Development of non-invasive biomarkers to direct individualised management of preschool wheeze

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Sponsor

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

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This protocol describes the “Non-invasive biomarkers for preschool wheeze” study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the UK Policy Framework for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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GLOSSARY OF ABBREVIATIONS

ICS	inhaled corticosteroids
PCR	polymerase chain reaction
BDR	bronchodilator reversibility
FOT	forced oscillation technique
WBC	White blood cell
BAL	Bronchoalveolar lavage
PHC	preschool healthy controls
PSW	preschool wheezers
GP	General practitioners
dBNs	dynamic Bayesian networks
BNs	Bayesian Networks

KEYWORDS
preschool children, recurrent wheezing, blood eosinophils, lung function, infection, inflammation, treatment, non-invasive tests

STUDY SUMMARY

TITLE Development of non-invasive biomarkers to direct individualised management of preschool wheeze

DESIGN

(i) Cohort observational study with collection of clinical details, blood samples, sputum, oropharyngeal swabs and airway samples from children.

(ii) Laboratory study using blood and airway samples collected to assess inflammation and infection.

(iii) Proof-of-concept feasibility study in a sub-group of the cohort to investigate biomarker guided targeted treatment for preschool wheeze. This will enable sample size calculation for a larger clinical trial

AIMS

Aim 1:

Investigate the sensitivity and specificity of:

(a) aeroallergen sensitisation and blood eosinophils in reflecting lower airway eosinophils;

(b) blood neutrophil phenotype and chemotaxis in reflecting lower airway neutrophils; and

(c) induced sputum and oropharyngeal and nasal swabs in reflecting lower airway infection

in preschool children with severe, recurrent wheeze undergoing clinically indicated bronchoscopy (n=50).

Aim 2:

Determine the feasibility of undertaking 3 to 6 monthly assessments of the proposed non-invasive biomarker tests listed below in preschool wheezers, and relate the results to symptoms, wheeze attacks and prescribed medication.

1. skin prick tests for aeroallergen sensitisation (will only be done once),
2. blood eosinophils (using a point-of-care test),
3. blood neutrophil function (using a chemotaxis assay and flow cytometry),
4. bacterial detection (pathogen specific PCR from oropharyngeal and nasal swabs) and induced sputum,
5. lung function with bronchodilator reversibility (using forced oscillation technique [FOT]). (n=100)

Aim 3: Undertake a proof-of-concept feasibility study to see if parents/caregivers will agree to take part in a stratified treatment clinical trial of biomarker guided therapy in recurrent preschool wheeze (n=15).

OUTCOME MEASURES

Primary outcomes: Specificity, sensitivity, longitudinal stability and acceptability of non-invasive tests (biomarkers) of eosinophilic inflammation, phenotype of airway neutrophils, airway infection and lung function in children with preschool wheeze.

Secondary outcomes: Relationship of the tests below to wheeze attacks and symptom control (TRACK questionnaire) when assessed 3 monthly for over one year in children with preschool wheeze.

1. Lung function assessed using FOT (Rrs: respiratory system resistance; Xrs: respiratory system reactance; AX: area of reactance) 3 monthly
2. Inhaled corticosteroid dose and oral prednisolone dose 3 monthly
3. Induced sputum cultures at baseline and 12 months
4. Blood eosinophil count 3 monthly
5. Neutrophil function and phenotype in relation to infection status, clinical symptoms, and prescribed medication 3 monthy
6. Nasal and oropharyngeal swab for infection 6 monthly
7. Assessment of symptom burden to caregivers using the validated Paediatric Asthma Caregivers Quality of Life Questionnaire: PACQLQ

POPULATION Preschool children with recurrent wheeze aged between 1-5 years

ELIGIBILITY Children aged 1-5 years with either

(i) ≥2 hospitalisations for acute wheeze; or

(ii) at least 1 admission requiring high dependency unit or intravenous bronchodilator therapy;

