Study details

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1 Trial Summary

Title: To determine the optimum series of investigations to diagnose asthma in

Short title: **RADicA (R**apid **A**ccess **D**iagnost**ic**s for **A**sthma)

Primary objectives:

1. Determine the optimum diagnostic pathway for asthma based on conventional tests of large airway function and novel tests of small airway function
2. Determine the optimum diagnostic pathway for "steroid-responsive airways disease" based on conventional tests of large airway function and novel tests of small airway function

Secondary objectives:

1. Evaluate the accuracy of the National Institute for Health and Care Excellence (NICE) asthma diagnostic algorithms.
2. Identify the best predictor(s) response to inhaled corticosteroids (ICS, at 8-weeks) from measurements taken at baseline and early treatment (1-2 weeks)
3. In healthy volunteers, establish reference intervals and calculate repeatability coefficients for MBW, and IOS where there is a lack of evidence on what threshold constitutes a 'normal' set of values
4. In healthy volunteers, establish reference values and calculate repeatability coefficients for PExA and VOC, where there is a lack of evidence on what threshold constitutes a 'normal' set of values.
5. Identify the profile of biomarkers in volatile organic compounds (VOCs), and particles in exhaled air (PExA) which best predict asthma diagnosis.
6. Evaluate whether markers of immune cell activation predict asthma and predict response to treatment.
7. Evaluate the predictive capacity of upper respiratory viral biomarkers
8. Determine the optimum diagnostic pathway based on conventional tests of large airway function and novel tests of small airway function in a) adults and older children (≥12 yrs) and b) younger children (<12 yrs)

Type of trial: Clinical study to determine appropriate diagnostic tests, open label

Trial participants:	Patients with symptoms consistent with asthma, not currently receiving regular treatment with inhaled corticosteroids will be recruited from primary care or secondary care.
Planned sample size:	up to 150 who have completed all core visits plus additional healthy controls (1:1 ratio)
Trial design and methods:	The study will use a prospective cohort design. Participants with one or more symptoms in keeping with asthma (i.e., cough, wheeze, chest tightness and breathlessness), not currently receiving inhaled corticosteroid treatment, will be recruited. Participants will undergo 4 core visits and up to 2 optional visits. At these visits a series of standard and novel lung function tests will be performed and tissue sample collected, before and following; 1 to 2 weeks (early follow up) and 6 to 8 weeks (late follow up) of standard asthma treatment. In addition we will recruit age/gender matched healthy controls at a ratio of 1:1 to attend two visits to collect data on normal ranges and reproducibility of the novel tests
Planned trial sites:	The Manchester University NHS Foundation Trust (Wythenshawe site) will house the asthma diagnostic centre. General practitioners, walk-in centres from the local area, secondary care centres (Greater Manchester and Cheshire) will refer patients for inclusion in the study.

2 Background, Rationale, Risks and Benefits

2.1 Background/Rationale

There is currently no single 'gold standard' test to confirm (or refute) a diagnosis of asthma. Asthma has largely been a clinical diagnosis based on a characteristic pattern of symptoms and signs in the absence of an alternative explanation. The British asthma management guidelines (British Thoracic Society/Scottish Intercollegiate Guideline Network) have been widely accepted and used throughout the United Kingdom [1]. These guidelines advocate a trial of treatment for patients suspected of having asthma at clinical assessment; patients who respond to treatment are then formally diagnosed with asthma. In a Canadian study of a large cohort of adults with a recent diagnosis of asthma, careful re-evaluation ruled-out the diagnosis of asthma in one third[2] This likely reflects a combination of asthma remission and over diagnosis, but in 2% of the population an alternative serious cardiorespiratory condition was diagnosed. The European Asthma Research and Innovation Partnership (EARIP) recently identified improving the diagnosis of asthma in their 15 key research priorities. [3] Due to concerns about the over- [2] and under-diagnosis[4] of asthma, UK experts have recently developed comprehensive guidance on the diagnosis of asthma incorporating objective tests, on behalf of the National Institute of Health and Care Excellence (NICE)[5] The algorithm incorporates the sequential use of five measures of lung function and inflammation, each applied as a dichotomous variable: (1) Spirometry, (FEV_1/FVC); (2) bronchodilator reversibility (BDR); (3) fractional exhaled nitric oxide (FeNO); (4) peak flow variability (PEFv) and (5) bronchial hyperresponsiveness testing (BHR) to methacholine or histamine. A minimum of two tests must be positive to confirm the asthma diagnosis. In children, only the first four tests are included in the algorithm. Much of the existing evidence for the use of individual tests has been collected in adults, with a paucity of data from children. Individually, these tests have sensitivity in the region of 50% with a

specificity ranging from 72-87% (in children). Although it is widely recognised that false positive or false negative results of diagnostic tests will have important consequences in patient management, the accuracy of these tests, in this specific sequence, for diagnosing asthma remains unknown, and the impact on patient care has not been tested prospectively. A series of similar diagnostic workups using BDR, PEFv, mannitol and methacholine have previously been shown to have either poor sensitivity or specificity in diagnosing asthma, when compared to a diagnosis made by a panel of experts.[6] We have recently demonstrated that in a population of adolescents the proposed algorithm performs poorly in diagnosing asthma when compared to current clinical practice.[7] Therefore there is an urgent clinical need to test the proposed diagnostic algorithm to understand the impact on patient care currently and to identify whether it can be improved by comparing alternative sequences of tests and/or alternative thresholds for positive tests to optimise asthma diagnosis now - a new 'gold standard'.

In addition, the published diagnostic algorithm only focuses on tests of large airways, whereas abnormalities in the small airways are known to contribute to the clinical expression of asthma. Tests to measure small airway function were first designed in the 1960s and include multiple breath washout (MBW, which is a technique that allows assessment of ventilation heterogeneity) and impulse oscillometry (IOS, which measures peripheral airway resistance)[8,9] These tests may be more sensitive in detecting early changes in lung function than spirometry and devices for these measurements are commercially available. Despite multiple efforts, normative ranges in adults for impulse oscillometric parameters are yet to be established.[10]Moreover, assessment in a symptomatic untreated population is required to measure their accuracy in asthma diagnosis. It is likely that these tests of small airway function will show better sensitivity and specificity than lung function tests currently used in clinical practice and included in the NICE algorithm, and it is necessary to identify their role in the diagnostic workup for asthma both in adults and children. Furthermore, an early predictor of response to inhaled corticosteroids would be valuable as this would shorten the duration of trials of treatment.

Advances in technology now allow for small airway biomarkers in exhaled breath to be measured non-invasively. Particles in Exhaled Air (PExA©), combines breathing manoeuvres with sophisticated analysis instrumentation to collect biological samples from the small airways.[11] Deep breathing opens the small airways and aerosolises the airway lining fluid, releasing microscopic particles, which are subsequently exhaled for collection and analysis. The PExA© device collects exhaled particles on a substrate and provides real time feedback on particle counts. The substrate can also be removed for further lipid and protein content analysis using mass spectrometry. Preliminary results have shown differences in exhaled particle numbers and protein profiles between people with small airway disease and those without.[12,13] Volatile organic compounds (VOCs- the product of metabolic processes occurring in the airways), and non-volatile proteins and lipids can be measured in breath and providing potential novel biomarkers for inflammatory airways disease. We have developed a validated method for the collection of breath for the capture and analysis of exhaled VOCs, and demonstrated that we can sample safely and reliably in patients with respiratory disease. We and others have shown that markers of oxidative stress are raised in people with asthma compared to healthy controls.[14-16] PExA and VOCs could potentially provide a range of biomarkers that will revolutionise asthma diagnosis, but testing in heathy populations is required to establish normal ranges, followed by testing in asymptomatic untreated populations to further develop knowledge of specific patterns in disease. As such, these test are experimental and some way off use in the clinic.

In addition to the identified gap in knowledge regarding asthma diagnosis, there is also a lack of understanding of the underlying inflammatory processes' in asthma patients. Further analysis of inflammatory cells present in the blood and lungs of early asthma patients could be vital to improve and refine asthma diagnosis, endotyping, and development of targeted treatments. Use of multi-parameter flow cytometry (BD Fortessa, 16+ parameters), mass cytometry (Helios CyTOF, 40 parameters), imaging flow cytometry (Imagestream) and FACS isolation of defined cell populations (BD Influx) will enable us to generate a state-of-the art understanding of the proportions and activation status of specific immune cell types in PBMCs, sputum and BAL from early asthma patients. This information has the potential to generate a high-resolution picture of how early asthma modulates the activation and function of the key inflammatory cells. Previously it has been shown that murine dendritic cell (DC) ability to promote allergic inflammation is controlled by methyl-CpG-binding protein Mbd2.[17] This experimental research will aim to define expression of Mbd2, and genes under the control of Mbd2, in DC subsets sampled from blood, sputum and BAL of early asthma patients. It has previously been shown that one of the major genes regulated in murine DCs by Mbd2 is CCL17[17]. The precise function of CCL17 in human allergic disease as well as the identity of the key human cellular sources of/responders to this chemokine are currently unclear. By delineating human inflammatory cell expression of Mbd2, CCL17 and its receptor CCR4 in PBMCs, sputum and BAL during asthma we will aim to determine how this relates to DC subset activation and function in early disease.

