The optimum series of investigations to diagnose asthma

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List of abbreviations

AE	Adverse Event
ACQ	Asthma Control Questionnaire
AGP	Aerosol generating procedure
AHR	Airway hyper responsiveness
Albumin	Human serum albumin
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AO	Airways Oscillometry
AO BDR	Airways Oscillometry Bronchodilator Reversibility
AOS	Airwave Oscillometry System
AQLQ	Asthma Quality of Life Questionnaire
ATS	American Thoracic Society
AUC	Area under curve
AUROCC	Area under the receiver operating characteristic curve
AX	area of reactance
BAL	Bronchioalveolar lavage
BDP	Beclomethasone dipropionate
BDR	Bronchodilator reversibility
BHR	Bronchial hyperresponsiveness
BHRc	Bronchial hyperresponsiveness challenge
BCTmann	bronchial challenge test with Mannitol
BCTmeth	bronchial challenge test with Methacholine
BCThist	bronchial challenge test with Histamine
BMI	Body mass index
BRC	Biomedical Research Centre
BTS	British Thoracic Society
BUD	Budesonide
CF	Cystic fibrosis
COPD	Chronic Obstructive Pulmonary Disease
CoV	Coefficient of variation
CRF	Case report form
CV	Core visit in RADicA study
DDP-4	Dipeptidyl peptidase-4
DPI	Dry powder inhalers
DPPC	Dipalmitoyl-phosphatidylcholine
DRR	Dose Response Ratio
DSeq	Equipment dead space
EARIP	European Asthma Research and Innovation Partnership
EBC	Exhaled breath condensate
EIB	Exercise induced bronchoconstriction
ELISA	enzyme-linked immunosorbent assay
EMIS	Egton Medical Information systems
eNose	electronic nose
Eos	Eosinophils
ERS	European respiratory society

EPOER	Expert Panel Objective Evidence Review
FEF$_{25-75}$	Forced expiratory flow (averaged from 25% to 75% of the FVC)
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
FFP	Filtering face pieces
FOT	Forced oscillation technique
FRC	Functional residual capacity
Fres	Resonant frequency
FVC	Forced vital capacity
GINA	Global Initiative for Asthma
GLI	Global lung function initiative
GP	General Practitioner
He	Helium
HTA	Human Tissue Act
HRCT	High resolution computer tomography
Hz	Hertz
IC	Informed consent
ICC	Intra-class correlation
ICS	Inhaled corticosteroid
IFN-γ	Interferon gamma
IgE	Immunoglobulin E
IL	Interleukin
ILD	Interstitial Lung Disease
INCA	Inhaler compliance assessment device
IOS	Impulse oscillometry system
ISAAC	International study of asthma and allergies in childhood
LABA	Long-acting β-agonist
LCI	Lung clearance index
LC-MS	liquid chromatography and *mass spectrometry*
LLN	Lower limit of normal
LTRA	Leukotriene receptor antagonist
MAAS	Manchester Asthma and Allergy Study
MBW	Multiple breath inert gas washout
MDC	Minimal detectable change
MDI	Metered dose inhaler
MEF 25-75	Maximum expiratory flow at 25-75% of vital capacity
MFT	Manchester University NHS foundation trust
MMEF	Maximal mid expiratory flow
mRNA	messenger ribonucleic acid
MS	Mass spectrometry
N$_2$	Nitrogen
NG80	Asthma Guidelines produced by NICE in 2017
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health Research
NO	Nitric Oxide

NPV	Negative predictive value
OV	Optional visit in RADicA study
O_2	Oxygen
PD_{15}	Dose of inhaled substance that provokes a 15% fall in FEV1
PD_{20}	Dose of inhaled substance that provokes a 20% fall in FEV1
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
PPV	Positive predictive value
Q	flow
R	Resistance
RAD	RADicA unique identifier number
RADicA	Rapid Access Diagnostics for Asthma Study
REC	Research ethics committee
ReCIVA	Breath sampler
ROC	Receiver operating characteristic curve
ROS	Reactive oxygen species
Rrs	Respiratory Resistance
RV	Residual volume
R5	Resistance at 5Hz
R20	Resistance at 20Hz
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
SEM	Standard error of measurement
SD	Standard deviation
SF6	0.2% Sulphur Hexafluoride
SIGN	Scottish Intercollegiate Guideline Network
SIII	Phase III slope
Sn	sensitivity
SnIII	Concentration-normalised phase III slope
SOP	Standard operating Procedure
Sp	specificity
SpE	Sputum Eosinophils
SPMs	specialised pro-resolving mediators

SPT	Skin prick test
SRAD	Steroid Responsive Airways Disease
TLC	Total lung capacity
TV	Tidal volume
TMF	Trial Master File
V_A	Alveolar volume
VC	Vital capacity
VH	Ventilation heterogeneity
VOC	Volatile organic compound
WHO	World Health Organisation
X	Reactance
Xrs	Respiratory system reactance
X5	Reactance at 5Hz
X5in	Reactance at 5Hz in inspiration phase
X5ex	Reactance at 5Hz in expiration phase
Zrs	Impedance of the respiratory system
ΔFEV1	Incremental change in FEV1

Abstract

Background: Asthma is frequently misdiagnosed attributed to lack of objective testing. The National Institute for Health and Care Excellence recommend diagnostic algorithms in attempt to improve diagnosis (NG80). Algorithms have not been validated. An evidenced based optimal approach to asthma diagnosis in steroid naïve symptomatic patients has yet to be established. The novel test Airways Oscillometry (AO) lacks evidence for its use in asthma diagnosis in steroid naïve symptomatic adults. The SARS-CoV-2 pandemic emphasises knowledge gaps in diagnosing asthma when aerosol generating procedures (AGPs) are not available. This thesis aims to address these gaps in knowledge.

Methods: Symptomatic untreated patients were referred into 'Rapid Access Diagnostics in Asthma' research clinic with clinician suspected asthma. Clinical consultation, all tests from the NG80 (FEV_1/FVC ratio, BDR, PEFv, FeNO, BCTmeth), and other tests (blood eosinophils, skin prick testing, and airways oscillometry) were measured pre- and post- trial of treatment with inhaled corticosteroids. Expert panel confirmed or refuted asthma diagnosis.

Results: NG80 algorithms confidently rule-in asthma but underdiagnose patients in a third of asthmatic adults and one in seven asthmatic children. Our recommended algorithm in adults (any one test positive of: wheeze auscultated, FEV_1/FVC <LLN, BDR ≥12 and 200ml, PEFv (alternative) >20%, Eosinophils >0.4×10^9/L, BCTmeth PD20 ≤0.2), and children (any two tests positive of: wheeze auscultated, FEV_1/FVC <LLN, BDR ≥12, PEFv (alternative) >20%, Eosinophils >0.4×10^9/L, FeNO ≥35ppb, BCTmeth PD20 ≤0.2), outperformed the current NG80. AO BDR (X5ex%change) was the only measure to discriminate asthma from non-asthma in symptomatic adults (p 0.014). Our non-AGP algorithm (any one positive of; auscultated wheeze, Eosinophils >0.4×10^9/L, PEFv(alternative) >20%) provided sensitivity 55%, specificity 100%, PPV 100%, NPV 60%, reducing the need for spirometry-based tests by one-third.

Conclusions: National asthma guidance (NG80) rules-in asthma but underdiagnosed asthma risking misdiagnosis or treatment delay. Our alternative diagnostic algorithms (with and without AGPs) performed better to 'rule-in' asthma but require further validation. The novel test AO BDR was able to discriminate asthma from symptomatic "not asthma" in steroid naïve patients showing a potential role in asthma diagnosis.

Declaration

I hereby declare that no portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or institute of learning.

<div align="right">Sarah Drake</div>

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Dedication

To my husband-to-be Guy Crouch, who has encouraged and supported me throughout this process, you have been the most amazing father to our one-year-old son Hunter Crouch enabling me to work long hours and weekends in order to complete my thesis.

Love you so much, Thank you

Acknowledgments

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The author graduated from The University of Manchester Medical School in 2008 with Bachelor of Medicine, Bachelor of Surgery (MBChB). She worked as a junior doctor in Manchester before moving to London to complete core training in the Acute Care Common Stem (ACCS) program at Guy's & St Thomas' NHS Foundation Trust and St George's Universities Hospitals NHS Foundation Trust (2011-2014). During this time she was awarded membership of the Royal College of Physicians (MRCP UK), completed a PGCert in Medical Simulation which was awarded by the University of Bedfordshire (2014), and became an Instructor of Advanced Life support (ALS). In 2014 she returned to the north-west after obtaining a training number in the Respiratory Speciality Training program. In 2017 she completed the Specialist Certificate Examination (SCE) in Respiratory medicine before taking time out of program to complete respiratory research at the University of Manchester, based in the National Institute for Health Research unit at Manchester University NHS Foundation Trust (Wythenshawe site). She hopes to obtain her Certificate of Completion of Training (CCT) in autumn 2022 and continue her career as a consultant in Respiratory medicine.

The authors' current publications related to this thesis:

1. *'Asthma Diagnosis: the changing face of guidelines,'* (2019) commissioned for and published in 'Pulmonary Therapy,' an international, peer reviewed journal.[1]
2. *'Diagnosing asthma with and without aerosol generating procedures,'* (2021) published in 'The Journal of Allergy and Clinical Immunology.'[2]

Outline of the thesis

The thesis is comprised of six chapters; 1) Introduction and literature review, 2) General methods for RADicA, 3) Study A 'Diagnosing asthma with standard tests,' 4) Study B 'Diagnosing asthma using airways oscillometry,' 5) Study C 'Diagnosing asthma during a pandemic,' 6) Final conclusions and future work. The introduction chapter consists of four sections and includes the background of the topic to be addressed, the literature review and the thesis objectives. The methods chapter describes the 'Rapid Access Diagnostics in Asthma' (RADicA) study. The three study chapters consist of: introduction, methods, statistical analysis plan, results, and discussion. The final conclusions chapter summarises each thesis objective highlighting the most important findings from the thesis and identifies future work to be completed.

1.   Introduction

This chapter comprises four sections. This first section outlines the chosen format of the thesis. The second section of this chapter outlines current issues surrounding asthma diagnosis. This section is adapted from my review article entitled *'Asthma Diagnosis: the changing face of guidelines,'*(2019) commissioned for and published in Pulmonary Therapy, an international, peer reviewed journal.(1) The section explores past and present methods for diagnosing asthma and describes what we currently know about the underlying pathogenesis and why this may be important when diagnosing asthma. After which, the more novel test Airways Oscillometry' is described in relation to its potential in asthma diagnosis. Current gaps in our knowledge relating to asthma diagnosis are then summarised. The third section of this chapter summarises the pre-existing literature on standard objective tests used in asthma diagnosis and the newer test 'Airways Oscillometry' (AO), performed in June 2018 before commencing recruitment to the 'Rapid Access Diagnostics in Asthma' (RADicA) study. The final section in this chapter describes the specific objectives covered by this thesis.

1.1. Thesis structure

This thesis is submitted in the alternative format. The format was chosen because the completed research takes the form of three separate studies linked together with the same diagnostic theme. Each study has been completed with the intention to be submitted for publication.

The introduction to the thesis is based upon my review article *'Asthma Diagnosis: the changing face of guidelines,'* (2019) commissioned for and published in 'Pulmonary Therapy,' an international, peer reviewed journal.(1) The article has been edited from its published format to be more encompassing of the second and third study. The first study in this thesis assesses performance of current asthma diagnostic guidelines produced by NICE. Following this we look at the best predictors for asthma and best diagnostic algorithm. The second study in the thesis investigates the role of the novel test Airways Oscillometry in asthma diagnosis. The third study is based upon my published paper 'Diagnosing asthma with and without aerosol generating procedures' (2021).(2) This paper was written with myself as first author under my supervisory professors. After being accepted by the journal and initial revisions made, following my absence whilst on maternity leave Dr Ran Wang joined the team and kindly made final revisions and included an additional data analysis using my collected data (see section 5.4.2.2), we are therefore co-authors on the paper.

1.2. Introduction to asthma diagnosis

1.2.1. History of asthma and diagnostic guidelines

Asthma is the most common chronic disease affecting people from childhood through to adulthood.(3) It is a characterised by variable expiratory airflow limitation, classically presenting with episodes of wheeze, shortness of breath, chest tightness and/or cough.(4) Asthma presents a significant global health burden. The World Health Organisation (WHO) published estimates suggesting that more than 235 million people worldwide are affected by asthma, and that over 380,000 deaths were attributed to asthma over a 12 month period.(5) In the United Kingdom (UK) on average three people will die from asthma every day.(6) Asthma has been shown to be underdiagnosed across all countries irrespective of the level of development.(5) In addition, a large population study in Canada demonstrated that up to 33% of people may have been incorrectly diagnosed and treated for asthma; this group were more likely to have received their initial diagnosis in the absence of objective testing.(7) As both over- and under-diagnosis are significant concerns, accurate diagnosis is vital in order to optimise health and improve quality of life and survival.

In order to establish a diagnosis, we must first understand asthma. The term originates from the Greek verb "*aazein*", meaning to pant or exhale with an open mouth.(8) Historically the word "asthma" was first documented as a medical term in the *Corpus Hippocraticum* (460-370 B.C.). The term was used to indicate a form of difficult breathing; it was a descriptive word to denote a symptom that was more severe than dyspnoea but less severe than orthopnoea.(9) Over time the word evolved to become the name of a disease that is now embedded within modern medical textbooks. Despite asthma being both well acknowledged and widespread, there was no guidance available on how to best diagnose or treat asthma until an epidemic of asthma deaths emerged in the 1960s.(10) The costs of not recognising and treating asthma correctly triggered a progressive increase in asthma related research driven by public health and health economics.

The first published national asthma guidelines were developed by the Thoracic Society of Australia and New Zealand in 1989(11). These were closely followed by guidance from the British Thoracic Society(12) and a Canadian practical guideline report(13) in 1990. The U.S

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Department of Health guidelines (EPR-1) followed in 1991(14), at the same time the International Study of Asthma and Allergies in Childhood (ISAAC) program was commenced, with a view to study the aetiology of asthma(15). Over subsequent years more comprehensive national and international guidance has evolved (figure 1), and in parallel there has been a decline in the age-adjusted death rate attributed to asthma. Despite this, overall, the asthma related mortality remains high. This has been attributed to an aging population(16); however, it would be naïve to assume that this is the only explanation, with ongoing debate still concerning optimum diagnostic and management strategies for this common disease.

1.2.2. The changing face of asthma diagnosis

Recent literature has taken us back to thinking about asthma by its original descriptive and symptom-focused roots rather than describing a discrete disease entity. (17, 18) There is a drive to determine the underlying cause of the "symptom" asthma in an individual, acknowledging that there are likely multiple aetiologies which may require different diagnostic and management pathways. A popular analogy compares asthma with "anaemia,"(18) both terms being used to describe manifestations of diseases reflecting several pathophysiological mechanisms. Whilst the analogy is useful in reflecting the potential complexity of asthma in an individual, its shortcomings exemplify one of the major issues in asthma care: whilst anaemia can be diagnosed with a simple blood test (i.e., haemoglobin level), no such single objective test exists to diagnose asthma.

Several approaches have emerged towards deconstructing asthma and categorising patients either by the underlying disease process or specific clinical characteristics. Endotypes refer to distinct groups with well-defined cellular or molecular biomarkers and a discrete underlying pathophysiology.(19) Evolution of endotypes has in part been a "reactive" process secondary to advances in asthma treatments, which are being developed to act upon specific pathophysiological abnormalities. There is now a need to highlight the underlying cause of the asthma symptoms experienced by a patient in order to prescribe the most effective drug. It would be neither appropriate nor cost effective to treat all patients that have the "symptom" asthma with a targeted drug unless it acts specifically

upon that patient's underlying pathophysiological abnormality. The process of deconstructing asthma into the underlying diseases by endotyping is important, but it is likely to evolve slowly over time as our understanding of airway pathophysiology continues to advance. In the interim defining a universal diagnostic pathway will be challenging; it is likely that multiple pathways with linked biomarkers may be required in the future.

Figure 1.   Evolution of asthma guidelines
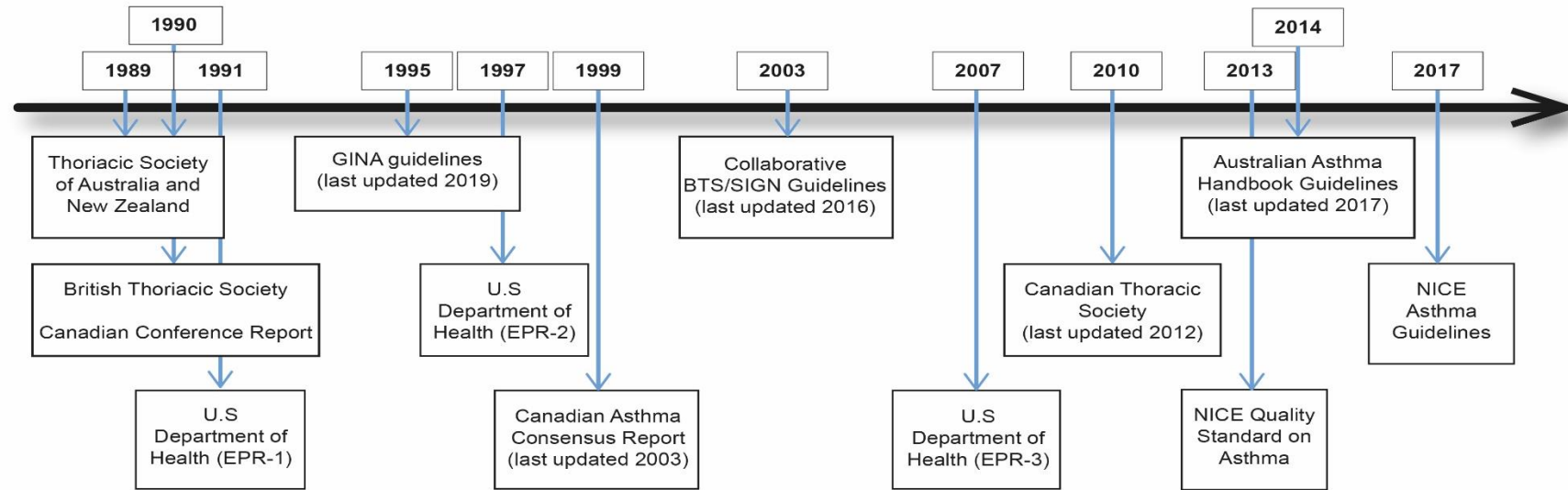
1.2.3. Pathogenesis underlying asthma

With current evidence suggesting that multiple aetiologies known as endotypes exist and lead to the clinical manifestation "asthma," understanding the underlying and perhaps co-existing aetiology in each patient is desirable in order to trigger the appropriate treatment regime. The "one-size-fits-all" treatment previously used for asthma is no longer thought to be the best approach. The last two decades have seen some progress in defining some of these underlying endotypes responsible for asthma symptoms.

To understand an endotype we must first appreciate the underlying pathology in the lower airways that may contribute to the "symptom" asthma. A commonly reported term in asthma is "airway remodelling." This term describes any deviation of cellular composition or the structural components of the airways compared to a healthy individual.(20) It is unclear if remodelling causes a predisposition to asthma exacerbations or if it is the result of airways subjected to asthma exacerbations. Common pathological changes seen in the airways of those with asthma symptoms include epithelial changes (goblet cell metaplasia, hyperplasia, increased mucin stores), and submucosal changes (subepithelial fibrosis, increase submucosal gland cells, smooth muscle hypertrophy and hyperplasia, blood vessel cells leading to increased blood vessels.)(21)

The most researched and described mechanism associated with "asthma" symptoms is type-2-inflammation-associated asthma. The literature reveals a majority of asthma patients, but not all, have evidence of type 2 inflammation.(21)Type-2 inflammation is not a fixed pathway and deciphering the predominant immunological abnormality will likely lead to the endotype. This endotype can then be targeted by appropriate drugs to control or prevent or cure asthma. More than one endotype may be present.

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Type-2 inflammation involves cytokines (IL4, IL5, IL13), and inflammatory cells (type 2 T helper lymphocytes, mast cells, basophils, eosinophils, IgE- producing plasma cells). Symptomatic patients with over production of any of these biomarkers have been shown to respond to steroids. However, it may be that certain endotypes respond better than others. Attempts to further identify and characterise these steroid responsive endotypes of asthma are underway. Targeting a more specific section of the immunological cascade has led to more targeted and patient specific therapy, allowing the reduction of steroids and their associated side-effects.

Identifying Biomarkers

Establishing the endotype of asthma in the initial diagnostic pathway would be the gold-standard approach to Asthma diagnosis. This knowledge would signpost patients to the most effective treatment regime sooner, reducing overuse and misuse of steroids. To achieve this, we need diagnostic tests that can identify biomarkers of each endotype. A biomarker is any molecule, gene, or characteristic by which the endotype of a disease such as asthma can be identified.(22) Current biomarkers for type-2 inflammation endotypes include: Blood IgE, sputum eosinophils, blood eosinophils, FeNO, serum periostin, blood DPP-4, sputum gene expression.(23)

Currently the only commercially available biomarker recommended in the recent asthma diagnosis guideline(24) is fractional exhaled nitric oxide (FeNO). Nitric oxide (NO) is a free radical that has both physiological and pathological effects in the airways. NO has some bronchoprotective effects such as airway smooth muscle relaxation and inhibition of smooth muscle proliferation. It has been speculated that in asthma a deficiency of local NO can account for some of the airway hyperresponsiveness (AHR).(25) High levels of exhaled NO have been associated with inflammatory diseases of the airways including asthma.(25) NO is produced from many different sources within the airways (i.e. epithelial cells, inflammatory cells (macrophages, neutrophils, and mast cells), vascular endothelial cells), its presence doesn't pinpoint a specific pathological pathway]. It is used as a biomarker for

type-2 inflammation, but it is a non-specific biomarker. Measuring FeNO levels in exhaled breath is useful because it has been widely demonstrated that corticosteroids can reduce FeNO levels and that this correlates with a reduction in respiratory symptoms. However, corticosteroids themselves are very non-specific and in addition have negative side effects. More specific biomarkers are being sought with the aim to provide more specific and targeted treatments. Eosinophil levels and IgE levels are other examples of non-specific biomarkers that are seen in type-2 inflammation.

Other biomarkers are being sought through novel techniques such as exhaled breath analysis i.e., volatile organic compounds (VOCs) in exhaled breath, and techniques that sample particles in expired air (PExA). The concept is driven by the knowledge airway inflammation and "oxidative stress" occurs in the airways of many patients with asthma.(26) The latter results from reactive oxygen species (ROS), a by-product of inflammatory cell activation. Reactive oxygen species play a role in degradation of polyunsaturated fatty acids and formation of volatile hydrocarbons.(26) Saturated hydrocarbons in exhaled breath formed during lipid peroxidation of fatty components of cell membranes are thought to reflect the degree of airway inflammation and oxidative stress and potentially predict the risk of an asthma exacerbation.(27) These volatile organic compounds excreted in exhaled breath can be local or systemic. Different patterns of VOCs have been called "breathprints" and have been shown to have the ability to diagnose asthma (sensitivity 87%, specificity 86%)(26) from healthy controls. Common groups of VOCs include alcohol, aldehyde, alkane, carboxylic acid, cycloalkanes and ketones.

Although most biomarkers currently do not feature in standard asthma diagnostic guidelines some are now used in severe asthma guidelines. Type 2 inflammation inhibitors have emerged over the last one to two decades including drugs that target IgE such as Omalizumab, and those that target IL-5 such as Mepolizumab, Reslizumab, and Benralizumab.(28) Whilst patients with high IgE are easy to determine through a simple blood test, patients that benefit from anti-IL-5, IL-4, or IL-13 inhibitors are less easily to pick out, this is where biomarkers for the type 2 cytokines have an important role. Whilst

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elevated levels of IL-5 mRNA on bronchial biopsy of asthma patients have been demonstrated (29), a less invasive test in the form of a biomarker is more clinically useful to detect patients with raised type 2 inflammatory cytokines. IL-5 has been shown to be the main cytokine for eosinophil activation (29) and high eosinophil levels are used as a biomarker for anti IL-5 therapy, however this biomarker is not specific for IL-5. IL-4 biomarkers include raised IgE, FeNO and sputum gene expression however these are also non-specific to IL-4.

Cytokines associated with asthma (figure 2):

Figure 2.   Role of key cytokines in asthma
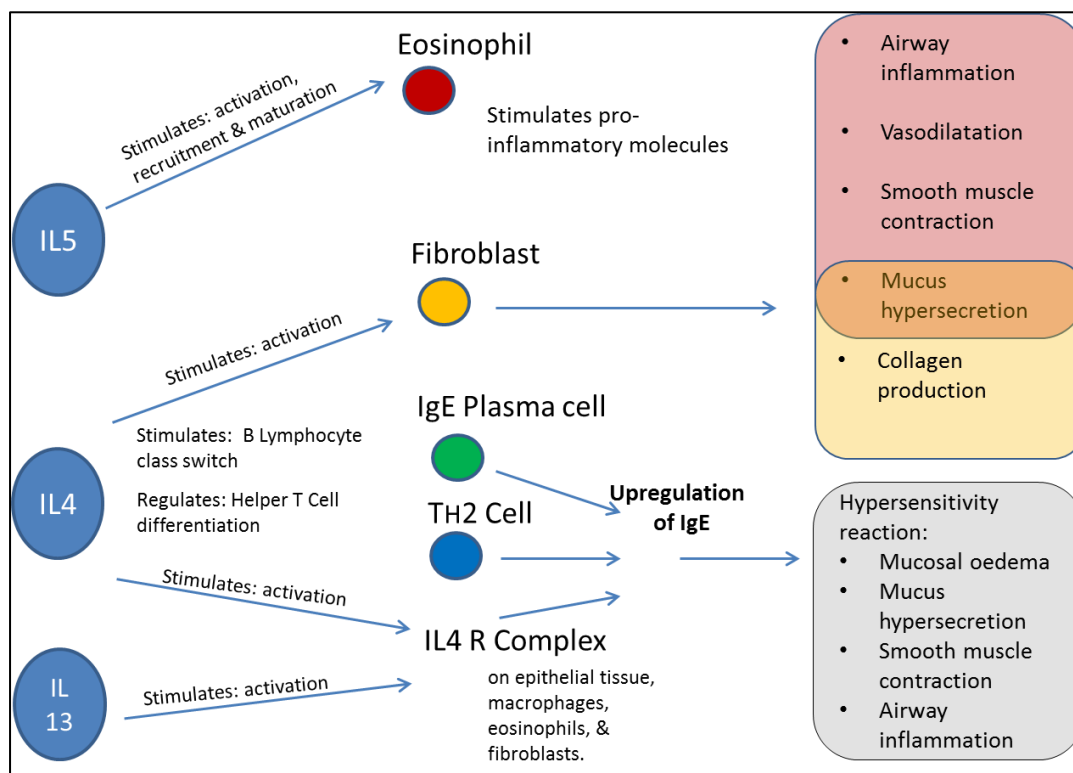
IL-5 plays a key role in activation, maturation, and recruitment of eosinophils. Eosinophils secrete pro-inflammatory molecules leading to airway inflammation, vasodilatation, smooth muscle contraction and mucus hypersecretion.(29) A Cochrane review (2017) supports the use of IL-5 inhibitors in patients with severe asthma and high eosinophil counts.(29)

The other major cytokines associated with asthma are IL 4 and IL13. Both of these cytokines are involved in activating the type 2 IL-4 receptor complex which is expressed in epithelial tissue, macrophages, eosinophils and fibroblasts.(30) IL-4 regulates helper T cell

differentiation to T_H2 cells, stimulates B lymphocyte cell class switch increasing IgE levels, and stimulates mucus hypersecretion and collagen production by fibroblasts.(31)IL-13 shares similar signalling pathways to IL-4, IL-13 alters expression of proteins involved in eosinophilic inflammation, increased airway reactivity, mucus secretion and collagen production.(32)

Other asthma endotypes have been described; these include patients with low type 2 cytokine levels and elevated non-type 2 cytokines including IL-17 and IFN-γ.(33) Other pathological abnormalities discovered in people with asthma include a deficiency in specialized pro-resolving mediators (SPMs), these molecules are involved in controlling the immune response.(33) A deficiency could explain the exaggerated immune response associated with asthmatic airways. It may be that future drugs could target these biomarkers and to increase SPM production.

Phenotypes of Asthma and "treatable traits"

Another way of deconstructing asthma is through phenotypes, defined by observable symptoms or disease characteristics. Phenotyping is possible through assessment of clinical, functional, radiological, or biological parameters.(34) This is distinct from endotypes, which requires knowledge of the underlying cellular or molecular pathology. Hence, identifying the phenotype may help to select drugs that improve the observed clinical presentation, whereas endotype-driven therapy will directly target an underlying mechanism.

A linked concept is that of treatable traits, defined as observable components that can be modified to improve well-being (34, 35). The concept can encompass both classification systems and is perhaps a more clinically useful way to classify asthma. It can be illustrated by the aforementioned comparison with "anaemia". A patient who presents with breathlessness due to anaemia may benefit symptomatically from a blood transfusion, irrespective of the underlying disease. Likewise, a patient who presents with breathlessness

and wheeze due to asthmatic bronchoconstriction will benefit from a bronchodilator inhaler irrespective of the underlying inflammatory mechanism.

With the emergence of phenotypes and endotypes and observation of their overlap, attempts have been made to unravel these in order to provide a more accurate prediction of an individuals' prognosis and forecast the most effective treatment plan.(36) Whilst continuing to explore the underlying endotypes and origins of asthma, an interim model is required for the present day. The "treatable trait" model is both easier to understand and currently more clinically useful. Common treatable traits can be found in table 1. Identifying some of these traits within the diagnostic algorithms has the potential to enable early and appropriate therapeutic management of asthma. *The Lancet* asthma commission (18) also advocated deconstructing asthma characteristics into treatable traits, supporting the concept of a precision approach and opposing the current "one-size-fits-all" approach to asthma management.

Table 1.   "treatable traits" that could prompt targeted intervention in asthma

Pulmonary	Symptom based	Wheeze Cough (productive / non-productive) Breathlessness
	Modifiable Exposures	Allergens Bacterial infection Viral infection Exercise Occupational
	Functional	Variable airflow limitation Bronchial hyperresponsiveness Fixed airflow obstruction
	Radiological	Air trapping Airway wall thickening
	Biological	Raised FeNO Blood / airway eosinophilia Raised total / specific IgE
	Pathological	Airway remodelling
Extra pulmonary		Obesity Obstructive sleep apnoea Rhinosinusitis Eczema Gastro-oesophageal reflux disease Dysfunctional breathing pattern Inducible laryngeal obstruction
Behavioural / psychosocial		Anxiety Depression Smoking Poor medication adherence

The result of this evolving perception of asthma, and the recognition that asthma is inadequately diagnosed across the world, has triggered recent changes in diagnostic guidelines. Guidelines have started to encompass more objective tests within the diagnostic algorithms. These objective tests will assist in grouping patients with the "symptom" asthma and enabling earlier exposure to appropriate treatments. However, different national and international diagnostic algorithms currently present conflicting advice.