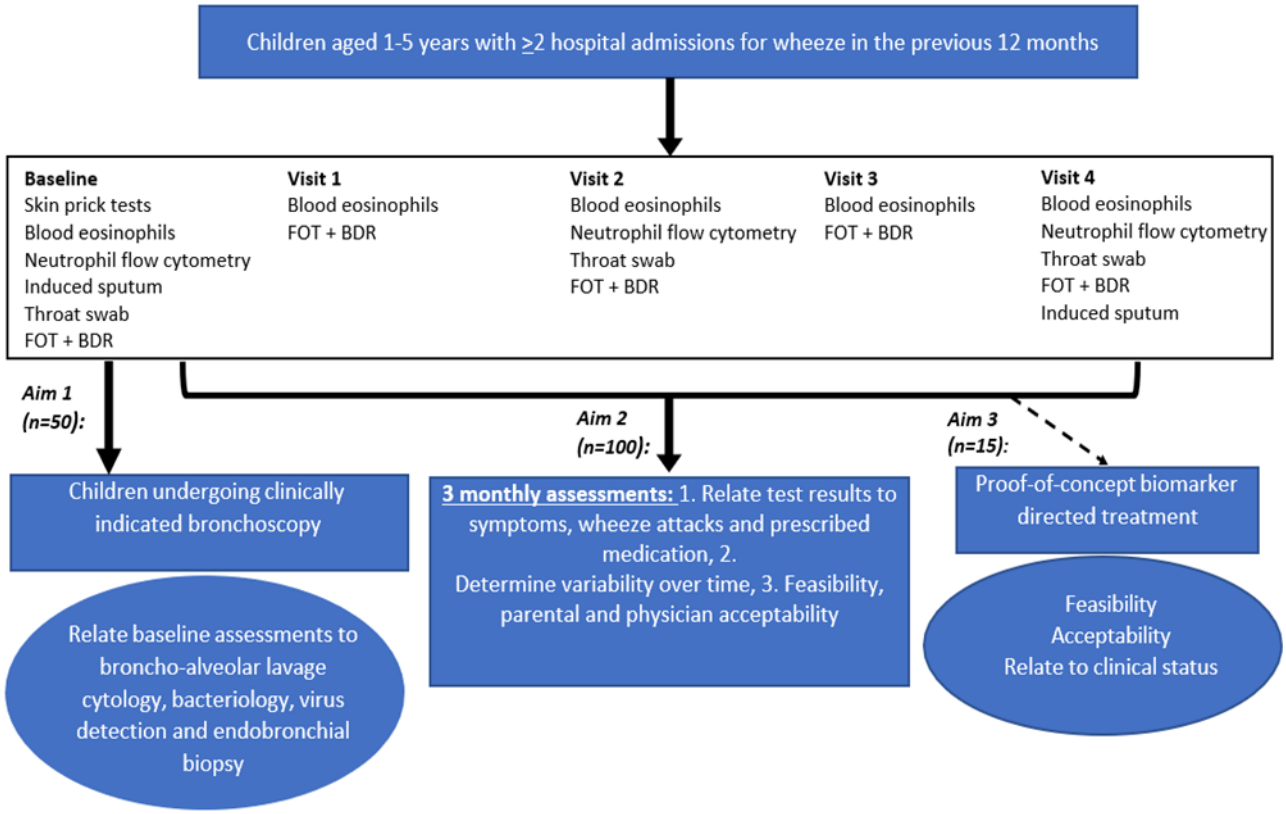
during the previous year.

For Aim 1 only - children undergoing clinically indicated bronchoscopy, as determined by their existing medical team, as part of their standard management

DURATION 36 months

REFERENCE DIAGRAM

FIGURE 1:



1. INTRODUCTION

1.1. BACKGROUND

Healthcare utilisation and morbidity in preschool children with wheezing: Wheezing illnesses and asthma are the most common non-communicable diseases of childhood worldwide. Approximately half of children in the UK experience at least one wheezing episode by age 6 years, and children under 6 years account for 75% of UK childhood hospital admissions for acute wheeze attacks. The prevalence of preschool wheeze in England was 8% in 2017, and of those initially presenting to primary care, 15.8% attended an emergency department, and 13.9% had a hospital admission over the subsequent 2 years. Recurrent symptoms, multiple attacks and hospital admissions in preschool wheezers account for one-third of healthcare costs for childhood asthma and are associated with long-term diminished lung function and increased morbidity and mortality in early adulthood4. The unchanged disease burden over the last 20 years, both in the UK and USA suggests current management is failing to reduce numbers of acute wheeze attacks in preschool age, and we urgently need a step-change in our treatment approach to prevent recurrent attacks of preschool wheeze.

1.2. RATIONALE FOR CURRENT STUDY

The poorly understood pathophysiology of preschool wheeze to date has led to a “one size fits all”, standardised treatment of all preschool wheezers using inhaled corticosteroids (ICS) as maintenance therapy to prevent attacks. The justification for this approach has been an extrapolation of the treatment used in school-age asthma. However, most school-age children have allergic, eosinophilic asthma associated with type 2 immunity. In contrast, only a sub-group of preschool wheezers have airway eosinophilia and aeroallergen sensitisation (~25%), and a randomised, double-blind clinical trial (INFANT study) in this age group has shown that these are the group most likely to benefit from ICS. However, children without these features have no or poor response to any currently available treatment. Preschool wheeze is now known to be heterogeneous, but the current management approach purely based on parental reporting of symptoms, which we know cannot distinguish between underlying airway pathologies, likely accounts for the ineffectiveness of this approach in preventing wheezing attacks.

Even when ICS are ineffective and could be causing harm, treatment is rarely discontinued because of symptom burden, physician insecurity and parental reluctance to stop therapy. We know that when they are targeted to the eosinophilic subgroup, ICS are highly beneficial in preschool wheezers. But our current approach only considers symptom patterns reported by parents, without objective tests to define the underlying airway phenotype leading to symptoms in each patient. Biomarkers and treatment options to identify and prevent wheeze attacks in preschool children without allergic sensitization or blood eosinophilia are lacking**.** Therefore, there is an urgent unmet need for appropriate and clinically feasible biomarkers that will allow identification of effective treatment strategies for individual patients.