The human virome encompasses all of the viruses found on or in the human body.[18] It is well documented that asthma attacks almost always follow upper respiratory virus infections. Viruses are also present at the initiation of asthma, as an episode of viral wheeze; furthermore, repeated episodes make asthma persist. It seems that patients with asthma have an immune defect in detecting and killing respiratory viruses.[19] Our preliminary data show that the respiratory virome (the viral microbiome) is considerably different between healthy children and children with asthma.[19] In fact, the virome appears to be most different in patients who have symptoms, rather than those who have controlled disease. We think that changes in the make-up of the virome composition may reflect symptoms of asthma. If this proves to be true, and the changes in the virome happen first, we may be able to predict when asthma symptoms will appear or get worse, based on virome 'snapshots', giving us the opportunity to intervene before patients get too sick. It is therefore important to study the virome in asthma, both in terms of pathophysiology and prognostic potential.

Other markers that have been demonstrated in asthma patients and may have a role in asthma diagnosis include chitinases and chitinase-like proteins (CLPs). CLPs are a family of molecules associated with inflammation and structural lung changes in asthmatic patients. YKL-40 is the best studied example of a CLP that is increased in the serum, BAL and sputum of asthmatics, with YKL-40 levels higher in patients with severe disease and greater oral corticosteroid use.[20]. Whilst CLPs may be potential biomarkers of certain asthma endotypes, murine models have shown that both chitinases and CLPs are also important drivers of allergic airway inflammation.[21] Therefore, we aim to examine the expression of chitinases and CLPs in asthmatic patients alongside in-depth analysis of PBMC, sputum and BAL inflammatory phenotypes to determine how chitinase and CLP expression relates to different immune cells and whether their expression during early asthma can predict patient responses to treatments.

The University of Manchester and partner NHS Trusts recently received a Biomedical Research Centre (BRC) award from the National Institute for Health Research (NIHR). One aspect of the successful bid was to set up the Rapid Access Diagnostic for Asthma (RADicA) study to determine the optimum series of

investigations to diagnose asthma in adults and children. Patients with asthma symptoms will be referred from primary and secondary care into the RADicA study to undergo a series of standard lung function tests, tests of small airway function and novel tests of biomarkers of small airway inflammation, before and after commencing treatment with inhaled corticosteroids. The overarching objective of the RADicA project is to provide data that can inform the development of diagnostic pathways for asthma in children and adults. This will include existing lung function tests (Spirometry, BDR, FeNO, PEFv, BHR), emerging tests of small airway function (MBW and IOS) and more exploratory tests of biomarkers of small airway inflammation (PExA and VOC) to optimise diagnostic pathways for adults and children with asthma. Secondary experimental objectives include the measurement of proteins and cells in blood and sputum (where available and dependent upon securing additional funding) and in BAL is a subgroup of patients who undergo bronchoscopy to gain novel insights into inflammatory processes in asthma development. We have chosen to study this symptomatic population in whom there is diagnostic uncertainty in line with recommendations of the European Medicines Agency Guideline[22] on clinical evaluation of diagnostic agent; our recruitment strategy aims to mirror the population in whom the algorithm is intended in clinical practice.

2.2 Risk and benefits

2.2.1 Potential Risks and Benefits

Summary of risks (core visits)

- Methacholine challenge - severe bronchoconstriction
- Fainting during blood tests
- Local/systemic response to skin-prick testing
- Following use of ICS – oral thrush, hoarse voice

Summary of risks (optional visits)

- Severe bronchoconstriction during mannitol challenge
- Bronchoscopy – pyrexia, infection, pneumothorax, haemoptysis, sedation risk

Summary of benefits (core +/- optional visits)

- Patients will benefit from a prompt and comprehensive assessment of lung function and diagnosis from respiratory specialists
- GPs will benefit from the option to refer patients for advanced respiratory and diagnostic assessment (including tests within the NICE algorithm which are not currently available in primary care), with rapid access to the research clinic

- Data will inform policies and guideline on asthma diagnosis in the future
- Healthy control data will contribute to future reference ranges for tests of small airway function

2.3 Trial Risk Category

The core visits of this trial is categorised as Type A = No higher than the risk of standard medical care.

For the subgroup of patients who undergo bronchoscopy this visit would also be categorised as type A

3 Trial Objectives/Design and Outcome measures

3.1 Trial Short Description

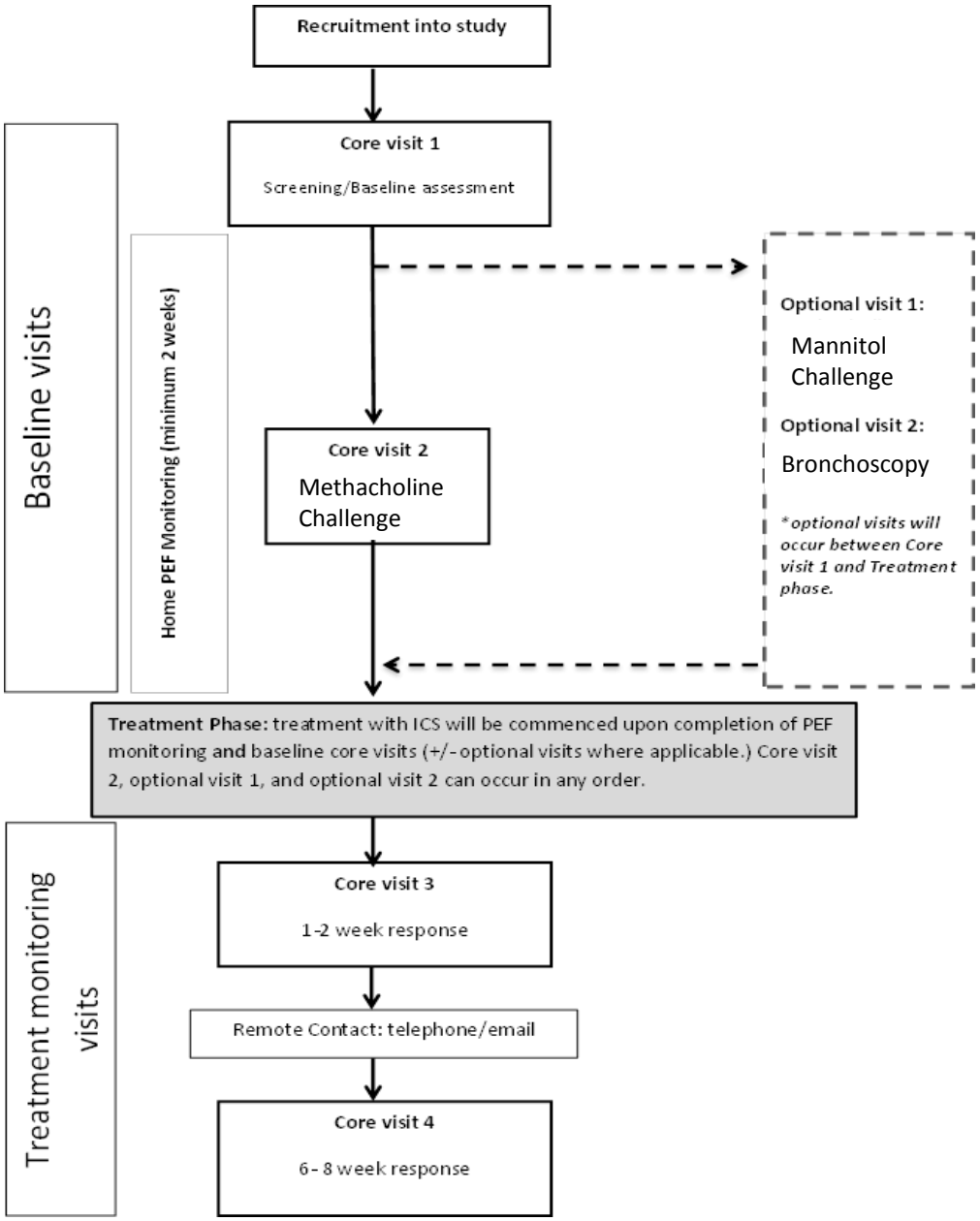
The study will use a prospective cohort design. Participants with one or more symptom suggestive of asthma (i.e., cough, wheeze, chest tightness and breathlessness), not currently receiving inhaled corticosteroid treatment, (or who has not been taking regular ICS for at least 2 weeks) and has not received systemic corticosteroids for at least 4 weeks will be recruited. Participants will undergo 4 core visits and up to 2 optional visits (section 5.2).

At Visit 1, following written informed consent, the participant will be thoroughly evaluated (clinical history and examination) following a structured proforma.

Participants in whom asthma is deemed low probability **and** in whom an alternative diagnosis is suspected (e.g. pneumonia) would be discussed with the supervising consultant or nominated deputy and would be withdrawn from the study and referred back to their GP to be evaluated further (GP letter 2). If appropriate due to clinical urgency and/or severity direct referral to a general respiratory clinic or on call team at Wythenshawe hospital or local clinic would be arranged.

Provided no alternative diagnosis is suspected to be more likely than asthma as the cause of their respiratory symptoms, they will continue in the study. Participants will then undergo a series of standard and novel lung function tests before starting standard asthma treatment with inhaled corticosteroids for 6-8 weeks. They will attend for follow up visits (section 3.1.1) at 1-2 and at 6-8 weeks after starting treatment and repeat the standard and novel lung function tests, as well as questionnaires and blood tests, to assess subjective and objective response to inhaled corticosteroids (section 5.2).