1.2.4. Asthma guidelines: are the new asthma guidelines recommended by NICE a step in the right direction?

At present two national guidelines are available for treating asthma in the UK, both aiming to recommend the best approach to diagnosing (and treating) asthma but contradicting one another in several key areas. These guidelines produced by the British Thoracic Society in partnership with the Scottish Intercollegiate Guideline Network (BTS/SIGN) (37), which cover the whole of the UK, and by the National Institute for Health and Care Excellence (NICE)(38), which cover only England, have led to confusion and significant concerns amongst healthcare professionals.(39-41)

Until recently the asthma guideline produced by BTS/SIGN (see figure 3) (37) has been widely accepted in the UK.(39)

Figure 3.   BTS diagnostic algorithm(37)

(this figure is reproduced from the BTS/SIGN British Guideline on the Management of Asthma by kind permission of the British Thoracic Society)
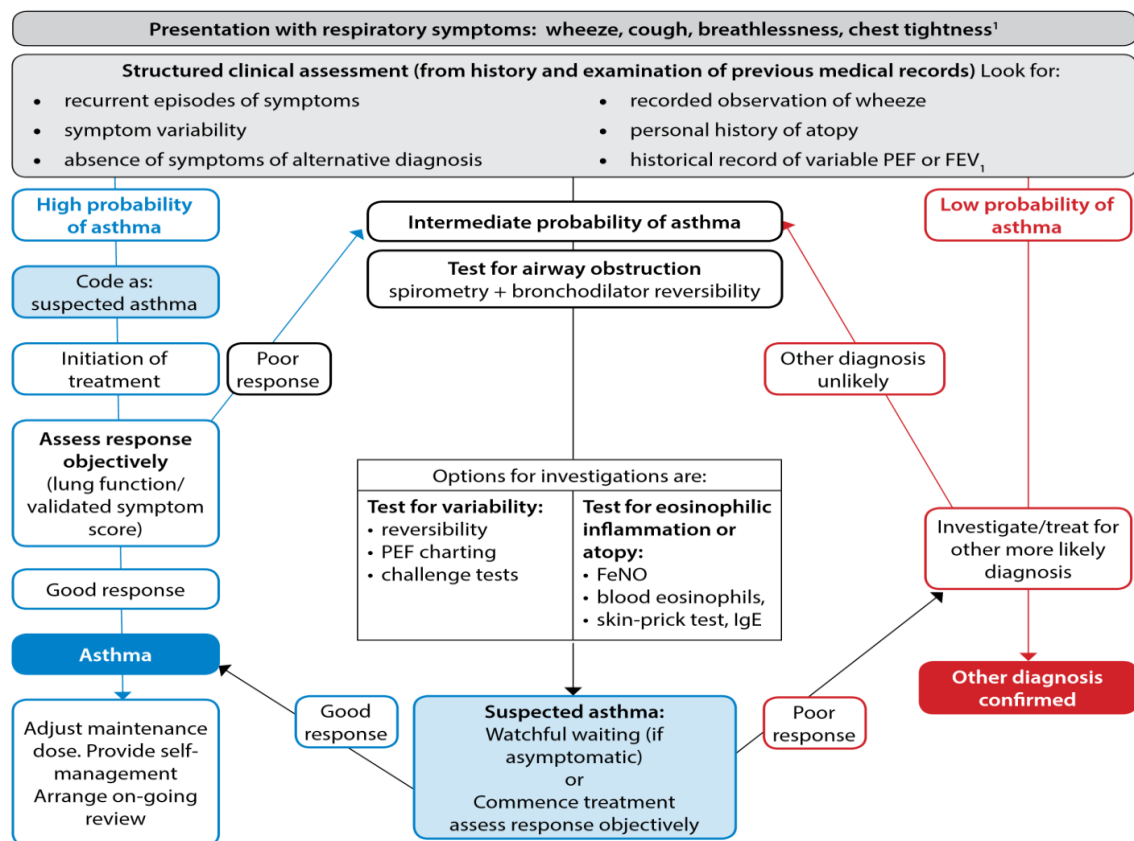
The first formal BTS guidelines were published in 1990. The guidelines evolved over the subsequent decade, and in 2003 the introduction of a more evidence-based methodology was formally introduced when BTS joined with SIGN to produce the British Guideline on the Management of Asthma. This guideline was formed in collaboration with Asthma UK, the Royal College of Physicians of London and the Royal College of Paediatrics and Child Health amongst others.(37) The latest version, updated in 2019, provides recommendations for asthma diagnosis in children and adults. The guideline recommends a clinical diagnosis based predominantly upon physician assessment and encourages the use of objective investigation to demonstrate variable airflow obstruction or bronchial hyperresponsiveness (BHR). However, objective tests are not a requirement for diagnosis. The guideline recommends that a patient having a "high probability" of asthma based upon structured clinical assessment alone is sufficient to commence asthma treatment and subsequently to confirm the diagnosis if there is a perceived treatment response.

A "high probability" of asthma is supported by evidence of episodic symptoms, auscultated wheeze, history of atopy and no suggestion of an alternative diagnosis. In this case, objective testing is not required, even though it has previously been demonstrated that diagnosing asthma in the absence of objective tests was associated with over-diagnosis of asthma.(7) Furthermore, by following this algorithm, the diagnosis (through both the "intermediate probability" and "high probability" routes) is based on response to a trial of low- to medium-dose inhaled corticosteroid treatment, a premise that could lead to diagnostic error. First, asthma and "corticosteroid-responsive respiratory symptoms" are overlapping but different entities. Second, a positive or negative response to treatment, whether based on symptoms only or including lung function, is not a robust test. Major causes of a positive response other than corticosteroid-responsive disease include placebo response (usually very high in studies of inhaled pharmacotherapy) and natural variability in the symptoms; the patients may well have presented at a nadir (for example following a recent exacerbation triggered by a viral infection or allergen exposure), which then could have improved spontaneously at the time of consultation. Conversely, a negative response could be due to poor adherence to regular therapy or to progression of disease.

Another recent guideline on diagnosis and management of asthma was produced by NICE (see figure 4-5).(38)

Figure 4.   NICE diagnostic algorithm in children(38):
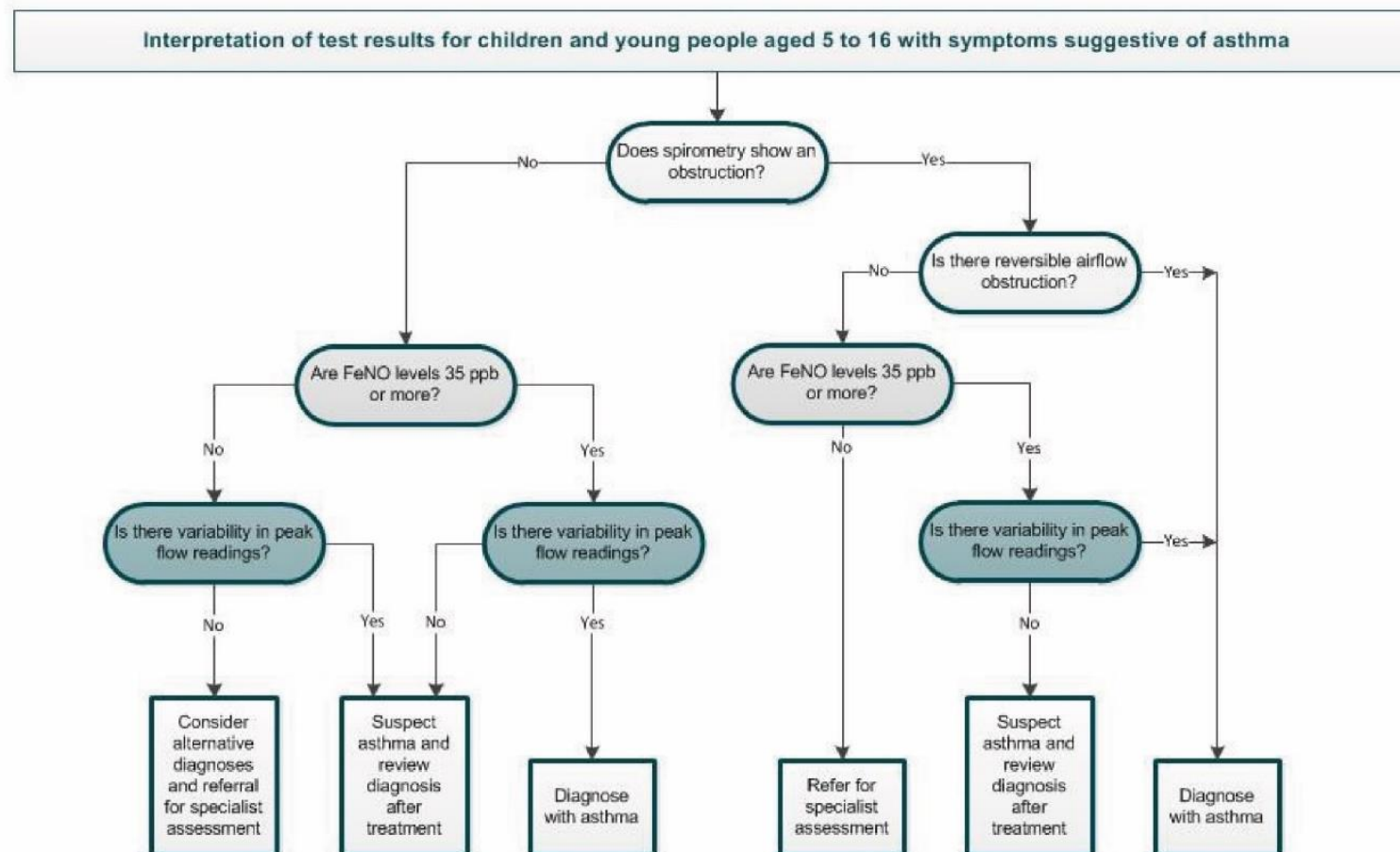
Figure 5.   NICE diagnostic algorithm in adults(38)
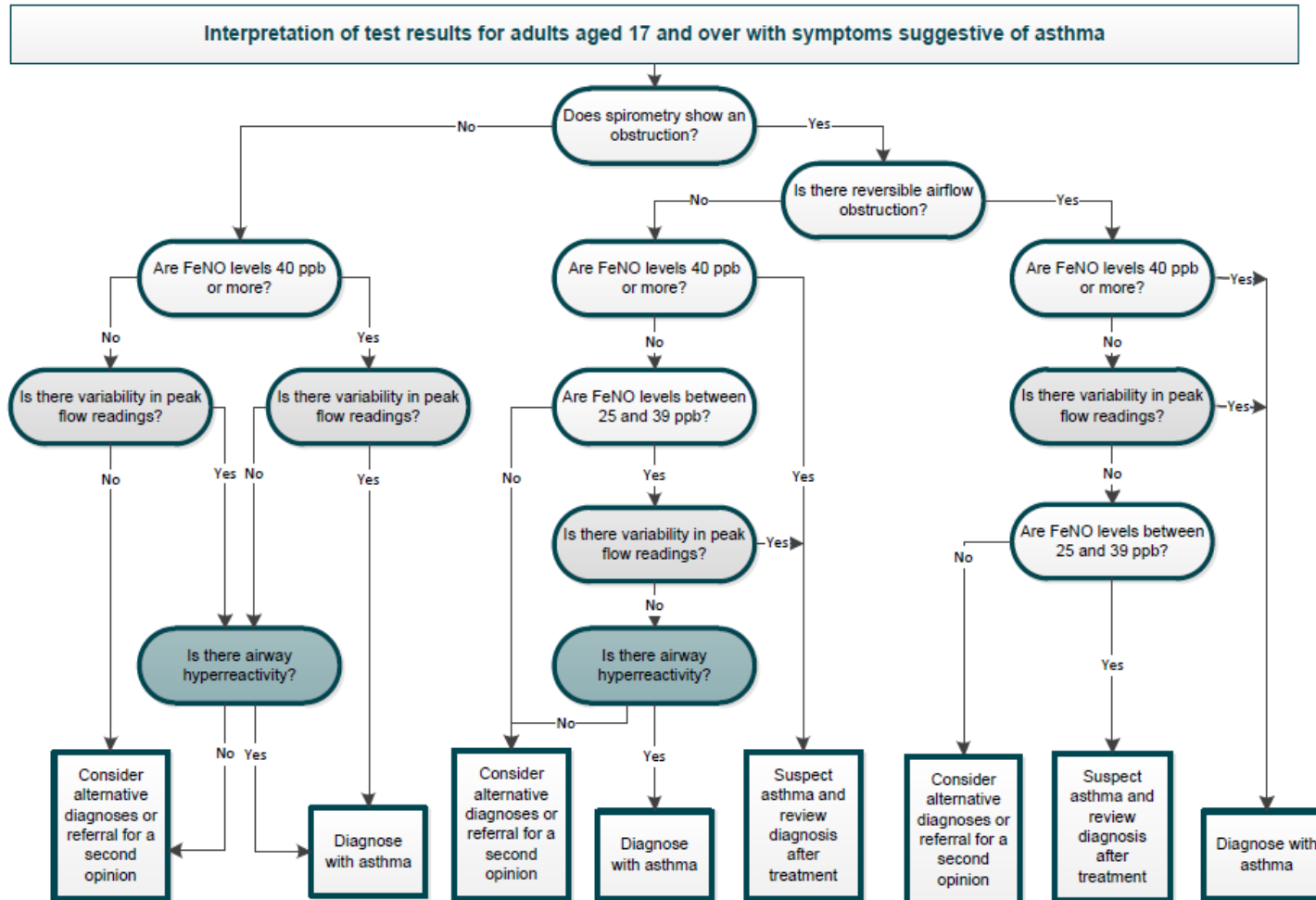
NICE methodology differs from BTS/SIGN in that, in addition to an evidence-based approach, the guideline places an emphasis on a health economics analysis. NICE guidelines critique the evidence on asthma diagnosis using clinical assessment alone (a strategy employed in one pathway of the BTS/SIGN algorithm), concluding that this approach was found to have poor specificity, and is likely contributing to over-diagnosis.(38) The guideline therefore recommends compulsory objective investigations for asthma diagnosis. Perhaps due to an emphasis on health economy, NICE recommend using an algorithm with sequential tests. The algorithm includes tests of airflow obstruction (i.e. spirometry), bronchodilator reversibility (BDR), airway inflammation (i.e. fractional exhaled nitric oxide (FeNO)), and airflow variability, plus bronchial challenge tests if results are inconclusive. The lack of a single gold-standard test necessitates combination testing and developing a reliable diagnostic pathway with as few investigations as possible makes sense, although the diagnostic performance of these tests in the sequence recommended has not been validated. The health economics weighting could perhaps mean tests such as peak expiratory flow variability (PEFv) are more likely to be recommended than other tests such as skin prick testing for atopy or bronchial challenge testing because they are cheap and have high positive predictive value, even if the negative predictive value is poor.

Interestingly, a study evaluating the NICE algorithm sequence in children, and a separate study reviewing a similar style of combination testing in adults, both demonstrate a lack of evidence as to the diagnostic reliability of the combination testing algorithms that were utilised.(42, 43) The study in the paediatric cohort used data from the Manchester Asthma and Allergy Study (MAAS), a prospective population-based cohort; the authors demonstrate that the suggested cut-offs which define positive values for spirometry, FeNO and bronchodilator reversibility recommended by NICE were all suboptimal in the cohort of children studied. Moreover, these values are not adjusted for age, height, or gender. Cut-offs are the same for all children between 5 and 16 years of age. The authors state that the algorithm should not be used in children. They propose more "realistic" cut-off values for the tests used within the algorithm. (43) Further work on this cohort looking at the children at an earlier time point is described later in the report.

The second study in the adult cohort looked at five diagnostic tests (four of which feature in the NICE guidelines), and the authors demonstrate the difficulties in producing a single sequence to diagnose asthma with both high sensitivity and specificity. They suggest it would be advantageous to first clinically ascertain whether the purpose of the tests is to confirm or exclude asthma.(42) It is important to highlight that both of these studies draw their final conclusions using a "clinical diagnosis" of asthma as the deciding outcome. It is controversial to critique an algorithm using a gold standard that has been criticised as being suboptimal. However, perhaps the take home message is that more research is required to establish a validated and efficient diagnostic pathway.

British guidelines *versus* international guidelines

Other national and international guidelines produced or updated over the past decade include Canadian Thoracic Society,(44) the Australian Asthma Handbook,(45) and the Global Initiative for Asthma (GINA)(46) guidelines. The latter are international guidelines with a focus on managing and diagnosing asthma across all health economies. All these guidelines recommend that diagnosis include both clinical impression of asthma through a detailed history and examination, and also objective tests. Recommended investigations include spirometry, bronchodilator reversibility, peak flow variability and bronchial challenge testing. None of these guidelines specify the most efficient sequence of tests to best confirm or refute the diagnosis. These guidelines are more in line with NICE recommendations, but in well-defined circumstances will allow a pragmatic diagnosis to be made in the absence of objective tests. The Australian guideline recommends trial of treatment with subsequent diagnosis guided by a suggestion of clinical improvement in children who are unable to perform spirometry. The Canadian guideline also allows for trial of treatment in preschool children. GINA guidelines specify a trial of treatment in anyone whom it is felt there is a more urgent clinical need to commence early treatment. However, there is the expectation that these individuals will return for objective diagnostic testing within 12 weeks. The GINA guidelines also now acknowledge that different subgroups of asthma exist. However, currently they do not recognise a strong enough correlation between the subgroup and the treatment response, and therefore state that tests assessing

bronchial hyperresponsiveness or inflammation are not necessary in asthma diagnosis. This contrasts with the emerging approach of sub-grouping asthma into treatable traits.(18) GINA recently updated the Pocket Guide for Asthma Management and Prevention(47) and have included guidance on phenotyping asthma; however, this is not considered until step 5 of the asthma management algorithm, in those whom asthma remains uncontrolled despite high-dose corticosteroids. The potential problem with this approach is that by this stage, the patient has already been subjected to high-dose corticosteroids, which may or may not have been appropriate and may alter the efficiency of subsequent testing and interpretation of results.

It should be recognised that the recommendations for diagnosing asthma in the absence of objective tests in certain patient groups is largely due to a deficiency in tests that can be performed by children. There is a clear need for novel tests that can assess small airways disease in this cohort of the population.

In addition to conflicts regarding the sequence and type of tests recommended across the different guidelines, the threshold used as a positive test also varies (table 2). The most marked discrepancies appear to be in spirometry, peak expiratory flow variability (PEFv), and exercise challenge testing. For some of these, the differences may appear trivial (e.g., using "≥" rather than ">"), but for others there are significant differences depending on the guideline used (e.g. the lower limit of normal (LLN) for FEV1/forced vital capacity (FVC) for a 20-year-old male is 86%, and for an 80-year-old female is 62%; for neither would a fixed cut-off of 70% be clinically appropriate). Recommendations for PEFv calculations are varied across guidelines.

Table 2.   Positive test thresholds for objective tests across international guidelines

	BTS(37)	NICE(38)	*GINA(46, 47)
Spirometry	Adults: FEV1/FVC ratio *<LLN* Children: as above	Adults: FEV1/FVC ratio *<70% (or <LLN if available)* Children: as above	Adults: FEV1/FVC <LLN Children: as above
BDR	Adults: FEV1 increase by *≥12% and ≥200mls* Children: *(≤16y): FEV1 increase by ≥12%*	Adults: FEV1 increase by *≥12% and ≥200mls* Children: *(≤16y): FEV1 increase by ≥12%*	Adults: FEV1 increase by *>12% and >200mls* of baseline Children: *(6-11y) FEV1 increase by >12% of predicted value*
FeNO	Adults: ≥40ppb Children: ≥35ppb	Adults: ≥40ppb Children: (≤16y): ≥35ppb	Not included
PEFv	Adults: >20%variability (using minimum 2weeks PEF diary – *calculating percentage of the average PEF*). Alternatively >20% variability when symptomatic vs non-symptomatic. *Children: not recommended*	Adults: >20% variability (using minimum 2weeks PEF diary – *calculating amplitude as a percentage of mean or highest value*) *Children: (≤16y) as above*	Adults: *>10% variability* (using minimum 2weeks PEF diary – *calculating days highest minus days lowest, divided by mean of days highest and lowest and averaged over the week*). *Children: (6-11y) >13% variability measured as above*
BHR Tests	Adults: Histamine or Methacholine PC$_{20}$ ≤8mg/ml Alternatively mannitol (positive defined as drop in FEV1 >15%) *Children: as above*	Adults: Histamine or Methacholine PC20 ≤8mg/ml *Children: (≤16y) not recommended*	Adults: Histamine or Methacholine dose PC$_{20}$ (guideline states 'using standard doses') Alternatively Eucapnic voluntary hyperventilation, hypertonic saline, or mannitol PC15 *Children: (≤16y) not recommended*
Exercise challenge test	Adults: fall in *FEV1 >15%* Children: *as above*	Not included	Adults: fall in *FEV1 >10% and >200ml from baseline* Children: *(≤16y) fall in FEV1 >12% predicted or PEF >15%*
* GINA 2018 guideline report is used plus updates have been extracted from 'The Pocket guide for Asthma Management and Prevention' (updated 2019). The official GINA Report for 2019 was not currently available at the point of publication of this review article.			

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1.2.5. What is the future of asthma diagnosis?

Despite some contradictions amongst current asthma diagnostic guidelines, the general trend is moving towards diagnosing asthma using objective tests. NICE guidelines are perhaps currently the most aggressive in this approach, driven in part by the consideration of health economics. With the emergence of stratified and biomarker-driven therapeutics, future diagnostics will need to move beyond "asthma," to enable identification of phenotypes and endotypes. The NICE algorithm is the first to move towards such an approach, by including a non-invasive type-II biomarker (high FeNO) that is predictive of corticosteroid responsiveness.

There are specific challenges in achieving an objective diagnosis of asthma in children and adults who cannot perform spirometry or FeNO. However, novel tests of airflow obstruction and airways inflammation (in the small and large airways) are in development and may have an emerging role in asthma diagnosis and phenotyping. Some of these tests are much easier to perform on young children and will potentially enable objective diagnosis of asthma in pre-school children (see table 3). (52)

Table 3.   Novel tests of airflow obstruction and airways inflammation

Test	Measures (e.g.)
Airway oscillometry(48)	- R5 (total airway resistance at 5Hz) - R20 (central airway resistance at 20Hz) - R5-20 (peripheral airway resistance: the difference between 5Hz and 20Hz) - X5 (total airway reactance at 5Hz) - X5in/X5ex (total airways reactance at 5Hz during inspiration or expiration respectively) - AX (reactance area under the curve) - Fres (resonant frequency)
Multiple Breath Washout (MBW)(49)	- LCI (lung clearance index) - Sacin (acinar ventilation heterogeneity) - Scond (conductive ventilation heterogeneity)
Novel tests: Tests of small airway pathology and inflammation	
Test	Measures (e.g.)
Volatile Organic Compounds (VOC)(50)	- Mass spectrometry - Electronic nose
Particles in Expired Air (PExA)(51)	- Number of exhaled particles - Protein analysis: surfactant protein A, albumin

At present, diagnostic investigations recommended in national asthma guidelines predominantly interpret large airway pathophysiology and fail to consider the small airways. This is likely due to the ease of access and also minimal invasiveness of large airway tests.

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Small airways are defined as airways without cartilage and <2mm in diameter.(53) Between the trachea and the alveoli there are 23 generations of branching tubes comprising large and small airways.(54) Historically, the small airways have been viewed as a "silent zone" because they account for less than 10% of total airway resistance,(55) and until recently, commonly used imaging and physiological tests have not been able to detect abnormalities in these airways. Accurate investigation of the small airways was only possible by invasive procedures such as transbronchial biopsy and post-mortem examination. Non-invasive investigations that can reflect small airways such as forced expiratory flow at 25-75% of pulmonary volume (FEF_{25-75}) have been accessible, but the results are highly variable due to its dependence upon the forced vital capacity (FVC).(56) It has now been accepted that the small airways in patients with asthma are a significant contributor to airflow limitation.(55, 57) Involvement of these airways is not detected by routine spirometry and peak flow monitoring.(55) Using these large airway tests alone may result in missed diagnosis of asthma in patients that have early disease with preserved large airways. It has been demonstrated that pathology can occur in the small airways of patients before changes are detected in spirometry and even before onset of asthma symptoms.(54) Recent advances in non-invasive tests that are able to assess small airway function and composition could potentially enable the detection of asthma at an earlier stage.

1.2.6. Is Airways Oscillometry the next big test for asthma diagnosis?

What is Airways Oscillometry?

Airways Oscillometry (AO) is the measure of airway impedance and allows the assessment of functional airway mechanics. Impedance represents all forces that oppose impulse propagation.(58) In airways disease, structural changes within the airway lumen and changes in the airway tissues have an impact on airflow and 'impulse propagation.' This will impact the impedance of the airways. Analysis of these differences and comparison with healthy non diseased lungs, may assist in diagnosis and monitoring of certain lung disorders.

The oscillometry technique involves calculating standard airway measures; pressure (P) and flow (V), however in contrast to current lung physiology testing (i.e. Spirometry, FeNO, and PEF) this technique does not require the patient to perform a specific or forced respiratory manoeuvre which can be difficult to attain in some groups including children, elderly, and those with disabilities or moderate symptoms. The technique involves the use of sound waves projected into the lungs during relaxed tidal breathing. Changes in pressure and airflow that result from these sound waves are recorded and from this, impedance can be calculated. It has previously been shown that this technique is sensitive at detecting mechanical changes in both proximal and distal airways and it may therefore be a good tool for early detection of small airways disease in asthma.

The technique was first described by DuBois et al in 1956. (59) The original system was called 'Forced oscillation technique' (FOT) and involved passing single frequency sinusoidal sound waves (created via a loudspeaker), into the lungs during tidal breathing to generate information on airway impedance. Over time different techniques have been developed which all used the same principle of superimposing pressure waves onto tidal breathing in order to measure respiratory impedance.(60)

Today two classic techniques dominate most of the literature. Single or multi-frequency forced oscillation technique (FOT) using sinusoidal waveforms of different frequencies transmitted sequentially into the lungs, and Impulse oscillometry (IOS) using an impulse that mathematically consists of all frequencies is projected into the lungs.(61) The literature often uses the terms FOT and IOS interchangeably or uses FOT as a blanket term to cover all forms of oscillometry, this can lead to confusion. Even though all techniques report the same measures of impedance (reactance and resistance), the values generated are not directly transferable and so comparison using standardised reference ranges is not currently feasible across all techniques.(62) The lack of standardisation in oscillometry technique, oscillometry terminology and the frequencies at which the impedance is analysed, has likely contributed to the slow progression of this test from research in to clinical practice.

Figure 6.   Oscillometry set up (60)
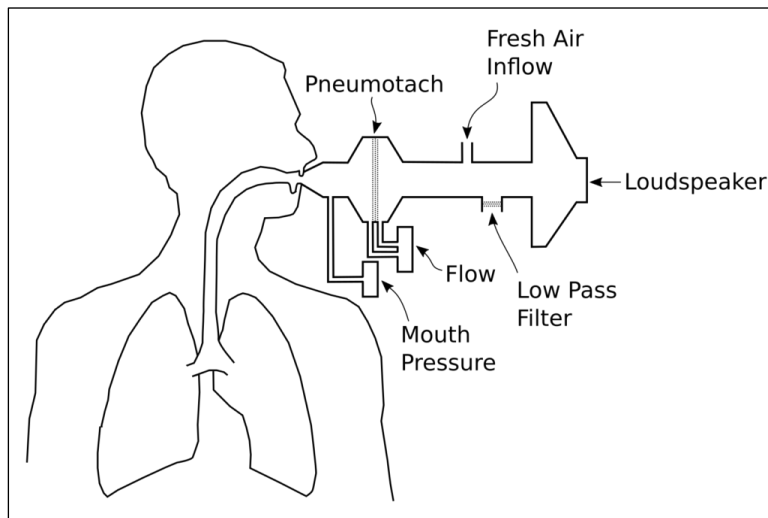
In all techniques the patient breathes through a mouthpiece attached to a pneumotach that calculates flow and pressure at the mouth during tidal breathing. Simultaneously, oscillatory pressures generated through a speaker are projected down into the airways. All systems incorporate a flow of fresh air and low pass filter to reduce interference from tidal breathing on the pressure waveform.(60)

All techniques use the method of "forcing." Forcing describes the concept of applying forced pressure into the airways, is does not imply the breathing manoeuvre required by the patient is forced (a common misinterpretation). Different techniques use different methods of forcing, each has strengths and weaknesses which have previously been well described by Smith et al (2005).(63)

FOT forcing: in this technique continuous spectra forcing using sinusoidal waves at a single frequency or multi-frequency are applied. The continuous nature allows a better overview and sensitivity to detect abnormality within all parts of the airways and may be more accurate in lungs with regional non-homogeneity.

Impulse Oscillometry System (IOS) Forcing: this technique uses impulse shaped "time-discrete" external forcing using pressure pulses opposed to the sum of several sinusoidal waves. This allows up to ten impulses per second (10 impedance spectra) which is greater than that possible through other forms of forcing.(63) The high impedance spectra of IOS

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gives better resolution and better intra-breath measurements. However due to the high impulse rate only short time constants are recorded reducing the ability to measure the entirety of the airways which can potentially miss-represent regional abnormalities. The 'impulse pressure forcing' exerts a higher impact to the airways compared to sinusoidal or PRN forcing, it has been reported to be poorly tolerated by patients.(63)

In essence, an analogy would compare IOS to High-Resolution Computer Tomography (HRCT) in which precise detail is provided on smaller slices throughout the airways opposed to FOT which would compare to a standard Computer Tomography (CT) therefore reflecting a greater proportion of the airways but providing less resolution and therefore perhaps less precise information on more detailed intra-breath analysis. Both techniques are suitable at providing measurements on respiratory impedance derived from resistance and reactance.

Oscillometry measurements: frequency, resistance and reactance

Irrespective of which type of forcing is used, the core measurements are the same. Associations in pressure and flow are measured during external forcing at set frequencies to produce information on the mechanical properties of the lungs.(64) Total Airway impedance (Zrs) is generally broken down into two domains at each frequency; an in-phase and out-phase component, this represents the respiratory system resistance (Rrs) and respiratory system reactance (Xrs) respectively. Lack of understanding of these two components has likely led to the slow transition of oscillometry into clinical practice. Differences in these components in healthy lungs versus different respiratory diseases have now been reported (see Literature review, section 1.3.1.4). There is developing interest in this technique as a tool for both diagnosing and monitoring common respiratory disease.