Review of existing literature

A novel approach for biomarker-guided management of preschool wheeze

Pathological clusters of preschool wheeze: The Saglani group have recently shown that preschool wheeze is a heterogenous condition by identifying 4 pathologically distinct clusters in children with severe, recurrent preschool wheeze: 1. Atopic, 2. Non-atopic with low infection rate and high ICS use, 3. Non-atopic with high viral and bacterial infection rate and airway neutrophilia, and 4. Non-atopic with low infection rate and not prescribed ICS. The variables which distinguished the preschool wheeze clusters included allergic sensitisation, blood eosinophils, lower airway neutrophils, and the pattern of airway bacterial infection[6]. However, these findings have their limitations as they were from invasive, lower airway bronchoscopic samples, which can only be obtained on a single occasion from very severe wheezers. Symptom pattern (episodic vs persistent), which is the current way of distinguishing pathology and determining treatment, was shown to not be predictive of lower airway inflammation or infection. These findings have now identified the need for a change in physician mindset, and that management strategies for preschool wheeze needs to be based on objective biomarkers which predict response to treatment. To facilitate clinical translation and targeted treatments for all preschool wheezers with disease of all severity and in all healthcare settings, these limitations need to be addressed by identifying non-invasive biomarkers of lower airway infection and inflammation, assessing their stability and feasibility over time, and comparing their accuracy to lower airway samples.

These newly described pathological preschool wheeze clusters, based on blood and airway inflammation and infection, suggest that a biomarker guided treatment approach may prove more successful than current standard care in preventing preschool wheeze attacks. My project aims to extend these findings to bedside clinical tests that can be used for targeted treatment. I will investigate the feasibility, sensitivity, specificity, and longitudinal variability of 1. skin prick tests (to detect allergen sensitisation), 2. blood eosinophils (using a point-of-care test), 3. blood neutrophil function (using a chemotaxis assay and flow cytometry), 4. bacterial detection (pathogen specific PCR from oropharyngeal and nasal swabs) and induced sputum, and 5. lung function with bronchodilator reversibility (forced oscillation technique [FOT]), as biomarkers that can be applied in future stratified interventional clinical trials.

Proposed non-invasive biomarkers:

1. Non-invasive tests for allergic sensitisation and eosinophilia

The previous analyses identified that 25% of wheezers were in the atopic cluster, and both observational and clinical trial data have identified atopy (aeroallergen sensitisation) and blood eosinophilia as biomarkers of preferential response to ICS[7]. Based on the known action of ICS targeting eosinophilia, I propose that ICS would be an appropriate treatment for children in the atopic cluster. This suggests every preschool wheezer who is considered for ICS treatment should have phenotyping with aeroallergen sensitisation and blood eosinophil as a minimum before a decision is made to commence ICS.

Skin prick tests for allergen sensitisation are simple, non-invasive and can be performed in clinic. However, performing blood test in preschool children is challenging. Venepuncture is a difficult procedure in this age group, time consuming and the result is not immediately available, and

frequently declined by parents. The Saglani group have obtained preliminary feasibility and acceptability data for a finger prick blood sample, using a point-of-care device (HemoCue WBC DIFF analyser for WBC) that measures differential white blood cell count, providing a result within a few minutes. Children and parents/ carers were approached in clinic and 84% agreed to have the test, with the main reason for refusal being needle anxiety. Of those that had the test, both parents (98%) and children found the test acceptable, and most were happy to have the test performed routinely (81%) (Table 1) suggesting this is a pragmatic approach that can be adopted.

Table 1. Feasibility and acceptability of finger prick blood eosinophil test in children with wheeze and asthma

Number of children whose parents/carers were approached	67
Number of children whose parents/carers agreed to participate	56 (84%)
Number of children who had the test (8 had to leave clinic before test was done)	48/56 (86%)
Age (years); median (range)	4.3 (1-5)
Median child rating score: How they found it*	2
Median parent rating score: Acceptable method to obtain blood sample**	5 (98% scored 5)
Median parent rating score: Acceptable for child to have this done routinely**	5 (81% scored 5)
Successful sample obtained first attempt (out of 48)	45 (94%)
Successful sample obtained >1 attempt (out of 48)	2 (4%)
Fingerprick blood eosinophil count (10^9/L); median (range)	0.40 (0-3.8)

*Children rated score from a 2-point smiley Likert scale (1 – sad face; 2 – happy face)

**Parent rated score from a 5-point Likert scale (1 – worst; 5 – best)

2. Blood biomarkers to identify phenotype and function of airway neutrophils

Biomarkers and treatment to prevent wheeze attacks in preschool children without allergic sensitisation / blood eosinophilia remain elusive. The Saglani group have obtained preliminary data showing that the phenotype of airway neutrophils is distinct in the different preschool wheeze clusters. Wheezers as a group had a predominance of $CD62L^{hi}$ regulatory neutrophils, however only those **without** bacterial infection had significantly more $CD62L^{hi}$ neutrophils. Those with bacterial infection in their airways had significantly more $CD62L^{lo}$ inflammatory neutrophils (Figure 2). A flow cytometry panel has been established in the lab to phenotype neutrophils from 200mcl of blood taken during finger prick sampling, and I will use this to relate blood-derived data to lower airway neutrophil phenotyping in order to identify peripheral biomarkers of neutrophil activation status.

