operations group and clinical work stream group (WP4) described in section 15 of this protocol will meet fortnightly and monthly respectively to discuss overall quality control and assurance of the clinical study. In addition the University of Leicester operates a risk based audit programme to which this study will be subject to.

13. CODES OF PRACTICE AND REGULATIONS

13.1 Ethics

The study will obtain ethical approval from an approved ethics committee and be registered on a trial database.

13.2 Sponsor Standard Operating Procedures

All relevant Sponsor SOPs will be followed to ensure that this study complies with all relevant legislation and guidelines

13.3 Declaration of Helsinki

The Investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki (last amended October 2000, with additional footnotes added 2002 and 2004).

13.4 ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

13.5 Approvals

Once Sponsor authorisation has been confirmed, the protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC) and host institution(s) for written approval.
Once Sponsor authorisation has been confirmed, the Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

13.6 Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participants ID number on the CRF and any electronic database. All documents will be stored securely and only accessible by trial staff and authorised personnel. The study will comply with the Data Protection Act which requires data to be anonymised as soon as it is practical to do so.

13.7 Other Ethical Considerations

The key ethical consideration specific to the MRC-EMBER study is the recruitment of acutely unwell patients that may not be able to provide informed consent. A hierarchical consent process will be adopted for the study with gold standard always being written patient informed consent at time point 1a, whilst accepting that for some patients and children personal legal representatives may be required to give written infirmed consent pending patient consent. In all cases written consent from the patients or parent in the case of young children will be obtained prior to time point 1b assessments.

14.DATA HANDLING AND RECORD KEEPING

EMBER study number

The participants in the study will be assigned a EMBER study number. This number will identify the participant through all stages of data collection and integration.
To reduce errors the EMBER study number will also contain a check digit and it will also be printed as a barcode whenever appropriate, so it can be scanned into data collection tools.

e-CRF data

The EMBER clinical data platform will be built using a bespoke informatics platform (ClinicLE) used previously in respiratory research [18].

Several features of this platform will ensure high quality data collection and integration including:

- user access control so users have permissions to enter/view only appropriate data forms
- integration of data from hospital systems including patient demographics, pathology test results and respiratory physiology
- data field validation (allowing specific types of data; specific ranges of values; or a specific set of selectable options)
- appropriate field design (e.g. using Y/N options instead of checkboxes to ensure the answer is not ambiguous)
- verification through e-signatures and record locking capability
- regular execution of data quality rules to ensure missing/incorrect data is identified quickly
- branching logic so that fields which are not relevant are not displayed to the user
- a form design and data entry audit log/history

A formal test plan will be written during the implementation of the e-CRFs and clarified with other collaborators. Following the completion of the e-CRF implementation, the test plan will be executed to ensure that data collection forms operate within the expected parameters.
A Standard Operating Procedure will be written documenting the use of the EMBER system, specifically for the purpose of entering and managing e-CRF data by the EMBER research staff.
The participants' name and any other identifying detail including date of birth will NOT be included in the final exports of e-CRF data used for research purposes.

Clinical data

Identifiable clinical data exports will be de-identified programmatically by:

- populating the EMBER study number from the recruitment database
- removing hospital numbers, NHS numbers and other patient identifiers
- removing name, address and GP details
- removing or shifting date values
- filtering out free text which may contain identifiable information

Experimental data

Data generated in laboratory experiments will be linked to EMBER study participants using the EMBER study number. No other identifiable data will be present in this data.

Data storage and backup

EMBER clinical data will be stored in an access-controlled database back-end located at the University Hospitals of Leicester. The data will only be available through the hospital network, via the EMBER web

application front-end. The database will be backed up informally every hour to a UHL shared drive in a process managed by the hospital's IT department.

Experimental data and subsequent analyses will be stored using the University of Leicester's research data storage (R) drive backup system (http://www2.le.ac.uk/offices/itservices/ithelp/services/rfs) will be used to maintain all raw experimental data and subsequent analyses.