3.1.1 Trial flow chart



Study Time line:

Visits	CV 1	(CV2/OV1/OV2)	Commence treatment period	CV 3	CV 4
Shortest study pathway	D 0	(Range D1- D14)	D 14	D 21	D 56
Longest study pathway	D 0	(Range D1 – D42)	D 42	D 56	D 98

3.2 **Trial Objectives**

3.2.1 Primary objectives

1a. Determine the optimum diagnostic pathway for asthma based on conventional tests of large airway function and novel tests of small airway function

1b. Determine the optimum diagnostic pathway for "steroid-responsive airways disease" (SRAD) based on conventional tests of large airway function and novel tests of small airway function

3.2.2 Secondary objectives

2a. Evaluate the accuracy of the National Institute for Health and Care Excellence (NICE) asthma diagnostic algorithms

2b. Identify the best predictor(s) response to inhaled corticosteroids (ICS, at 8-weeks) from measurements taken at baseline and early treatment (1-2 weeks)

2c. In healthy volunteers, establish reference intervals and calculate repeatability coefficients for MBW, and IOS where there is a lack of evidence on what threshold constitutes a 'normal' set of values

2d. In healthy volunteers, establish reference values and calculate repeatability coefficients for PExA and VOC, where there is a lack of evidence on what threshold constitutes a 'normal' set of values.

2e. Identify the profile of biomarkers in volatile organic compounds (VOCs), and particles in exhaled air (PExA) which best predict asthma diagnosis.

2f. Evaluate whether markers of immune cell activation predict asthma and predict response to treatment.

2g. Evaluate the predictive capacity of upper respiratory viral biomarkers

2h. Determine the optimum diagnostic pathway based on conventional tests of large airway function and novel tests of small airway function in a) adults and older children (≥12 yrs) and b) younger children (<12 yrs)

3.3 Outcome measures

Table 1

	Test	Outcome measures	Established threshold for positive results
Symptoms	Asthma Control Questionnaire (ACQ)	ACQ-5	Change of 0.5[23]
Tests included in NICE algorithm[5]	spirometry	FEV_1/FVC FEV_1, FVC, MEF_{25-75}	FEV_1/FVC <70% or below LLN
	BDR	Δ FEV_1 or FVC following 400mcg inhaled Salbutamol	≥ 12% and 200mls increase in FEV_1 and/or FVC
	FeNO	NO ppb	>40 ppb in adults (35 in children)
	PEFv	PEF variability measured twice daily for 2 weeks	≥20% variability in PEF over at least 3 days Measured as daily amplitude percentage mean: [(PEFhighest – PEFlowest) % PEFmean]x100
	BHR_{mann}	Mannitol PD15	Dose causing 15% fall in FEV_1
	BHR_{meth}	Methacholine PD20	Dose causing 20% fall in FEV_1
Tests of small airway function	IOS	R_{rs}5Hz, R_{rs}20Hz, R5-20, X_{rs}5Hz, X_{rs} 20Hz, X5-20	To be established
	MBW	LCI, Scond, Sacin	To be established
Experimental biomarkers of small airway inflammation	PExA	N^o of exhaled particles	To be established
	VOC	Mass spectrometry	To be established
Other	Blood - eosinophils	Blood eosinophil count	> 0.4 × 10^9/L[24]
	Blood – other	Cell culture, immune cell count, measures of immune cell activation (Mbd2, CCL17, CCR4) YKL-40 (CHI3L1), AMCase (CHIA), Chitotriosidase (CHIT1)	Experimental (funding dependent)
	Sputum	Sputum eosinophil %, Sputum	≥2% eosinophils[25]

		neutrophil %	
	Sputum - other	Cell culture, immune cell count, measures of immune cell activation (Mbd2, CCL17, CCR4) YKL-40 (CHI3L1), AMCase (CHIA), Chitotriosidase (CHIT1)	Experimental (funding dependent)
	Skin prick tests	To inhalant allergens	Atopic if 1 or more positive
	Serum specific IgE	allergen specific IgE to common inhalants (mite, cat, dog, grass, tree)	Atopic if 1 or more positive
	BAL	Cell culture, immune cell count, measures of immune cell activation (Mbd2, CCL17, CCR4)	experimental
	BAL - other	YKL-40 (CHI3L1), AMCase (CHIA), Chitotriosidase (CHIT1)	Experimental – funding dependent
	Nasopharyngeal swabs	Virology profile	experimental
	Stored blood	Other biomarkers	

3.3.1 Primary measures/endpoints

Primary objective 1a. Determine the optimum diagnostic pathway for asthma based on conventional tests of large airway function and novel tests of small airway function.

Asthma definition i: Asthma will be diagnosed based on clinical symptoms and signs alone, recorded in a standardised format and three clinicians will be asked to score asthma as high probability, intermediate probability or low probability asthma, based on information collected in the structured clerking proforma. When two out of three scored an individual as high or intermediate probability asthma, the individual was classified as "asthma". In the event of 2 or more scoring low probability the subject was classified as "not asthma".

Asthma definition ii: Asthma will be defined on the basis of symptom consistent with asthma **and** objective evidence of variable airflow obstruction, (determined by observation of PEF chart, spirometry pre- and post-salbutamol, bronchial challenge results)

Primary objective 1b: Determine the optimum diagnostic pathway for "steroid-responsive airways disease" (SRAD) based on conventional tests of large airway function and novel tests of small airway function

Steroid-responsive airways disease will be defined as improvement in symptoms, airway physiology, inflammatory profiles and clinical impression following 6-8 weeks of ICS treatment. Patients will be categorised as symptom responsive (Primary endpoint), physiology responsive (secondary endpoint), and clinically responsive (secondary endpoint), as follows:

Steroid-responsive airways disease i: Symptom responsive (0.5 unit improvement in ACQ-5)

Steroid-responsive airways disease ii: Physiological responsive (12% improvement in FEV1 or FVC and 200ml, or 1 DD improvement in PD15Mann or PD20MCh

Steroid-responsive airways disease iii: Clinically responsive (clinical impression of "ICS responsive" from the patient and 2 of 3 clinicians who have access to spirometry, bronchial challenge tests and FeNO pre- and post-ICS treatment)

Univariate and multivariate logistic regression analysis (binary and linear) will be used to determine the prediction probability of asthma following investigation with outcome measures listed in Table 1 (section 3.3). Please see section 8.3 for detailed data analysis plan.

3.4 Trial Design

The study will be a prospective cohort study. A symptomatic population with suspected asthma will be recruited to ensure that it is representative of the population in whom the asthma diagnostic algorithm would be used in clinical practice. Participants with symptoms of asthma (i.e., cough, wheeze, chest tightness and/or breathlessness), not currently taking inhaled corticosteroid treatment will be recruited through primary and secondary care sources (section 5.2.1). Participants will undergo 4 core visits, 1 telephone/email contact and up to 2 optional visits (section 5.2). At these visits a series of standard and novel lung function and bronchial challenges will be performed and tissue samples collected; full details of these procedures are presented below (section 5.2.14). Following the final baseline visit, participants will commence treatment with an inhaled corticosteroid for daily use during the 6 - 8 week treatment period. Participants will also be provided with a bronchodilator for use as required throughout the duration of the study, this will be provided at core visit 1. (section 5.2.8).

3.4.1 Study Setting

Manchester University NHS Trust (MFT): The physiological assessment and tissue collection will take place at Manchester University NHS Trust (MFT). The majority of the work will take place on the Wythenshawe Hospital site.

Manchester Institute for Biotechnology (MIB): The analysis of breath sample composition i.e., VOCs, will take place in the Manchester Institute for Biotechnology.

3.4.2 End of trial

The sponsor will notify the REC within 90 days of the completion of the study. This will be the date of the last visit/data item of the last patient undergoing the trial.

4 Selection and Withdrawal of Subjects

4.1 Informed consent

The Principal Investigator (PI) retains overall responsibility for the informed consent of participants and will ensure that any person delegated responsibility to participate in the informed consent process is duly authorised, trained and competent to do so.

A 'participant information sheet' (PIS) and an 'informed consent' (IC) form will be presented to the participants detailing: the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. Where appropriate, participants under 16years old will be presented with an 'assent sheet' and the consent form will be completed by the parent or legal guardian. Participants will be allowed as much time as wished to consider the information, and the opportunity to question a member of the research team to decide whether they wish to participate in the study.

Written informed consent will be obtained from all participants (or via parent/ legal guardian) by the principal investigator or by a delegated member of the research team. The consent process will involve five stages, listed below:

1. Giving the participants a written information sheet
2. Giving time to consider the information
3. Providing the opportunity to ask questions
4. Receiving the written consent
5. Reminding participants that they are free to withdraw at any time

Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the informed consent (or their parent or legal guardian as appropriate) and assent to participate will also be obtained from the participant where appropriate. A copy of the signed Informed Consent will be given to the participants. The original signed form will be retained at the study site.

4.2 Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable

The participant information sheet (PIS) will include details of the biological samples that will be used in the current study and those that may be stored for future use. Participants will be able to choose whether

they allow for their samples to be used for future respiratory and allergy studies and indicate their decision on the consent form, by initialling next to the corresponding option.