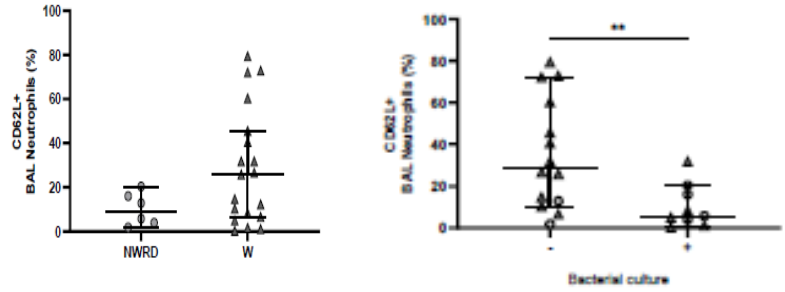


Figure 2. Proportion of CD62L^{+} neutrophils in bronchoalveolar lavage (BAL) from preschool wheeze (W) compared to non-wheeze related disease (NWRD), and wheezers with (+) and without (-) airway bacterial infection.

The Saglani lab has also investigated the **function of blood neutrophils** with the use of TAXIScan chemotaxis apparatus which allows dynamic measurements of leukocyte migration with as few as 500 cells/data point. The assay generates a rich data set regarding the migration of individual neutrophils along a gradient of chemoattractant. Analysis of the cell tracks allows measurement of the velocity (V) and directionality (D) of each cell and to combine this in a V-D plot. Setting relevant threshold values for both parameters directional migration amongst the population of neutrophils can be accurately measured. Using this assay, preliminary data show reduced chemotaxis of blood neutrophils to CXCL8 from preschool wheezers compared to healthy controls (Figure 3).

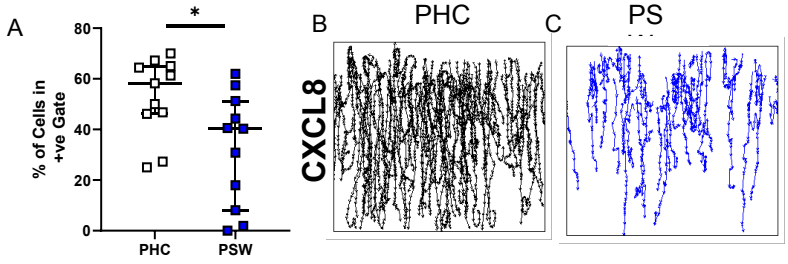


Figure 3. Chemotaxis of blood neutrophils from preschool healthy controls (PHC) compared to preschool wheezers (PSW) to CXCL8. (A) reduced % chemotaxis, (B&C) cell tracks towards CXCL8.

In this study, using small 100mcL blood samples, I will correlate neutrophil function and phenotype with infection status, clinical symptoms, and prescribed medication in children with recurrent preschool wheeze.

3. Non-invasive tests for airway bacterial infection

Preschool wheeze Cluster 3 (non-atopic, high infection rate) had the highest prevalence of airway bacterial and viral infection, and *Moraxella catarrhalis, Haemophilus influenzae* and *Streptococcus pneumoniae* made up 80% of the bacteria identified, suggesting these children may benefit from targeted antibiotics. Induced sputum testing is feasible and safe in young children but is time consuming and requires specialist training. However, a pathogen specific PCR to detect these three bacteria is also available. Bacterial detection in induced sputum, or by PCR, may be an additional biomarker for stratification of targeted antibiotic treatment. It has been shown that oropharyngeal swabs reflect lower airway microbes in children with different chronic lung diseases and nasopharyngeal samples also reveal *Moraxella catarrhalis, Haemophilus influenzae* and *Streptococcus pneumoniae* as the commonest organisms in preschool children's respiratory systems during wheeze episodes.

Preliminary data from the Saglani group comparing the pathogen specific PCR in oropharyngeal swabs to broncho-alveolar lavage (BAL) PCR and BAL culture results have shown complete agreement between BAL culture and oropharyngeal swab PCR. *Additional* organisms were detected by the PCR compared to culture in 26/64 (41%) samples, and the PCR failed to detect a cultured organism in 3/64 (4%) patients. This suggests that the PCR test is accurate, and I propose that throat and nasal swabs will prove to be feasible as non-invasive tests to detect lower airway bacterial infection and identify children who may benefit from targeted antibiotics.