Risks
Unauthorised access to data: Users will be given appropriate access to databases/data shares for their role, based on recommendations by the PI and other investigators. **Identification of research subjects**: Research subjects will be identified only through their EMBER study number. The connection between this ID and the patient's demographics will only be available to authorised personnel. **Data corruption:** In the unlikely event that data becomes corrupted, it will be restored from a backup of the server which is less than 24 hours old. In the case of the EMBER database this means a small amount of data may be missing, and it will have to be re-entered. To mitigate this, paper copies of CRFs should be kept for at least a week after entry into the EMBER clinical data platform. **Erroneous data entry:** Data entered from paper forms will be verified and if required corrected by a separate person. Data records will then be electronically signed and locked.

EXTERNAL DATA SHARING

Data and samples may be analysed by other investigators/organisations collaborating with the study investigators. In such cases, patient confidentiality will be strictly maintained.

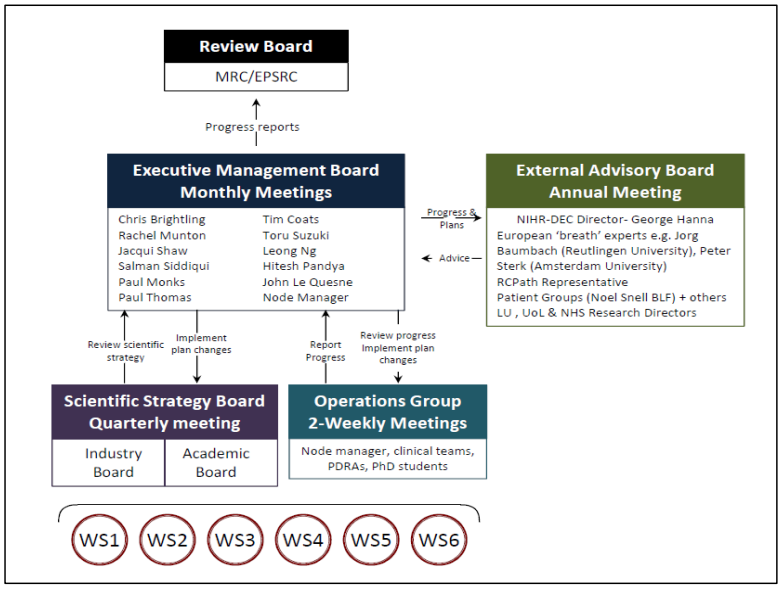
15. STUDY GOVERNANCE

15.1 Clinical Steering Committee (TSC)

The clinical study will be managed through the clinical work package (WP4) of the MRC-EMBER molecular pathology node. The core clinical group (Professor Siddiqui, Dr Pandya, Dr Greening and Professor Coates) all have membership within the EMBER Executive steering group.

The Executive Group comprises the work strand leads and other key PIs. A Scientific Strategy Board comprising all Co-PIs and industrial partners will guide the Executive Board. An operations group chaired by the EMBER's manager will oversee delivery of work. An External Advisory Board with membership derived from experts in the field, Leicester and Loughborough Universities and NHS Research Directors will also oversee the clinical delivery and progress. The Sponsor will be sent minutes from the Scientific Strategy Board and External Advisory Board Meetings. An overview of the governance procedure within EMBER that the clinical work package (WP4) contributes towards is outlined in Figure 6 below.

Figure 6: MRC EMBER program management organogram



15.2 Data Safety Monitoring Committee (DSMC)

The operations group within EMBER will report any unexpected serious adverse events to the core clinical group (Professor Siddiqui, Dr Gaillard Dr Greening and Professor Coats). All unexpected SAEs will be discussed by the core clinical group at monthly EMBER executive board meetings.

16.FINANCING AND INSURANCE

Direct Research Costs: Direct research costs required to deliver the stated research: including research staff, technicians, breath sampling, breath analysis and bioinformatics are fully funded by the UK Medical Research Council (MRC) and The Engineering and Physical Sciences Research Council (EPSRC) grant number MR/N005880/1. Additional funding has been received from the Midlands Asthma and Allergy Research Association (MAARA) and the Leicester National Institute for Health Respiratory (NIHR) Biomedical Researh Unit, for the EMBER clinical program.