Frequency: multi-frequency oscillometry classically uses frequencies ranging from 5-40Hz, this is above the frequency of spontaneous breathing.(65) In a healthy person resistance

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(Rrs) is often frequency independent, however airway reactance (Xrs) is more negative at lower frequencies becoming more positive at higher frequencies.(66) Positive and negative values are assigned for mathematical purposes and describe the relationship of pressure and flow and their relationship with inertance and elastance within the airways (described in more detail in 'reactance' subsection below). It is possible to pin-point the part of the airways being measured at different frequencies through existing understanding of sound waves. Higher frequency pressure waves exhibit more energy but travel less distance; they reflect the mechanical properties of the more proximal airways. Lower frequency pressure waves exhibit less energy and move further; they reflect the whole lung mechanics.(65) When considering resistance, subtracting the reactance value of the higher frequencies (R20) from lower frequencies (R5) is thought to reflect lower airway mechanics. Higher resistance within lower airways has been shown to correlate with obstructive airways disease in asthmatic lungs.(67)

Resistance: perhaps considered the easier of the two measurements to understand. Resistance in simple terms is sum of the change in pressure divided by flow.(60)

Counterintuitively, due to the non-linear structure of the respiratory tract, resistance usually decreases in the lower lungs due to doubling of airways resulting in increased surface area which outweighs the increase in resistance expected from the reducing airway diameter (Figure 7).(68) In asthma, due to a combination of airway narrowing and obstruction leading to ventilation inhomogeneity within the distal airways, the same pressure is exerted across a lesser volume which leads to a characteristic increase in Rrs in distal airways (i.e., R5). Any conditions causing upper airways obstruction would be demonstrated by an increase in Rrs starting more proximally. Therefore the higher frequencies would reflect this change

Figure 7.   Lung architecture and resistance (Davies et al, 2010)(68)
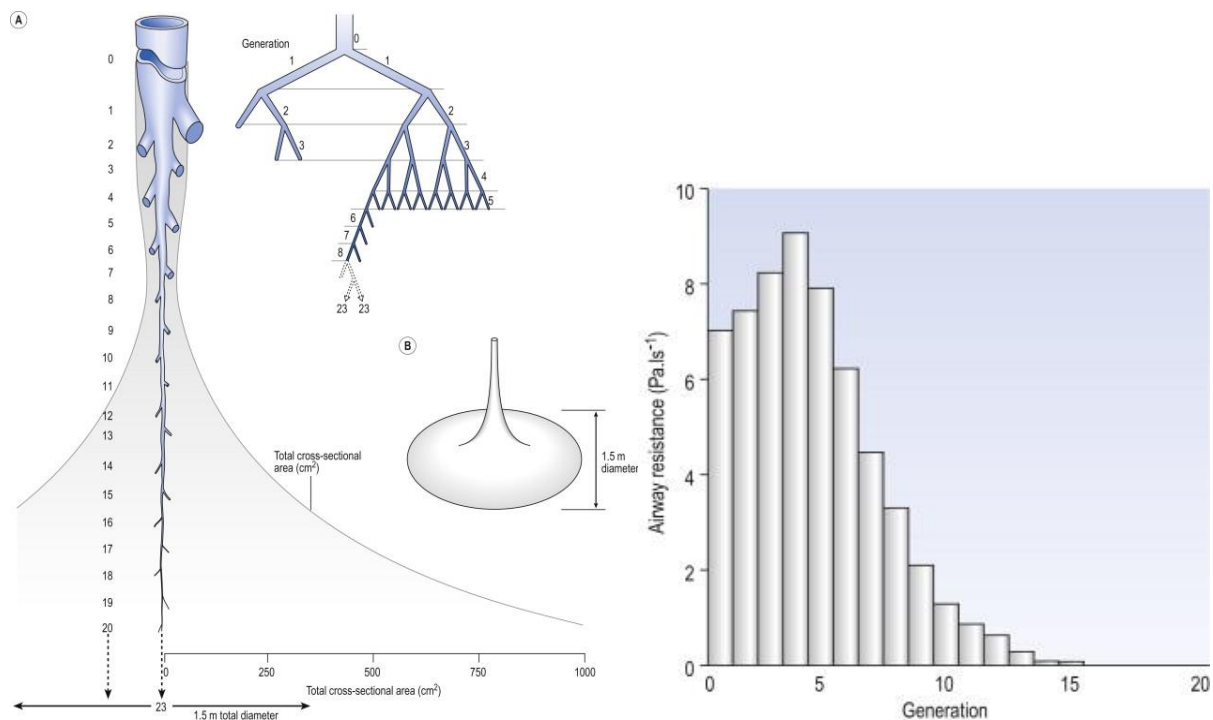
Reactance: Reactance (the more complicated component), is referred to as the out-phase component of impedance. Reactance relates to movement of airway walls and is derived from the effect of inertia (inertance) and airway elasticity (capacitance) on the moving gas column. For mathematical purposes when the inertial forces dominate (i.e. flow peak lags pressure peak) it is recorded with a positive sign and when capacitance dominates (flow peak leads pressure peak) it is recorded with a negative sign.(60, 64) (See figure 8)

At higher frequencies (which is representative of the more proximal airways) inertance is the main component of reactance (Xrs) and results from the relationship between pressure and air flow acceleration, at lower frequencies (representative of the distal airways) capacitance is the main component of Xrs and results from the relationship of pressure and volume.(66)

Figure 8.   Relationship of capacitance and inertance (figure from Johnston (2017)(60)
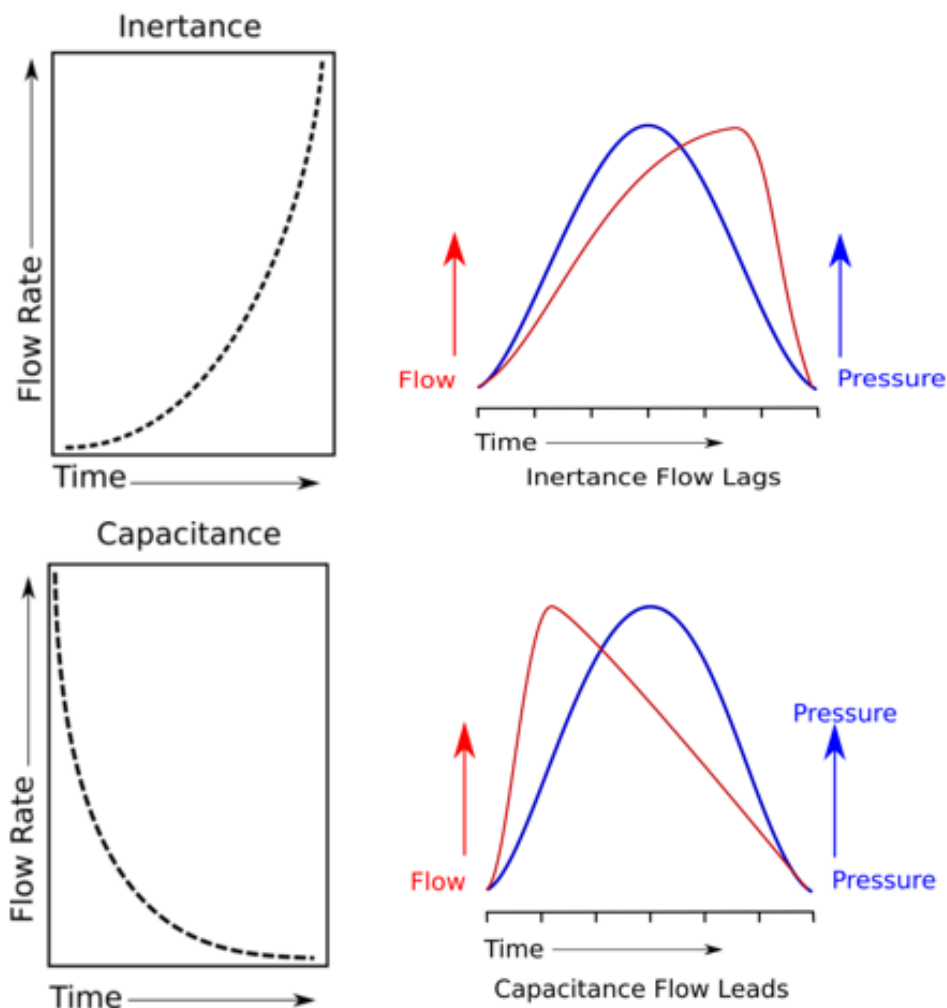
Inertia reflects the ease of air to pass through the airways. The energy to move a column of air through the airways increases exponentially as pressure is applied, it can be explained by the analogy of pushing a car. Air acts like the car, it resists the force most at the start but then resistance decreases as the car starts to move. In this sense, the flow lags pressure initially leading to greater inertance (reflected in the upper airways), moving down the airways the inertance decreases becoming less positive as airflow increases. Additionally, in significant airways disease total lung volume maybe reduced due to obstruction or fibrosis, in these patients inertance plays even less of a role in airways impedance because a smaller column of air creates less resistance to acceleration and capacitance instead dominates the reactance portion of impedance.

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Capacitance always dominates the lower airways due to elastic properties of these airways. Capacitance reflects the "energy return" properties of the airways but is frequently misinterpreted to reflect the "stiffness" of lungs on inflation.(61) As the air column moves, pressure is exerted on the airway walls. The airways act in a similar way to a spring which has least resistance when initially extended but resistance increases as pressure is applied, therefore the flow peak leads the pressure peak.(60) In disease (i.e. hyperinflation, fibrosis) the 'elastic return' properties of the lung are reduced therefore less energy return results in less airflow resistance, and so pressure lag behind flow is more marked and capacitance is more negative. As a result, overall reactance is more negative and the Fres (frequency where capacitance and inertance forces are equal) occurs at a higher frequency. Higher Fres is associated with underlying lung disease. This principle is exaggerated in disease that leads to ventilation inhomogeneity when portions of the lung are not ventilated and so the same pressure is applied to a smaller volume which therefore further exaggerates the out-phase pressure changes due to capacitance.(65)

Further complex mathematical processing of the measurements through the software is performed and the superimposed signals are discriminated from the underlying respiratory pressure and flow.(63) Impedance measurements are usually an average of the measurements made during the testing period after rejection of measurements that do not meet mathematically defined reliability criteria, including segments from during the transition of inhalation and exhalation, due to reduced accuracy in periods of rapidly changing tidal flow.(60, 69) Importantly, the clinical application of FOT does not require this knowledge of the underlying 'complex' mathematical processing, however for further detail please refer to Smith et al (2005).(70)

Interpretation of the Oscillogram:

Figure 9.   Standard Oscillogram
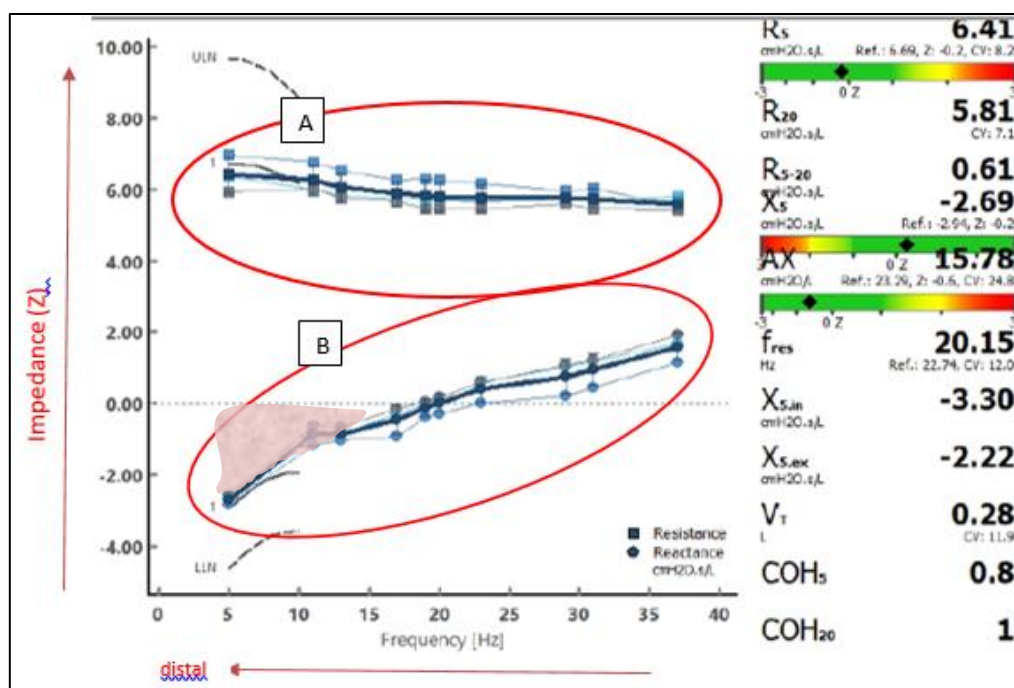
The example Oscillogram (figure 9) is the report that would be interpreted if this test was used in clinic. It reports information on the different mechanical components of the airways. The 'x' axis represents frequency [Hz], y axis represents impedance. As discussed previously the lower frequency pressure wave's travel further and are most representative of the lower airways. The line in marked area 'A' reports the resistance (R) part of impedance, the line in marked area 'B' reports the reactance (X) part of impedance. The point where the reactance line crosses 0 is the resonant frequency (Fres) and represents the frequency at which inertance and capacitance forces cancel each other out, and so impedance is made up of resistance alone.(65) The shaded area within the area marked 'B' is the 'area of reactance' (AX) and is the sum of frequencies where capacitance dominates over inertance.(60) Both Fres and AX values are derivatives of reactance (X) and have been demonstrated to correlate with asthma control and inhaled corticosteroid use in patients with known asthma.(65)

Reference ranges for oscillometry are starting to be defined and should take into account sex, age, height and gender. Lundblad et al (2019) has previously suggested the following

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approximation reference ranges for healthy adults in the absence of the above information: R5 ≤3 cmH2O.s.L^{-1}, R5-20 close to zero, X5 ≥-2.0, Fres 8-15Hz, and AX <10cmH2O. L^{-1}. Further information on oscillometry and its potential role in asthma is presented in the literature review below.

1.2.7. Can we diagnose asthma when Aerosol Generating Procedures are not available?

On 30th January 2020 the World Health Organisation (WHO) declared a Public Health Emergency of International Concern (PHEIC) regarding the SAR-CoV-2 outbreak. In May 2020, after a prolonged period of non-emergency patient care services being put on hold, a phased plan to restart services commenced. During this time, Aerosol generating procedures (AGPs) were not available in primary care and there was a limited capacity in secondary care due to concerns over spread of SARS-CoV-2.

World-wide asthma guidelines at the time of the pandemic recommended that where possible, in those in whom there is a clinical suspicion, asthma diagnosis should be confirmed with objective tests.(38, 71-73) The Global Initiative for Asthma (GINA) and the National Institute for Health and Care Excellence (NICE, England) both recommended the use of spirometry-based tests in the algorithms for asthma diagnosis, requiring temporal variability in lung function, bronchodilator reversibility and/or bronchial provocation challenges.(24, 74) Whilst lung function testing forms a crucial step in asthma diagnosis, the forced expiratory manoeuvre during testing frequently triggers cough and it is therefore considered potentially aerosol generating.(75) During the SARS-CoV-2 pandemic, additional precautions were required in order to limit the spread of virus, the use of filtering face-pieces (FFP3) and rooms with six air changes per hour are recommended.(76) Although helping to reduce risks of transmission of infection, these measures increase the duration of each test whilst reducing capacity within the healthcare system, resulting in significant backlog in respiratory physiology services.(76) Together with a substantial demand for lung function testing during the pandemic,(76) the accessibility and prolonged waiting times for

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spirometry-based tests impede prompt asthma diagnosis and treatment in symptomatic patients.

Whilst most diagnostic tests for asthma require clinic-based aerosol generating manoeuvres, others can either be done at home (such as peak flow monitoring for the measurement of diurnal variability) where risks of transmission of infection are minimal, or have little or no aerosol generating potential [such as measurement of fractional exhaled nitric oxide (FeNO), blood eosinophils and physical examination for wheeze].(77-80)

This raised the question "Can an asthma diagnostic algorithm that is based on non-aerosol generating procedures (AGPs) and home-based peak flow monitoring be used to "rule-in" asthma in some patients presenting with symptoms?" Such an algorithm could facilitate prompt diagnosis and treatment in a subset of symptomatic patients, reducing the demand for spirometry-based tests (which may not be immediately available), and therefore be more feasible in primary care. This algorithm could be compared to the performance of the current asthma algorithm in the UK (NICE) and international diagnostic pathways (GINA).

There is a clear need to develop an alternative algorithm for asthma diagnosis based on tests that would be available for use by primary care during a pandemic, when usual tests of lung function are not readily available. In addition to using established tests, it may be useful to consider the addition of the novel tests that are non-AGPs such as Airway Oscillometry (AO). AO has received significant attention over the last decade. Recent literature already supports AO as a complimentary test for asthma control monitoring in adults when used in conjunction with spirometry.(81) Good association between spirometry and AO has previously been demonstrated,(82) this implies that AO may be a good alternative to our current first line test if Spirometry is contra-indicated. The role of AO incorporated into an algorithm with other non-AGPs to predict asthma has not yet been investigated. The test is easy to perform and is not aerosol generating. Further research is required to investigate the potential role of AO within a diagnostic algorithm for asthma when non-AGPs are required.

1.2.8. Summary of current gaps in our knowledge regarding asthma diagnosis

Given the complexity of asthma, it is likely that in the future, a hybrid approach utilising both established and novel tests will be required in the optimal diagnostic pathway. The best practice pathway has yet to be established. The goal is to develop a diagnostic pathway that is able to discriminate between both phenotypes and endotypes and therefore not only identify asthma patients but also signpost them to the most effective treatment pathway. However, at this time, underlying endotypes are still being defined, and whilst research continues in this area, it is important to take a more pragmatic approach to diagnosing and treating asthma in the present.

It is likely that we will see the emergence of novel investigations of the small airways enter the asthma diagnostic pathway. More evidence is required before these novel tests can be fully established into clinical care. In addition, following the recent global pandemic it may be necessary to have an alternative back up pathway for asthma that does not use AGPs. Below is a review of the literature available on both standard and novel tests used in asthma diagnosis.

1.3. Literature review

Due to the complexity of asthma, to derive the best diagnostic algorithm it is important to review the literature on how each individual test performs in asthma diagnosis. In addition to reviewing standard tests, we also include the novel test airways oscillometry. The literature review was carried out in June 2018 before commencing the RADicA study with the intent to identify gaps in the literature concerning asthma diagnosis. Unfortunately, due to the current lack of consensus in defining asthma it was not possible to only look at studies using a single diagnostic test standard because at present multiple definitions exist across the literature. Some authors define asthma subjectively, others objectively, and others use a combination. Additional information on population, patient demographics, and test standards used in the studies included in the review, is shown in summary tables (see appendix A). This literature review highlights the evidence base underpinning asthma diagnosis, and it was subsequently used to help generate the thesis objectives.

1.3.1. Established tests

1.3.1.1. Tests of airway physiology

Spirometry

Spirometry is a test that calculates lung function by measuring inspired and expired air against time. It is reported to be the most useful test for assessment of lung function that is readily available to patients.[83] It is validated, non-invasive, and can provide reproducible information on pulmonary function.[84] The ratio of Forced Expiratory Volume in one second (FEV_1) and Forced Vital Capacity (FVC) provides useful information on the presence of airflow obstruction and/or airflow restriction. Airflow obstruction can be seen in patients with asthma due to the underlying bronchoconstriction and airway hyper responsiveness.[85] Airflow obstruction can be appreciated visually on spirometry graphs when compared to spirometry from patients with normal lung function (figure 10)[86].

Figure 10. Airflow obstruction in asthma (Gibbs et al, 2015)(86)
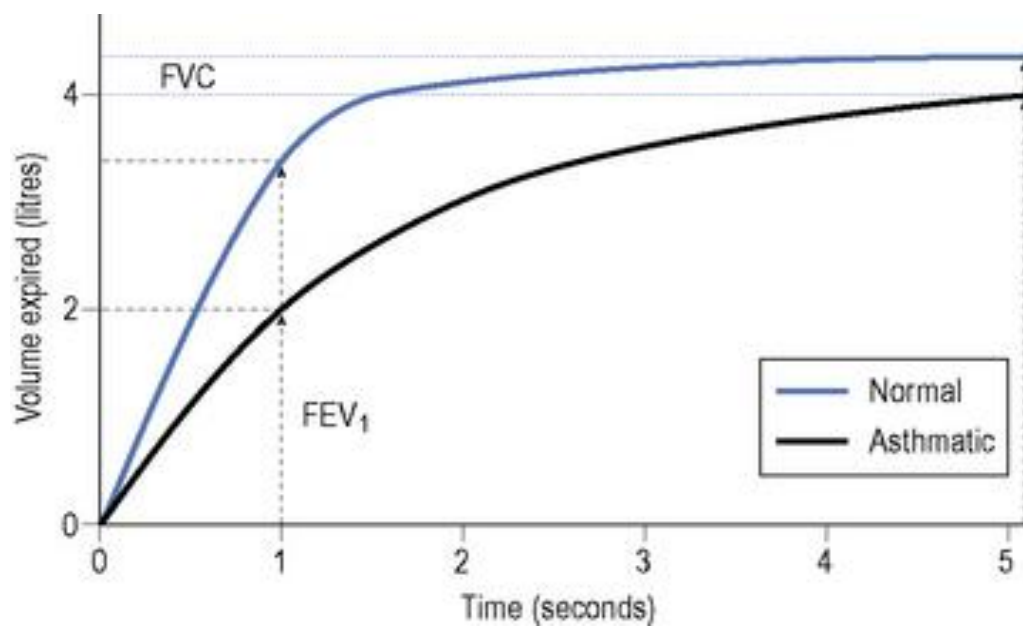
Spirometry coupled with "clinical impression" is frequently used as an objective test in the diagnosis of asthma. Recently it has been highlighted to be neither specific nor sensitive enough to diagnose asthma independently and accurately.(87) At present the exact role of spirometry in asthma diagnosis is still to be defined. One concern regarding spirometry in asthma diagnosis relates to the use of a fixed cut off value to confirm airflow obstruction. Cerveri et al(88) suggest that the fixed cut off value to define airflow obstruction (FEV$_1$/FVC <70%) is oversimplified and inaccurate. This is supported by studies which show spirometry underestimates (88) and overestimates(89) airflow obstruction in younger and older populations respectively. It has been demonstrated that when using this fixed cut off value in young adults more than 50% of patients would be missed when compared with using the lower limit of normal (LLN) value.(88) Lower limit of normal (LLN) calculations are based on predicted values for that individual and take account of age, height, gender and ethnicity.(90) Murray et al(43) demonstrate that in an adolescent paediatric population (mean age 15.5 years) using the FEV$_1$/FVC ratio <70% is a poor predictor of asthma because lower limit of normal in children is much higher than in adults (figure 11).(91)

Figure 11.   Spirometry: lower limit of normal in children (Murray et al, 2017)(91)
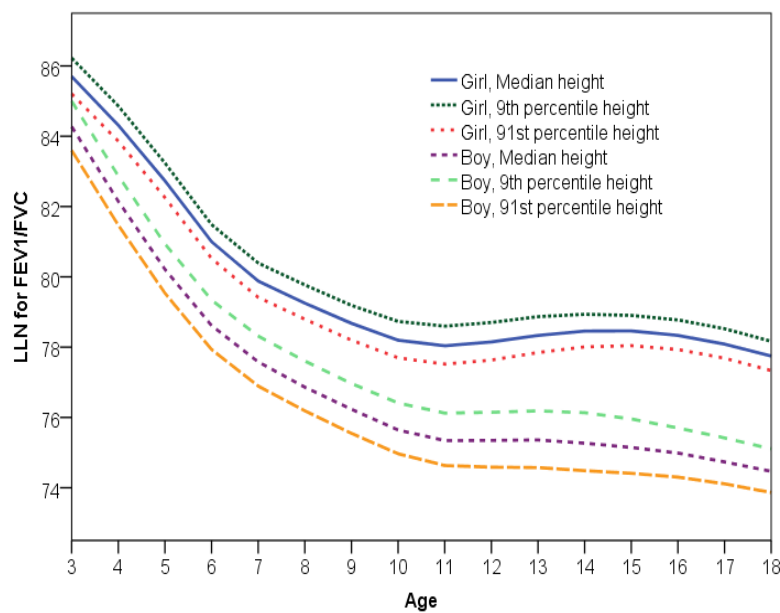
British asthma guidelines(37) put emphasis on clinical impression opposed to an objective test in the diagnosis of patients with high clinical suspicion for asthma. The guideline reviews literature on spirometry in asthma diagnosis and concludes that due to airflow obstruction variability in asthma, the interpretation of an isolated test is unhelpful. The guideline highlights a study that demonstrate in patients with asthma symptoms, spirometry confirmed obstruction was higher in those admitted to hospital in comparison to those managed in the community. The study implies that patients less unwell with their asthma at the time of testing may demonstrate less obstruction resulting in false negative diagnosis if interpretation is based upon an isolated spirometry result. The guideline expresses equal concerns regarding poor specificity of spirometry leading to the potential for over diagnosis.

In contrast, the alternative national guideline produced by NICE (38) recommends spirometry (recorded as FEV_1/FVC ratio) to be the first test in the diagnostic algorithm for both adults and children. The guideline clearly puts weight on this test by recommending that confirmation of obstructive spirometry together with bronchodilator reversibility (BDR) in symptomatic children is sufficient to diagnose asthma without any additional tests. The rationale behind this recommendation is difficult to decipher given that at the time the guidelines were published only one study considered the diagnostic ability of spirometry in a paediatric cohort and this study did not review children and adults separately, so it is

difficult to draw conclusions (92). Following publication of the draft guidance, a subsequent study specifically assessed FEV_1/FVC ratio in a paediatric population.(43) The authors demonstrate that only 2.7% of asthmatic children in their cohort had $FEV_1/FVC<70\%$. The ability of the FEV_1/FVC ratio at this cut off value to predict asthma was poor (sensitivity 3%, PPV 29%). Using a 70% cut-off for FEV/FVC gives a high specificity – 99%, indicating that at this threshold it may be a useful 'rule in' test but would miss 97% of the asthma cases. Reanalysing data using the lower limit of normal, more children in this population had evidence of obstruction (increase from 2.7 to 14%). The authors emphasise the importance of using lower limit of normal for FEV_1/FVC ratio because it is more sensitive, with minimal change in specificity (97%). The authors calculated (using Youden's statistic) using a higher cut off value in children ($FEV_1/FVC \leq 83.8\%$) was better in their cohort of children. This cut off improved the specificity however despite this change it remained a poor diagnostic test in this population (sensitivity 54%, specificity 81%, PPV 35%). The NICE guidelines show agreement that in the adult population, due to low sensitivity, obstructed spirometry even in the context of BDR is not sufficient. The adult algorithm requires additional tests such as positive FENO or peak flow variability before the label asthma can be documented.

Key studies concerning accuracy of spirometry in asthma diagnosis in adults reveals a discrepancy between studies on which parameters are to be measured. Some studies assess FEV_1 whilst others assess FEV_1/FVC ratio. Fortuna et al(93) review the diagnostic reliability of $FEV_1<80\%$ and/or FEV_1/FVC ratio $<75\%$ to predict asthma in symptomatic patients. In this cohort 77% of patients diagnosed with asthma by a Methacholine challenge had normal spirometry and would have been incorrectly labelled as non-asthma if using spirometry in isolation. Popovic et al(94) also demonstrate that spirometry is a poor diagnostic tool when used without other objective tests. The authors only report FEV_1 opposed to FEV_1/FVC ratio. There is a lack of studies that assess the national guideline recommended measure (FEV_1/FVC ratio), this could potentially be a better marker of obstructive airways disease and may therefore yield a better sensitivity. Further research looking specifically at the FEV_1/FVC ratio as a predictor of asthma in older and younger populations is warranted before it is possible to comment on its role in asthma diagnosis. In addition to FEV_1 and FEV_1/FVC ratio, maximal mid-expiratory flow (MMEF) has previously been reported to be

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reproducible and have the same ability to detect abnormality in asthmatic children as FEV$_1$.(95) Further research investigating spirometry markers of small airways in asthma diagnosis and comparison of MME with standard FEV1/FVC ratio is needed.

Peak Expiratory Flow Variability (PEFv)

Serial Peak Expiratory Flow (PEF) monitoring to look for airflow variability (PEFv) has been utilised to assist in asthma diagnosis for many decades. PEF is a measure of forced expiratory flow and is reduced in obstructive airways disease. The test is easy to access, non-invasive and cheap. It was previously thought to be useful in distinguishing asthma from other respiratory diagnoses. By definition, asthma is a disease of variable airflow obstruction which is unique from most other common lung diseases. It would make sense therefore that a test of airflow variability would be useful in asthma diagnosis. There are only five key studies identified that have directly attempted to assess the role of PEFv in asthma diagnosis.(92, 96-99) All studies show PEFv has poor sensitivity. However, there is a discrepancy in the method and analysis applied across the studies making comparisons difficult.