4. Lung function tests to assess airway obstruction and bronchodilator reversibility (BDR)

For recurrent wheezers without atopy, peripheral eosinophilia, or airway infection, the treatment of choice may be as required bronchodilators and/or regular long-acting muscarinic antagonists. However, to understand the benefit of these treatments, lung function testing and demonstration of bronchodilator reversibility is required. The Saglani group have generated preliminary data in fifteen preschool children showing feasibility of the forced oscillation technique (FOT), a recognised test for airway function, and have demonstrated BDR (Figure 4). However, feasibility in a larger number and for longitudinal assessments needs to be determined.

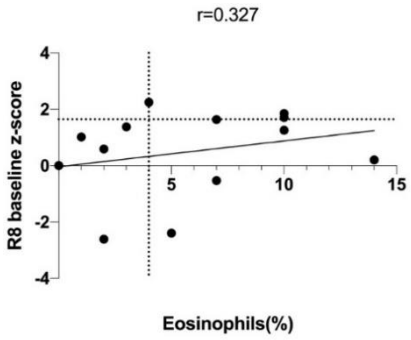


Figure 4: Correlation between respiratory resistance at 8 Hz (R8) z-score obtained using the forced oscillation technique and blood eosinophils using finger prick testing (%) in preschool children aged 1-5 years.

Research question:

Can non-invasive tests (biomarkers) of allergen sensitisation, eosinophilic inflammation, phenotype and function of airway neutrophils, airway infection and lung function be used to personalise therapy for recurrent preschool wheeze?

Hypothesis:

Non-invasive biomarkers are stable, feasible to measure longitudinally, with high sensitivity and specificity, and can be used to personalise therapy of recurrent preschool wheeze.

2. STUDY OBJECTIVES

Primary Objectives:

Aim 1. Investigate the sensitivity and specificity of: (a) aeroallergen sensitisation and blood eosinophils in reflecting lower airway eosinophils; (b) blood neutrophil phenotype and chemotaxis in reflecting lower airway neutrophils; and (c) induced sputum and oropharyngeal and nasal swabs in

reflecting lower airway infection in preschool children with severe, recurrent wheeze undergoing clinically indicated bronchoscopy.

Aim 2. Determine the feasibility of undertaking 3 to 6 monthly assessments of the proposed non-invasive biomarker tests listed below in preschool wheezers, and relate the results to symptoms, wheeze attacks and prescribed medication.

Aim 3. Undertake a proof-of-concept ***feasibility*** study to see if parents/caregivers will agree to take part in a stratified treatment clinical trial of biomarker driven therapy in recurrent preschool wheeze.

Secondary Objectives:

1. To determine the severity of preschool symptoms using a validated symptom questionnaire (TRACK) and relate the results to the tests above and assessment of symptom burden to caregivers using the validated Paediatric Asthma Caregivers Quality of Life Questionnaire: PACQLQ.

2. To determine how much treatment is required for preschool wheeze, including calculating the steroid burden (inhaled corticosteroid and oral prednisolone) the child requires, and the number of unscheduled healthcare visits.

3. To determine lung function in the preschool wheeze cohort, including bronchodilator response.

4. To explore the relationship between airway inflammation and infection and serum inflammation and inflammatory markers.

5. To explore the accuracy of oropharyngeal and/or nasal swabs in detecting lower airway infection in preschool wheezers.

3. STUDY DESIGN

Study design:

1. Single centre, basic science study with human biological sample collection.

2. Laboratory studies of biological samples collected.

3. Proof-of-concept, feasibility study in a subgroup of the whole cohort.

This study has three aims and the children may be recruited into one or more of the aims.

Recruitment:

Recruitment will be from the Royal Brompton Hospital which is a tertiary referral centre for respiratory paediatrics, and also sees patients who are directly referred by their GPs from Northwest London. This target population includes children from all sociodemographic and ethnic backgrounds as referrals are received from across London and the Southeast region.

Children eligible for participation will first be identified in clinic by their existing clinical care teams. The potential participants' parents or guardian will only be approached by members of the research team: Clinical Fellows, Research Nurses or the Principal Investigator (PI), face to face in the clinic to explain the study. If they are interested, then parents will be given the Patient information Sheet (PIS). We will then give them at least 48 hours and up to two weeks to decide. We will schedule a time to make a phone call, if they consent, to answer any potential queries. We will then follow this up within two weeks, but this can be extended by mutual agreement if families feel they need longer to consider.

If they agree to take part, we organise the first study visit to fit in with their next outpatient appointment, where the consent form will be signed. All subsequent visits will be scheduled to tie in with their outpatient appointments, so no extra study visits will be required. Research participants will not receive any payments, reimbursement of expenses or any other benefits or incentives for taking part in this study during their outpatient appointments. However, in the event that additional study appointments are required, travel and car parking expenses will be reimbursed. Individual researchers will not receive any personal payment over and above normal salary, or any other benefits or incentives, for taking part in this research.