4.3 Inclusion and exclusion criteria

4.3.1 Inclusion Criteria

i. Males and females ≥5years and <70 years
ii. Clinical suspicion of asthma from GP or referring health care professional
iii. One or more symptom in keeping with asthma (i.e., cough, wheeze, chest tightness and/or breathlessness)
iv. Capable of giving informed consent or where under 16 years attends with parent or legal guardian.

4.3.2 Exclusion Criteria

i. Current ICS (used within previous 2 weeks) or oral steroid treatment (within the previous 4 weeks)
ii. Former/Current smokers **if** >10 pack year smoking history
iii. Other relevant comorbidities (e.g., other lung disease; CF, COPD, ILD or bronchiectasis)
iv. Recent antibiotic treatment within previous 2 weeks (these participants may be able to enter the study at a later date)
v. Pregnant

4.4 Screening and Eligibility Assessment

Participants referred to this study will be assessed for eligibility by a member of the research team against the defined inclusion and exclusion criteria (Section 4.3).

4.5 Identification and selection of Participants

Potential participants will be identified from participating GP surgeries, community teams, and secondary care hospitals across Greater Manchester. Patients with symptoms consistent with asthma (i.e. cough, wheeze, chest tightness and/or breathlessness) that have not been taking inhaled corticosteroids in the previous 2 weeks or oral steroids within the previous 4 weeks will be eligible. Any patients identified as potential participants but who have received recent steroids may be eligible for re assessment at a later date.

4.6 Randomisation Procedure / Code Break

Not applicable.

4.7 Withdrawal of Subjects

Participants may be withdrawn from the research at any stage, at the request of the participant, in the event of a SAE and/or in the event of the development of significant new medical condition. The reason for withdrawal will be documented. If withdrawal is due to an AE/SAE follow up will be as stated (Section 6.2.3)

4.8 Expected Duration of Trial

The duration of trial will be between 8-14 weeks for each participant and will be dependent upon which visits the participant opts to complete and the time between visits. The proposed end date for the research is 2022.

5 Trial Procedures

5.1 Visit details

Participants will undergo 4 core visits (Table 2), 1 telephone contact and up to 2 optional visits (section 3.1.1). At these visits a series of standard and novel lung function and bronchial provocation challenges will be performed and tissue samples collected as shown in the visit schedule (section 5.2). Full details of the procedures are presented below (section 5.2.14). Departmental Standard Operating Procedures (SOPs) will be followed for each test.

5.2 Visit schedule

Core visit 1 will include almost all of the lung function tests, however as it is not possible to do tests of bronchodilator reversibility on the same day as a methacholine challenge test, the methacholinechallenge test will occur at least 1 day later as core visit 2. In addition, as peak flow monitoring takes 2 weeks to complete, although this starts at Core visit 1 these measurements will be continued at home for 2 weeks. Participants will be given the option of completing 2 additional visits (optional visit 1 for a mannitol challenge and optional visit 2 for bronchoscopy). Core visit 2 and optional visit 1 and 2 can occur at any time and in any order prior to starting treatment with ICS (section 3.1.1).

5.2.1 Recruitment

Recruitment will occur both at GP centres in the Greater Manchester area and from secondary care referral. Secondary care referral will include patients identified in outpatient clinics, accident and emergency or acute assessment units, and inpatient wards. Other referral sources may include community teams ie community respiratory nurses and district nurses. Potential participants meeting the study criteria will be provided with information (i.e. verbal, poster, participant information leaflet (PIL), or patient information sheet (PIS)) if they wish to proceed into the study they will be referred by their health care professional.

General Practice surgeries in Manchester: GP surgeries in Manchester will be invited to identify patients to this research. A list of the participating surgeries will be held in the TMF. Patients with symptoms consistent with asthma (i.e., cough, wheeze, chest tightness and/or breathlessness), not currently receiving ICS treatment, will be eligible for recruitment. Patients presenting acutely unwell that have received a course of steroids within the last four weeks may still be referred for eligibility review at a later date.

Secondary care hospitals in Manchester: Secondary care hospitals in Manchester will be invited to identify patients into this research. A list of the participating hospitals will be held in the TMF. Patients with symptoms consistent with asthma (i.e., cough, wheeze, chest tightness and breathlessness), not currently receiving ICS treatment, will be eligible for recruitment. Patients presenting acutely unwell that have received a course of steroids within the last four weeks may still be referred for eligibility review at a later date.

5.2.2 Eligibility check and booking (phone call)

Eligibility will be checked by a member of the research team against the inclusion and exclusion criteria (Section 4.3). Eligible participants will be booked for Core Visit 1. A copy of the patient information sheet (PIS) will be provided to the patient if they do not already have a copy. This will be done by email, by post or by offering access through the website (www.radica.org.uk).

Participants invited to attend core visit 1 will be asked to refrain from the following prior to all clinical visits:

- Short acting bronchodilators: 8 hrs
- Smoking: 1hr
- Caffeine: 8 hrs

5.2.3 Core visit 1(screening/baseline assessment)

This visit is a *core visit* and will be completed by all participants

The procedures conducted at this visit are stated below. Participants in whom asthma is deemed low probability **and** in whom an alternative diagnosis is suspected (e.g. pneumonia) would be discussed with the supervising consultant or nominated deputy and would be withdrawn from the study and referred back to their GP to be evaluated further (GP withdrawal letter). If appropriate direct referral to a general respiratory clinic or on call team at Wythenshawe hospital or local clinic could be arranged.

1. Informed consent
2. Pregnancy test (if applicable)
3. Demographics / Clinical history
4. Symptoms (including ACQ-5)
5. Clinical Examination
6. Skin prick test - if the patient is on antihistamines, this test will be deferred to a future baseline visit (see Section 3.1.1 for baseline visits) and medication withheld as indicated)
7. Blood test (all participants - can be completed at any baseline visit)
8. Nasopharyngeal swabs
9. FeNO
10. VOCs
11. IOS (pre-salbutamol)
12. PExA (pre-salbutamol)
13. MBW (pre-salbutamol)
14. Spirometry (pre-salbutamol)
15. Administration of inhaled salbutamol for bronchodilator reversibility testing
16. IOS (post-salbutamol)
17. Spirometry (post- salbutamol)

Following the visit all patients continuing in the study will be issued with an inhaled short acting bronchodilator to use as required in case they become symptomatic between visits. For participant convenience they will also receive their inhaled corticosteroid treatment but not commence treatment

until instructed following baseline visits and 2 week PEF monitoring (baseline visits include: core visit 1 and 2 plus optional visits if applicable). However, if following any baseline visit, the clinician decides that it is in the patient's interest to start treatment (likely to be oral corticosteroids and/or inhaled corticosteroids and/or antibiotics) for asthma immediately, after discussion with the supervising consultant this will be commenced. These patients will omit any pending baseline visits but will be invited to attend for core visits 3 and 4 and will be offered additional support through NHS clinics as appropriate.

5.2.4 Home lung function monitoring

All participants will receive a peak flow meter and record twice-daily (morning and evening) PEF measurements for 2-weeks. In addition they will be instructed to note down any use of their salbutamol inhaler and where possible record pre and post bronchodilator PEF measures.

5.2.5 Core visit 2- Methacholine challenge visit

This visit is a *core visit* and will be completed by all participants providing they are eligible with no contra-indications (appendix 3). Participants can attend for the methacholine challenge visit at any point between core visit 1 and entering the treatment period phase providing there is a gap of ≥24hrs from completing bronchodilator reversibility challenge (done in core visit 1), or ≥48hrs between challenge tests and/or bronchoscopy

The procedures conducted at this visit is as follows:

1. Symptoms (ACQ-5)
2. Collection of PEF measurements (if participant has not completed 2 weeks this can be collected at the next visit)
3. IOS (pre-methacholine)
4. PExA (pre-methacholine)
5. MBW (pre-methacholine)
6. Spirometry (pre-methacholine)
7. Methacholine provocation challenge
8. IOS (post-methacholine)
9. Sputum will be collected where expectoration is voluntarily achieved at any point during the baseline visits, however we would most likely expect sputum during/following the methacholine challenge
10. Participants will be instructed to commence their treatment with ICS (standard asthma treatment) once they have completed their last baseline visit **and** 2weeks PEF monitoring.

5.2.6 Optional visit 1- Mannitol challenge visit

This visit is an optional visit which anyone enrolled on the study will be given to option to attend if they wish providing they are eligible with no contra-indications (see appendix 4). Eligible and willing participants can attend for the Mannitol challenge visit at any point between core visit 1 and entering the treatment period phase providing there is a gap of ≥24hrs from completing bronchodilator reversibility

challenge (done in core visit 1), or ≥48hrs between challenge tests and/or bronchoscopy. Participants attending optional visit 1 will also be asked to refrain from any antihistamines for 72hrs before the appointment.

1. Symptoms (ACQ)
2. IOS (pre-Mannitol)
3. Spirometry (pre-Mannitol)
4. Mannitol provocation challenge
5. IOS (post-Mannitol)
6. Participants will be instructed to commence their treatment with inhaled corticosteroids once they have completed their last baseline visit and 2weeks PEF monitoring.