NHS Treatment Costs: Not applicable for acute research studies as by definition patient care will often continue after the R&D activity has ceased due to the fact that patients will enter chronic care pathways.

NHS Support Costs: None anticipated as the research in question is a discovery biomarker campaign.

Insurance will be provided by the University of Leicester.

17. PUBLICATION POLICY

Publications will be prepared according to the MRC-EMBER consortium agreement and the University of Leicester publications policy. In brief all intended publications will be submitted to the EMBER executive board for review and comments within 60 days of journal submission. Authorship will be according to contribution and internationally recognised guidance on journal authorship.

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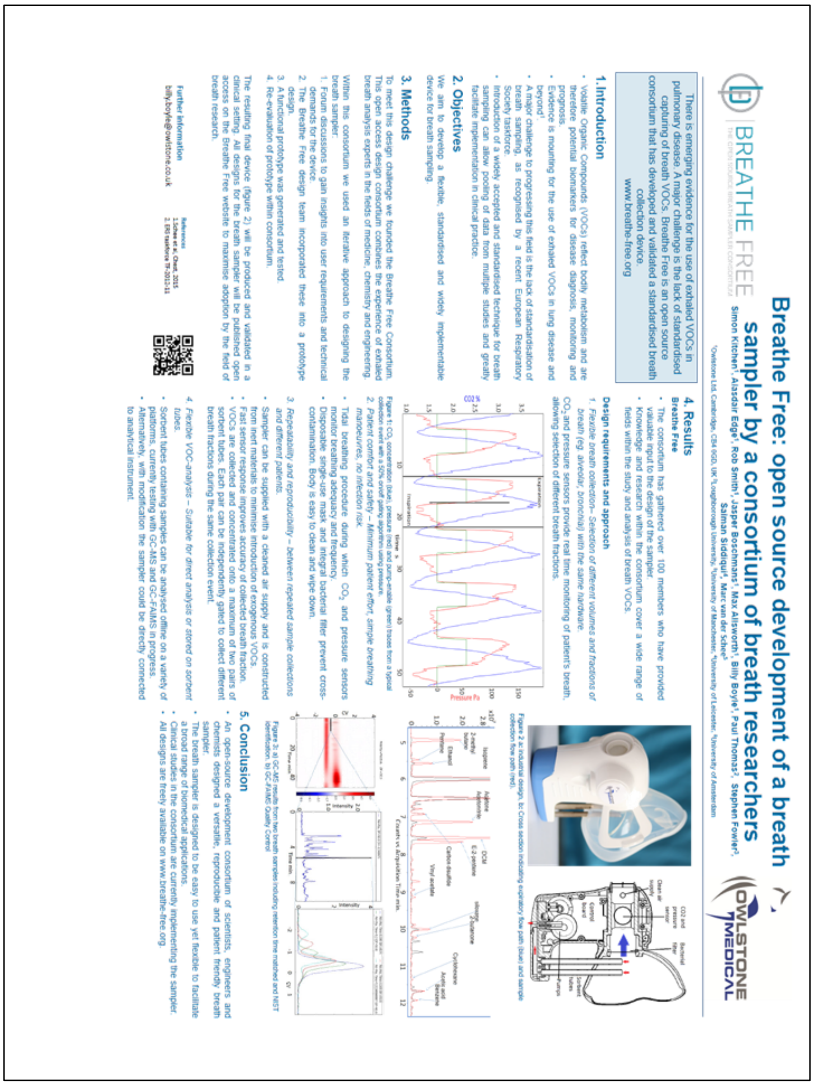
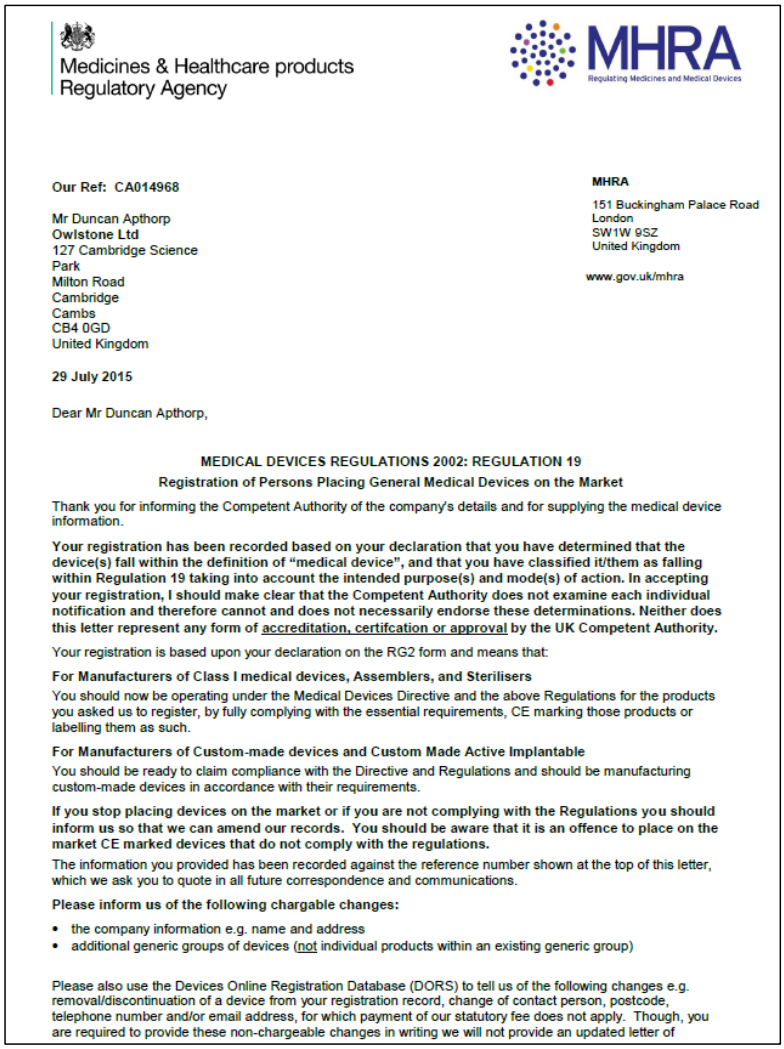
APPENDIX A: RECIVA breath sampling device