Aim 1: Children participating in this aim are recruited from the cohort of preschool wheezers who have been identified by their existing clinical teams as needing a bronchoscopy, as part of their standard clinical management. We will aim to use surplus samples taken during the bronchoscopy and endobronchial biopsy for the study, with parental consent. Number of children: 50.

Aim 2: Children participating in this aim are recruited from the respiratory clinics who meet the criteria listed below. Children recruited for Aim 1 can be recruited into Aim 2. Number of children: 100

Aim 3: Children participating in this aim can be recruited from Aims 1 or 2, as this is a small proof-of-concept study to investigate biomarker-driven targeted therapy for preschool wheeze. Number of children: 15

Duration:

Over 36 months, with each child being followed-up for 12 months after recruitment.

3.1. STUDY OUTCOME MEASURES

Primary outcomes:

Specificity, sensitivity, longitudinal stability and acceptability of non-invasive tests (biomarkers) of allergen sensitisation, eosinophilic inflammation, phenotype and function of airway neutrophils, airway infection and lung function (FOT) in objective phenotyping of preschool wheeze and investigating the relationship between these objective tests and symptom control (TRACK questionnaire) and wheeze attacks.

Secondary outcomes:

Relationship of the tests below to wheeze attacks and symptom control, using the respiratory and asthma control in kids (TRACK): a caregiver-completed validated questionnaire for preschool-aged children, 3 monthly for a year.

1. Lung function (Rrs: respiratory system resistance; Xrs: respiratory system reactance; AX: area of reactance) 3 monthly
2. Inhaled corticosteroid dose and oral prednisolone dose 3 monthly
3. Induced sputum cultures at baseline and 12 months
4. Blood eosinophil count 3 monthly
5. Neutrophil function and phenotype in relation to infection status, clinical symptoms, and prescribed medication
6. Nasal and oropharyngeal swab for infection 6 monthly
7. Assessment of symptom burden to caregivers using the validated Paediatric Asthma Caregivers Quality of Life Questionnaire: PACQLQ

4. PARTICIPANT ENTRY

4.1. PRE-REGISTRATION EVALUATIONS

Participants do not need any pre-registration evaluations before being considered for recruitment.
Parent / guardian must be able to give informed consent to participate in the study.
Child and accompanying parent / guardian are contactable by telephone and able to attend 3 monthly visits, which will be scheduled at the same time as their existing clinic appointment.
Child and parent/guardian are able to comply with the requirements and restrictions listed in the consent form.

4.2. INCLUSION and EXCLUSION CRITERIA

Table 2: Inclusion and Exclusion criteria for each aim

Aims	Inclusion Criteria	Exclusion Criteria
Aim 1	Children aged 1-5 years, undergoing clinically indicated flexible bronchoscopy, for recurrent preschool wheeze, as determined by their existing clinical team as part of their routine medical management	Children with a known alternative respiratory diagnosis such as cystic fibrosis or bronchiectasis. A child will not be eligible for inclusion in this study if following medical assessment, physical examination or initial investigations, the physician
Aim 2	Children aged 1-5 years attending the paediatric respiratory clinics at the Royal Brompton Hospital with either (i) >/=2 hospitalisations for acute wheeze; and/or	
Aim 3		

	(ii) at least 1 admission requiring high dependency unit or intravenous bronchodilator therapy; during the previous year.	considers the child to be unfit for the study.

Withdrawal criteria: If the person with parental responsibility loses capacity, the child will be withdrawn from the study. Any identifiable data or tissue already collected with consent will be retained and used for the study, but no further data collection or procedures would be undertaken.

5. ADVERSE EVENTS

5.1. DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject 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 hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5.2. REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

5.3.1 Non serious AEs

All such events, whether expected or not, should be recorded

5.3.2 Serious AEs

An SAE form should be completed and emailed to the Chief Investigator within 24 hours. However, relapse and death due to preschool wheeze, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the <name of REC> where in the opinion of the Chief Investigator, the event was:

- 'related', ie resulted from the administration of any of the research procedures; and

- 'unexpected', ie an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all related and unexpected SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting SAEs
RGIT@imperial.ac.uk
Prof Sejal Saglani
s.saglani@imperial.ac.uk
Fax:02075943119, attention Sejal Saglani
Please send SAE forms to: Sejal Saglani
Tel: 0207 5943167 (Mon to Fri 09.00 – 17.00)

6. ASSESSMENT AND FOLLOW-UP

Children participating in Aim 1: One-off assessment carried out on the day of the bronchoscopy and no follow-up needed. Assessments carried out are listed below in Table 4. We will aim to do the oropharyngeal swab, skin prick testing (if not done in the last 6 months) and finger prick blood test under general anaesthetic, during the bronchoscopy procedure.

A bronchoscopic programme which enables research samples to be collected, with parental informed consent, from children undergoing clinically indicated bronchoscopy for severe recurrent wheeze is established at the Royal Brompton Hospital. The Saglani group have shown that it is safe to take additional samples and this does not add significant time.