5.2.7 Optional visit 2- Bronchoscopy visit

This visit is an *optional visit* and will be completed by up to 20 adults. Eligible patients will be invited to attend at any stage between core visit 1 and entering the treatment period phase providing there is a gap of ≥48hrs between challenge tests and/or bronchoscopy. Exclusions from completing the bronchoscopy will be in accordance with the SOP.

1. Informed consent
2. Bronchoscopy
3. Participants will be instructed to commence their treatment with inhaled corticosteroids once they have completed both their last baseline visit and 2weeks PEF monitoring.

5.2.8 Treatment period

Inhaled corticosteroids

Flixotide Accuhaler will be prescribed in all patients. Adults and children age 16 years and over will be prescribed 250 mcg twice daily. Children aged 5 to 15 years will be prescribed 100 mcg twice daily. These dosages are in line with the dosages recommended in the Summary of Product Characteristics[26].

Instructions will be provided for missed doses of Flixotide, as follows:

- If it is almost time for next dose (within 4 hours), skip the missed dose and take the next dose when it is due.
- Otherwise, take it as soon as it is remembered, and then go back to taking the medicine as usual.
- Do not take a double dose to make up for the missed dose.

Medication adherence will be monitored using the INCA device. The INCA device creates time stamped acoustic recordings of an individual's inhaler use, in which empirical evidence of temporal and technique adherence in inhaler use can be monitored over time.

In the unlikely event of a participant not tolerating the Flixotide Accuhaler we will offer the Flixotide Evohaler at the equivalent dose as an alternative option (this is what would happen in standard care). If a

participant is switched to the alternate option we will not be able to generate data on compliance through an INCA device but we will record their self-evaluation of compliance, and information from the dose-counter, at each visit.

Reliever medication

An inhaled short acting bronchodilator will be prescribed for use PRN. A Ventolin Accuhaler will be prescribed and participants instructed to take 200mcg (1 puff) as required, with a maximum daily dose of 800mcg. As above, medication usage will be monitored using the INCA device. Doses are in line with the summary of product characteristics for the Ventolin Accuhaler[26]. In the unlikely event of a participant not tolerating the Ventolin Accuhaler we will offer the Salbutamol metered dose inhaler at the equivalent dose (I.e. 100mcg 2 puffs) as an alternative option (this is what would happen in standard care). If a participant is switched to the alternate option we will not be able to generate data on compliance through an INCA device but we will record their self-evaluation of compliance at each visit.

Medication withhold times

Where possible, participants will be asked to withhold certain medication or drugs prior to each visit, in line with department SOPs and international guidelines, as below:

- Short-acting beta-2 agonists for 8 hours
- Inhaled corticosteroids for 12 hours
- Smoking for 1 hour
- Caffeine for 8 hours
- Antihistamine 72hours (prior to skin pick testing and mannitol challenge only)

5.2.9 Core visit 3- 1-2 week ICS response

This visit is a ***core visit*** and will be completed by all participants 1-2 weeks after commencing ICS treatment:

1. Symptoms (ACQ)
2. Clinical examination
3. Check adherence
4. Blood test (in adults, also offered in children >12yrs)
5. FeNO
6. VOCs
7. IOS
8. PExA
9. MBW
10. Spirometry
11. Participants will receive their next study inhalers and instructions as to when to start them.
12. Participants will be issued with a GP Letter and non-urgent prescribing form so that they can organise ongoing treatment for when they complete the study (this will prevent any treatment

delays at the end of the study, but is only to be commenced in patients that are given a diagnosis of asthma at the end of Core visit 4).

5.2.10 Remote contact

Telephone/text/email contact will be made to all participants to check compliance and symptoms between core visit 3 and core visit 4.

5.2.11 Core visit 4- 6-8 week ICS response

This visit is a *core visit* and will be completed by all participants 6-8 weeks after commencing ICS treatment:

1. Symptoms (ACQ)
2. Clinical examination
3. Nasopharyngeal swabs
4. Blood test (in adults, also offered in children >12yrs)
5. FeNO
6. VOCs
7. IOS
8. PExA
9. MBW
10. Spirometry
11. Methacholine challenge
12. IOS (post mannitol)
13. GP Summary of results letter

Following core visit 4 the results of the investigations will be reviewed and a diagnosis of 'asthma' or 'not asthma' will be based upon clinical assessment and objective tests. A letter summarising the diagnosis and a recommended treatment plan will provided to the GP and copied to the patient.

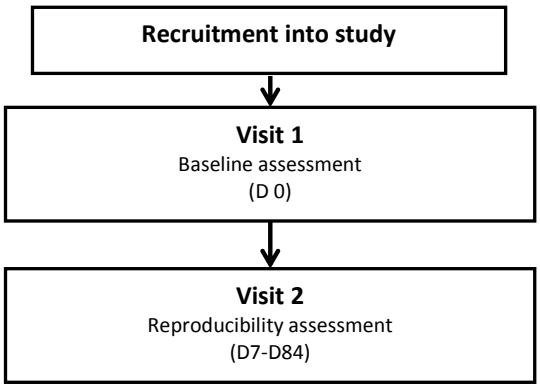
5.2.12 *Table 2. Visit schedule*

	Baseline Visits					Treatment Monitoring Visits		
	Core Visit 1	Core Visit 2	Optional Visit 1	Optional Visit 2		Core Visit 3	Remote contact	Core Visit 4
Visit name	Screening/ baseline/ dispense treatment	Methacholine challenge	Mannitol challenge	Bronchoscopy		1-2week response		6-8 week response
Demographics/ Clinical history	✔				Treatment Phase starts: (commence ICS)			
Symptoms (ACQ)	✔	✔	✔	✔		✔		✔
examination	✔							✔
VOCs	✔	✔				✔		✔
PExA	✔	✔				✔		✔
MBW	✔	✔				✔		✔
FeNO	✔	✔				✔		✔
Skin Prick test	✔*							
Blood	✔*					✔ †		✔ †
Pregnancy test (if applicable)	✔							
Spirometry	✔	✔	✔			✔		✔
IOS	✔	✔	✔			✔		✔
BDR	✔							
Mannitol			✔					
Methacholine		✔						✔
Sputum		✔*						✔
BAL				✔				
Nasopharyngeal swab	✔							✔
GP letter	✔					✔		✔
Dispense treatment	✔					✔		
Start prn reliever	✔							
Start ICS					✔			

*Can be completed at any baseline visit † In adults and offered in children >12yrs

5.2.13 Healthy control visit schedule

5.2.13.1 Trial Flow Chart



Visits	V 1	V2
Shortest study pathway	D 0	D7
Longest study pathway	D 0	D84

5.2.13.2 Recruitment

Participants will be recruited using posters and flyers which will be displayed (for example in hospital waiting rooms, staff rooms), or distributed through social media (for example facebook). Participants meeting the study criteria will be provided with a participant information sheet which will also contain contact information for the research team. Those wishing to be involved can contact the research team and will be booked for eligibility assessment. A summary of the visits is shown in Table 3

5.2.13.3 Eligibility check and booking (Phone call)

Eligibility will be checked against the inclusion and exclusion criteria (Section 5.2.13.4). Eligible participants will be booked for Visit 1.

Participants invited to attend visit 1 will be asked to refrain from the following prior to the appointment:

- Smoking: 1hour
- Caffeine: 8 hours

5.2.13.4 Inclusion and Exclusion Criteria

Inclusion Criteria:

i. Males and females ≥5years and <70 years
ii. Capable of giving informed consent or where under 16years attends with parent or legal guardian.

Exclusion criteria:

i. Diagnosis or repeat prescription of asthma treatment past or present
ii. Significant respiratory, cardiac or other medical co-morbidity
iii. More than one course of antibiotics for chest infection in the last 12 months
iv. Pregnant women
v. >10 pk yr smoking history
vi. Recent antibiotic treatment for any cause within previous 4 weeks
vii. Active symptoms of rhinitis (within last 2 weeks)

Comment [SD1]: review on monday

5.2.13.5 Visit 1- Baseline assessment

This visit will be completed by all participants.

Tests conducted at this visit is as follows:

1. Informed consent
2. Pregnancy test (if applicable)
3. Demographics/Clinical history
4. Symptoms
5. Clinical Examination
6. Skin prick test (if the patient is on antihistamines, this test will be deferred to visit 2 and medication withheld as indicated)
7. Blood test
8. Nasopharyngeal swabs
9. FeNO
10. VOCs
11. IOS (pre-salbutamol)
12. PExA (pre-salbutamol)
13. MBW (pre-salbutamol)
14. Spirometry (pre-salbutamol)
15. Administration of inhaled salbutamol for bronchodilator reversibility testing
16. IOS (post-salbutamol)
17. Spirometry (post-salbutamol)

5.2.13.6 Visit 2- reproducibility assessment (1 – 12 weeks)

This visit will be completed by all participants and can occur at any time from one to twelve weeks following visit one.

1. Symptoms
2. Clinical examination
3. FeNO
4. VOCs
5. IOS
6. PExA
7. MBW

8. Spirometry
9. GP summary of results letter

5.2.13.7 Table 3: Visit schedule (healthy volunteers)

Visit name	Visit 1 baseline assessment	Visit 2 Reproducibility assessment
Demographics/ Clinical history	✔	
Symptoms	✔	✔
Examination	✔	✔
VOCs	✔	✔
PExA	✔	✔
MBW	✔	✔
FeNO	✔	✔
Skin prick test	✔*	
Blood	✔	
Pregnancy test (if applicable)	✔	
Spirometry	✔	✔
IOS	✔	✔
BDR	✔	
Nasopharyngeal swab	✔	
GP Letter	✔	✔

*Can be completed at any visit (visit 1 or visit 2)

5.2.14 *Specific procedures (core visits)*

Clinical History and Demographics: Patient demographics and clinical history will be recorded in the CRF as source data. Measurements will include; height, weight, ethnic origin, age, smoking history and will be collected according to the department SOPs.