 A lack of guidance on how to interpret PEF and calculate variability may explain the lack in solid evidence base for this test to predict asthma. The most commonly used analysis to determine PEFv include calculation of the amplitude percentage mean [(PEFhighest – PEFlowest) % PEFmean]x100 or amplitude percentage maximum [(PEFhighest – PEFlowest)%PEFhighest]x100.(100) Results from these daily calculations are then averaged over seven to fourteen days. The calculations are complex (figure 12) and as a result they are infrequently performed in general practice due to practicality and time restraints.(100)

Figure 12. PEFv Calculation (amplitude % highest)(100)

Calculation of peak flow indices

$$\text{Diurnal variability} = \left(\frac{(660-620)\times100}{(660+620)/2} + \frac{(660-640)\times100}{(660+640)/2} + \frac{(600-590)\times100}{(600+590)/2} + \frac{(610-590)\times100}{(610+590)/2} \right.$$

$$\left. + \frac{(660-580)\times100}{(660+580)/2} + \frac{(590-580)\times100}{(590+580)/2} + \frac{(690-610)\times100}{(690+610)/2} \right) /7$$

$$= (6.3 + 3.1 + 1.7 + 3.3 + 12.9 + 1.7 + 12.3)/7$$

$$= 5.9\%$$

$$\text{Lowest PEF as \% of personal best value} = \frac{570}{710} \times 100 = 80\%$$
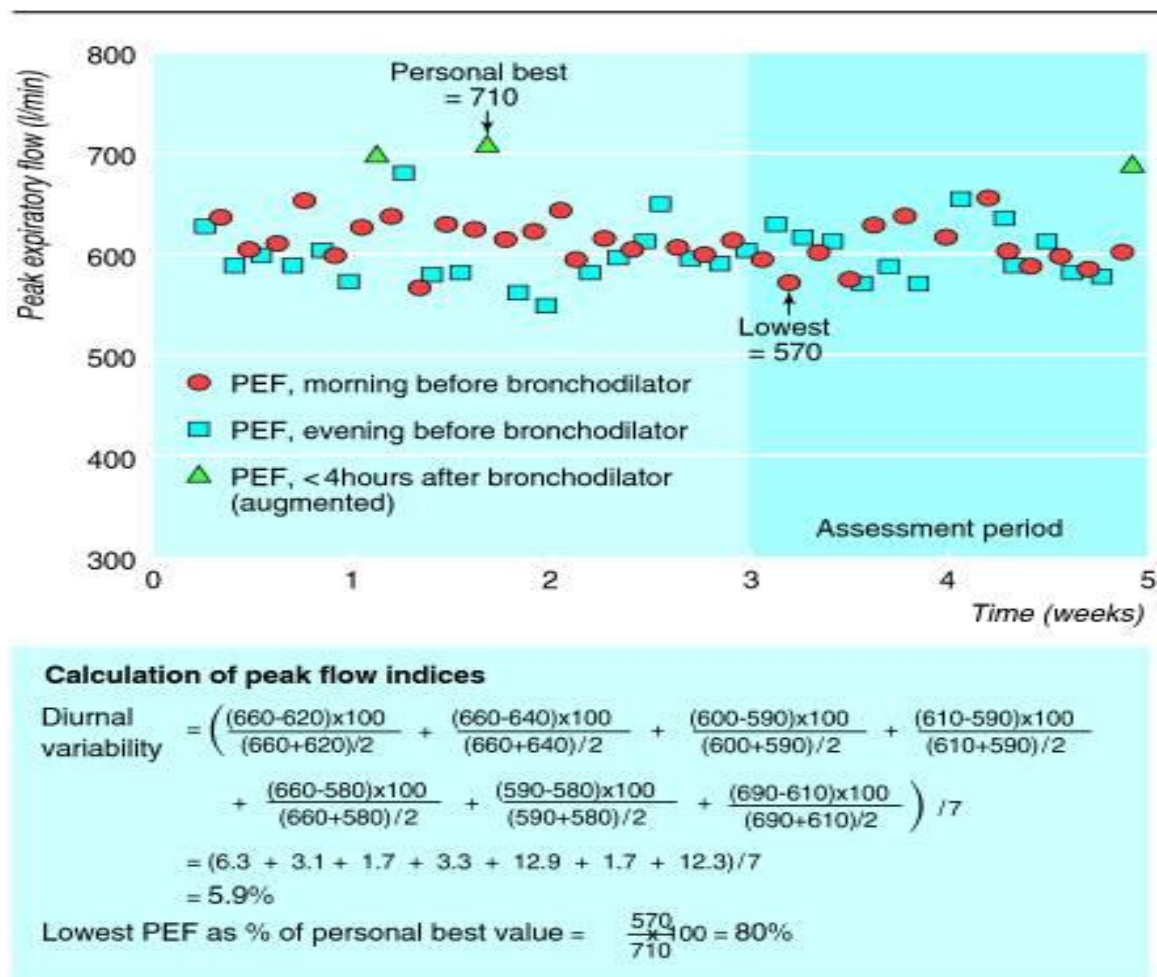
In addition national guidelines on recording PEFv produced by the BTS are vague.(37) Recording PEFv as measure of airflow variability in an optional test in the BTS guidelines, if used, the guidelines recommend PEF measurements are recorded in symptomatic patients as the best of three attempts twice daily for two to four weeks (minimum 14 days). Variability ≥20% is considered positive. The guideline recommends recording the 'mean variability' however it does not specify the best calculation to record the mean variability lending its self to diversity in clinical practice. The guideline also accepts variation to be defined by recording a difference in a PEF reading in a patient when symptomatic versus not symptomatic. Alternatively, the NICE guideline recommends using PEFv >20% to be positive, which is almost the same parameter as BTS guidance. These guidelines also recommend a minimum of two weeks monitoring and do not specify which calculation is used, however only accept either amplitude in variation as percentage of mean or highest value. In clinical

practice to record this result would be time consuming and is reliant on the patient being both able and compliant.

Den Otter et al (1997)(97) conducted a large cross sectional observational study and demonstrated that 38% of symptomatic patients had no significant PEFv despite receiving a diagnosis of asthma through a positive BHR challenge. Similarly, Thiadens et al (1998)(98) show >40% of patients categorised as asthma through BHR testing had negative PEFv. This cohort of patients had cough predominant symptoms and opposed to wheeze. It is possible that PEFv is less sensitive in cough variant asthma and perhaps highlights that a different diagnostic work-up may be required for different phenotypes of asthma. The authors show that in those who did demonstrate variability, the number of days with variability demonstrated in a two week monitoring period was more reliable than calculating the %amplitude change.

The studies in adult only populations both analyse PEFv ≥15% to be a positive test. This is lower than currently recommended in UK guidelines. Using a lower value would likely increase sensitivity and perhaps be preferable in screening patients for a diagnosis of asthma. However, at present there are no studies that support PEFv as a diagnostic tool when used in isolation. More studies are required to decipher the best method to analyse PEF to determine variability and the best cut off to define a positive test before it can be validated within the diagnostic algorithm.

Bronchodilator Reversibility (BDR)

Asthma by definition is characterised by reversible airflow obstruction. Previously bronchodilator reversibility (BDR) testing was thought to be a specific test for asthma. Studies now demonstrate this pattern is seen to a lesser extent in patients with chronic obstructive pulmonary disease (COPD).(101, 102) In addition there are a group of patients who will have evidence of both asthma and COPD, in which the relevance of performing BDR is beneficial to direct treatment but not helpful in assigning a diagnostic label. The

validity and usefulness of BDR testing in the diagnostic pathway has therefore come to question.

Three key studies assess the ability of quantifying airflow reversibility (a measure of change in FEV1 following an inhaled bronchodilator) to differentiate asthma from other lung diseases. Chhabra et al(101) specifically sought to test the ability of BDR to differentiate asthma and COPD and conclude BDR is a poor test to distinguish the two diseases. They suggest the most useful method is to report absolute change in FEV_1 following bronchodilation. They use a cut off >0.2L to give a sensitivity of 73%, specificity 80% and positive predictive value (PPV) 82%. Using this cut off the negative predictive value (NPV) was low (69%) suggesting that this test may underdiagnose asthma. The study did not exclude patients taking corticosteroids. Partially treated airways may explain the low reversibility observed. In addition, all patients in this study had abnormal spirometry (FEV_1/FVC ratio ≤70%). This could represent a more severe asthma cohort with fixed airflow disease and could explain difficultly in distinguishing asthma from COPD. Two other studies review the role of BDR in patients with abnormal spirometry.(103, 104) Brand et al(103) show ΔFEV_1%predicted following bronchodilation was the most useful predictor to establish asthma from COPD. Quadrelli et al(104) also show ΔFEV_1%predicted can differentiate these two diseases and show ΔFEV_1%initial to be least useful. All these studies use a very specific population cohort only recruiting participants with abnormal spirometry. Most patients presenting with early symptoms of asthma have a normal baseline spirometry. Further studies are required in a less selected population cohort before these finding can be utilised in clinical practice.

Only one study review BDR as an independent predictor for Asthma in children.(43) The study demonstrates that when using the cut off (≥12%) recommended by NICE guidelines BDR is a poor diagnostic predictor for asthma (sensitivity 16%, PPV 32%). The authors suggest a lower value of ≥3.48% would improve the diagnostic ability of this test (sensitivity 77%), however the specificity and PPV remained poor (45% and 21% respectively). More studies are essential in children and adults given that this test forms part of the national

asthma diagnosis guidelines. These studies must include patients who have normal baseline spirometry to review the ability of this test in both patient groups.

Bronchial Challenge Test (BCT)

Despite the absence of a gold standard diagnostic test in asthma diagnosis, BCT (also known as Bronchial Hyperresponsiveness Challenge (BHRc)) is often implemented as a surrogate gold standard. This is implied by: 1) a positive test is often used as the deciding diagnostic test in patients that are not diagnosed by first line investigations 2) studies attempting to validate alternative diagnostic tests in asthma have commonly used BCT as the reference standard test to assign or exclude asthma diagnosis. It is possible that this practice results from early work by Hargreaves et al(1982)(105) who reported bronchial hyperresponsiveness to Histamine and Methacholine can be used to diagnose asthma. This practice has subsequently been challenged,(106, 107) however BCT testing has continued to be utilised. Interestingly if BCT is not sensitive and specific for asthma, the validity of other studies that have used it as a reference standard may be questioned.

Review of the literature shows positive BCT is not specific to asthma.(108, 109) It has presumably been adopted as a reference standard because it has previously been found to outperform other available tests. Popovic et al (2003)(94) conducted an observational study to determine the most useful tests in asthma diagnosis, BCT had highest diagnostic accuracy (93%) compared to skin prick testing, sputum eosinophils and IgE measurements. The authors demonstrate that all of the other tests analysed in their study had a diagnostic accuracy <65%. The specificity of BCT in this study was still only 85%, which could lead to false positive diagnoses. Nieminen et al (1992)(108) also demonstrate low specificity (76%) of the BCT with Methacholine (BCTmeth). They show that non-asthmatic patients with alternative diagnoses (bronchitis, allergic rhinitis, COPD) had a positive BCT. In addition, the authors found only 89% of asthmatic patients demonstrated positive BCT. If this cohort was reflective of the general population and used in isolation it would lead to some false negative diagnoses. Some participants in the study were taking steroids which may impact upon the results. In a typical population attending for investigation of possible asthma, most

patients would be steroid naïve. More research on BCT would be useful to determine its NPV in a steroid naïve population.

BCT can be conducted with different agents. Common challenge tests use: Methacholine (BCTmeth), histamine (BCThist), or mannitol (BCTmann). The former two are described as 'direct' bronchial challenge tests because the agents act directly on airway receptors on bronchial smooth muscle. The latter test is described as an 'indirect' bronchial challenge test because the agent triggers release of mediators from inflammatory cells within the airways, these mediators then act upon airway receptors on bronchial smooth muscle.(110) Both NICE and BTS guidelines only recommend the use of 'direct' bronchial challenge testing, however it has been suggested that indirect tests are more specific at detecting eosinophilic airway inflammation and have a superior role in asthma diagnosis compared to direct tests.(110) Bobolea et al (2012)(111) demonstrates that BCTmeth underdiagnosed one quarter of their patients that were later diagnosed as asthma following BCT with adenosine. Koskela et al (2003)(112) compare BCTmann (indirect) and BCThist (direct) in steroid naïve patients, they conclude that only 51% of patients labelled with asthma had positive BCTmann, which was less reliable than BCThist. It is important to note that the patient population in this study were older (age range 44-54yrs), more than half were current or ex-smokers, and the most common symptom was cough. It does not represent the typical demographic and clinical presentation seen in most new asthma referrals. Anderson et al(113) are the only study to my knowledge that specifically look at BHRc as a diagnostic tool in adults and children, however only the combined data is published. They found BCTmeth and BCTmann to have similar ability to predict asthma, sensitivity (sn) 80%, specificity (sp) 65%, PPV 78%, NPV 46 and sn 69%, sp 62%, PPV 79%, NPV 48% respectively. They conclude that both Methacholine and Mannitol challenges performed more poorly in patients with asthma than had previously been documented. In the study they exclude patients 'extremely likely' and 'extremely unlikely' based on clinical impression to have asthma, this would impact on the overall findings and change the sensitivity of the test. Four other key studies specifically compare BCTmann and BCTmeth in asthma diagnosis,(114-117) all studies found similar agreement between the tests, however BCTmann may have higher specificity(116, 117), although it was found to be less well tolerated. The standard for

classifying asthma in patients varied across each study, so the accuracy of the results to predict asthma could be questioned.

On review of the literature, more research is needed to establish the exact role BCT should have in the diagnostic pathway for asthma. The most effective agent for BCT testing is also unclear based on current literature.

1.3.1.2. Tests of airway inflammation

Fractional Expired Nitric Oxide (FeNO)

Measurement of the forced expired nitric oxide (FeNO) has received significant attention across the last decade. This compound is present in the breath of all humans and high levels have been associated with asthma.[118] Guidelines produced by the American Thoracic Society (ATS)[118] recommend the use of this test in monitoring asthma and suggest it may also have a role in asthma diagnosis. More evidence on FeNO specifically as a diagnostic tool would be required to validate its role in the diagnostic pathway for asthma. Recent UK guidelines produced by NICE[38] have included this test early in the diagnostic algorithm. The algorithm was generated with the cost efficiency in mind and may not fully represent best clinic practice. A review of the literature analysing the role of FeNO specifically in the diagnosis of asthma is presented.

Fortuna et al[93] compare the diagnostic accuracy of FeNO with other common asthma investigations including spirometry, and sputum eosinophil count. The authors report FeNO was superior to spirometry as a diagnostic tool. FeNO was shown to have a sensitivity of 77% and NPV of 78%. These values were only achieved by using a low cut off cut off for a positive test (FeNO ≥20ppb) which was low compared to other studies and ATS guidelines.[118] At this value specificity was compromised (Sp 64%). High FeNO was seen in 35% of the non-asthma patients. It is not surprising that using this low cut off resulted in the illusion of FeNO being a sensitive test for asthma when you look at the literature reporting normal FeNO values. Toren et al[119] report standardized FeNO values in a

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general population study involving >5000 people (Figure 13). The use of a higher positive cut off value of >30 has been shown to improve specificity of this test within the range 82-91%(120-123)

Figure 13. FeNO variance with age and gender in general population study (Toren et al, 2017)(119)
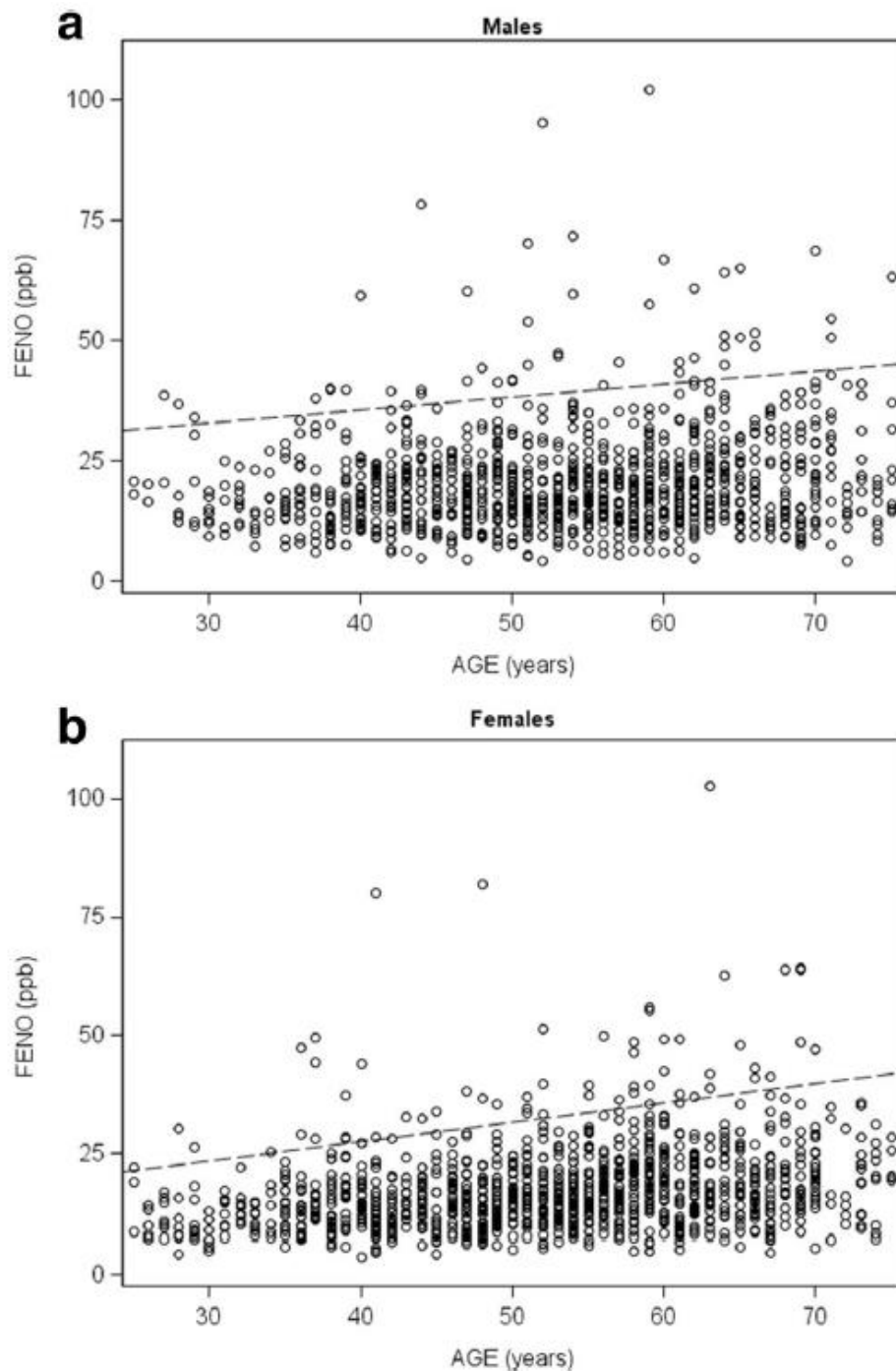
Chatkin et al(120) reviewed the use of FeNO in a population of asthmatic and non-asthmatic patients with chronic cough. They demonstrate that FeNO is significantly higher in the

asthma group, this illustrates that raised levels are triggered by something specific to asthmatic airways that is not necessarily seen in all patients with chronic cough. However, the authors demonstrate low PPV and conclude that FeNO is not a good test when used in isolation in the diagnostic pathway for asthma. This result was supported by Fukuhara et al(121) who demonstrate that FeNO used in isolation underdiagnosed asthma in one out of five patients. Chatkin et al(120) reveal a NPV of 93% suggesting that perhaps FeNO may be a better 'rule-out' test in patients suspected to have asthma. Several other studies support the use of FeNO as a 'rule out' test.(122, 124-126) Heffler et al(126) found that in steroid naïve patents with levels <25ppb likelihood of asthma is almost non-existent. However, it is important to note that it is likely this would only be transferable to a steroid naïve non-smoking population.

Much of the data on FeNO in asthma diagnosis relates to a very specific group of patients that include steroid naïve and non-smoking populations. A few studies have started to review FeNO in a wider population. It is likely that the reason many studies exclude patients that smoke is because early studies show smokers have lower levels compared to non-smokers therefore potentially reducing its diagnostic ability in this population.(127-129) This finding has been challenged by an increasing number of studies.(123, 130-132) It is important to know whether FeNO is accurate in this cohort of people because it has been reported that up to one third of patients with asthma smoke (132). Malinovschi et al (132) look at the diagnostic ability of FeNO in smoking and non-smoking patients. They show that levels in asthmatic smokers remained significantly higher when compared with non-asthmatic smokers and so support its use as a diagnostic tool. Lower cut off values were suggested in order to optimise sensitivity and specificity. Verleden et al(129) also found no statistically significant difference between smoking and non-smoking healthy volunteers and demonstrate significantly higher levels in asthma patients that smoke compared with healthy controls that smoke. Sato et al(123) found that levels were actually higher in smoking asthmatics compared to non-smoking asthmatics. This contrasts most other studies, and the authors speculate the smoking group were more atopic and that this may account for the difference. However, Cordeiro et al(124) specifically reviewed FeNO levels in atopic patients with and without asthma and demonstrate that atopy is not significant

factor. They show that FeNO (positive cut off >38ppb) is a useful diagnostic tool to diagnose asthma in patients with atopy. Berylne et al(133) similarly show no significant difference in FeNO levels between atopic and non-atopic healthy subjects. Considering multiple contrasting studies there is still a gap in our knowledge in how we can interpret FeNO results in the wider population. A single cut off value may be over simplistic. Perhaps at present FeNO should only be considered as a 'rule out' test until more research is conducted. Out of sixteen studies that report on a specific cut of value, ten different values were used. Toren et al (2017)(119) acknowledge this variation in the literature and set out to establish reference values for FeNO. The authors review >5000 people in a population study aiming to establish reference ranges of FeNO in smokers, ex-smokers and never-smokers. The authors show FeNO is significantly influenced by sex, height, age, smoking and atopy (Figure 8). They recommend the use of individual reference values determined by reference equations opposed to the current fixed value approach. Further research is essential to determine reference equations and validate their use in asthma diagnosis.

Sputum Eosinophils

Analysis of sputum eosinophil levels are not routine practice in asthma diagnosis in the UK. This test is not available at present in primary care. Normal sputum eosinophil count has been reported to be <1.9%.(134) A raised count has been associated with asthma, allergic rhinitis, eosinophilic bronchitis and atopic cough.(135) The cut off for a 'significantly' raised count is yet to be agreed upon and has varied throughout the literature. Review of the literature on sputum eosinophils as a predictor for asthma diagnosis highlights three key studies in adults (two including children), and one in children. (92, 93, 122, 136, 137) Fortuna et al(93) used a cut off ≥3%, they show in isolation the utility of this biomarker is inferior to other standard tests that have been recommended in the latest UK Asthma guidelines.(38) However, they demonstrate a raised sputum eosinophil count in combination with raised FeNO outperforms spirometry or FeNO in isolation. This biomarker may therefore have a role in a combination testing algorithm. This diagnostic combination was also demonstrated in other studies.(92, 122) Smith et al(92) review adults and children

and report sputum eosinophilia to have a diagnostic sensitivity, specificity, and PPV of 86%, 88%, and 80% respectively. Louhelainen et al(136) also looked at adults and children but they analysed data on the population groups separately. They show %eosinophils were higher in both adults and children but only achieved statistical significance in asthmatic adults. Siven et al(137) assess diagnostic value of Sputum eosinophilia in a paediatric population, they recommend using 2.7% as the cut off value to predict asthma and demonstrate sensitivity, specificity, and PPV to be 81%, 92%, and 89% respectively. Given the variability across the studies and the fact that induced sputum to obtain samples is often poorly tolerated, more research would be useful to define where (if at all) in the diagnostic algorithm this test should be placed.

1.3.1.3. Tests of atopy

Skin Prick Testing (SPT)

Skin prick testing (SPT) for common aeroallergens has been described as an essential test in confirmation of IgE mediated allergic diseases. One such disease is 'atopic' asthma. However the exact role of SPT to assist in diagnosis of asthma is still undefined. It has been utilised as an adjunct in asthma diagnosis but it was not incorporated into the latest diagnostic algorithm(38). The guidelines recommend SPT is not to be used in the diagnosis of asthma but could be performed in patients with a diagnosis of asthma in order to identify triggers. This recommendation may be because literature looking at the use of SPT specifically as a diagnostic tool is sparse. However, given that there is now a new drive to determine the exact phenotype of asthma from the outset in order to match patients to targeted treatment regimes, more research assessing SPT in asthma diagnosis would be useful.

Tschopp et al(138) show that SPT compared to IgE; an alternative marker of allergy, was more sensitive (65% vs 56%) at diagnosing allergic asthma. This was supported by Popovic et al(94) who show SPT has the second highest diagnostic accuracy when looking at common diagnostic tests in asthma. Soriano et al(139) specifically look at atopy in determining asthma. Indirectly their study shows SPT (panel of five common aeroallergens) and raised IgE (>0.35U/l) to be independent predictors of BHR, a marker of asthma. Only one study in

children reviewed the ability of SPT to differentiate asthma from non-asthma. The authors demonstrate SPT had >90% sensitivity in differentiating children with asthma from those without asthma.

Reviewing of the literature suggests that SPT does have a role in diagnosing a specific subgroup of asthma. However, it is not clear if this should be a first line test or an adjunct after initial diagnosis is established. The current literature demonstrates sensitivity and specificity as high as 91% and 78% respectively, however this is on a selected group of atopic patients, and sensitivity would likely be much lower in a general population.

Immunoglobulin E Measurements (IgE)

Several studies show correlation between raised IgE and asthma.(124, 138-144) Only five studies appear to directly review the diagnostic value of IgE. Obaidi et al(144) show total IgE was predictive of asthma. Using a cut off value of >200IU/ml they show sensitivity, specificity, PPV, and NPV to be 93%, 91%, 97% and 86% respectively. The authors conclude that IgE is a good diagnostic tool in asthma and suggest that it could be utilised in conjunction with other biomarkers. Linneberg et al(142) assessed the diagnostic ability of the allergy screen assay "ADVIA Centaur" and SPT. The authors conclude IgE assay has a good concordance with SPT (sensitivity 85%, increasing to 100% when ≥4 positive on SPT) and has value in predicting allergic asthma. They suggest it may be superior to SPT as a diagnostic tool due to better accessibility. Tschopp et al(138) argue IgE is less effective compared to SPT in diagnosis of allergic respiratory diseases. However, when looking specifically at allergic asthma they demonstrate similar overall efficiency of both tests. Drkulec et al(141) is the only study to look at this specific question in a paediatric population. They use IgE >116 to differentiate asthma from non-asthma (sensitivity 96%, specificity 77%). They show both total and specific IgEs are significantly higher in allergic asthma group. When deciphering between these two tests, they concluded specific IgE (3 panel analysis: dust mite/ragwort/timothy grass) had better diagnostic value than total IgE and of the allergens dust mite was the strongest predictor for asthma.

1.3.1.4. Novel tests

Airways Oscillometry (AO)

Over the last decade more literature is emerging in relation to airways oscillometry (AO) and asthma. AO was initially suggested to have a potential role in children or those who cannot easily perform spirometry.(145, 146) As a result many studies analysing oscillometry are in children. More recently it has been demonstrated to be a sensitive test at evaluating lung function in the distal airways compared to spirometry, therefore may benefit all patients under investigation for respiratory symptoms and not just children or those unable to perform spirometry. Oppenheimer et al(147) reviewed a group of patients following dust and fume exposure who had normal spirometry but ongoing unexplained respiratory symptoms, using AO they demonstrated evidence of small airways dysfunction that was not detected during assessment of the larger air passages. Spirometry performed in these patients was normal because it does not reflect distal airways. The authors demonstrated improvement in small airways function following bronchodilator medication, this highlights that when using tests of the upper airways alone, it is possible to miss distal pathology that is amenable to treatment. Investigating the small airways in patients with asthma may therefore pick up treatable pathology and improve asthma management. At present there are limited studies that directly look at AO in asthma diagnosis, leaving gaps in our knowledge that warrants further research.

Literature on airways oscillometry using Impulse Oscillometry Technique:

Recent work has shown that airway reactance is a sensitive measure of distal airways dysfunction (145) and may therefore have a potential role in the early diagnosis of asthma. Currently only one study reviews IOS performance as a diagnostic tool for asthma. However, ten additional studies were identified that review IOS performance in known asthma patients. Mondal et al(148) assess oscillometry performance as an alternative to spirometry and show that this test has good reproducibility and would be a potential alternative to

spirometry in BHR testing. This use of IOS as an alternative to spirometry in children is supported by several other studies.(149-151).

Manoharan et al(152) assess IOS pre and post treatment period with small particle inhalers in an adult population. The authors show a significant IOS response that is not demonstrated in spirometry (FEV1 and FEF 25-75%) and indirectly imply IOS is superior at assessing smaller distal airway pathophysiological responses. This is supported by Short et al(153) who show IOS is superior to spirometry in detecting bronchodilator reversibility and bronchoconstriction. This would potentially imply that oscillometry should have a place in the current diagnostic algorithm. However, it should be noted that the authors use FEV_1 and FEF_{25-75} as a marker of spirometry, opposed to FEV_1/FVC ratio which is recommended in the NG80. Further studies comparing IOS and FEV_1/FVC ratio in patients with suspected asthma would be useful to further assess which test is superior and in which context.

Other studies evaluating IOS in asthma patients demonstrate the ability of the test to distinguish physiological changes in both proximal and distal airways.(154) IOS has been shown to have the ability to differentiate between severe and mild-moderate asthma $(p<0.01)$(155)

Literature on airways oscillometry using Forced Oscillation Technique:

Whilst there is a reasonable amount of literature on FOT, very few studies specifically assess the ability of FOT to diagnose asthma. Only two key studies were identified that specifically set out to identify this question; one in children, one in a mixed population of adults and children.(62, 156)

Evens et al(156) review the ability of FOT to distinguish between an asthma (n=99) and a healthy control group (n =200) in children aged three to seven years old. They found that Fres performed the best $(P<0.0001)$ to discriminate healthy control from asthma. However, Fres, AX and X6 were all able to distinguish asthma from healthy control group. Resistance

at R6 and R4-R24 was unable to distinguish asthma from control group. FOT was not statistically able to differentiate asthma from other lung disease. They report children born pre-term had more abnormal FOT results compared to the asthma group. This study used children on inhaled corticosteroids; the ability of FOT to diagnose asthma in a steroid naïve population was not tested.

The main barriers to developing reference ranges for FOT is that most studies are using different parameters at different frequencies and with different FOT devices. The largest reference dataset produced by Calogero et al in 2013 (157), use R and X at 8Hz, however more recent studies are using 5Hz.

Starczewska-Dymek et al(2018)(158) review the ability of FOT to predict asthma using R8 and X8 measurements in children aged 2 to 6 years (n= 53 controlled asthma, n= 53 uncontrolled asthma, n= 45 controls). They demonstrate both resistance and reactance differentiated between the three groups.

In addition to using mean differences in resistance and reactance values between groups, BDR cut-offs have also been reported in the paediatric population using cut-offs based upon the lower 5th or upper 95th percentile of a healthy population reference group aged between two to thirteen years old.(157) (Table 4) More research is needed in an adult population. Starczewska-Dymek et al(2019)(159) look at the role of FOT with BDR testing to detect bronchoconstriction in a paediatric population aged 2-6years and demonstrated 71.6% asthma patients had a positive test opposed to 7.7% healthy controls using the Calogero reference ranges. The study suggests that FOT may have a place in asthma diagnosis in children.

Table 4.   AO BDR cut off for normal (adapted from Calogero et al(157))

Relative change (%baseline) Cut off for normal (adapted from Calogero et al(157))					
	AX	Fres	Rrs6	Xrs6	Xrs6
Relative change %baseline	-81	-47	-34	50	74

Heijkenskjold rentzhog et al (2017)(62) review oscillometry as a diagnostic tool for asthma and assess the association of oscillometry and spirometry. They reviewed 234 asthma patients and 60 healthy control subjects (age range, 13-39 years). All patients were on asthma treatment. They report higher resistance in both proximal and distal airways in the asthma group. They suggest high Fres was associated with asthma. Worse X5, Fres and R5-R19 were correlated with poorly controlled asthma (P <0.05) Significant differences were demonstrated between groups even in treated patients, this may further support the potential role of oscillometry in a steroid naïve population that have not received prior treatment with disease modifying drugs.

Summary of airways oscillometry in asthma

Whilst AO has been shown to be sensitive in detection of small airways dysfunction, detection of bronchodilator reversibility in known asthma patients, and its ability to differentiate severe asthma from mild moderate asthma. There is a gap in the literature concentrating on the use of AO in an adult population or use of AO to diagnose asthma from a group of steroid naïve symptomatic patients (i.e., the group the test would be used on in clinical practice). AO has been compared with spirometry-based measurements in a small number of studies but further evidence is sought in order to confirm or refute this finding.

1.4. Thesis objectives

Questions still to be answered

The literature review highlights the paucity of quality data in asthma diagnosis. Whilst there is a consensus that objective tests are needed to diagnose asthma, there is a gap in our knowledge regarding, which tests and in what sequence, are required to accurately diagnose asthma. The novel tests AO is receiving increasing attention, however more research looking at the potential role within asthma diagnosis in adults is needed. In addition, following the recent SARS-CoV-2 outbreak, an additional knowledge gap in asthma diagnosis when AGPs are not available has arisen.