20. APPENDIX B: RECIVA CE Marking Authorisation



Medicines & Healthcare products Regulatory Agency

MHRA
Regulating Medicines and Medical Devices

Our Ref: CA014968

Mr Duncan Apthorp
Owlstone Ltd
127 Cambridge Science
Park
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CB4 0GD
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MHRA
151 Buckingham Palace Road
London
SW1W 9SZ
United Kingdom

www.gov.uk/mhra

29 July 2015

Dear Mr Duncan Apthorp,

MEDICAL DEVICES REGULATIONS 2002: REGULATION 19
Registration of Persons Placing General Medical Devices on the Market

Thank you for informing the Competent Authority of the company's details and for supplying the medical device information.

Your registration has been recorded based on your declaration that you have determined that the device(s) fall within the definition of "medical device", and that you have classified it/them as falling within Regulation 19 taking into account the intended purpose(s) and mode(s) of action. In accepting your registration, I should make clear that the Competent Authority does not examine each individual notification and therefore cannot and does not necessarily endorse these determinations. Neither does this letter represent any form of accreditation, certifcation or approval by the UK Competent Authority.

Your registration is based upon your declaration on the RG2 form and means that:

For Manufacturers of Class I medical devices, Assemblers, and Sterilisers

You should now be operating under the Medical Devices Directive and the above Regulations for the products you asked us to register, by fully complying with the essential requirements, CE marking those products or labelling them as such.