Table 3: Assessments and investigations for Aim 1 only:

Assessments/ Investigations	Study visit
Vital Signs measurements (Temp, BP, Pulse and respiration)	X
Height and weight	X
Physical Examination	X
Medical History	X
Informed consent	X
Validated symptom questionnaire (TRACK)	X
Paediatric Asthma Caregivers Quality of Life Questionnaire: PACQLQ	X
Forced oscillation technique and bronchodilator reversibility test	X
Oropharyngeal and nasal swabs for pathogen specific PCR to detect Moraxella/Streptococcus/Haemophilus	X
Skin prick test or blood aeroallergen IgE (house dust mite, cat, dog, grass, tree pollen and mixed moulds)	X

Sputum induction for bacterial culture and pathogen specific PCR to detect Moraxella/Streptococcus/Haemophilus	X
Blood sample collection (Finger prick) for point of care testing and laboratory studies	X
Bronchoscopy – bronchoalveolar lavage and endobronchial biopsy (part of routine clinical management and surplus sample used for study with parental consent)	X

Children participating in Aim 2 and Aim 3: Children in these two aims do **not** need bronchoscopy. They will have the panel of non-invasive tests listed in Table 5, at the intervals below. These children will be seen for the research study at the same time as their routine outpatient clinic follow-up visits, which will be every three months over one year (Visits 1-4), and therefore no separate research visits are required. The tests listed below in Table 5 will be undertaken at the times indicated.

Table 4: Assessments and Investigations for Aims 2 and 3.

Assessments/ Investigations	**Study visits (every 3 months for 12 months)**				
	Baseline	1	2	3	4
Vital Signs measurements (Temp, BP, Pulse and respiration)	X	X	X	X	X
Height and weight	X	X	X	X	X
Physical Examination	X	X	X	X	X
Medical History	X	X	X	X	X
Informed consent	X	X	X	X	X
Validated symptom questionnaire (TRACK)	X	X	X	X	X
Paediatric Asthma Caregivers Quality of Life Questionnaire: PACQLQ	X	X	X	X	X
Forced oscillation technique and bronchodilator reversibility test	X	X	X	X	X
Oropharyngeal and nasal swabs for pathogen specific PCR to detect Moraxella/Streptococcus/Haemophilus	X		X		X
Skin prick test (house dust mite, cat, dog, grass, tree pollen,and mixed moulds)	X				
Sputum induction for bacterial culture and pathogen specific PCR to detect Moraxella/Streptococcus/Haemophilus	X				X
Blood sample collection (finger prick) for point of care testing and laboratory studies	X	X	X	X	X

Children participating in Aim 3 only: Our experience is that intervention studies are challenging in preschool wheezers. Parents are often hesitant about proposed changes to treatment, even though existing treatment may be ineffective, and they may not adhere to prescribed therapy. Aim 3 is to undertake a proof-of-concept feasibility study for using biomarker results to determine management. Children in this Aim will be recruited from the cohort of children in Aim 2, and therefore

will have all the tests carried out at the same intervals. If parents consent to taking part, children will have the following treatment adjustments based on the results of their tests (Table 5):

Table 5: Proposed biomarker guided management for proof-of-concept feasibility study

Biomarker result at baseline assessment	Treatment
Allergen sensitisation +/- blood eosinophilia ($\geq$0.3x10^9/L)	Regular inhaled corticosteroids
Non-atopic*; positive bacterial detection in sputum	4 weeks of targeted antibiotics
Non-atopic*; negative bacterial infection	Short acting bronchodilators as required

*non-atopic is defined as blood eosinophil of <0.3x10^9/L + no evidence of allergen sensitisation

This proof-of-concept study will be undertaken to understand whether parents will agree to their children taking part in an interventional trial, and to assess the feasibility of the proposed targeted treatment approach in a subgroup of wheezers. Wheeze attacks and symptoms over the course of a year will be compared to the children in Aim 2 as a reference control cohort. This aim will enable a more accurate sample size calculation and design for a future interventional trial.

The sputum samples, oropharyngeal swabs and finger prick blood test will be collected by a clinical research fellow or research nurse. Bronchoscopy samples (bronchoalveolar lavage and endobronchial biopsy) will be collected by a clinician responsible for the patient, as part of the samples collected for the patient's clinical management and surplus samples will be used for this study, with parental consent.

In all three aims, **parents and the child can refuse any test at any visit** and continue to take part in this study. This will be made very clear at recruitment and during each study visit. One of our key aims is to look at the acceptability of the proposed non-invasive tests to both parents and the child, and therefore, the reason for refusal will be recorded. This will help us understand the acceptability of particular tests and the frequency of testing that is deemed acceptable. We will also perform a visual analogue score for every test done on the child to assess tolerance and acceptability.

The participants will have a thorough respiratory assessment (including lung function tests, skin prick allergy testing) and the results will be made available immediately to the participants and caregivers, as part of the study. Incidental findings, during the course of the assessment, would be communicated to the families, and their GP and clinical care team - examples may include incidental bacterial infection, for which the participants will be prescribed appropriate treatment.