The RADicA study has a series of primary and secondary objectives (see appendix B, RADicA protocol). However, this thesis will cover the following three objectives separated into three studies (chapters 3, 4, and 5).

1.4.1. Study 1: Asthma diagnosis using standard tests

A study to determine the best investigations and diagnostic algorithm to predict EPOER asthma (see section 2.6.1) using standard tests. The following will be addressed.

- Assessment of the ability of the NICE (NG80) diagnostic algorithm to predict EPOER confirmed asthma.
- Investigation of which measurements; using standard tests, best predict EPOER confirmed asthma individually and in combination (i.e., an algorithm).

1.4.2. Study 2: Asthma diagnosis using Airways Oscillometry

A study to determine the predictive power of 'Airways Oscillometry' in asthma diagnosis. The following will be addressed.

- Assessment of the repeatability of AO in healthy volunteers and effect of confounding variables on baseline measurements.

-   Investigation to determine the ability of AO to predict EPOER 'asthma' from EPOER 'not asthma' in symptomatic patients (exploratory analysis in adults 17+).
-   Investigation to determine if AO measurements correlate with current tests used in asthma diagnosis.

1.4.3. Study 3: Asthma diagnosis during a pandemic

A study to determine the best investigations and diagnostic algorithm to predict EPOER asthma when Aerosol Generating Procedures (AGPs) are not available. The following will be addressed.

-   Investigation to determine the best diagnostic test or algorithm to predict EPOER asthma when AGPs are not available (exploratory analysis in adults, 16+ years).
-   Assessment of how the proposed non AGP algorithm compares to current standard UK (NICE) and international (GINA) guidelines.

2.   Methods

This chapter describes the Rapid Access Diagnostics for Asthma (RADicA) study using the following format: study design, study participants, study procedure, procedures for measurements and procedures for recruitment, study outcome definitions, and statistical analysis. Not all of the measurements collected in the RADicA Study will be analysed in this thesis. Details regarding measurements that were collected in the RADicA study but not analysed as part of this thesis will be described separately (see appendix C).

2.1. Study design

The Rapid Access Diagnostics in Asthma Study (RADicA) is a prospective observational cohort study that was designed to investigate how best to diagnose asthma. A symptomatic population with clinician-suspected asthma was recruited. Symptomatic patients were selected to ensure that the study participants were a representative sample of the population in whom asthma diagnosis would be sought. In addition, matched asymptomatic healthy controls were also recruited to assess normal reference ranges and repeatability of novel breathing tests. The research clinic was situated at Manchester University NHS Foundation Trust (Wythenshawe site), U.K. Our protocol was submitted to Greater Manchester East Research Ethics Committee and received favourable opinion on 8th January 2019. Recruitment opened for healthy volunteers on 14th February 2019, and for patients on 10th May 2019. Recruitment was paused on 23rd March 2020 due to the SARS-CoV-2 pandemic. Planned recruitment was 150 patients and 150 matched healthy controls, however this was not achieved due to the international pandemic. This thesis reviews the 115 patients and 66 healthy controls that were recruited prior to the recruitment pause.

2.2. Symptomatic patient group:

2.2.1. Study participants

Referral was through local primary care practitioners or secondary care outpatient units. See detailed list of recruiting general practices (appendix D). Clinicians within the greater Manchester area were eligible to refer into the study; methodology on the recruitment procedure is described later in the methods chapter (section 2.5). All symptomatic patients referred with clinician suspected asthma were screened by a member of the research team using inclusion and exclusion criteria outlined in table 5. A detailed patient information sheet (PIS) was sent to potential participant; children under 16 years were presented with age appropriate PIS and were required to attend with their next of kin/guardian. A minimum of twenty-four hours was given for the participant to decide whether to take part before formal written consent was completed. In the event the referring clinician felt there was a clinical need for us to see the patient more urgently this option was approved by the ethics committee.

Table 5.   'Symptomatic patient' inclusion and exclusion criteria (RADicA study)

Inclusion Criteria:	- Males and females ≥5years and <70 years - Clinical suspicion of asthma from GP or referring health care professional - One or more symptom in keeping with asthma (i.e., cough, wheeze, chest tightness and/or breathlessness) - Capable of giving informed consent or where under 16 years attends with parent or legal guardian.
Exclusion Criteria:	- Current ICS (used within previous 2 weeks) or oral steroid treatment (within the previous 4 weeks) - Former or current smokers **if** >10 pack year smoking history - Other relevant comorbidities (e.g., other lung disease; CF, COPD, ILD or bronchiectasis) - Recent antibiotic treatment within previous 2 weeks (these participants may be able to enter the study at a later date) - Pregnant

2.2.2. Study procedure

Eligible participants underwent four core visits and if suitable were offered one optional visit. The first two visits (+/- optional visit) were called 'baseline visits' and were completed before a trial of inhaled corticosteroids were offered. The last two visits were called 'steroid-response visits' and were performed after the patient was commenced on inhaled corticosteroid treatment. A simplified patient flow diagram with timeline is presented below (Figure 14). All data was recorded in a structured workbook and then uploaded onto an electronic database.

Figure 14. Flow diagram and timeline for 'symptomatic patient group' (RADicA study)
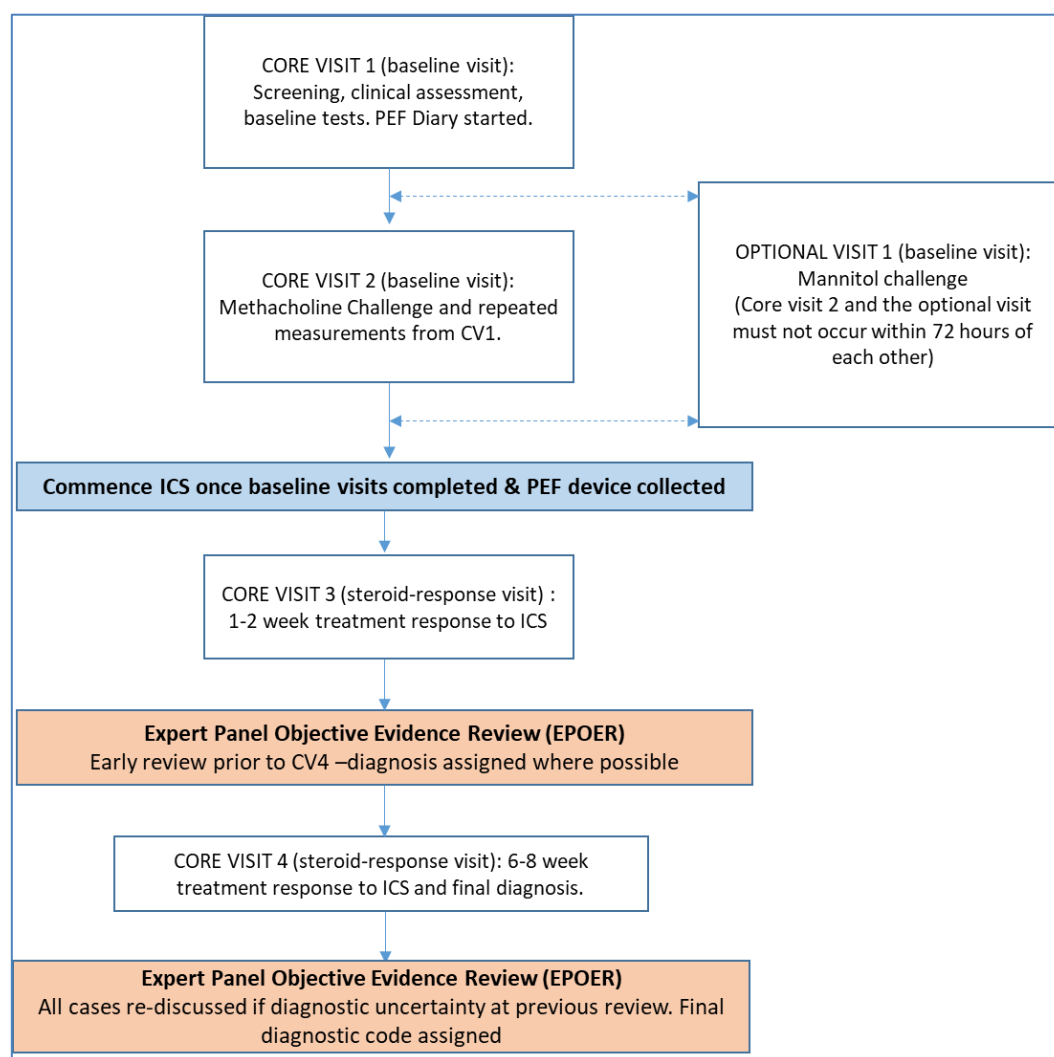
Visits	CV 1	(CV2/OV1)	Commence treatment period	CV 3	CV 4
Shortest study pathway	D 0	(Range D1- D14)	D 14	D 21	D 56
Longest study pathway	D 0	(Range D1 – D41)	D 42	D 56	D 98

2.2.2.1.   Core visit 1 (baseline assessment visit)

At core visit one (CV1) the study doctor took written informed consent. All participants then underwent detailed clinical history and examination, and completed a questionnaire regarding symptoms, past medical history, and medications. Participants also completed the Asthma control questionnaire (ACQ). Following the clinical consultation, the clinician

recorded their clinical impression using one of the four options: 'high possibility,' 'Intermediate possibility,' 'Low possibility,' or 'Alternate diagnosis more likely.' Participants coded 'alternate diagnosis more likely' were discussed with the supervising consultant, withdrawn from the study and either referred back to their GP or directly into a clinical service if appropriate. All other participants continued in the study and completed a series of objective tests outlined below (table 6). Detailed description of how each test was carried out is described in the next section of the methods chapter (section 2.3). At the end of CV1 participants were provided an electronic Peak expiratory Flow (PEF) meter and instructed to complete two weeks of twice daily monitoring. Patients also received inhaler technique training using an 'Incheck device' and were then provided with an inhaler for symptom relief (Ventolin Accuhaler 200mcg prn, maximum recommended dose of 800mcg per twenty-four hours). An alternative was offered to young children if clinically appropriate after assessing technique (Salbutamol MDI 100mcg two puff prn).

At the end of CV1, the second baseline visit (core visit 2, CV2) was scheduled for approximately two weeks later. Participants were also informed about the optional visit (OV1) where they could attend for an additional test called a 'Mannitol Challenge Test' (BCTmann). Interested participants were provided with a PIS. The optional visit was scheduled if the participant wanted to attend, unless any specific contra-indication were identified (see section 2.2.2.3).

If during CV1 the study clinician had significant concerns about delaying definitive asthma treatment with inhaled corticosteroids for example if a participant appeared very symptomatic or unwell, the participant was discussed with the supervising consultant and a decision could be made to expedite treatment, i.e., start ICS immediately and omit CV2.

Table 6. Summary of tests performed by 'symptomatic patient group' at each visit

	Baseline Diagnostic Visits			Treatment Response Visits	
	Core visit 1 (CV1)	Core visit 2 (CV2)	Optional visit 1 (OV1)	Core visit 3 (CV3)	Core visit 4 (CV4)
Physical exam	☑				☑
Asthma control questionnaire (ACQ-5)	☑	☑	☑	☑	☑
Fractional Expired Nitric oxide (FeNO)	☑	☑		☑	☑
Airways Oscillometry (AO)	☑	☑	☑	☑	☑
Volatile Organic Compounds (VOC)	☑	☑		☑	☑
Multiple Breath Washout (MBW)	☑	☑		☑	☑
Particles in Expired Air (PExA)	☑	☑		☑	☑
Spirometry	☑	☑	☑	☑	☑
Bronchodilator Reversibility (BDR)	☑				
Bronchial Hyper-responsiveness to Methacholine (BCTMeth)		☑			☑
Bronchial Hyper-responsiveness to Mannitol (BCTMann)			☑		
Airways Oscillometry (post BDR or BHRc)	☑	☑	☑		☑
Skin Prick Testing (SPT)	☑*				
Blood Eosinophils (Eos)	☑*			☑**	☑
*can be completed at CV2as alternative if needed. **only offered in children >12years					
Note: a detailed description of all tests can be found in the "procedures of measurements" section					

2.2.2.2. Core Visit 2 (Methacholine challenge (BCTmeth) and repeated measurements visit)

At CV2 the participant repeated some of the objective tests from CV1 (see table 6) and in addition they completed BCTmeth. The PEF meter was collected, if compliance was noted to be poor and there was no clinical urgency to start treatment, patients were encouraged to complete further measurements with a target of 14 days data collection.

2.2.2.3. Optional visit 1: Mannitol challenge test

All eligible participants were invited to attend the optional visit 1 (OV1) to complete a BCTmann challenge test. Eligibility was decided on by the absence of any clinical contra-

indications (see section 2.3.6) to perform a Mannitol challenge test, ability of patient to perform reliable spirometry, and absence of clinical need to commence treatment more urgently. The visit was not scheduled within 72hours of the any other bronchial challenge test to ensure no bias in the test result was encountered.

2.2.2.4. Initiation of inhaled corticosteroids

Inhaled corticosteroids (ICS) were commenced after all applicable baseline visits were completed and following collection of the PEF monitor. Inhaler teaching and technique was checked in clinic. Treatment included Flixotide Accuhaler 250mcg twice daily (Flixotide Accuhaler 100mcg twice daily in children <16years old). A digital 'inhaler compliance assessment device' (INCA) was attached to each steroid inhaler to monitor patient technique and compliance. Patients were supplied with six to eight weeks of treatment. A second option of Flixotide Evohaler 125mcg two puff twice daily (Flixotide Evohaler 50mcg two puff twice daily in children <16 years old) and salbutamol MDI two puff as required via volumatic spacer device were available in the event of a participant not tolerating or being able to operate the dry powder inhaler. It was not possible to attach an INCA device to these inhalers, so no objective measure of adherence for these patients was possible.

2.2.2.5. Core visit 3 (early steroid response visit)

All patients were invited back after 1-2 weeks of ICS use to complete a series of tests (see table 6) and ACQ.

2.2.2.6. Expert Panel Objective Evidence Review (EPOER) discussion

Before the final visit all patients were discussed individually at a panel consisting of members from the clinical team. The panel included: two respiratory consultants, one paediatric respiratory consultant, and clinical doctors from the RADicA team. If all members were not available, a minimum of two were required (at least one to be of consultant level). In the event of any uncertainty the case was further discussed at a full expert panel

meeting. In all events the panel had access to all clinical notes and results of all subjective and objective information. However, results of the novel tests were not taken into consideration because not enough information was yet known about these tests to guide the diagnosis. A clinical impression was recorded at this interim panel; however the final diagnosis was not decided until after the final visit. The following clinical impression was recorded where possible prior to CV4 using the following definitions.

- **Asthma**: panel feel "definite asthma" based upon review of clinical consultation and all available objective evidence pre and post treatment.
- **Not Asthma**: panel feel "definitely not asthma" based upon review of clinical consultation and all available evidence pre and post treatment.
- **Possible Asthma**: panel can't confirm definite 'asthma' or 'not asthma' despite all baseline tests completed (with the exception of BCT in children) and/or following review of objective testing following trial of inhaled corticosteroids.
- **Insufficient Evidence**: panel can't confirm definite 'asthma' or 'not asthma,' however not all objective baseline tests completed and/or missing objective tests post trial of treatment. The panel feel that if the missing data from pre and post treatment were available this would enable a definitive diagnosis of 'asthma' or 'not asthma' to be confirmed.

2.2.2.7.   Core visit 4 (final steroid response visit and diagnosis)

All patients were invited back at six to eight weeks from commencing inhaled corticosteroids (up to a maximum of twelve weeks). Patients completed objective tests post treatment period (see table 6) and clinical examination. The consultant panel clinical impression was considered along with the final consultation and core visit four results. An EPOER diagnosis was recorded for all patients: 'asthma,' 'not asthma,' 'possible asthma,' or 'insufficient information'. In the event of any clinical uncertainty the case was discussed with the on-call senior consultant. If a patient was withdrawn before CV4, that patient was still reviewed in the final 'expert panel' and assigned EPOER diagnosis based upon all available information prior to withdrawal.

2.3. Procedures of measurements

Tests featuring in NICE Algorithm

2.3.1. Spirometry

Spirometry was measured with the JAEGER^{TM} Vyntus^{TM} PNEUMO (vyaire medical, USA), in accordance with the American Thoracic Society / European Respiratory Society (ATS/ERS) guidelines.[160] Equipment was calibrated using three-flow volume calibration daily prior to the commencing procedures, successful calibration was achieved when measured volumes are in +/- 10% limits. Patient demographics were imputed into the software; gender, ethnicity, age, height, and weight, to calculate Global Lung Index (GLI) spirometry reference equations.[161]. The participant was instructed to tidal breath, then inhale deeply followed by maximal exhalation as quick and fast as possible from total lung capacity (TLC) to residual volume (RV). A minimum of three technically acceptable measurements (i.e., free of artefact, slow starts, and coughing) were required. Up to nine attempts were permitted if required. The best two measurements were required to be within 5% of each other for the test to be considered technically acceptable (see example, figure 15). In children a visual animation using the Vyntus^{TM} PNEUMO software was used if required to encourage optimal technique.

Figure 15. Example of technically acceptable flow volume Loop

Spirometry Flow-Volume

	Pred	Best	%(B/P)	1	2	3	-3 -2 Z-Score 2 3
VC MAX		4.59					
FVC	3.88	4.59	118	4.40	4.59	4.59	●
FEV1	3.30	4.07	123	3.99	4.07	4.06	●
FEV1%M		88.56		86.86	88.56	88.37	
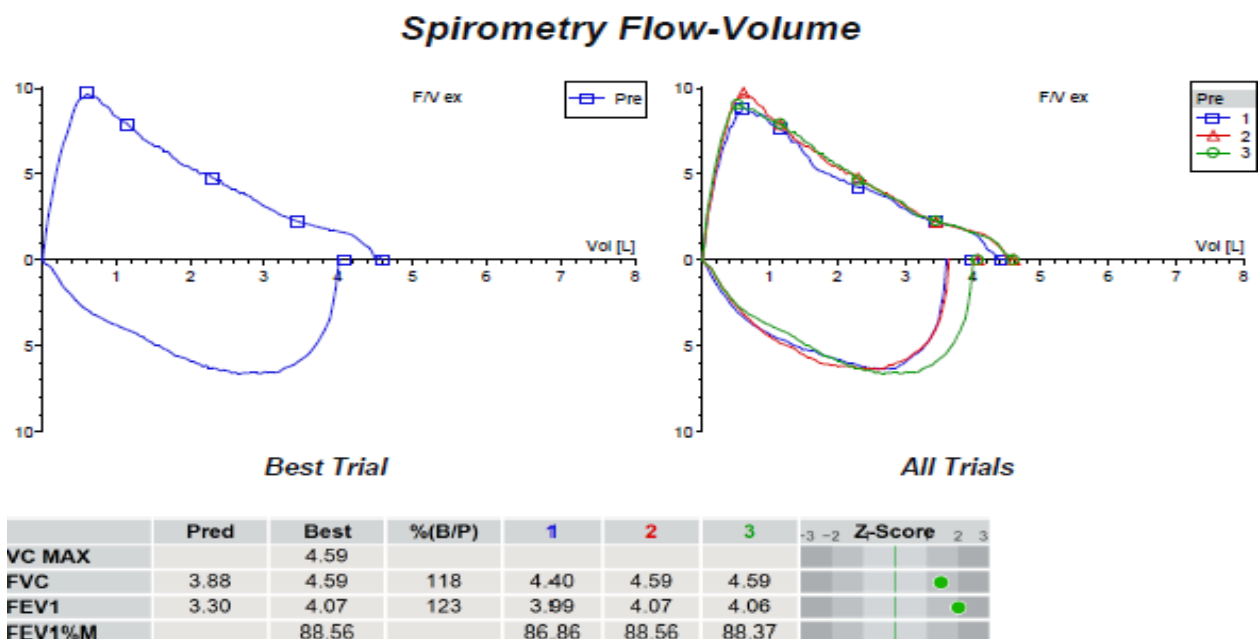
Spirometry outcome measures:

Forced vital capacity (FVC), expiratory flow rates (FEV$_1$, MMEF and PEF), and FEV1/FVC ratio were recorded for each attempt. Attempt with the best FVC was recorded. All results were recorded in litres and also expressed as percentage predicted using Global Lung Function Initiative (GLI) prediction equations.(161) Predicted FEV$_1$/FVC ratio and Lower limit of Normal (LLN) was also recorded. The GLI equations take into account: age, height, gender, and ethnicity. In addition, data was expressed as dichotomous variables with a positive test denoted as 1) FEV$_1$/FVC ratio of less than 70% or 2) FEV$_1$/FVC ratio less than LLN.

2.3.2. Bronchodilator reversibility (BDR)

Following the first spirometry measurement participants were instructed to take one maximal inhalation of salbutamol (100mcg) via a spacer device and hold their breath for ten seconds. This was repeated three times (i.e., total salbutamol dose 400mcg). Fifteen minutes following the final inhalation of Salbutamol, spirometry was repeated (method as above, section 2.3.1). Reversibility was calculated as percentage change in FEV$_1$ fifteen minutes following administration of 400mcg of Salbutamol via spacer. Bronchodilator

reversibility testing was performed on all patients even if baseline spirometry in the normal range. In addition, Airways Oscillometry (AO) (see section, 2.3.10) was performed pre and post salbutamol.

Bronchodilator reversibility outcome measures:

Absolute (in litres) and percentage change in FEV_1 was calculated and a positive challenge was recorded if FEV_1 and/or FVC increased by ≥12% and 200mls in adults, or if FEV_1 and/or FVC increased by ≥12% in children.

2.3.3. Fractional Expired Nitric Oxide (FeNO)

FeNO measurement was recorded using the NIOX VERO (Circassia) in accordance with the manufacturer's instructions. Sensors are calibrated directly against chemiluminescence methods during manufacturing and therefore did not require re-calibration by the user. The operational lifetime of the instrument was considered maximum of five years in use or 15,000 measurements or the expiration date, whichever comes first. The sensor has an operational lifetime of twelve months after installation or the expiration date (whichever comes first) and the breathing handle could be used for 1000 measurements or one year (whichever comes first).(162)

The measurement was performed prior to tests with forced expiratory maneuverers. Participants were asked to refrain from smoking for one hour and caffeine for eight hours prior to testing. If a participant had consumed caffeine within the preceding eight hours, this was recorded. In the unlikely event a participant had smoked within the preceding hour, the test would be delayed. The test was performed with the patient in a seated position. The participant was then asked to perform an exhalation to residual volume (RV), followed by an inhalation through the device filter to total lung capacity (TLC). Participants were then instructed to make a controlled exhalation for ten seconds at a standardised flow rate (50 ml/s +/- 10%), guided by a visual animation to ensure that the flow rate was optimal (Figure 16).

Figure 16. Performing FeNO measurement

Fractional expired Nitric Oxide outcome measures:

FeNO level was recorded measured in parts per billion (ppb) and documented in the workbook. A level of 40ppb or greater was interpreted as high in adults (35ppb or greater was interpreted as high in children).(24) In addition predicted FeNO, and %predicted FeNO was recorded for each participant using reference values according to Toren et al.(119)

2.3.4. Peak Expiratory Flow Variability (PEFv)

PEFv was measured using the e-Mini Wright digital flow meter (Clement Clarke Ltd. Harlow, UK) in accordance with manufacturer's instructions. Participants were instructed to inhale fully, then to seal their mouth around the mouthpiece and perform an exhalation as hard and fast as possible from TLC. This was repeated twice more, and the highest recording was selected by the e-mini wright software. The device has on-screen zoning system that shows a visual indicator if PEF is within red (<50 predicted), yellow (50-79% predicted), or green (≥80 predicted) zones. Participants were asked to complete this twice per day (morning and evening), for a minimum of fourteen days where possible. If salbutamol was required due to patient symptoms around the time of the measurement (within eight hours), where possible participants were instructed to perform PEF measurement prior to taking their inhaler or document if this was not possible.

Diurnal peak flow variability (DPV) was recorded using the 'daily amplitude percentage mean' (97, 99):

Daily Amplitude Percentage Mean = [(PEFhighest – PEFlowest) / PEFmean] x 100

From this calculation the PEFv was calculated:

PEFv = (Σ Daily amplitude percentage mean) / Number of days

The number of days PEF was measured morning and evening was recorded. The Daily Amplitude Percentage Mean for each day where two measurements were successfully recorded was calculated. Where fourteen days was not achieved PEFv was still recorded if a minimum of three days was achieved.(98) If less than three days were achieved the test was invalid and recorded as missing data.

Peak Expiratory Flow variability outcome measures:

PEF was defined as a positive test if either of the two classifications were true:

1. PEFv: PEFv > 20% (using above equation).
2. PEFv(alternative): 'daily amplitude percentage mean' > 20% on three or more days. The exact number of days meeting this criterion was also recorded.

2.3.5. Methacholine challenge test (BCTmeth)

Bronchial hyper-responsiveness was measured through a methacholine challenge test (BCTmeth) using the Vyntus APS Nebulizer system with integrated dosimeter (Vyaire medical, USA). The test was not performed if defined contra-indications were present (table 7) or if salbutamol medication was taken within preceding eight hours.

Table 7.   Absolute and relative contra-indications to Methacholine challenge test

Absolute	Relative
FEV1 <50% predicted or <1.0L)	Spirometry induced airways obstruction
Recent myocardial infarction or cerebrovascular accident (<3months)	FEV1 < 70% predicted or <1.5L
Uncontrolled hypertension (SBP >200, DBP >100)	
Aortic aneurysm	

Baseline spirometry was measured according to ATS/ERS guidelines(160), FEV_1 was repeated ninety seconds after each inhalation of methacholine (Stockport Pharmaceuticals, UK). A quadrupling dose protocol was administered (cumulative dose; 0.015mg, 0.060mg, 0.240mg, 0.960mg) using single concentration of methacholine (table 8).

Table 8.   Methacholine challenge protocol

Dose number	Methacholine dose (mg)	Cumulative Dose (mg)
1	0.015	0.015
2	0.045	0.060
3	0.180	0.240
4	0.720	0.960

The challenge was completed when either a 20% or greater reduction in FEV_1 occurred (see equation 1 below), or the maximum methacholine dose had been administered. Dose of inhaled Methacholine to provoke 20% decrease in FEV_1 (PD_{20}) was reported automatically by the Vyntus APS Nebulizer system software (see equation 2 below). All participants received salbutamol 400mcg via a spacer device at the end of the test, if bronchoconstriction failed to return to within 10% of baseline further doses were administered. In addition, AO was performed pre and post Methacholine challenge (see section 2.3.10, Airways Oscillometry). The test was not performed within seventy-two hours of BDR or BCTmann test.

Equation 1: Percentage fall in FEV_1

% fall = <u>Lowest FEV_1 post baseline – lowest FEV_1 post challenge</u> x 100
       Lowest FEV_1 post-baseline

96

Equation 2: PD_{20} for dosimeter method

PD_{20}= antilog [log D_1+$\frac{(\text{log } D_2 - \text{log } D_1)\ (20 - R_1)}{(R_2 - R_1)}$]

D_1 = second to last methacholine dose (preceding D_2)
D_2 = final cumulative dose of methacholine (resulting in a 20% or greater fall in FEV_1)
R_1 = % fall in FEV_1 after D_1
R_2 = % fall in FEV_1 after D_2

(PD_{20} was calculated automatically by the Vyntus APS Nebulizer system software)

Methacholine Challenge Test Outcome Measures:

PD_{20} and cumulative dose was recorded. Additionally, the percentage fall in FEV_1 was also recorded. A positive test was recorded if the percentage fall in FEV_1 was 20% or greater with a PD_{20} of ≤0.2mg (*equivalent of PC20 ≤8mg/ml*).(163) Where a fall in FEV1 did not occur after the highest dose had been given, a censored PD_{20} of twice the highest cumulative dose was recorded (PD_{20} 1.92mg) for the purpose of analysis. We also calculated a dose response ratio using the following equation:

DRR = %decline in FEV1 post challenge / total cumulative methacholine dose

Other tests featuring in UK and international asthma guidelines
2.3.6. Mannitol challenge test (BCTmann) (optional test)

Baseline spirometry was measured according to ATS/ERS guidelines(160). The test was not performed if defined contra-indications were present (Table 9) or if salbutamol medication was taken in the preceding eight hours.

Table 9.   Absolute and relative contra-indications to Mannitol challenge test

Absolute	Relative
FEV1 <50% predicted or <1.0L)	Spirometry induced airways obstruction
Recent myocardial infarction or cerebrovascular accident (<3months)	FEV1 < 70% predicted or <1.5L
Uncontrolled hypertension (SBP >200, DBP >100)	
Aortic aneurysm	

FEV_1 was repeated sixty seconds after completion of each dose of mannitol. The protocol consisted of increasing doses of Mannitol, delivered through a dry power delivery device (Osmohale, pharmaxis Pharmaceuticals Limited) using the following regime: 0, 5, 10, 20, 40, 80, 160, 160, 160mg (164). The participant was instructed to tilt their head to a forty-five-degree angle whilst wearing a nose clip, then seal their lips around the mouthpiece and take a controlled deep inhalation. The participant must then hold their breath for five seconds before exhalation. The challenge was stopped when a reduction in FEV_1 of 15% or greater was recorded or the maximum cumulative dose of 635mg was delivered. In addition, baseline AO was performed prior to the Mannitol Challenge test, and repeated immediately after the challenge (see subsection below: Airways oscillometry). All participants received salbutamol 400mcg via a spacer device at the end of the test irrespective of FEV_1 change as a precaution. Where applicable, if bronchoconstriction failed to return to baseline (within 10% of baseline) a further dose was administered, and spirometry repeated until a satisfactory response was recorded.

Mannitol challenge test outcome measures:

Provoking dose (PD_{15}) (equation 1)(165), and percentage fall in FEV_1 (equation 2) was recorded through APS software. A positive test was recorded if the percentage fall in FEV_1 was 15% or greater.

Equation 1: $PD_{15}= \log_2(d_{j-1})+ \dfrac{(\log_2(d_j) - \log_2(d_{j-1}))*(15 - (\%FEV_1 \textit{ fall at } d_{j-1}))}{(\%FEV1 \text{ fall at } dj) - (\%FEV_1 \text{ fall at } d_{j-1})}$

(where dj is the last cumulative dose, and d$_{j-1}$ is the second last cumulative dose)

Equation 2: Percentage Fall = [FEV$_1$Baseline – FVE$_1$Post challenge*] % [FEV$_1$Post challenge*]

*(*FEV$_1$Post challenge = FEV$_1$ following each concentration of Mannitol)*

2.3.7. Skin Prick Testing

Skin prick testing was used to identify IgE-mediated sensitisation to common aeroallergens. Where applicable, antihistamine medicine was held for seventy-two hours prior to the test. The test was performed on the volar aspect of the forearm, at least five centimetres above the wrist and five centimetres below the elbow. The site for allergen introduction was marked with a pen, each marked site was at least two centimetres apart. The panel consisted of a positive and negative control and eight allergens:

- Allergopharma®, Germany: Histamine (positive control), saline solution (negative control), Birch, Grass mix, Dermatophagoides pteronyssinus
- Lofarma®, Italy: Aspergillus Fumigatus, Alternaria Alternata, Cladosporium, Gatto epitelio, Epitelio di cane

A single droplet was dropped onto the skin at each site, the dropper did not have contact with the skin. The skin was then pierced with a disposable lancet through the droplet at a 90-degree angle. Once all the droplets had been pierced, they were blotted with a clean tissue. The patient was instructed not to scratch the area. The reactions to the allergens were read after fifteen minutes. The size of the wheal was measured with a transparent ruler. Only the wheal was measured, the surrounding erythema (redness) was not measured. If the wheal was misshapen, the longest diameter and the midpoint orthogonal diameter were measured and recorded. If the negative control showed a wheal, dermatographism was considered likely and the test was considered invalid.