For Manufacturers of Custom-made devices and Custom Made Active Implantable

You should be ready to claim compliance with the Directive and Regulations and should be manufacturing custom-made devices in accordance with their requirements.

If you stop placing devices on the market or if you are not complying with the Regulations you should inform us so that we can amend our records. You should be aware that it is an offence to place on the market CE marked devices that do not comply with the regulations.

The information you provided has been recorded against the reference number shown at the top of this letter, which we ask you to quote in all future correspondence and communications.

Please inform us of the following chargable changes:

- the company information e.g. name and address
- additional generic groups of devices (not individual products within an existing generic group)

Please also use the Devices Online Registration Database (DORS) to tell us of the following changes e.g. removal/discontinuation of a device from your registration record, change of contact person, postcode, telephone number and/or email address, for which payment of our statutory fee does not apply. Though, you are required to provide these non-chargeable changes in writing we will not provide an updated letter of

registration. As the updated information does not affect your regulatory obligations or the information published on our Public Access Registration Database (PARD).

Thank you for registering the following generic groups of devices:

Class I Devices:
Sampling And Cell Collection Devices (Patient Contact - Not IVDDs)

Custom Made Devices:
None

Products Covered By Article 12:
None

Confidentiality

Please note that in accordance with Directive 2007/47/EC as of 21st March 2010 information on the registration of persons responsible for placing devices on the market will no longer be treated as confidential and the Competent Authority will provide third parties with information on the name and address of manufacturers and authorised representatives and their devices that have been registered. However the names of individuals, their telephone numbers and email addresses will remain confidential unless you have chosen to trade using personal details. This change only applies to medical devices and does not affect In Vitro Diagnostic devices registration, which remain confidentiality under Article 19 of the In Vitro Diagnostic Directive 98/79EC.

If your company name or that of a manufacturer that you represent is based on an individual's personal name it will be published unless you inform the MHRA that you would like the company name to remain confidential.

Likewise, if your company address or that of a manufacturer that you represent is the personal home address of an individual it will be published unless you inform the MHRA that you would like the company address to remain confidential.

Should you have any queries regarding your registration please do not hesitate in contacting us.

Yours sincerely

Jasu Patel

Jasu Patel

020 3080 7195
020 3118 9809

Jasu.Patel@mhra.gsi.gov.uk

21. APPENDIX C: Interim analysis and sample size calculation

21.1 EMBER Sample Size Estimates

An interim analysis was conducted in September 2017 to determine the sample size of the ember acute study.

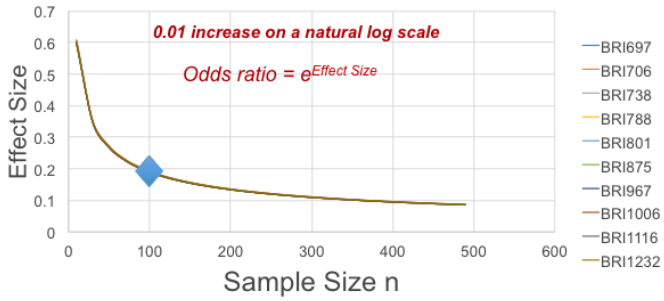
21.2 Closed formula for logistics regression models

82 acutely breathless adults (asthma:35, community acquired pneumonia :16, COPD:26) and 17 healthy controls were utilised for the analysis.

A panel of 10 pre specified aldehyde biomarkers derived from GCMS analysis were supplied. The biomarkers were normalised to a common standard and were not background subtracted.

A closed formula from Hsieh *et al*, Statis med 1998, was utilised to calculate sample sizes from logistic regression models of the 10 aldehydes with acute breathlessness as the outcome measure. The sample size estimates are also relevant to acute class comparions vs. the sum of other acute classes.

The results of the analyses are outlined in the figure below (each BRI number annotates a specific aldehyde).



Based upon the sample size estimates we would have an 80% power at the 5 % significance level to detect an odds ratio of association of 1.2 for a given disease class with 55 patients per disease class. Given the fact that MRC EMBER seeks to discover and replicate breath VOCs amongst 5 adults classes (CAP, heart failure, COPD, asthma and healthy aged matched subjects).