In some cases, children may have an incidental finding during the study, such as identified as having respiratory disease or symptoms that requires further referral or treatment, and in such cases their families and their usual health care providers (GP and clinical care team) will be informed.

End of study is defined as last research visit of the last subject recruited.

7. STATISTICS AND DATA ANALYSIS

Sample size calculations:

Aims 1 and 2 are observational, thus sample size is opportunistic and pragmatic.

Aim 1: Approximately 4 wheezers/month undergo bronchoscopy, with recruitment over 24 months and based on past experience, a conservative 60% acceptance rate for inclusion, we estimate 50 children will be included in Aim 1.

Aim 2: Based on previous data, a sample size of 44 participants (assuming 25% positive cases) will allow the detection of a moderate level of agreement, with expected sensitivity at 67% (95%CI: 23%-95%), at a significance level of 5% with 80% power. Children for Aims 2 and 3 need to be recruited in the first 2 years to allow 12 months follow-up. From the number of preschool wheezers seen in the clinic, we estimate recruitment of 4 children per month, allowing approximately 100 children to be included in Aim 2 over 24 months.

Aim 3: 20% of families agreed to be included in a previous interventional trial undertaken by the Saglani group, therefore n=15 is my target for Aim 3. An important outcome of Aim 3 will be to understand how many agree to be included and reasons for declining, this will enable a realistic sample size calculation for a future interventional clinical trial. A sample size of 15 participants will allow the detection of a high level of agreement, with expected sensitivity at 95% (95%CI: 45%-100%), and at a significance level of 5% with 80% power.

Data Analysis:

Aim 1
To assess the diagnostic accuracy of aeroallergen sensitisation and blood eosinophils in reflecting lower airway eosinophils; of blood neutrophil phenotype and chemotaxis in mirroring lower airway neutrophils; and of induced sputum and oropharyngeal swabs in replicating lower airway infection, I will compute several accuracy measures, such as sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios. Furthermore, to verify the validity of the standard cutoff and to eventually identify more appropriate cutoff values for the considered diagnostic tests, I will perform Receiver Operating Characteristic (ROC) curve analysis.

Aims 2 and 3
Information related to the feasibility of the intervention will be gathered using a combination of quantitative and qualitative methods. Descriptive statistics will be used to summarise the baseline demographics of all patients included in the feasibility analysis and to evaluate parental and children's satisfaction with the various diagnostic tests. To identify factors responsible for dropout, baseline characteristics and questionnaire scores of patients who agreed or did not agree to undergo follow-up will be compared using parametric and non-parametric tests, where appropriate.

To understand the robustness of the proposed diagnostic approach, I will investigate the temporal variation in biomarker trajectories and their relation to clinical status by adopting longitudinal generalised linear models and dynamic Bayesian networks (dBNs). Bayesian networks (BN) are probabilistic graphical models that, using Bayes' theorem, represent knowledge about a certain domain/disease by encoding conditional dependences among random variables in directed acyclic

graphs. A dBN is the extension of a BN applied to longitudinal data. The model describes the dependencies among the variables over time. This allows for encoding cycles and feedbacks between variables to represent their relationships over different time windows. The heterogeneity of the joint dynamics of wheeze recurrence and biomarker variation will be explored using joint latent class models for recurrent events, which will allow me to identify subgroups of children characterised by different disease risk profiles linked to specific biomarker variations. The groups are formed to minimise the within-class variability both with respect to the evolution of longitudinal markers and to the occurrence of an event.

To identify differences between patients in the intervention study compared to those in the observational arm, I will perform differential longitudinal analysis. I will combine simple parametric and nonparametric hypothesis testing techniques with time-varying models based on time-series analysis and smoothing splines. Symptom scores will be recorded using a validated questionnaire at each visit for those in the intervention study and adjustments will be made for season.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

8. REGULATORY ISSUES

8.1. ETHICS APPROVAL

The Study Coordination Centre has obtained approval from the xxx Research Ethics Committee (REC) and Health Research Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

8.2. CONSENT

Consent to enter the study must be sought from each participant's parents or care giver (with parental rights) only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

8.3. CONFIDENTIALITY

The samples will be stored in a linked pseudonymised form. The Chief Investigator will preserve the confidentiality of participants taking par in the study and is registered under the Data Protection Act.

Data will be pseudonymised.

8.4. INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

8.5. SPONSOR

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

8.6. FUNDING

Action Medical Research and The Masonic Charitable Trusts are funding this study.

8.7. AUDITS

The study may be subject to audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Frame Work for Health and Social Care Research.

9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through the National Heart & Lung Institute, Imperial College London and department of respiratory paediatrics, Royal Brompton Hospital, London.

10. PUBLICATION POLICY

All results will be published in open access journals in Line with Imperial College London's open access policy, and in line with the finder's publishing policy.

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