Skin prick test outcome measures:

A wheal diameter of three millimetres or greater (using the longest dimension) than the negative control was recorded as a positive reaction. All patients with one or more positive skin prick test were recorded as sensitised.

2.3.8. Blood eosinophil levels (Eos)

Venepuncture was performed using a butterfly needle with a vacutainer (or alternatively a blue/green needle attached to a 10ml syringe). Topical anaesthetic using 1g of 4% lidocaine

cream (LMX4®, Ferndale Pharmaceuticals Ltd, UK) was offered to all paediatric patients. Approximately 10mls of blood was collected for haematology and serum save (biochemistry tube). Samples were labelled and transported to the lab within sixty minutes. The purple top tube was processed immediately (Sysmex XN Haematology Analysers, Sysmex Ltd, UK) for blood eosinophil levels and full blood count. The gold top was frozen and stored for future analysis.

Blood eosinophil level outcome measures:

Blood eosinophil count was recorded and a level greater than $0.4x10^9$/L was recorded as positive.

2.3.9. Asthma Control Questionnaire (ACQ-5)

The validated asthma control questionnaire 'ACQ-5' was administered in order to record symptom perception.(166, 167) The ACQ-5 consists of the first five symptom based questions, each question has six possible answers that demonstrate the participants symptom perception relating to the preceding seven days. A total out of thirty was recorded, and the mean score by dividing the total by the number of questions answered (i.e., five). A score between zero and six was recorded.

Asthma control questionnaire outcome measures:

A score between zero and six was recorded with zero reflecting no symptoms and six maximal symptoms. A reduction in the score of greater than 0.5 points was considered a significant improvement (minimal important difference).(167)

Novel tests of small airway function:

2.3.10. Airways oscillometry (AO):

Airways Oscillometry was performed using the commercially available device, TremoFlo C-100 (Airwave Oscillometry System AOS). The device was calibrated daily with a standard adult calibration test load (a paediatric calibration test load was used for children 13 years or younger). TremoFlo C-100 uses Forced Oscillation technique (FOT). Multi-frequency

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sinusoidal waveforms comprising prime frequency signals (non-harmonic composite airwaves: 5, 11, 13,17, 23, 29, 31, & 37 Hz) form a composite air waveform, this is then superimposed onto normal tidal breathing.

The patient was instructed to be seated with legs uncrossed to reduce extra-thoracic pressure, a nose clip was applied, and the head positioned in a slightly extended position to reduce upper airway compression. The participant was then asked to support their cheeks using the palms of their hands, this was to reduce extra-thoracic impedance. In young children, a second member of staff or the parent provided the support across the cheeks. The participant was then instructed to maintain a good seal around the mouthpiece while the operator performed the test. The patient was instructed to carry out normal tidal breathing during the AO procedure. Each test recorded 20 seconds of tidal breathing. A minimum of three tests were completed (figure 17).

Figure 17. i) Patient performing AO ii) Oscillogram (pre- and post- bronchodilation)
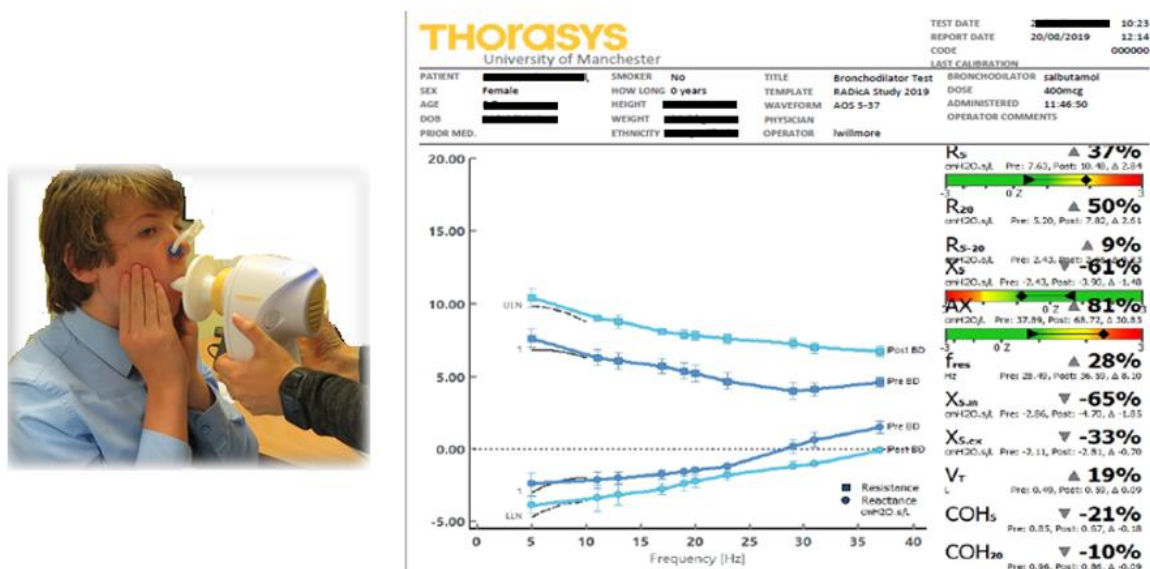
Coherence was recorded to reflect reproducibility of the tests and provide a quality control. Target coherence at 5 Hz of >0.8 was used, if this was not achieved a further two measurements were attempted. The overall results reported were the average of all of the measurements performed. AO was performed as a standalone test. AO was also performed

fifteen minutes following bronchial reversibility with 400mcg of salbutamol, and immediately following completion of bronchial challenge testing with Methacholine and Mannitol using the challenge test end points described in the relevant subsections above.

Airway oscillometry outcome measures:

The following parameters were routinely reported: R5, R20, R5-R20, X5, (X5in, X5ex), Fres, AX, and tidal volume. Where Fres does not cross 'y = 0' the value 40Hz was allocated. Where available, percentage predicted of each measurement was also recorded using published population reference ranges that are utilised within the Tremflo C-100 software (168-171). In addition, percentage change of each parameter post bronchodilator was documented.

2.4. Summary of outcome measures

A summary of tests and their outcome measures in the RADicA study (table 10). Tests performed but not used within this thesis are reported in appendix C.

Table 10. RADICA outcome measurements

	Test	Outcome measures	Established threshold for positive results
Symptoms	Asthma Control Questionnaire (ACQ)	ACQ-5	Change of 0.5(172)
Tests included in NICE algorithm(38)	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 (PD_{20} ≤0.2)
Tests of small airway function	IOS	R5, R20, R5-20, X5, X5in, X5ex, AX, Fres	To be established
Other	Blood - eosinophils	Blood eosinophil count	> 0.4 × 10^9/L(173)
	Skin prick tests	To inhalant allergens	Atopic if 1 or more positive

Outcome measures of tests performed in RADicA but not analysed in this thesis are summarised in Appendix C

2.5. Procedures for recruitment

General practices from within the NHS Manchester Clinical Commissioning group were contacted through email to inform them about the RADicA research clinic including indications for referral and exclusion criteria. Ninety-three practices were emailed. The email outlined the referral process and included a link to the RADicA Webpage (https://www.radica.org.uk/), a copy of the fax referral form, and advertisement leaflets for distribution to the practitioners. In addition, our local Accident and Emergency department and Paediatric department were informed about the study. The patient referral procedure included two possible referral pathways: fax referral or online referral via the RADicA website. The fax referral form was also available via the RADicA website or through an EMIS (Egton Medical Information systems) 'pop-up'. The latter required authorisation from the individual general practices. Thirteen practices gave authorisation for the RADicA 'pop-up' to be installed on their electronic patient software system (Egton Medical Information systems – EMIS). This is described in more detail below.
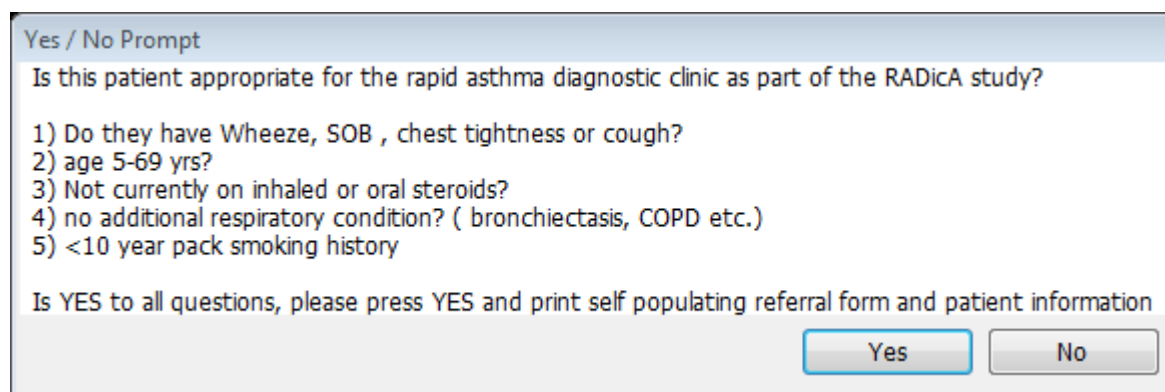
2.5.1. Installation of window 'pop-up' boxes into EMIS software in General Practices

We trailed a novel recruitment process using the EMIS electronic patient software system that is available at all General practices within the NHS Manchester Clinical Commissioning group. The 'pop up' was designed to raise awareness of the study and also assist general practitioners select appropriate patients that were eligible for the RADicA study.

The 'pop-up' was programmed to appear on EMIS if a specific trigger phrase was selected by the clinician i.e., 'suspected asthma.' However, the 'pop-up' was only programmed to appear if no contra-indications existed on the patient file (see Installation Instructions below). This helped to prevent the 'pop-up' appearing inappropriately. The 'pop-up' prompted the clinician to consider making a referral into the RADicA study (figure 18: EMIS Pop-up). If the clinician selected 'YES', a word document entitled 'RADicA_PATIENT INFORMATION LEAFLET _V1' loaded up with the attached fax referral form. In most

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practices the referral form self-populated with the patient's details. If the clinician selected 'No', the template disappeared allowing the clinician to continue with their normal consultation.

Figure 18. EMIS pop-up

Installation Instructions for RADicA 'pop-up' on EMIS Software:

1. Open EMIS software on the computer desktop.

2. Select '**EMIS configuration'** from the drop-down menu in the top left corner.

3. Select '**Resource Publisher**.'

4. Select '**Concepts**' – Click on '**create folder**,' name the folder 'RADicA.'

5. Select to insert new concepts by clicking **'Insert Concept'** – a menu will appear enabling you to create patient concepts (i.e., patient filters). When each concept is created save it into the RADicA folder.

   Patient concepts for RADicA:

   - Concept 1: Select 'Age,' Select 'Rule based concept,' Select 'Filter,' Type in "5 – 69"

   - Concept 2: Select 'Lung Condition,' Filter out all lung conditions except asthma by 'ticking' next to condition (e.g., Cystic Fibrosis, Pulmonary Fibrosis, COPD, Lung cancer). DO NOT tick 'Asthma.'

   - Concept 3a: Select 'Inhaled medication,' Filter out contra-indicated inhaled medication by 'ticking' next to medication (e.g., Preparation >> Ingredients >> Beclomethasone >> All). Repeat for all common inhaled corticosteroids.

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- Concept 3b *(put this into same 'concept folder' as inhaled medication):* Select 'Medication Course,' Filter out contra-indicated medication by ticking next to medication (e.g., Prednisolone). Click on each medication to add specific detail (e.g., Prednisolone >. Time period >> attribute >> Type in "after today's date minus one month"). Untick topical medications.
- Concept 4: Select 'clinical code,' Filter out pregnant patients by 'ticking' next to pregnant. Click on 'pregnant' to add specific detail (e.g. Pregnant >> Time period >> attribute >> Type in "After today's date minus 9 months." Untick all other boxes.

6. Select 'Protocols and Templates,' Select 'Add Folder', Select 'Radica Folder.'

7. Select 'Protocol Builder,' Add each concept from the RADicA Folder onto the protocol map by dragging each Concept into the Protocol Builder Window (e.g. Age, Underlying Lung Condition, Medication, Pregnant patient.) Then add a 'Yes/No Prompt' Box and add text "Is the Patient eligible for the rapid access diagnostic clinic as part of the RADicA study?"

8. Connect all concept boxes to the <u>correct arm</u>, if the concept is not met add 'end.'

9. Click 'Activate all concepts' and review protocol (figure 19)

10. Save the RADicA Referral form 'RADicA PATIENT INFORMATION LEAFLET _V1' from the USB drive onto the computer desktop.

11. Open EMIS software on the computer desktop. Select 'Template manager,' Select 'document templates,' Select 'create folder,' Select 'import document,' Select 'Non EMIS document.'

12. Select 'Action,' Select 'Launch Document,' Then select 'Radica protocol map,' Right click on properties and select 'Activate.'

13. Next Add Triggers: Select 'Trigger manager,' Select 'Properties,' Select 'Manage Triggers,' Select 'Add Code,' Select, 'Always Run.'
- Add Triggers by double clicking on all relevant triggers (the triggers selected were dependent on the individual surgery, we selected common codes used by the local clinicians) e.g., ***"Suspected asthma," "Peak Flow PEFR," "Asthma screening***."

Test the Protocol: Log into EMIS software, select 'Patient search,' select 'dummy patient,' (each practice manager had a list of 'dummy patient' names for testing).

Select 'new consultation,' Select a trigger code i.e. suspected asthma. Check that the pop-up is only activated in appropriate patients.

Figure 19. 'Pop-up' protocol builder
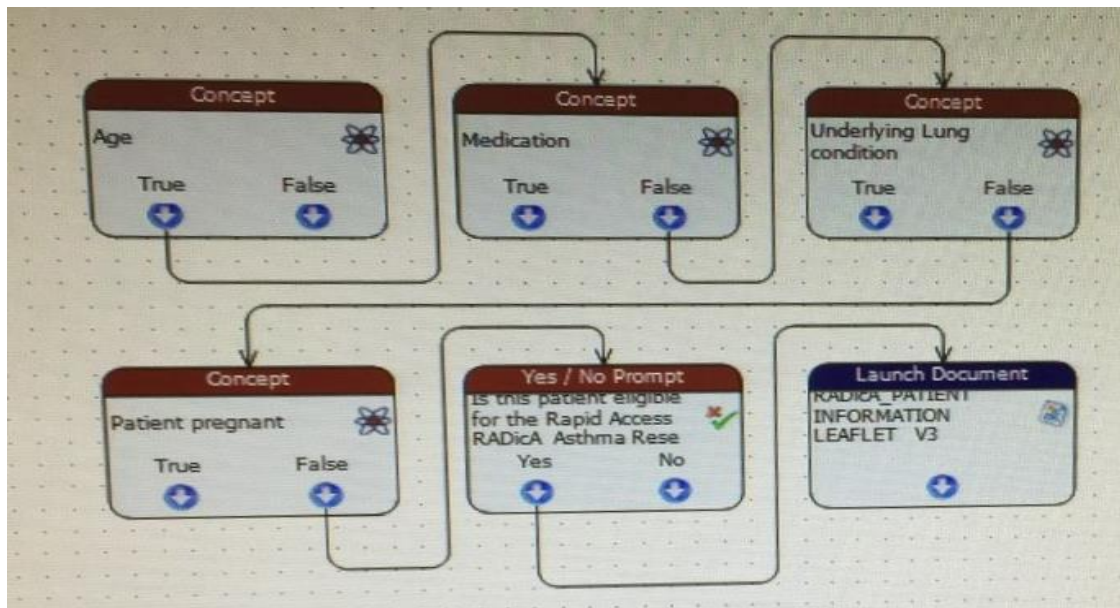
2.5.2. RADicA website design

The RADicA Website (http://www.radica.org.uk) was designed by me and implemented by our data manager Mike Porter (see figure 20). The purpose of the website was to assist with recruitment. Primary and secondary care clinicians were able to refer directly into the study through the webpage or given an option to print and fax through a patient referral form if preferred. Clinicians and patients could access the Patient Information Sheets (PIS) to learn more about the study.

Webpage design for recruitment:

Figure 20. RADicA website

i) RADicA Portal accessed through www.radica.org.uk
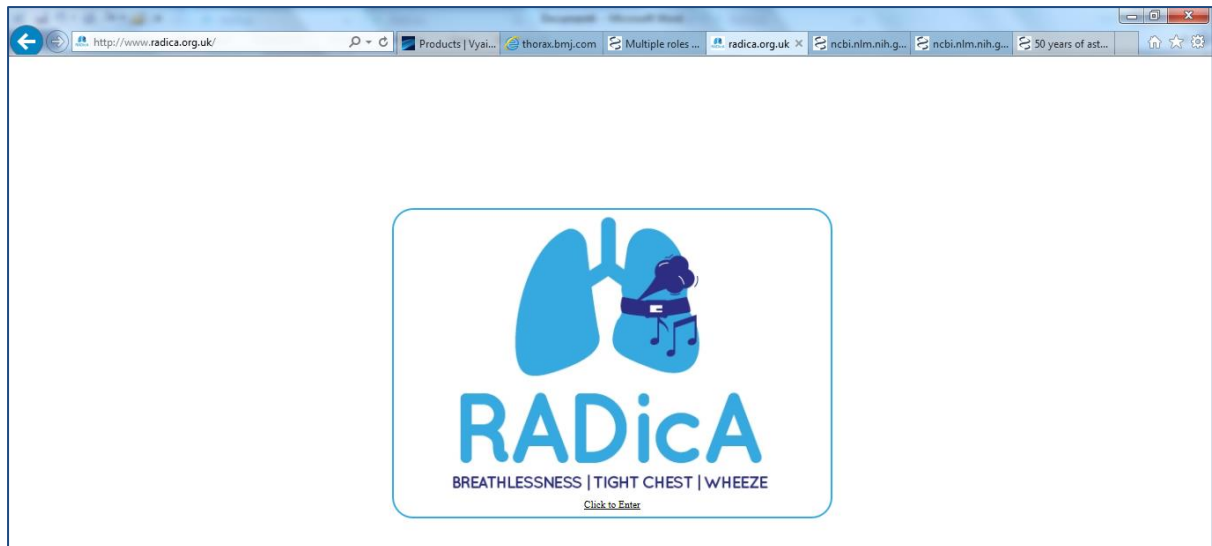
ii) RADicA webpage Home screen
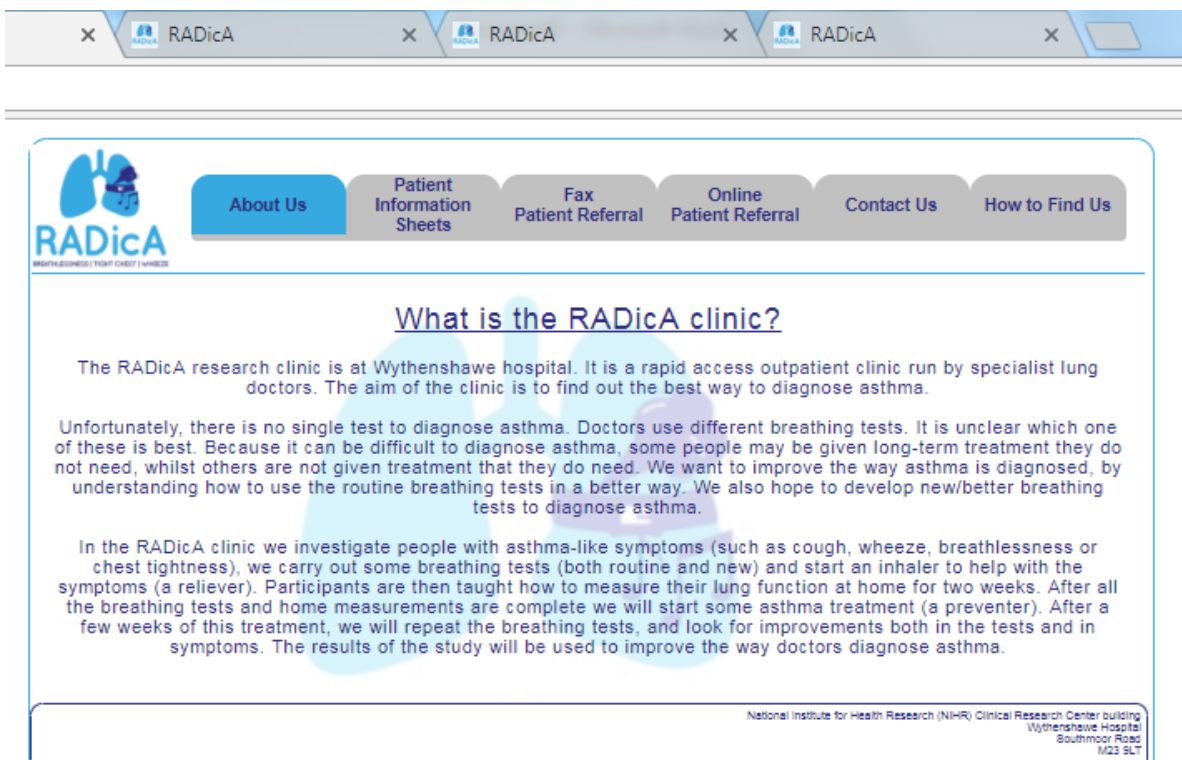
iii) RADicA referral form (fax version)

PLEASE NOTE: Patient's must have not taken oral corticosteroids for 4 weeks, inhaled corticosteroids for 2 weeks and antibiotics for 2 weeks prior to their first appointment. Therefore patients on regular inhaled steroids are not suitable for RADicA.

REFERRAL FORM

Please print and fill out the referral form above and give the attached information to the patient.

To refer, return the form to:

Email: radica@mft.nhs.uk
Fax: 0161 291 3513

For any other queries please contact the research team on:

Telephone: 07790911507 / 0161 291 3553

National Institute for Health Research (NIHR) Clinical Research Center building
Wythenshawe Hospital
Southmoor Road
M23 9LT

iv) RADicA referral form (email version)

FOR CLINICIAN USE ONLY

DO YOU THINK YOUR PATIENT HAS ASTHMA?

DO THEY HAVE A WHEEZE, SHORTNESS OF BREATH, CHEST TIGHTNESS OR COUGH?

If they are:

- Between 5 and 69 years old.
- Have no other lung disease that you know of.
- Either be a non-smoker OR an ex-emoker(≤10 pack year history) OR current smoker (≤10 pack year history).
- Not pregnant.
- Have not taken oral corticosteroids for 4 weeks, inhaled corticosteroids for 2 weeks and antibiotics for 2 weeks prior to their first appointment. Therefore patients on regular inhaled steroids are not suitable for RADicA.

Please download and give the patient a copy of the Patient Infomation Leaflet
Click here to download the
Patient Information Leaflet
PLEASE FILL IN THIS FORM AND CLICK SUBMIT
Any queries ring: 07790911507 / 0161 291 3553

Patient Name	
Date of Birth	
NHS Number	
Patient Contact Number(s)	
Patient Contact Email	
Referring Clinician Name	
Referring Clinician Contact Number	
GP Practice	
GP Contact Number	

Submit

Reset

2.6. Study outcome definitions

The RADicA study protocol (appendix B) has defined primary endpoints and outcome measures for asthma and steroid responsive airways disease (SRAD). This thesis will use the following outcome measure for asthma diagnosis which was assigned by expert panel consensus.

2.6.1. Expert Panel Objective Evidence Review (EPOER)

At the end of the study all patients were reviewed by an expert panel consisting of two respiratory consultants with specialist interest in Asthma, one consultant paediatrician, and a clinical research fellow (respiratory trainee with minimum 5 years' experience in respiratory medicine). The expert panel reviewed: clinical history, clinical examination, patient questionnaires, markers of variable airflow obstruction (determined by observation of PEF chart, spirometry pre- and post-salbutamol, bronchial challenge results), blood eosinophils, FeNO, and SPT. Where available, data from both pre and post treatment was considered. Data from novel and experimental tests **did not** form part of the decision process. One of the four diagnoses were allocated to all patients (table 11).

Table 11. EPOER diagnostic outcomes

Code	Criteria
EPOER confirmed 'Asthma'	Expert panel agree "definite asthma" based upon review of clinical consultation and all available objective evidence pre and post treatment.
EPOER confirmed 'Not Asthma'	Expert panel agree "definite not asthma' based upon review of clinical consultation and all available evidence pre and post treatment.
EPOER 'Possible Asthma'	Expert panel can't confirm definite 'asthma' or 'not asthma' despite all baseline tests completed (with the exception of BCT in children) and/or following review of objective testing following trial of inhaled corticosteroids.
EPOER 'Insufficient Evidence'	Expert panel can't confirm definite 'asthma' or 'not asthma,' however not all objective baseline tests completed and/or missing objective tests post trial of treatment. The panel feel that if the missing data from pre and post treatment were available this would enable a definitive diagnosis of 'asthma' or 'not asthma' to be confirmed.

2.7. Asymptomatic healthy group

2.7.1. Study participants

Participants were recruited using local advertisement through posters and flyers, social media, and 'word of mouth.' Participants were then able to self-refer into the study through telephone or email. All referrals were screened for eligibility by the RADicA team (table 12).

Table 12.   'Asymptomatic healthy group' inclusion and exclusion criteria (RADicA)

Inclusion Criteria:	Males and females ≥5years and <70 years
	Capable of giving informed consent or where under 16 years attends with parent or legal guardian.
Exclusion Criteria:	Diagnosis or repeat prescription of asthma treatment past or present
	Significant respiratory, cardiac or other medical co-morbidity
	More than one course of antibiotics for chest infection in the last 12 months
	Pregnant women
	>10 pack year smoking history
	Recent antibiotic treatment for any cause within previous 4 weeks
	Active symptoms of rhinitis (with 2 weeks)

2.7.2. Study procedure

Eligible participants underwent two visits. This first visit (baseline assessment visit) was booked at a time convenient to the participant, and the follow up visit (reproducibility assessment visit) was scheduled between one and twelve weeks later. Participants completed a series of objective tests (table 13).

Table 13.   Summary of tests performed by 'asymptomatic healthy group' at each visit

	Visit 1 (Baseline assessment visit)	Visit 2 (reproducibility visit)
Physical exam	☑	
FeNO	☑	☑
OA	☑	☑
VOC	☑	☑
MBW	☑	☑
PeXA	☑	☑
Spirometry	☑	☑
BDR	☑	
AO (post BDR)	☑	
SPT	☑	
Blood Eosinophils	☑	

Procedure for each measurement is described (see section 2.3). A simplified patient flow diagram with timeline is presented below along with summary of all tests performed (figure 21). Data was recorded in a workbook and transferred to an electronic database.

Figure 21. RADicA flow diagram and timeline for 'asymptomatic healthy group'

Visits	Visit 1	Visit 2
Shortest study pathway	D 0	D7
Longest study pathway	D 0	D84
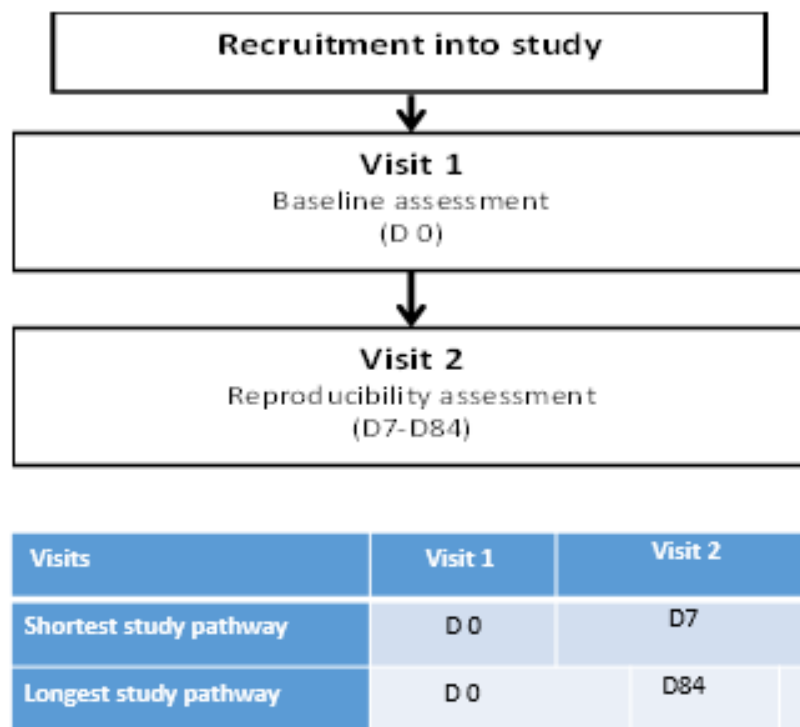
2.7.3. Procedures of measurements

See section 2.3 'symptomatic patient group.'

2.7.4. Summary of outcome measures

See section 2.4 'symptomatic patient group.'

2.7.5. Procedures for recruitment

Healthy volunteers were recruited through local advertisement and word of mouth. The website described above (section 2.5.2, 'RADicA website design'); http://www.radica.org.uk, also provided information for the public regarding self-referral into the 'asymptomatic healthy' arm of the study. All participants who showed interest in taking part were sent a 'patient information sheet.' Children under 16 years were sent and age-appropriate

information sheet and there next of kin/guardian was provided with a parental information sheet.

2.8. Statistical analysis plan

Statistical analysis plan for PhD thesis using preliminary data from the ongoing study; Rapid Access Diagnostics in Asthma (RADicA) (ISRCTN 11676160).

Sample size calculation for RADicA

Our department statistician, Philip Foden, provided guidance on sample sizes and data analysis. It was anticipated that approximately 60% of participants would fulfil the criteria of asthma. Sample size was based on the minimum of 10:1 events-to-variable ratio for logistic regression in order to avoid overfitting. With 120 (72 with and 48 without asthma) participants in the study, a multivariable logistic regression analysis, the primary analysis, could include five variables. In order to account for potential drop outs (estimated 10-20% maximum), the aim to recruit up to 150 participants was set. However, the power calculation required analysis of data from adults and children together. An amendment was made after we commenced recruitment when it became apparent these populations would need to be analysed separately. A corrected sample size of 150 adults and 150 children was recommended.

Thesis Analysis (interim analysis)

The thesis analysis is based on the data set from the first 115 patients admitted into the patient group of the RADicA Study and the first 65 healthy volunteers admitted into the control group of the RADicA study. The statistical analysis methods used for each specific thesis objective are described in detail at the start of each results chapter.