Doctor examination: A doctor will perform a cardiorespiratory examination and participants presenting contraindications for the study will be withdrawn.

Skin Prick Test: A standard skin prick test (SPT) will be performed to determine the allergy status of the participants following the departmental SOP. In short, a drop of different allergen extracts will be placed on participant's inner forearm. The skin will be pricked through the drop using the tip of a lancet. Fifteen minutes later, the diameter of the 'wheal' will be measured with a ruler to check for positive reaction(s).

The size of the reaction weal will be recorded in the CRF as source data. Skin prick tests will be performed when the patient has been off antihistamines for 72 hrs.

Blood: Venepuncture will be conducted according to the department SOP.

Nasopharyngeal Swabs: Nasopharyngeal swabs will be collected according to the departmental SOP.

FeNO: FeNO will be determined using the NIOX VERO (Circassia) according to the manufacturer's instructions and the department SOP. In short, the procedure includes an exhalation to RV, followed by an inhalation through the device filter to TLC. Participants then make a controlled exhalation for 10 s at a standardised flow rate. FeNO will be recorded in the CRF as source data.

VOCs: Breath samples will be collected using the ReCIVA breath sampler (Owlstone, Cambridge, UK) following the department's SOP. Participants will wear the mask and breathe tidally during the sampling period, which typically takes 6-10 minutes. Samples are collected onto 10cm long steel tubes packed with adsorbent material (Tenax GR) that traps VOCs. These tubes are stored at 4^{o}C until analysis (within two weeks). Samples will be analysed at the Manchester Institute of Biotechnology using our existing methodology[27]

IOS: Airways resistance and reactance will be measured using the THORASYS tremoFlo® C-100 Airwave Oscillometry System™ (AOS), in accordance with manufacturer's instructions and department SOP. The TremoFlo device waveform consists of a multi-frequency composite oscillatory pressure waveform of about 0.5 to 1 cm H_20 amplitude (1 to 2 cmH20 peak-peak) generated by a self-actuated oscillating mesh-screen piston within the device. This pressure waveform was superimposed on the subject's normal breathing and consists of oscillatory components at 5, 11, 13, 17, 19, 23, 29, 31 and 37 Hz. In brief, participants will be seated with their head in the neutral position. Wearing a nose-clip, participants will be instructed to firmly hold their cheeks, as to minimize the upper airway shunt artefact, and breathe tidally through the device. Measurements consist of 16 seconds recordings and the procedure will be repeated in triplicate. Data will be saved electronically and printed for the CRF as source data.

PExA: PExA will be assessed using the PExA 2.0 device according to the manufacturer's instructions and the department SOP. In short, participants are required to exhale to residual volume (RV) (thereby closing the small airways), inhale to total lung capacity (TLC) (vaporising the liquid particles in the airway fluid), and exhale through a mouthpiece into the device. Exhaled particles are then collected on a substrate surface within the device. The PExA 2.0 provides real-time analysis of the number of collected particles in the range of 0.5-4.5 microns, which will be recorded in the CRF as source data. Samples will be stored for future analysis.

Multiple Breath Washout (MBW): MBW is performed using a modified INNOCOR gas analyser (Gonem et al, 2014). Participants wear a nose clip and breathe a known concentration (0.2%) of an inert and non-absorbed gas, sulphur hexafluoride (SF6), via a mouthpiece connected to the INNOCOR device, until the concentration in their exhaled breath reaches a steady state (the wash-in phase). Participants are then switched to breathing room air and encouraged to maintain a steady respiratory frequency of 12 breaths per minute and a tidal volume of approximately 1 litre, making use of a real-time display of these parameters. The concentration of SF6 in exhaled breath is recorded during this 'wash-out' phase until it reaches 1/40 of the original concentration (0.005%). A number of parameters are derived from the raw MBW data using custom software, including Scond and Sacin. Scond is thought to represent ventilation inhomogeneity arising from conductive airway disease, while Sacin represents ventilation inhomogeneity arising from acinar airspace disease.

Spirometry / bronchodilator reversibility: Spirometry will be conducted according to the department SOP and the American Thoracic Society / European Respiratory Society (ATS/ERS) guidelines.[28] This involves the participant maximally exhaling from TLC into to a spirometer. Lung volume (FVC) and expiratory flow rates (FEV_1, FEF_{25-75} and PEF) will be recorded and used for analysis. A printout of the results will be filed in the CRF as source data. A bronchial reversibility challenge will also be conducted according to the department SOP. In short, baseline spirometry will be recorded, as described above. Participants will then be provided with a Salbutamol inhaler and spacer. Participants will be instructed to take one maximal inhalation of salbutamol (100mcg) via the spacer and hold their breath at TLC for 10s. The process repeated until 400mcg salbutamol has been delivered. Fifteen minutes following the final inhalation of Salbutamol, post-BD spirometry will be performed. The absolute and percentage change in lung function will be calculated and a positive challenge is considered if FEV_1 and/or FVC increases by ≥12% and 200mls. Printouts of the spirometry data will be stored in the CRF as source data.

Peak flow monitoring: Peak flow will be measured by participants in the morning and evening. Data will be collected and stored in the CRF as source data..

Methacholine challenge: A methacholine challenge will be used to test for BHR in a subset of participants. The challenge will be conducted according to the department's SOP. In short, quadrupling doses of methacholine will be inhaled using the tidal breathing protocol (Crapo et al., 2000). After each dose spirometry will be measured. The challenge will stop when a reduction in FEV_1 of 20% is noted or the maximal dose of 16mg/ml is delivered. The provoking dose and fall in FEV_1 will be recorded in the CRF as source data. If bronchoconstriction fails to return to baseline (within 10% of baseline) or the fall in FEV1 > 45% after methacholine and/or the participant reports respiratory distress, a standard dose of the bronchodilator salbutamol (200 ug) will be administered by inhalation via a spacer device.

Sputum: Sputum will be collected at any point where it is expectorated voluntarily as per departmental SOP. This will most likely occur at core visit 2 mannitol challenge but could be collected at any visit.

5.2.15 Specific Procedures (optional Visits)

Mannitol: The Mannitol challenge will be conducted in accordance with the manufacturer's guidelines (Aridol, Pharmaxis) and department SOP. Following the baseline spirometry, participants will inhale doubling doses of mannitol. After each dose, lung function will be measured with spirometry, until a reduction in FEV1 of 15% is recorded or the maximum dose achieved. The provoking dose and fall in FEV1 will be recorded in the CRF as source data. If bronchoconstriction fails to return to baseline (within 10% of baseline) or the fall in FEV1 > 45% after mannitol and/or the participant reports respiratory distress, a standard dose of the bronchodilator salbutamol (200 mcg) will be administered by inhalation via a spacer device.

Bronchoalveolar lavage (BAL) fluid: BAL will be collected during bronchoscopy according to department SOP.

5.3 **Analysis of samples**

Following analysis results will be retained in the CRF as source data. Various research teams will be responsible for the analysis of different aspects relating to the specific study objectives, as detailed below:

5.3.1 Blood

Analysis will include blood eosinophil count, IgE and blood corticosteroid measurement using standard methods. Additional analysis will include: metabolomics, Cell culture, immune cell count, measures of immune cell activation (including Mbd2, CCL17, CCR4), YKL-40 (CHI3L1), AMCase (CHIA), and Chitotriosidase (CHIT1) (pending funding). Where consent is obtained blood will be stored for future use.

5.3.2 Nasopharyngeal Swabs

Nasopharyngeal swabs will be collected and analysed for metagenomics/viromics at the University of Manchester.

5.3.3 VOCs

VOCs will be analysed using mass spectrometry at the Manchester Institute for Biotechnology as previously described[29]

5.3.4 PExA samples

Particles counts will be available at the time of collection. The substrate membranes will then be stored in Eppendorfs at -80°C for use in future research projects. It is anticipated that phospholipids and proteins content will be analysed using LC-MS/MS and/or ELISA.

5.3.5 Sputum

Sputum will be analysed for differential cell count and for cell surface markers. Additional analysis will include: Cell culture, immune cell count, measures of immune cell activation (Mbd2, CCL17, CCR4), YKL-40 (CHI3L1), AMCase (CHIA), and Chitotriosidase (CHIT1) (pending funding). Where consent is obtained sputum will be stored for future use.

5.3.6 BAL

BAL will be analysed for FACS (immune cell count), cell culture, immune cell count, and measures of immune cell activation (Mbd2, CCL17, CCR4). Additional analysis will include: YKL-40 (CHI3L1), AMCase (CHIA), and Chitotriosidase (CHIT1) (pending funding). Where consent is obtained specimens will be stored for future use.