We would require 110 patients per class – 550 patients across the program to achieve these aims.

Commented [CSB1]: This is not a complete sentence. There is a clause missing, or else the punctuation needs adjustment.

21.3 Closed formula for sensitivity and specificity of disease biomarkers

The closed formulae in Tilaki-Hajjan *et al*, Journal of Biomedical Informatics 48(2014), 193-2014, were also utilised to understand the power that the samples size above would provide with respect to biomarker sensitivity and specificity.

The following assumptions were made:

- That a sensitivity of 80 % with a precision (d) of between 5-8% would provide a useful biomarker capable of 'ruling out' an acute class. The same target was applied to specificity.
- We assume a prevalence of acute breathlessness of 80% as the MRC EMBER campaign uses acute breathlessness as the initial stratification tool for recruitment and 1:5 patients recruited will be non-breathless healthy controls. We aim to balance group sizes across classes equally.
- A type 1 error rate of 0.05 and power of 95% CI level, 90% CI level
- *Note for a prevalence ≥ 50%* $Nspec \geq Nsens$ *is always the case, so* N_{spec} *is the number we need to consider.*

95% CI, precision ± 5%

$N_{sens} = 1.96^2 \times 0.8 \times 0.2 / 0.05^2 \times 0.8 = 307$

$N_{spec} = 1.96^2 \times 0.8 \times 0.2 / 0.05^2 \times 0.2 = 1,230$

90% CI, precision ± 5%

$N_{sens} = 1.64^2 \times 0.8 \times 0.2 / 0.05^2 \times 0.8 = 215$

$N_{spec} = 1.64^2 \times 0.8 \times 0.2 / 0.05^2 \times 0.2 = 861$

To obtain *n* near 550, increase the maximum marginal error d (i.e. decrease the precision)

95% CI, precision ± 8%, Prevalence = 80%, Sensitivity and Specificity=80 %

N_{sens} = 120

N_{spec} = 480

21.4 Summary

550 patients (110 per class: healthy, acute asthma, acute COPD, community acquired pneumonia, acute heart failure) will be required for combined discovery and replication to (i) detect a minimum odds ratio for association of breath VOCs and acute breathlessness of 1.2 or between acute breathlessness class (e.g. pneumonia with remaining categories as a reference).

This sample size and group allocation will give us sufficient power to discover a disease specific VOC over a 95% CI with a specificity of 80% ± 8%, sensitivity of 80% ± 8% at an α = 0.05. The sample size will give us sufficient power to replicate based upon biomarker sensitivity but not specificity.

The power calculation assume that ALL acute patients have one or more of the following (i) patient defined acute breathlessness or (ii) a unit change in eMRC (assuming that they can quantify their usual baseline eMRC).

21.5 Recruitment Plan: Acute Adult Study

Disease Category	Discovery	Replication
Acute Adult Asthma	**55**	**55**
Acute COPD	**55**	**55**
Acute Heart Failure	**55**	**55**
Community Acquired Pneumonia	**55**	**55**
Adult healthy volunteers	**55**	**55**

21.6 Paediatric Recruitment Plan: Acute Study

Acute childhood wheeze and or confirmed asthma.

Revised to 50 Discovery and 25 replication, based upon current recruitment.

21.7 Stable Disease Recruitment Plan

This should be opportunistic and support the following ongoing initiatives

- Mologics COPD study
- Mepolizumab severe asthma early response study
- Sushila Natarajan ACF: project April 2018 (small healthy volunteer repeatabillty study n=12 healthy adults baseline x2, 2 weeks, 3 months GC-MS ReCIVA, PTRMS (Salman to discuss at Exec).
- Severe asthma clinical patient samples if possible/samples from aligned BRC studies (agreed at the exec)

Planned acute study recruitment last patient visit 1a

1st Nov 2018 for combined discovery and replication