3. Asthma diagnosis using standard tests.

3.1. Introduction

Asthma diagnosis has recently come under scrutiny following reports of misdiagnosis, with both under- and over-diagnosis being highlighted as an issue.(5, 7) The National Institute for Health and Care Excellence (NICE) now recommend a series of objective tests are performed to determine the likelihood of asthma before commencing treatment (the NG80 algorithm).(24) Following completion of the algorithm, patients are coded 'diagnose with asthma,' 'suspect asthma' (both groups receive treatment), or 'consider alternate diagnosis/referral for second opinion' (no treatment recommended). These guidelines were developed despite limited studies; or in some instances no studies, available on the performance of recommended asthma tests using specified thresholds (individually or in combination) in the population the algorithm was required (i.e., symptomatic patients attending primary or secondary care), during the literature review completed by NICE prior to developing the guideline. The diagnostic performance of the NG80 algorithm has not been prospectively investigated since it was developed in 2017.

3.2. Aim

We set out to determine the best approach to diagnose asthma using standard tests currently available in clinical practice. The aim of this analysis was to i) investigate the performance of the NG80 diagnostic algorithm in a population of symptomatic patients, ii) determine the diagnostic power of common tests used in asthma, and iii) determine multivariate algorithms that can 'rule in' a diagnosis of asthma and compare performance with current NICE guidelines.

3.3. Methods

Patients (5-70 years) with clinician-suspected asthma (symptoms of cough, wheeze, chest tightness and/or breathlessness) referred from primary care to the Rapid Access Diagnostics in Asthma (RADicA) research clinic were included (see methods, chapter 2). Only patients with the EPOER diagnosis 'asthma' or 'not asthma' were included in this analysis (see section 2.6.1), those without definitive diagnosis ('insufficient evidence' or 'possible asthma') are not included but are described in detail (section 3.4.1.3). Participants underwent a structured clinical history and physical examination before asthma diagnostic tests were performed over two visits (see section 2.2.2 for details). Participants were then commenced on ICS (Flixotide Diskus, 250mcg twice daily) for six to eight weeks after which time the diagnostic tests were repeated. Participants were classed as asthma or not asthma during an expert panel objective evidence review (see EPOER criteria, section 2.6.1). The performance of the NG80 diagnostic algorithms (figures 22 and 23) to rule in asthma was assessed (e.g., agreement between EPOER asthma and NG80 'diagnose with asthma'). The performance of individual tests, and tests used in combination, to predict EPOER asthma was investigated.

3.3.1. Statistical analysis

3.3.1.1.   Descriptive analysis of all patients referred into RADicA

The following introductory analysis was performed:

- A consort diagram of all patients referred into the RADicA study was generated alongside a diagram showing final diagnostic outcomes.
- Tables presenting demographics and clinical characteristics of all four EPOER groups were displayed for completeness. The tables report clinical results from visit one for all tests; except BCTmeth and PEFv, which are reported from visit two. If data was unavailable at visit one, visit two data was used to provide a comprehensive overview of each patient group.

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- Baseline measurements comparing the 'asthma' and 'not asthma' groups were reported in the introductory tables using independent samples T Test for comparison of normally distributed data, Mann-Whitney U test for non-normally distributed data, and chi squared (or Fisher's exact test) for categorical data. A p value of ≤0.05 was considered significant.

- All participants from the "possible asthma" or "insufficient evidence" groups (that were excluded from the analysis) are described in detail for completeness.

- Feasibility of carrying out the NICE recommended tests is explored in adult and paediatric populations separately.

- We explored similarities (using scatter plots, bar charts and boxplots) between routine asthma diagnostic tests and other common tests that have potential (but are not currently recommended) in asthma diagnosis.

- A consort diagram is shown of patients used in the main analysis (i.e., asthma and not asthma groups).

3.3.1.2. Ability of the NICE diagnostic algorithm (NG80) to predict asthma

This analysis, set out below, includes the dataset as described in section 2.8. The analysis only included patients with the EPOER outcome "asthma" or "not asthma." We did not include patients coded as "possible asthma" or "insufficient evidence" because asthma could not be confirmed or excluded in these two groups. Healthy controls are not analysed. The results are presented in two ways; i) adults only (≥17 years), and ii) children only (<17 years). The following was performed:

- The number (%) of cases where the NG80 algorithm could not be completed was reported, the reason that the algorithm could not be followed to completion is explored.

- A cross tabulation was performed comparing NG80-diagnosed asthma with EPOER-diagnosed asthma.

- The sensitivity, specificity, PPV, and NPV of the NICE diagnostic algorithm to correctly diagnose EPOER confirmed "asthma" and EPOER confirmed "not asthma" was computed by the following method:

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Performance of the NG80 as a "Rule in" algorithm for asthma was determined by computing the sensitivity, specificity, PPV, and NPV, of the NG80 to correctly identify EPOER asthma. For this analysis NG80 "asthma" rules in asthma, and NG80 "not asthma" or "possible asthma" is grouped together because neither outcome "rules in" asthma.

- A descriptive (exploratory) analysis was performed to look at i) which pathways through the NG80 were associated with the most 'true-positive' and 'true-negative' cases, and ii) which pathways through the NG80 were associated with the most 'false-positive' and 'false-negative' cases.

- The above analysis was repeated in children.

- False-positives, false-negatives, and indeterminate results (i.e., "possible asthma") were explored for performance of the NG80 compared to expert diagnosed (EPOER) asthma (adults and children combined). A table displaying patient characteristics for those with false-positive and false-negative results was compiled to compare patient characteristics of EPOER asthma and EPOER not asthma confirmed patients. Individual cases were described to explore why EPOER diagnosed asthma and NG80 diagnosis did not agree.

Where a test was attempted at more than 1 visit (e.g., FeNO at CV1 and CV2) only data available from the first visit where this was attempted was used when assessing NG80 performance, to mimic what would happen in clinical practice, where patients are unlikely to be offered further visits to try again if they failed first time.

Each of the pathways through the NICE algorithms has been assigned a number, this allowed us to quantify which pathways were most and least used (table 14, figure 22-23).

Table 14.   Pathways through NG80, linking to figures 22 and 23

NICE Path	NICE Test sequence	NICE Classification	Num Tests
Adult algorithm (17+ years)			
Path 1	Spirometry yes > BDR Yes > FeNO Yes	Asthma	3
Path 2	Spirometry yes > BDR Yes > FeNO No > PEFv Yes	Asthma	4
Path 3	Spirometry yes > BDR Yes > FeNO No > PEFv No > FeNO25-39 Yes	Possible asthma	4
Path 4	Spirometry yes > BDR Yes > FeNO No > PEFv No > FeNO25-39 No	Not asthma	4
Path 5	Spirometry yes > BDR No > FeNO Yes	Possible asthma	3
Path 6	Spirometry yes > BDR No > FeNO No > FeNO25-39 Yes > PEFv Yes	Possible asthma	4
Path 7	Spirometry yes > BDR No > FeNO No > FeNO25-39 Yes > PEFv No > BHR Yes	Asthma	5
Path 8	Spirometry yes > BDR No > FeNO No > FeNO25-39 Yes > PEFv No > BHR No	Not asthma	5
Path 9	Spirometry yes > BDR No > FeNO No > FeNO25-39 No	Not asthma	3
Path 10	Spirometry No > FeNO Yes > PEFv Yes	Asthma	3
Path 11	Spirometry No > FeNO Yes > PEFv No > BHR yes	Asthma	4
Path 12	Spirometry No > FeNO Yes > PEFv No > BHR No	Not asthma	4
Path 13	Spirometry No > FeNO No > PEFv Yes > BHR yes	Asthma	4
Path 14	Spirometry No > FeNO No > PEFv Yes > BHR No	Not asthma	4
Path 15	Spirometry No > FeNO No > PEFv No	Not asthma	3
Path 16	Spirometry Yes > BDR No >FeNO No > Feno25-39 Yes >PEFv No >BHR unavailable	Possible asthma	4
Path 17	Spirometry No > FeNO Yes > PEFv No > BHR unavailable	Possible asthma	3
Path 18	Spirometry No > FeNO No > PEFv Yes > BHR unavailable	Possible asthma	3
Paediatric Algorithm (<17 years)			
Path 19	Spirometry yes > BDR Yes	Asthma	2
Path 20	Spirometry yes > BDR No > FeNO Yes > PEFv Yes	Asthma	4
Path 21	Spirometry yes > BDR No > FeNO Yes > PEFv No	Possible asthma	4
Path 22	Spirometry yes > BDR No > FeNO No	Not asthma	3
Path 23	Spirometry No > FeNO Yes > PEFv Yes	Asthma	3
Path 24	Spirometry No > FeNO Yes > PEFv No	Possible asthma	3
Path 25	Spirometry No > FeNO No > PEFv Yes	Possible asthma	3
Path 26	Spirometry No > FeNO No > PEFv No	Not asthma	3

Figure 22. Adapted from NICE (2017)(24) diagnostic guideline (algorithm C)

NICE Diagnostic Algorithm Pathways (17+ years)
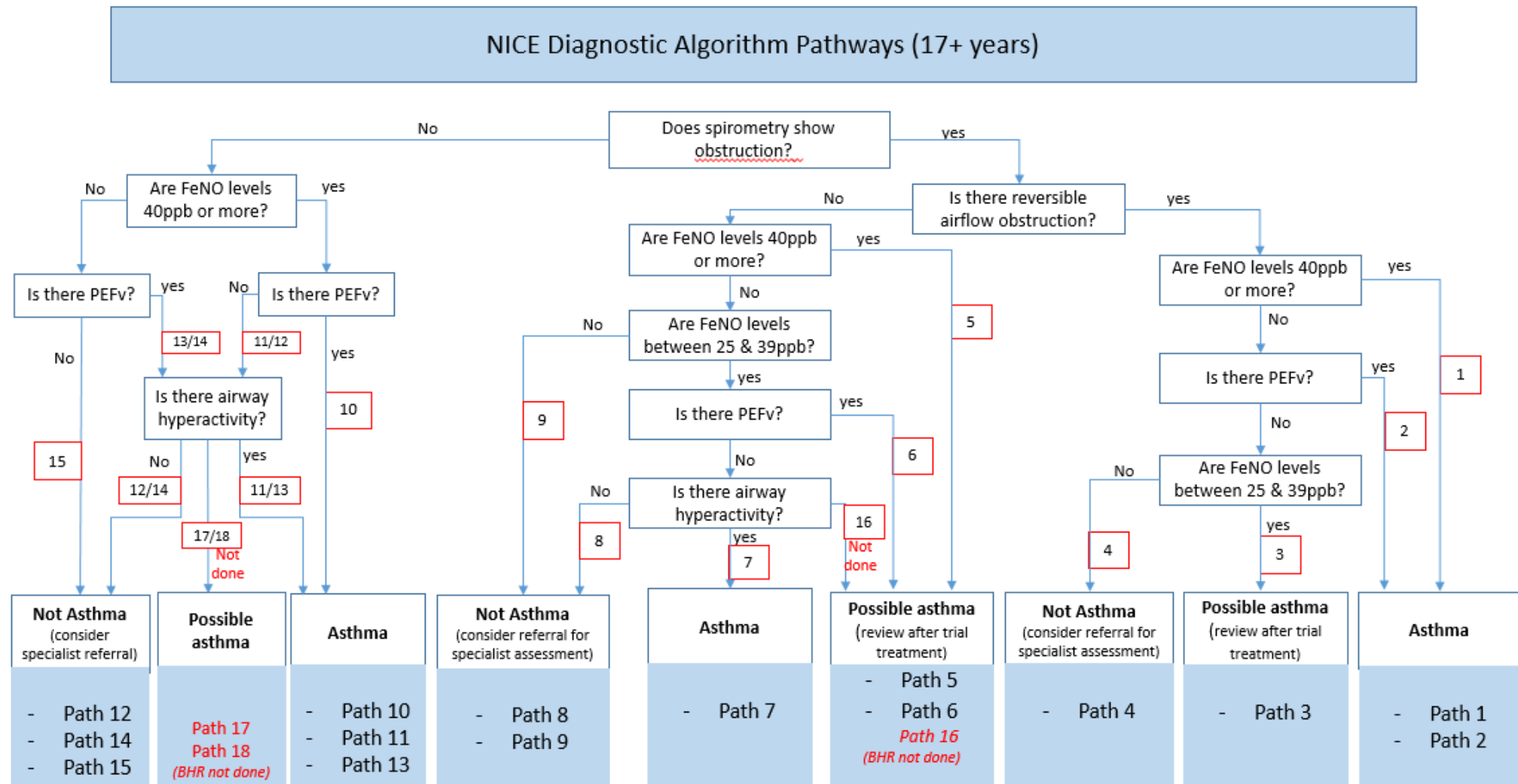
Figure 23. Adapted from NICE (2017)(24) diagnostic guideline (algorithm B)

NICE Diagnostic Algorithm Pathways (5-16 years)

Does spirometry show obstruction??
- No → Are FeNO levels 35ppb or more?
- yes → Is there reversible airflow obstruction?

No → Are FeNO levels 35ppb or more?
- No → Is there PEFv?
  - No → [26] → **Not Asthma** (consider referral for specialist assessment) — Path 26
  - yes → [25] → **Possible asthma** (review after trial treatment) — Path 25 / Path 24
- yes → Is there PEFv?
  - No → [24] → **Possible asthma** (review after trial treatment)
  - yes → [23] → **Asthma** — Path 23

Is there reversible airflow obstruction?
- No → Are FeNO levels 35ppb or more?
  - No → [22] → **Not Asthma** (Refer for specialist assessment) — Path 22
  - yes → Is there PEFv?
    - No → [21] → **Possible asthma** (review after trial treatment) — Path 21
    - yes → [20] → **Asthma** — Path 19 / Path 20
- yes → [19]

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Each pathway leads to one of four outcomes:

Table 15. NG80 outcomes

NG80 abbreviation	NICE Outcome Description	Treatment decision
Asthma	"Diagnose with Asthma"	Start ICS
Possible Asthma	"Suspect asthma and review diagnosis after treatment"	Start ICS and review the diagnosis after six to ten weeks by repeating spirometry and objective measures of asthma control and reviewing symptoms
Not Asthma	"Consider alternative diagnosis or referral for a second opinion" "Consider alternative diagnosis and referral for specialist assessment" "refer for specialist assessment"	Not for ICS
Unable to classify	Patients who had missing tests which resulted in the inability to complete the NICE algorithm sequence	

Depending on results of up to five tests (the sequence of which is clearly specified), there are 26 possible pathways through the NG80 algorithms; pathways one to eighteen are in the adult algorithm (including three in which BCT is not available), and pathways nineteen to twenty-six are in the paediatric algorithm (table 14, figures 22 and 23).

3.3.2. Which tests and what diagnostic combination of tests (algorithm) best predict asthma?

The following tests will be included in this section of the analysis (Table 16 and 17):

Table 16. Potential predictors of asthma (continuous data)

Test	Variables recorded
Spirometry	FEV_1, FVC, FEV_1/FVC, MMEF, FEV_1%pred, FVC%pred
BDR	FEV_1%change, FVC%change, FEV_1/FVC%change, MMEF%change
FeNO	FeNO level, FeNO%pred
PEFv	Mean daily amplitude percentage mean: [(PEFhighest – PEFlowest) % PEFmean]x100
PEFv(alt)	Days >20%
BCTmeth	PD_{20}
Blood	Eosinophils (Eos)

Table 17. Potential predictors of asthma (dichotomised and ordinal data)

Test	Cut Off for Positive
FEV_1/FVC (%)	<70, <LLN
BDR (FEV_1%change)	≥12 and 200mls (≥12% in children)
FeNO (ppb)	≥40 (≥35 in children <17yrs)
Eos (x10^9/L)	>0.4
PEFv (%)	>20%
PEFv(alt) (days>20%)	≥3
Clinical wheeze	Present
BCTmeth (PD_{20}, mg)	PD20 ≤0.2
SPT (Number allergens sensitised)	≥1
ACQ (Δpoints)	>0.5 reduction

The following analysis will be performed for each population i) adults (≥17 years) ii) children (<17 years):

- A univariate analysis was performed to determine if each test (described above) was significantly associated with the probability of predicting EPOER asthma (reference group EPOER not asthma.) For the normally distributed continuous data, comparison for differences between the two groups (EPOER asthma and EPOER not

asthma), was analysed using an independent samples T Test. However, where data is non-normally distributed, comparison for differences between the groups was analysed using the Mann-Whitney U Test for non-parametric data with two or more independent samples. A p value ≤0.05 was statistically significant.

- Boxplots were generated for each of the above variables that were able to differentiate between 'asthma' and 'not asthma,' this will visually demonstrate the relationship between EPOER asthma and EPOER not asthma for these tests.

- Correlations between diagnostic measurements used in the NG80 algorithm and alternative measurements shown to be significant predictors of asthma, were investigated using scatter plots and box plots.

- Correlation between FeNO (the NG80 algorithm recommended test for airways inflammation), and other markers often associated with airways inflammation, were investigated using scatter plots and box plots.

- The predictive power of each quantitative test to predict EPOER asthma was be reported using AUROC.

- Univariate analysis was performed on tests with dichotomised data using already established cut-offs to determine the ability of each test to predict EPOER confirmed asthma from EPOER confirmed 'not asthma. Chi-squared analysis; or Fishers Exact tests for groups with less than five patients, was be computed. The sensitivity, specificity, PPV, and NPV was computed for dichotomous data.

- Exploratory analysis was performed using regression models to explore the potential for a 'rule in asthma' diagnostic algorithm

- Exploratory analysis was then performed looking at the role of MMEF (a spirometry-based measure), as a diagnostic test for asthma. Youden's Index was computed to explore best cut-off values for this test. We explored the potential for this test to replace conventional spirometry measurements in the diagnosis of asthma.

3.4. Results

3.4.1. Descriptive analysis

3.4.1.1. Consort diagram of all patients referred into the RADicA study and their EPOER diagnostic outcome.

The consort diagram (Figure 24) shows the flow of all patients referred into the RADicA study (n= 196) and visits attended of all patients that were subsequently enrolled (n= 115) to completion or the point at which patients were withdrawn. The final diagnostic outcome of each patient using the EPOER diagnosis (asthma, not asthma, possible asthma, insufficient evidence) is shown (Figure 25).

Figure 24. Consort diagram of the point at which patients were withdrawn (all patients)

Referred: N = 196

Withdrawn:
-Screen Fail: N = 26
-Subject lost to follow up: N = 26

Enrolled (passed screening): N = 144

Withdrawn:
-Declined study (subject decision): N = 16
-Recruitment Paused (COVID pandemic): N = 11

CV1 Consent: N = 117

Withdrawn:
-Withdrawn as per Protocol (alternate diagnosis more likely at initial consultation): N = 1
-Not Eligible (>10 pack year discovered at initial consultation): N = 1

CV1 : N = 115

Withdrawn:
Subject Lost to follow up: N = 8
Clinical deterioration (started on ICS by GP): N = 3

CV2: N = 99

Withdrawn:
Adverse Event (Possible medication SE): N = 1

Withdrawn:
Subject Lost to follow up : N = 1

OV1: N = 52

Withdrawn:
Subject Lost to follow up: N = 2
Patient withdrew (patients decision) : N = 2

Withdrawn:
Subject Lost to follow up: N = 4
Patient withdrew (patients decision): N = 3
Adverse event (unrelated to study): N = 1

CV3: N = 65

Withdrawn:
Subject Lost to follow up: N = 2
Adverse Event (possible medication SE): N = 1

CV4: N = 87

Footnote: of the 115 patients enrolled at CV1, 87 remained in the study through to the final visit CV4. 28 patients were withdrawn between the first and last visit. Not all patients in the study completed every visit. 99 of the 104 patients remaining in the study at CV2 attended. 65 of the 90 patients remaining in the study at CV3 attended. Not attending a visit was not an indication for withdrawal.

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Figure 25. Diagram showing RADicA patient outcomes (Expert Panel Objective Evidence (EPOER) diagnosis)
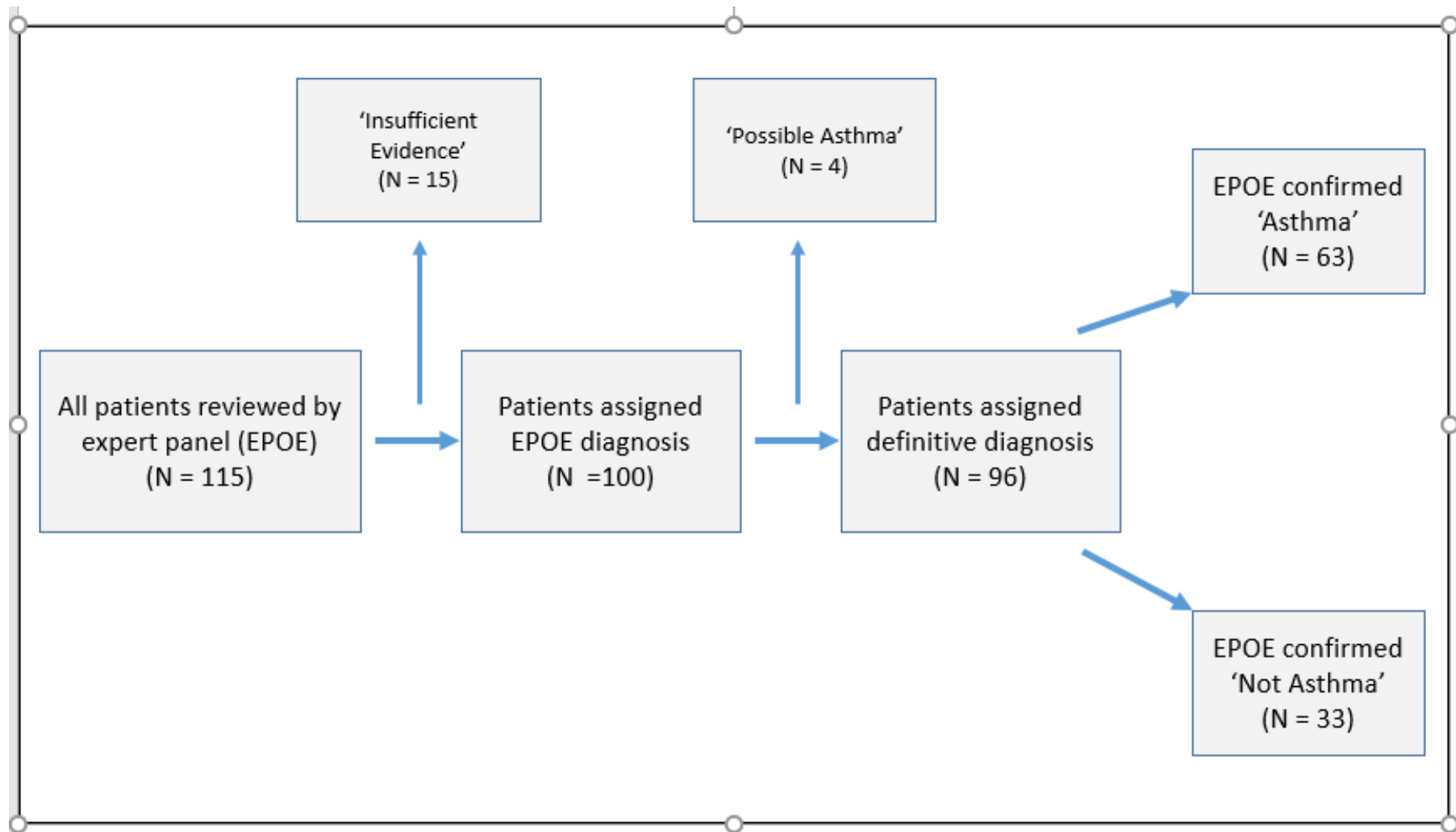
3.4.1.2. Demographics and baseline clinical characteristics of all patients enrolled into the RADicA study and EPOER asthma status.

Demographics and baseline clinical characteristics of all patients enrolled into the study (n= 115) are presented (Table 18-23) for group comparisons. All subsequent analysis is performed only on patients with EPOER asthma (n= 63) or EPOER not asthma (n= 33). The remaining 19 patients coded as EPOER possible asthma (n= 4) or EPOER insufficient evidence (n= 15) were excluded from the analysis and are described in detail below (*Section 3.4.1.3 'Patients excluded from the analysis.'*)

Table 18.   Demographics of all patients enrolled into RADicA:

Demographics	All Patients (N = 115)	Patient split into sub groups (N 115)				*P Value
		EPOER Asthma (N = 63)	EPOER Not Asthma (N = 33)	Possible Asthma (N = 4)	Insufficient Evidence (N = 15)	
Age, mean (SD) years	**23.33 (15.57)**	**22.71 (15.38)**	**29.76 (16.09)**	**11.25 (9.85)**	**15.00 (9.95)**	**0.039**
Gender, n (%) females	64 (55.7)	33 (52.4)	22 (66.7)	3 (75.0)	6 (40.0)	0.179
Ethnicity, n (%) white	79 (68.7)	43 (68.3)	26 (78.8)	2 (50.0)	8 (53.3)	0.276
BMI, mean (SD) kg/m^2	24.59 (6.95)	24.23 (6.62)	26.59 (7.37)	20.25 (7.13)	22.89 (6.80)	0.114
Normally distributed (mean, SD) reported, categorical data (number, %)						
* P values refer to the difference between the EPOER asthma and EPOER not asthma groups. T-test for normally-distributed data; chi squared (or Fisher's exact test [1]) for categorical data. Abbreviation: BMI, Body mass index,						

The mean age of patients referred into the Rapid Access Diagnostics in Asthma (RADicA) research clinic was 23 years. Two-thirds of all patients referred were of white ethnic origin. We had a similar proportion of male (44%) and female (56%) referrals. Average BMI was at the upper limit of normal. Patients with confirmed asthma were significantly younger than

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those who had a diagnosis EPOER 'not asthma' (mean(SD)) 22.7(15.4) versus 29.8(16.1) years respectively. The mean BMI of the asthma group was in normal range, and the mean BMI of the 'not asthma' group was "overweight," however on comparing the two groups this was not a significant difference.

Table 19.   Clinical history of all patients enrolled into RADicA

Clinical History	All Patients (N 115)	Patient split into sub groups (N 115)				*P Value
		Asthma (N 63)	Non Asthma (N 33)	Possible Asthma (N 4)	Insufficient Evidence (N 15)	
Current or ex-smokers, n (%)	21 (18.3)	10 (15.9)	8 (24.2)	1 (25.0)	2 (13.3)	0.318
Pack years, median (IQR)	0.00 (0.00-0.00)	00.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.0-0.0)	0.348
Duration symptoms, median (IQR) years	2.00 (1.00-7.00)	2.00 (0.60-7.00)	2.00 (1.00-6.50)	6.00 (2.75-7.00)	2.00 (0.60-5.00)	0.543
Eczema in last 12mnths, n (%)	29 (25.2)	18 (28.6)	5 (15.2)	2 (50.0)	4 (26.7)	0.143
Hay fever/Rhinitis in last 12mnths, n (%)	61 (53.0)	34 (54.0)	18 (54.5)	3 (75.0)	6 (40.0)	0.957
Anxiety in last 12 months, n (%)	**21 (18.3)**	**7 (11.1)**	**9 (27.3)**	**1 (25.0)**	**4 (26.7)**	**0.044**
Damp in the home (current or past), n (%)	40 (35.7) N = 112	19 (31.7) N = 60	13 (39.4)	2 (50.0)	6 (40.0)	0.453
Pets in the Home (current or past), n (%)	85 (75.9%) N = 112	47 (79.3) N = 60	26 (78.8)	2 (50.0)	10 (66.7)	0.959
Born Premature, n (%)	8 (7.2) N =111	4 (6.7) N = 60	4 (12.5) N = 32	0 (0.0)	0 (0.0)	0.298
Family Member with Asthma, n (%)	58 (50.4%)	28 (44.4)	15 (45.5)	4 (100.0)	11 (73.3)	0.585
Family Member with Allergy, n (%)	51 (44.3)	24 (38.1)	16 (48.5)	2 (50.0)	9 (60.0)	0.4552

not normally distributed (median, IQR) reported. Categorical data (number, %)

*P values refer to the difference between the 'asthma' and 'not asthma' groups. Mann Whitney U for non-normally distributed data, chi squared for categorical data. N = 'x' refers to the number of patients with data (only used when data set incomplete)

One in five of all patients referred reported a current or past history of cigarette use, but median pack year usage was low (> 10 pack years was an exclusion for the study). Past medical history of eczema, hay fever, or premature birth was not significantly different between groups. Higher proportion of self-reported anxiety over the preceding year was seen in those without asthma (p 0.004). Family history of asthma or allergy, household exposure to pets, and exposure to damp were not significantly different between EPOER asthma and EPOER not asthma groups.