6 **Assessment of Safety**

6.1 **Safety reporting**

All Adverse events (AE) or serious adverse events (SAE) (see definition, section 6.1.1) will be reviewed by the PI or an authorised member of the research team and causality will be categorised as defined below (c.f. Section 6.1.1)

6.1.1 Definitions

Adverse Event (AE): An AE is any untoward medical occurrence in a participant taking part in a clinical trial which does not necessarily have to have a causal relationship with the study drug under investigation. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the study drug, whether or not this has a causal relationship with the drug under investigation.

Serious Adverse Event (SAE): A serious adverse event is any untoward medical occurrence that: results in death; is life-threatening (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; is a congenital anomaly/birth defect; results in 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 participant and may require medical or surgical intervention to prevent one of the outcomes listed above.)

Causality: The relationship between an adverse event and the study drug or procedure will be assessed and categorised as below. The assessment will be based upon the PIs clinical judgement to determine the relationship, considering alternative causes, such as natural history of the disease process, concomitant therapy and other risk factors. Results may be: not related; unlikely to be related; possibly related; probably related; definitely related.

6.2 **Recording and reporting of AE/SAE**

6.2.1 Recording Adverse Events and serious adverse events

All AEs and SAEs will be recorded from the time the participant is recruited into the study until the completion of the final visit or the subject has been withdrawn. AEs will be assessed by the local PI for causality, intensity, seriousness and expectedness. All AEs will be recorded on the AE log. All SAEs must be recorded on the SAE form and the SAE form must be faxed to the RADicA Trial Manager and Sponsor within 24h. Research Ethics committee will also be informed. All SAE forms will have an assessment of relatedness and expectedness. When an AE/SAE occurs, it will be the responsibility of the Investigator to review all documentation (e.g. hospital notes, laboratory and diagnostic reports) related to the event. The Investigator will then record all relevant information in the AE log and on the SAE form (if the AE meets the criteria of serious). This responsibility may be delegated to a member of the research team. Assessment of events may be delegated to other suitably qualified physicians in the research team who are trained in recording and reporting AE/SAEs.

6.2.2 Reporting of SAEs

Once the Investigator becomes aware that an SAE has occurred, they will report the information to the Trial Manager within 24 hours. If the Investigator does not have all information regarding a SAE, they will not wait for this additional information before notifying the Trial Manager. The form will be updated when the additional information is received. All reported SAEs if considered by the PI to be possibly,

probably or definitely related to the study procedures will be expedited to the Sponsor, REC within 7 days of becoming aware of the event. Listings of adverse events will be provided to the Sponsor when requested.

6.2.3 Follow-up of subjects after adverse events

After initially recording and reporting an AE/SAE, the Investigator will be required to follow each AE/SAE until resolution or death of the subject. Follow up information on a SAE will be reported to the Trial Manager. AEs still present in subjects at the last study visit will be monitored until resolution of the event or until no longer medically indicated.

6.3 Notification of deaths

All deaths will be reported to the PI irrespective of whether the death is related to underlying disease, the IMP or an unrelated event. All deaths, including deaths deemed unrelated to the IMP, if they occur earlier than expected will be reported to the sponsor".

6.4 Pregnancy reporting

Post pubescent females of child-bearing potential will be asked to confirm that they are not pregnant. Where there is any doubt urine pregnancy testing will be undertaken and they will be advised to notify the investigator immediately if they become pregnant at any stage of the study, at which point we would withdraw the participant from the study and seek GP advice. Pregnancy is not considered an AE unless a negative or consequential outcome is recorded for the mother or child/foetus. If the outcome meets the serious criteria, this would be considered an SAE. Complications in relation to pregnancy must be reported as AEs.

6.5 Overdose

For the purpose of this study an overdose is defined as the consumption of a drug in quantities greater than recommended in the summary of product characteristics information. The PI must be notified if any overdose has occurred and information regarding the overdose must be recorded on the deviation log. Overdoses can be observed from the medication adherence INCA device, drug charts, or patient comments. If an SAE is associated with the overdose this must be reported and recorded as described in section 6.2 and 6.3 respectively.

6.6 Treatment Stopping Rules

If a patient develops a significant oral thrush or an unforeseen adverse effect to the inhaled corticosteroid happens, the treatment will be stopped and the patient withdrawn from the study. The trial might be prematurely discontinued by the sponsor, CI or regulatory authority on the basis of any new safety information.

7 Data Handling

7.1 Data monitoring plan

This study will be monitored by the Sponsor.

7.2 Baseline data

Data from this study will be collected onto paper CRFs and later inputted onto an electronic database. The data will be entered by a trained site investigator/research staff in accordance to guidelines. All CRFs must be completed in English.

7.3 What to record in the medical records

Information about the study, time and day of consent will be recorded. A copy of the PIS and GP letter will be attached. Procedures will be documented for each visit.

7.4 Access to data

The completed CRFs are the property of the sponsor and must not be made available in any form to third parties (except for authorised representatives of appropriate governmental health or regulatory authorities) without written permission of the sponsor.

7.5 Archiving

All research related documentation including the site file, case record forms etc will be kept with the Principal Investigator until all the data has been analysed. Then it will be boxed and archived offsite. When the documents are over 20 years old they can be destroyed, but prior to destruction, a log will be made of all the documents that are being destroyed.

8 Statistics and data analysis

The department statistician, Philip Foden, has provided guidance on sample sizes and data analysis.

8.1 Sample size calculation

It is anticipated that approximately 60% of participants will fulfil the criteria of asthma[30] and 50% will fulfil the criteria of steroid-responsive airways disease.[31,32] Sample size is based on the minimum of 10:1 events-to-variable ratio for logistic regression in order to avoid overfitting [33]. With 120 (72 with and 48 without asthma) participants in the study, a multivariable logistic regression analysis, the primary analysis, can include 5 variables. The co-primary outcome of SRAD (60 with and 60 without SRAD) can include 6 variables. A 1% significance level will be used due to there being two primary outcomes (each with a number of definitions) and to account for the number of candidate variables of interest. In order to account for potential drop outs (estimated 10-20% maximum), we aim to recruit up to 150 participants.

In relation to secondary objectives 2c and 2d, approximately 150 age and gender matched healthy controls will be recruited on a 1:1 ratio (with the 150 suspected asthma patients), to calculate reference intervals for small airways and experimental biomarkers. For a parametric analysis, Altman recommends a minimum of 100 participants.[34] In the case where there are 150 participants, the width of the 95% confidence intervals would be 0.56s, where s is the standard deviation of the observations.

8.2 Planned recruitment rate

We anticipate 1-2 participants will be recruited per week.

8.3 Statistical analysis plan

8.3.1 Primary outcome analysis

Primary objective 1a. Determine the optimum diagnostic pathway for asthma based on conventional tests of large airway function and novel tests of small airway function

Asthma definition i: Asthma will be diagnosed based on clinical symptoms and signs alone, recorded in a standardised format and three clinicians will be asked to score asthma as high probability, intermediate probability or low probability asthma, based on information collected in the structured clerking proforma. When 2 out of three scored an individual as high or intermediate probability asthma, the individual was classified as "asthma". In the event of 2 or more scoring low probability the subject was classified as "not asthma".

Asthma definition ii: Asthma will be defined on the basis of symptom consistent with asthma **and** objective evidence of variable airflow obstruction, (one of: diurnal PEF variability ≥ 20% measured over two weeks, bronchodilator reversibility (FEV1 ≥ 12% and 200ml to 400mcg salbutamol, bronchial hyper-responsiveness to methacholine and/or mannitol)

Single variable logistic regression analysis will be used to determine the relationship between asthma (definition i or ii) and the following outcome measures:

- Spirometry: FEV_1/FVC
- BDR: ΔFEV_1
- FeNO: ppb
- PEFv: %
- BHR mannitol: PD_{15}, or DRR
- BHR methacholine:PD_{20}, or DRR
- IOS
- MBW
- Blood eosinophils
- Skin prick tests
- Specific IgE tests

The variables will be included in the logistic regressions as continuous and categorical variables to determine the form of their relationship with asthma. When the variables are included as categorical or dichotomous, previously defined cut points will be used (c.f. section 3.3. Table 1). For all variables, inspection of the area under the receiver operating characteristic curve (AUROC) and the cut point that gives the best combination of sensitivity and specificity will be used to define an appropriate dichotomisation. We will report positive and negative predictive values, taking into account prevalence in the population sample.

Two approaches to creating an optimum series of investigations to predict asthma in adults and children will be used. The first will be to use multivariable logistic regression. In the primary analysis, age will be included alongside four key variables of interest (PEF variability, bronchodilator reversibility, FENO and blood eosinophils). As secondary analyses, age will be included as an interaction term with the variables of interest that may have a different relationship with asthma based on age. Potential confounders such as gender and smoking status will be considered for inclusion in additional analyses. Further secondary analyses will consider other variables of interest, such as the bronchial challenge and small airway function data. The results of the regression analysis can be used to create a scoring system, using either continuous or categorical versions of the variables. This scoring system can then be used to define risk groups for asthma. A second approach will be to use a classification measure (such as a decision tree analysis) to determine the best way of discriminating between asthma and non-asthma participants. This will attempt to determine the best way of correctly identifying asthma using the variables of interest.

Primary objective 1b. Determine the optimum diagnostic pathway for SRAD in adults and children based on conventional tests of large airway function and novel tests of small airway function

SRAD will be defined as improvement in symptoms, airway physiology, inflammatory profiles and/or clinical impression following 6-8 weeks of ICS treatment. Patients will be categorised as symptom responsive (Primary endpoint), physiological responsive (secondary endpoint), and clinically responsive (secondary endpoint), as follows:

SRAD i: Symptom responsive (0.5 unit improvement in ACQ-5 OR)

SRAD ii: Physiological responsive (12% and 200mls improvement in FEV1 and/or FVC, or 1 DD improvement in PD15Mann)

SRAD iii: Clinically responsive (clinical impression of "ICS responsive" from the patient and 2 of 3 clinicians who have access to all investigational data)

Single variable logistic regression analysis will be used to determine the relationship between steroid responsive airways disease and the following outcome measures:

- Spirometry: FEV_1/FVC
- BDR: ΔFEV_1
- FeNO: ppb
- PEFv: %
- BHR mannitol: PD_{15}, or DRR
- BHR methacholine: PD_{20}, or DRR
- IOS
- MBW
- Blood eosinophils
- Skin prick tests
- Specific IgE tests

Analysis will be conducted as per primary objective 1a

8.3.2 Secondary outcome analysis

2a. Evaluate the accuracy of the National Institute for Health and Care Excellence (NICE) asthma diagnostic algorithms

- The proposed NICE diagnostic algorithm will be followed to categorise patients and compare this to our classifications form the primary outcome, asthma (definitions i and ii) or SRAD (definitions i-iii). AUROC analysis will calculate the sensitivity, specificity, positive predictive value and negative predictive value of the NICE diagnostic pathway to identify asthma. We will compare the NICE algorithm to algorithms developed in objective 1

2b. Identify the best predictor(s) response to inhaled corticosteroids (ICS, at 8-weeks) from measurements taken at baseline and early treatment (1-2 weeks)

- As per analysis for Primary objective 1b, but with outcomes collected at 1-2 weeks of treatment.

2c. In healthy volunteers, establish reference intervals and calculate repeatability coefficients for MBW, and IOS where there is a lack of evidence on what threshold constitutes a 'normal' set of values

- Healthy controls will be recruited to calculate reference intervals for small airways parameters, which lack clear evidence on which threshold constitutes a 'normal' value[10,35-37] and to calculate repeatability coefficients, to determine what constitutes an important change in asthma following ICS treatment.

2d. In healthy volunteers, establish reference values and calculate repeatability coefficients for PExA and VOCs, where there is almost no evidence on what threshold constitutes a 'normal' set of values.

- Healthy controls will be recruited to calculate exploratory reference and to calculate repeatability coefficients, to determine what constitutes an important change in asthma following ICS treatment

2e. Identify the profile of biomarkers in VOCs, and PExA which best predict asthma diagnosis.

- Principal component analysis and multivariable logistic regression of VOCs in exhaled breath will be used to calculate the prediction probability for asthma and steroid responsive asthma (definitions as above)

2f. Evaluate whether markers of immune cell activation predict asthma and predict response to treatment.

2g. Evaluate the predictive capacity of upper respiratory viral biomarkers

2h. Determine the optimum diagnostic pathway based on conventional tests of large airway function and novel tests of small airway function in a) adults and older children (≥12 yrs) and b) younger children (<12 yrs)

- Separate subgroup analyses for adults and older children (≥12 yrs) and younger children (<12 yrs) will be performed. The analysis will be conducted as per primary objective 1a. The analyses will be considered exploratory due to the limited numbers in each subgroup.

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10 Appendix

10.1 Appendix 1: Abbreviations

AE	Adverse Incident
ACQ	Asthma Control Questionnaire
AHR	Airway hyperresponsiveness
Albumin	Human serum albumin
AOS	Airwave Oscillometry System
AQLQ	Asthma Quality of Life Questionnaire
ATS	American Thoracic Society
AX	Reactance area
BAL	Bronchioalveolar lavage
BDP	Beclomethasone dipropionate
BDR	Bronchodilator Reversibility
BHR	bronchial hyperresponsiveness
BHR_{mann}	bronchial hyperresponsiveness to Mannitol
BHR_{meth}	bronchial hyperresponsiveness to Methacholine
BMI	Body mass index
BRC	Biomedical Research Centre
BTS	British Thoracic Society
BUD	Budesonide
CF	Cystic fibrosis
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case report form
DPI	Dry powder inhalers
DPPC	Dipalmitoyl-phosphatidylcholine
DSeq	Equipment dead space
EARIP	European Asthma Research and Innovation Partnership
EBC	Exhaled breath condensate
ELISA	enzyme-linked immunosorbent assay
eNose	electronic nose
FEF 50	Forced expiratory flow at 50% of vital capacity

FeNO	Fractional exhaled nitric oxide at 50ml/s
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GINA	Global Initiative for Asthma
GP	General Practitioner
He	Helium
HTA	Human Tissue Act
IC	Informed consent
ICS	Inhaled corticosteroid
IgE	immunoglobulins
ILD	Interstitial Lung Disease
INCA	Inhaler compliance assessment device
IOS	Impulse oscillometry
LABA	Long-acting β-agonist
LCI	Lung clearance index
LLN	Lower limit of normal
MBW	Multiple breath inert gas washout
LC-MS	liquid chromatography and ***mass spectrometry***
MDI	Metered dose inhaler
MEF_{25-75}	Maximum expiratory flow at 25-75% of vital capacity
MFT	Manchester University NHS foundation trust
MS	Mass spectrometry
N_2	Nitrogen
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health Research
NO	Nitric Oxide
O_2	Oxygen
PD15	15% fall in FEV1 from baseline during challenge test
PD20	20% fall in FEV1 from baseline during challenge test
PEF	Peak expiratory flow
PEFv	Peak expiratory flow variability
PEx	Particles in exhaled air
PExA	Particles in exhaled air method

PI	Principal Investigator
PIS	Patient Information Sheet
PPB	parts per billion
Q	flow
R	Resistance
REC	research ethics committee
ReCIVA	
R_{rs}	Respiratory Resistance
R20Hz	Resistance at 20Hz
R5Hz	Resistance at 5Hz
R5-R20	Frequency dependence of resistance
RADicA	Rapid Access Diagnostic for Asthma
Raw	Airway resistance measured by body plethysmography
Rc	Central airway resistance
RL	Total lung resistance
ROC	Receiver operating characteristic
RV	Residual volume
RTLF	Respiratory tract lining fluid
SABA	Shot-acting β-agonist
Sacin	Acinar ventilation heterogeneity
SAE	Serious Adverse Event
SAO	Small airway obstruction
Scond	Conductive ventilation heterogeneity
SD	Standard deviation
SF6	Sulphur Hexafluoride
SIGN	Scottish Intercollegiate Guideline Network
SIII	Phase III slope
SnIII	Concentration-normalised phase III slope
SOP	Standard operating Procedure
SPT	Skin Prick Test
SRAD	Steroid Responsive Airways Disease
TLC	Total lung capacity
TMF	Trial Master File
VA	Alveolar volume

VC	Vital capacity
VH	Ventilation heterogeneity
VOC	Volatile organic compound
VT	Tidal volume
WHO	World Health Organisation
X	Reactance
X_{rs}	Respiratory reactance
X5	Reactance at 5Hz
Z	Impedance
∆FEV1	incremental change in FEV1

10.2 **Appendix 2: Asthma Control Questionnaire (ACQ-5)**

ASTHMA CONTROL QUESTIONNAIRE (ACQ)

ENGLISH VERSION FOR THE UK

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ID5805 / ACQ_AU2.0_eng-GB.doc

(Right click to open document; ACQ-5 based on general ACQ).

10.3 **Appendix 3:** Criteria and Contraindications for mannitol challenge testing

Criteria:

- Subjects must be able to perform technically acceptable and reproducible spirometry before commencing the mannitol challenge test.
- Confirm that the patient has not used any bronchodilators for the specified duration of action, or taken any short acting anti-histamines for 72 hours or long-acting anti-histamines prior to the test.

Absolute contra-indications:

- Severe airways obstruction (FEV1 < 50% predicted or < 1.0 L)
- Recent myocardial Infarction (< 3 months)
- Recent cerebral vascular accident (< 3 months)
- Uncontrollable hypertension, systolic BP > 200, or diastolic BP > 100
- Known aortic aneurysm
- Inability to understand procedure and implication of test
- Pregnancy

Relative Contra-indication

- Spirometry induced airway obstruction
- Airway obstruction (FEV_1< 70% predicted or <1.5 L)
- Current upper respiratory tract infection
- Exacerbation of asthma
- Inability to perform accurate and reproducible spirometry

10.4 **Appendix 4:** Contraindications for Methacholine challenge testing

Absolute

a) Severe airways obstruction (FEV_1 < 50%

predicted or <1.0 L).

b) Recent myocardial infarction (< 3 months).

c) Recent cerebral vascular accident (< 3 months).

d) Uncontrollable hypertension, systolic BP > 200, or diastolic BP > 100.

e) Known aortic aneurysm

f) Inability to understand procedure and implication of test

Relative

a) Spirometry induced airway obstruction.

b) Moderate airway obstruction. (FEV_1 < 60% predicted or <1.5 L).

c) Recent upper respiratory tract infection (< 2 weeks).

d) Exacerbation of asthma.

e) Inability to perform reliable quality spirometry.

f) Pregnancy.