Table 20.   Presenting features of all patients enrolled into RADicA

Presenting features	All Patients (N 115)	Patient split into sub groups (N 115)				*P Value
		Asthma (N 63)	Non Asthma (N 33)	Possible Asthma (N 4)	Insufficient Evidence (N 15)	
Cough (in absence of LRTI) in last 12mnths, n (%)	92 (80.0)	50 (79.4)	25 (75.8)	4 (100.0)	13 (86.7)	0.685
Wheeze (in absence of LRTI) in last 12mnths, n (%)	**85 (73.9)**	**52 (82.5)**	**20 (60.6)**	**2 (50.0)**	**11 (73.3)**	**0.018**
Breathlessness (in absence of LRTI) in last 12mnths, n (%)	102 (88.7)	53 (84.1)	32 (97.0)	4 (100.0)	13 (86.7)	0.091 ⱡ
Chest Tightness (in absence of LRTI) in last 12mnths, n (%)	80 (69.6)	47 (74.6)	23 (69.7)	4 (100.0)	8 (53.3)	0.607
Number of Symptoms (from cough/wheeze/SOB/tightness), median (IQR)	3 (3-4)	3 (3-4)	3 (2-4)	3.00 (2.25-3.75)	3.00 (2.00-4.00)	0.347
Present with all four symptoms in last 12 months (from cough/wheeze/SOB/tightness) n (%)	47 (40.9)	28 (44.4)	12 (36.4)	1 (25.0)	6 (40.0)	0.446
More than one respiratory symptoms in last 12mnths (from cough/wheeze/SOB/tightness) n (%)	110 (94)	60 (95.2)	31 (93.9)	4 (100.0)	13 (86.7)	1.00 ⱡ
Number of triggers to symptoms, median (IQR)	3.00 (2.00-5.00)	3.00 (2.00-5.00)	3.00 (1.50-5.50)	3.50 (2.25-4.00)	2.00 (1.00-5.00)	0.610
Exercise triggers symptoms, n (%)	83 (74.8) N = 111	43 (71.7) N = 60	23 (71.9) N = 32	4 (100.0)	13 (86.7)	0.983
Temp change triggers symptoms, n (%)	62 (55.9) N = 111	33 (55.0) N = 60	20 (62.5) N = 32	4 (100.0)	5 (33.3)	0.488
Aerosol exposure triggers symptoms, n (%)	34 (30.6) N = 111	17 (28.3) N = 60	11 (34.4) N = 32	0 (0.00)	6 (40.0)	0.549

ACQ Points, mean (SD)	1.51 (1.18) N = 114	1.55 (1.16) N = 62	1.37 (1.14)	0.75 (0.57)	1.87 (1.37)	0.482
ACQ Q1 How often woken with "asthma" symptoms at night, median (IQR) score	1.00 (0.00-2.00) N = 114	0.50 (0.00-2.00) N = 62	1.0 (0.00-2.00)	1.50 (0.25-2.00)	2.00 (0.00-2.00)	0.920
ACQ Q2 How bad "asthma" symptoms in morning, median (IQR) score	1.00 (0.00-2.00) N = 114	2.00 (0.00-3.00) N = 62	1.0 (0.00-2.00) 2.0	0.50 (0.00-1.00)	2.00 (1.00-3.00)	0.225
ACQ Q3 Limited in activities due to "asthma" symptoms, median (IQR) score	0 .00 (0.00-2.00) N = 114	1.00 (0.00-2.00) N = 62	2.0 (0.00-2.00)	0.00 (0.00-0.75)	1.00 (0.00-3.00)	0.392
ACQ Q4 Breathlessness due to "asthma" symptoms, median (IQR) score	2.00 (0.00-3.00) N = 114	2.00 (0.00-3.00) N = 62	3.0 2.00 (0.00-4.00)	1.00 (0.25-2.50)	3.00 (1.00-4.00)	0.588
ACQ Q5 How much of the time did you wheeze, median (IQR) score	1.00 (0.00-3.00) N = 114	1.50 (0.00-3.00) N = 62	4.0 1.00 (0.00-2.00)	0.00 (0.00-1.50)	2.00 (0.00-3.00)	0.219
Respiratory clinician suspect "high possibility asthma), n (%)	**56 (48.7)**	**41 (65.1)**	**7 (21.2)**	**3 (75.0)**	**5 (33.3)**	**<0.001**

Normally distributed (mean, SD) reported, Not normally distributed (median, IQR) reported. Categorical data (number, %)

* P values refer to the difference between the 'asthma' and 'not asthma' groups. T-test for normally-distributed data; Mann Whitney U for non-normally distributed data, chi squared (or Fisher's exact test [1]) for categorical data. N = 'x' refers to the number of patients with data (only used when data set incomplete). Abbreviation: ACQ, Asthma control questionnaire

The four classic asthma symptoms (wheeze, breathlessness, chest tightness, cough) were commonly reported amongst those with and without EPOER asthma, and only wheeze was more common in EPOER asthma compared to EPOER not asthma groups (P<0.05). Both groups had median of three symptoms. More than one third of patients in whom asthma was excluded reported all four symptoms that are classically associated with asthma. Common symptom triggers (i.e., exercise, temperature change, aerosol exposure) were reported in both EPOER 'asthma' and EPOER 'not asthma' patients, these were not different between groups. Exercise was the most common symptom trigger reported in three quarters of all patients. The asthma control questionnaire (ACQ, a validated questionnaire intended for use on patients diagnosed with asthma), did not differentiate between asthma and not asthma groups. During the first visit, immediately following the clinical history and

examination (but before any objective tests are performed) the doctor completes a clinical impression score (high possibility, intermediate possibility, low possibility, or alternate diagnosis more likely). Despite presenting features being similar in each group when recorded dichotomously (as present or absent), two thirds of those who were subsequently diagnosed with EPOER asthma were recorded as high probability asthma by the doctor, compared to one in five of those who were subsequently found not to have asthma (p<0.001).

Table 21. Clinical tests results (conventional) in all patients enrolled into RADicA

| Clinical Tests (conventional) | All Patients (N 115) | Patient split into subgroups (N 115) | | | | *P Value |
		Asthma (N 63)	Non-Asthma (N 33)	Possible Asthma (N 4)	Insufficient Evidence (N 15)	
FEV1, mean (SD) L	2.71 (1.12) N = 114	2.64 (1.16)	3.07 (1.03)	1.79 (0.98)	2.48 (1.02) N = 14	0.077
FEV1, mean (SD) %predicted	**96.45 (15.95) N = 114**	**91.70 (16.81)**	**103.64 (13.41)**	**94.50 (7.33)**	**101.43 (11.90) N = 14**	**0.001**
FVC, mean (SD) L	3.47 (1.50) N = 114	3.52 (1.58)	3.71 (1.30)	2.15 (1.35)	3.04 (1.44) N = 14	0.549
FVC, mean (SD) % predicted	103.55 (13.56) N = 114	102.64 (14.60)	104.70 (13.72)	98.50 (6.40)	106.43 (9.26) N = 14	0.505
FEV1/FVC Ratio, mean (SD) %	**79.69 (8.37) N = 114**	**76.52 (8.73)**	**83.37 (4.12)**	**85.21 (5.12)**	**83.64 (9.39) N = 14**	**<0.001**
BDR, median (IQR) %	**7.00 (102.75-111.25) N = 114**	**9.00 (5.00-15.00)**	**4.00 (1.00-7.00)**	**8.00 (7.25-8.75)**	**2.00 (-0.25-7.25) N = 14**	**<0.001**
FeNO, median (IQR) ppb	**21.00 (11.00-63.25) N = 112**	**50.00 (17.50-93.50) N = 61**	**14.00 (9.50-21.50)**	**8.00 (7.00-10.50)**	**16.00 (11.75-21.50) N = 14**	**<0.001**
PEFv Calculation, median (IQR) %	**10.56 (6.13-16.02) N = 91**	**11.64 (8.47-19.55) N = 54**	**6.24 (3.51-11.02) N = 25**	**14.85 (7.58-18.53)**	**7.58 (5.02-18.59) N = 8**	**<0.001**
PEFv Number Days >20%, median (IQR) days (data from returned PEF meters with minimum 4 days data)	**1.0 (0.00-3.00) N = 91**	**2.00 (0.00-4.00) N = 54**	**0.00 (0.00-1.50) N = 25**	**3.00 (0.75-4.50)**	**0.50 (0.00-2.50) N = 8**	**<0.001**

PEFv days recorded, median (IQR) days (If not returned recorded as 0 days)	9.00 (4.00-12.00) 2.0 N = 113	10.00 (7.00-12.00) N = 54	8.00 (3.00-11.50)	9.50 (4.50-16.00)	4.00 (0.00-9.00) N = 15	0.088
PEFv 14days Completed, n (%)	**11 (9.7) N = 113**	**10 (16.4) N = 61**	**0 (0.0)**	**1 (25.0)**	**0 (0.0) N = 15**	**0.013 ⌐**
Eos, median (IQR) x10^{9}cells/L	**0.22 (0.12-0.45) N = 103**	**0.33 (0.18-0.72) N = 55**	**0.12 (0.09-0.20) N = 32**	**0.14 (0.13-0.39)**	**0.24 (0.12-0.51) N = 12**	**<0.001**
SPT (Number allergens sensitised), mean (SD)	**1.48 (1.45) N = 113**	**1.76 (1.46)**	**1.09 (1.36)**	**1.50 (2.38)**	**1.08 (1.19) N = 13**	**0.031**
BCTmeth PD20, median (IQR)	**0.54 (0.07-1.92) N = 84**	**0.11 (0.02-0.32) N = 47**	**1.92 (1.92 – 1.92) N = 30**	**1.92 (1.92-1.92) N = 2**	**0.61 (0.28-1.92) N = 5**	**<0.001**
BCTmeth DRR, median (IQR)	**25.83 (9.78-142.33)**	**119.58 (32.63-505.00)**	**8.97 (4.64-17.17)**	**13.68 (9.57-17.78)**	**17.84 (5.85-453.60)**	**<0.001**
Wheeze auscultated, n (%)	**12 (10.4)**	**12 (19.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0.007 ⌐**

Normally distributed (mean, SD) reported, not normally distributed (median, IQR) reported. Categorical data (number, %)

* P values refer to the difference between the 'asthma' and 'not asthma' groups. T-test for normally-distributed data; Mann Whitney U for non-normally distributed data, chi squared (or Fisher's exact test [1]) for categorical data. N = 'x' refers to the number of patients with data (only used when data set incomplete). Abbreviation: DRR, dose response ratio

All conventional tests associated with asthma, including spirometry, PEFv, BDR, FeNO, allergic sensitisation, blood eosinophilia, and BCTmeth, demonstrated significant differences between 'asthma' and 'non asthma' patients. This is discussed in more detail in section 3.4.3 of the analysis.

Table 22.   Clinical tests results (dichotomised outcomes) of all patients enrolled into RADicA:

Clinical Tests (dichotomised outcomes)	All Patients (N 115)	Patient split into sub groups (N 115)				*P Value
		Asthma (N 63)	Non Asthma (N 33)	Possible Asthma (N 4)	Insufficient Evidence (N 15)	
FEV1/FVC <70%, n (%) positive	15 (13.2) N = 114	13 (20.6)	0 (0.0)	0 (0.0)	2 (14.3) N = 14	0.004 ⬆
FEV1/FVC <LLN, n (%) positive	26 (22.6) N = 114	24 (38.1)	0 (0.0)	0 (0.0)	2 (14.3) N = 14	<0.001 ⬆
FeNO, n (%) positive (adult: FeNO ≥40ppb, child: FeNO ≥35ppb)	39 (34.8) N = 112	33 (54.1) N = 61	4 (12.1)	0 (0.0)	2 (14.3) N = 14	<0.001 ⬆
BDR, n (%) positive (adult: ΔFEV1 >12% and 200ml, child ΔFEV1 >12%)	27 (23.7) N = 114	26 (41.3)	0 (0.0)	0 (0.0)	1 (7.1) N = 14	<0.001 ⬆
PEFv >20%, n (%) positive	15 (16.5%) N = 91	13 (24.1) N = 54	0 (0.0)	0 (0.0)	2 (25.0) N = 8	0.007 ⬆
PEFv(alt), n (%) positive	30 (33.0) N = 91	24 (44.4) N = 54	1 (4.0) N = 25	1 (25.0)	2 (25.0) N = 8	<0.001 ⬆
PEFv days recorded, median (IQR) days	10.00 (8.0-12.0) N = 91	10.5 (8.0-13.0) N = 54	9.0 (8.0-12.0) N = 25	9.5 (4.5-16.0)	9.0 (6.5-10.5) N = 8	0.343
Eos >0.4x10⁹/L, n (%) positive	28 (27.2) N = 103	23 (41.8) N = 55	1 (3.1) N = 32	1 (25.0)	3 (25.0) N = 12	<0.001 ⬆
Atopic (SPT ≥1 positive), n (%)	75 (65.2) N = 114	48 (76.2)	17 (51.5)	2 (50.0)	8 (57.1) N = 14	0.014
Atopic (SPT positive to HDM), n (%)	55 (48.2) N = 114	39 (61.9)	10 (30.3)	1 (25.0)	5 (35.7) N = 1	0.003
Atopic (SPT positive to grass), n (%)	48 (42.1) N = 114	31 (49.2)	11 (33.3)	1 (25.0)	5 (35.7) N = 1	0.136

BCTmeth PD20, n (%) positive (<0.2mg threshold)	32 (38.1) N = 84	30 (63.8) N = 47	1 (3.3) N = 30	0 (0.0) N = 2	1 (20.0) N = 5	<0.001 ⸕
BCTmann PD15, n (%) positive	18 (36.7) N = 49	15 (62.5) N = 24	2 (10.0) N = 20	1 (50.0) N = 2	0 (0.0) N = 3	0.001 ⸕

*Categorical data (number, %). * P values refer to the difference between the 'asthma' and 'not asthma' groups, chi squared (or Fisher's exact test [⸕]) for categorical data. N = 'x' refers to the number of patients with data (only used when data set incomplete)*

Abbreviation: HDM, house dust mite

Using conventional tests with dichotomised outcomes (spirometry, PEFv, BDR, FeNO, allergic sensitisation, blood eosinophilia, and BCT), we demonstrated significant differences between 'asthma' and 'non asthma' patients. This is discussed in more detail in section 3.4.3 of the analysis.

Table 23. Performance of NICE algorithm and completion of NICE recommended tests in all patients referred to RADicA

| | All Patients (N 115) | Patient split into sub groups (N 115) | | | | *P Value |
		Asthma (N 63)	Non Asthma (N 33)	Possible Asthma (N 4)	Insufficient Evidence (N 15)	
Completed all four tests from NICE Algorithm, n (%) (not including BCT)	90 (79.6%) N = 113	53 (86.9) N = 61	25 (75.8)	4 (100)	8 (53.3) N = 15	0.171
Number Tests Completed from NICE Algorithm, median (IQR) (i.e. not including BCT)	4 .00 (4.00 – 4.00) N = 113	4.00 (4.00-4.00) N = 61	4.00 (3.50-4.00)	4.00 (4.00-4.00)	4.00 (3.00-4.00)	0.189
NICE Algorithm completed, n (%)	91 (79.1)	54 (85.7)	25 (75.8)	4 (100.0)	8 (53.3)	0.225

not normally distributed (median, IQR) reported. Categorical data (number, %)

* P values refer to the difference between the 'asthma' and 'not asthma' groups. Mann Whitney U for non-normally distributed data, chi squared (or Fisher's exact test [1]) for categorical data. N = 'x' refers to the number of patients with data (only used when data set incomplete)

It was possible to complete the NICE algorithm in four fifths of our patients. This is presented in more detail below (section 3.4.1.4, Feasibility of performing tests).

3.4.1.3. Patients excluded from the analysis

In patients in whom the expert panel could not agree a definite diagnosis of 'asthma' or 'not' asthma, a clinical code of 'possible asthma' (n= 4) or 'insufficient evidence' (n= 15) was given. Possible asthma was assigned if a definite diagnosis was unclear despite a patient completing all baseline tests (excluding BCT in children) and objective testing following a trial of inhaled corticosteroids. However, if baseline tests or treatment response data was missing and it was felt that this information would allow a definitive diagnosis, insufficient evidence was recorded.

Demographics and clinical characteristics of patients excluded versus patients included:

The section above, '*Demographics and baseline clinical characteristics of patient group,*' (Table 18-19) reports baseline demographics and clinical characteristics of patients excluded from (possible asthma, insufficient evidence) and patients included (asthma, not asthma) in the analysis. The mean age of the excluded group was younger; 11.2 years and 15.0 years versus 22.7years and 29.8 years respectively. In both groups, 'possible asthma' and 'insufficient evidence', approximately three quarters were under 17 years old. A pie chart reporting the reason tests were not performed resulting in patients receiving the code 'insufficient evidence' is shown in figure 26.

Figure 26. Reason for missing tests in 'insufficient evidence' group

Reason for missing tests in 'insufficient evidence' group

Legend:
- non-compliance
- DNA
- withdrawn
- covid-related data gap
- non-compliance and unable to perform
- non-compliance and DNA
- contra-indication and withdrawn
- DNA and Covid related data gap
- unable to perform, non-compliance and Covid related data gap
- unable to perform and covid related data gap
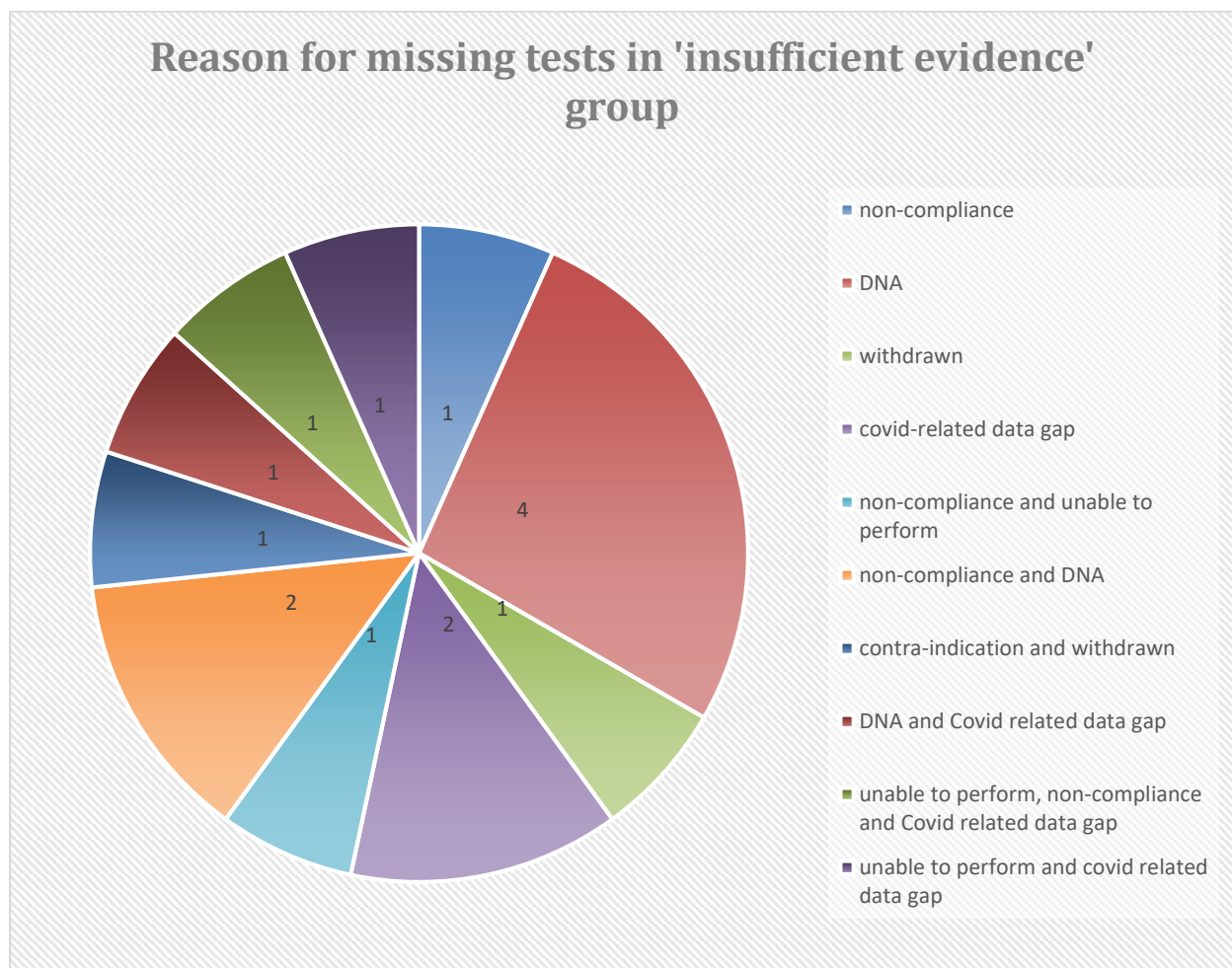
Patients coded as possible asthma and insufficient evidence:

All patients coded as possible asthma (n= 4) and insufficient evidence (n= 15) are described in detail in summary tables (Appendix E).

3.4.1.4.   Feasibility of performing tests recommended in the NICE algorithm

Feasibility in adult's ≥17 years:

Feasibility of performing technically acceptable tests was explored in all adults (n= 63) (Table 24). We reviewed all NICE recommended tests (spirometry, BDR, FeNO, PEFv, and BCTmeth).

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Table 24. Feasibility of performing NICE tests (adults)

	Number Completed (%)
spirometry	62/62 (100)
BDR	62/62 (100)
FeNO	63/63 (100)
PEFv	49/62 (79)
BCTmeth	48/54 (89)

One patient left the study and did not return after completing FeNO but before attempting spirometry and BDR. All patients who attempted were able to perform spirometry, BDR, and FeNO with good technique. Of 62 patients issued with PEF monitor, all patients demonstrated satisfactory technique in performing the required breathing manoeuvre whilst in clinic. The digital PEFv diary (with at least 3 days of completed measurements) was returned in four out of five patients. Of those who returned the diary (n= 49), only seven patients completed the recommended number of days (≥14 days). Of the thirteen patients who didn't complete monitoring; two patients were unable due to a clinical need to commence early treatment. In the remaining patients, poor compliance to return the monitor (n= 10) or not achieving the minimum four days of recordings (n= 1) was the cause of missing data. Therefore, we have demonstrated that although a single measure of peak flow during the clinic visit is a feasible test, lack of compliance with the requested repeated measures at home meant this test was not feasible as recommended, with the highest proportion of missing data. Of 54 adults attempting BCT, 48 (89%) completed it successfully; five patients had a contra-indication due to low baseline FEV_1 and one patient declined the test because of anxiety.

Feasibility in children <17 years:

Feasibility of performing technically acceptable tests was then explored in all children (n= 52) (Table 25). We reviewed all NICE recommended tests (spirometry, BDR, FeNO, and PEFv) and BCTmeth.

Table 25. Feasibility of performing NICE tests and BCTmeth (children)

	Number Completed (%)
spirometry	52/52 (100)
BDR	52/52 (100)
FeNO	42/52 (81)
PEFv	42/52 (81)
BCTmeth	38/45 (84)

Feasibility of performing technically acceptable tests was explored in all children. Of 52 children attending core visit one, all managed to perform spirometry and bronchodilator reversibility testing to a technically acceptable standard. Ten children (all <10 years old) did not complete FeNO due to inability to perform the test; when given a second opportunity at another visit, only three children (all under seven years old), failed to complete FeNO. Digital PEFv diary was returned in 42 children, of whom only 4 children successfully completed all 14 days monitoring. Of the ten children (mean age 8yrs (range 5-16)) who didn't complete monitoring, one child was unable due to a clinical need to commence early treatment. In the remaining children, poor compliance to return the monitor (n= 7) or not achieving the minimum four days of recordings (n= 2) was the cause of missing data. Of 45 children attending the BCT visit, 38 children successfully completed the test. Of the 7 children (mean age 8yrs (range 8-14)) that did not complete the test; five children were unable to complete due to concerns over poor concentration that may lead to inconsistent technique. The remaining two children were not offered a challenge because FEV_1/FVC ratio was too low, and the test was contra-indicated.

3.4.1.5. Overview of routine diagnostic measurements in asthma and correlations with novel asthma diagnostic measurements (whole population)

We investigate for similarities and differences between standard guideline recommended tests (FeNO, PEFv, FEV1/FVC ratio, FEV1%change) and other tests associated with asthma (blood eosinophils, FEV1%predicted MMEF%predicted, FEV1/FVC%change, and MMEF%change) not currently used in the diagnostic algorithm (NICE, 2017). We wanted to see if these other tests perform better in asthma diagnosis. We also wanted to know if presence of 'auscultated wheeze' was correlated with current recommended tests.

First, we looked at the relationship between standard FEV1/FVC ratio with novel spirometry output measurements, and standard post bronchodilation spirometry (FEV1%change) with novel post bronchodilation spirometry outputs (figure 27).

Figure 27. Relationship between i) baseline spirometry measurements and ii) post bronchodilation spirometry measurements

i)
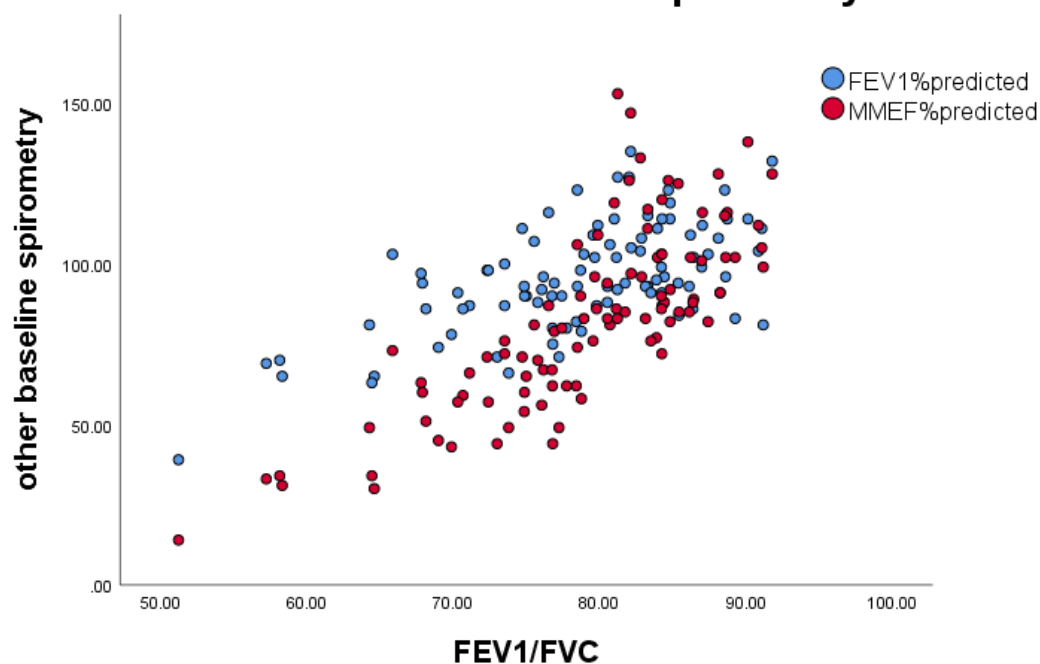
ii)

FEV1%change and other post bronchodilator spirometry measurements
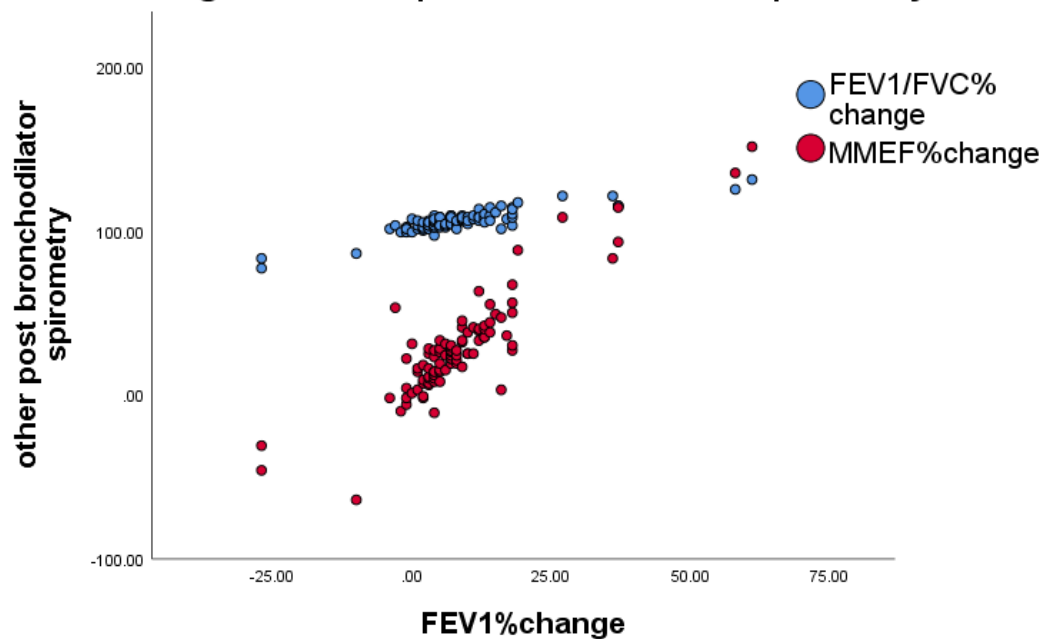
Current asthma diagnostic guidelines produced by NICE recommended spirometry output measurements FEV$_1$/FVC ratio and FEV$_1$%change. When we plotted other spirometry output measurements with these standard measurements, we show positive correlation between the tests indicating that these other measurements may be useful as an alternative; but not a replacement, test.

We then investigate standard asthma tests with the other common measurements (not currently featured in NICE diagnostic guidelines)in order to explore relationships between tests (Figure 28).

Figure 28. Relationship between standard asthma tests and potential novel diagnostic

tests

a) Comparison with FEV₁/FVC ratio
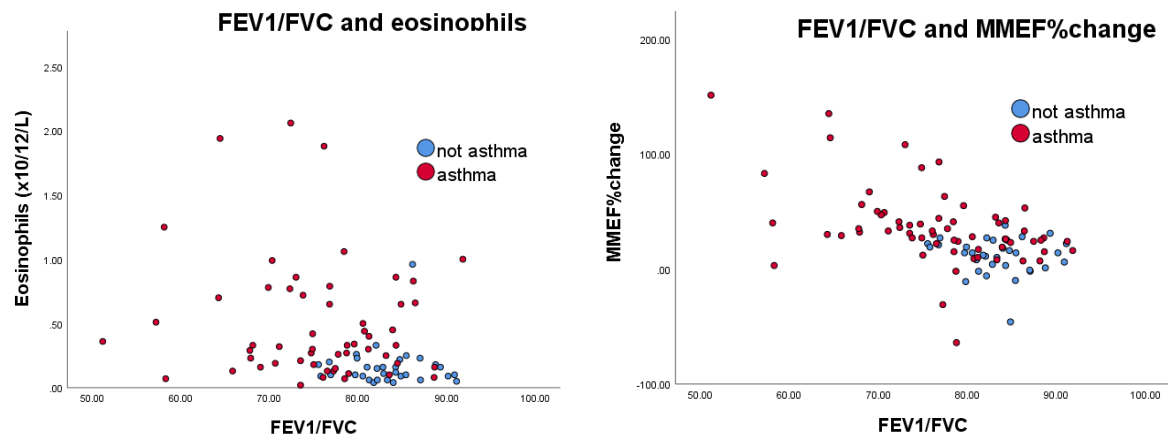
b) Comparison with FEV1%change

c) Comparison with PEFv

PEFv and eosinophils

PEFv and MMEF%predicted

PEFv and MMEF%change

d) Comparison with FeNO

FeNO and eosinophils

FeNO and MMEF%predicted

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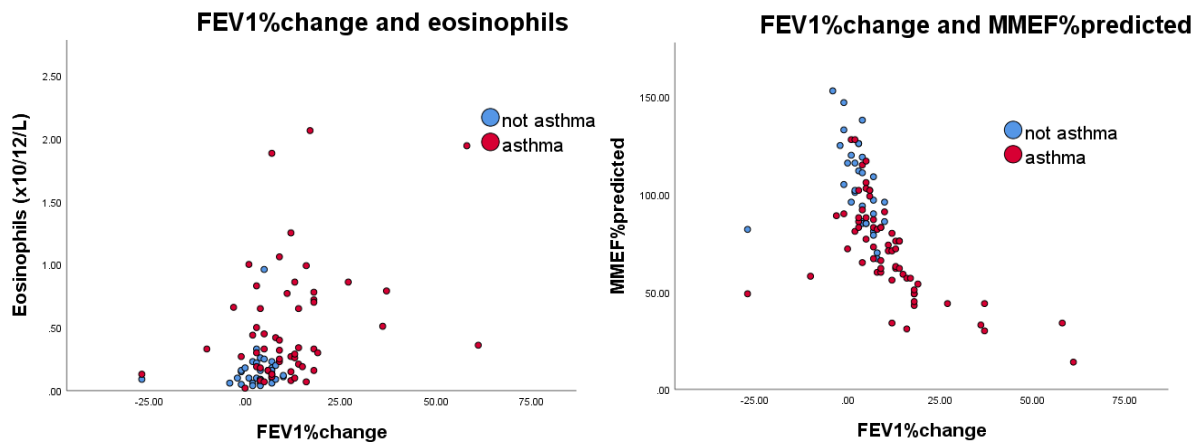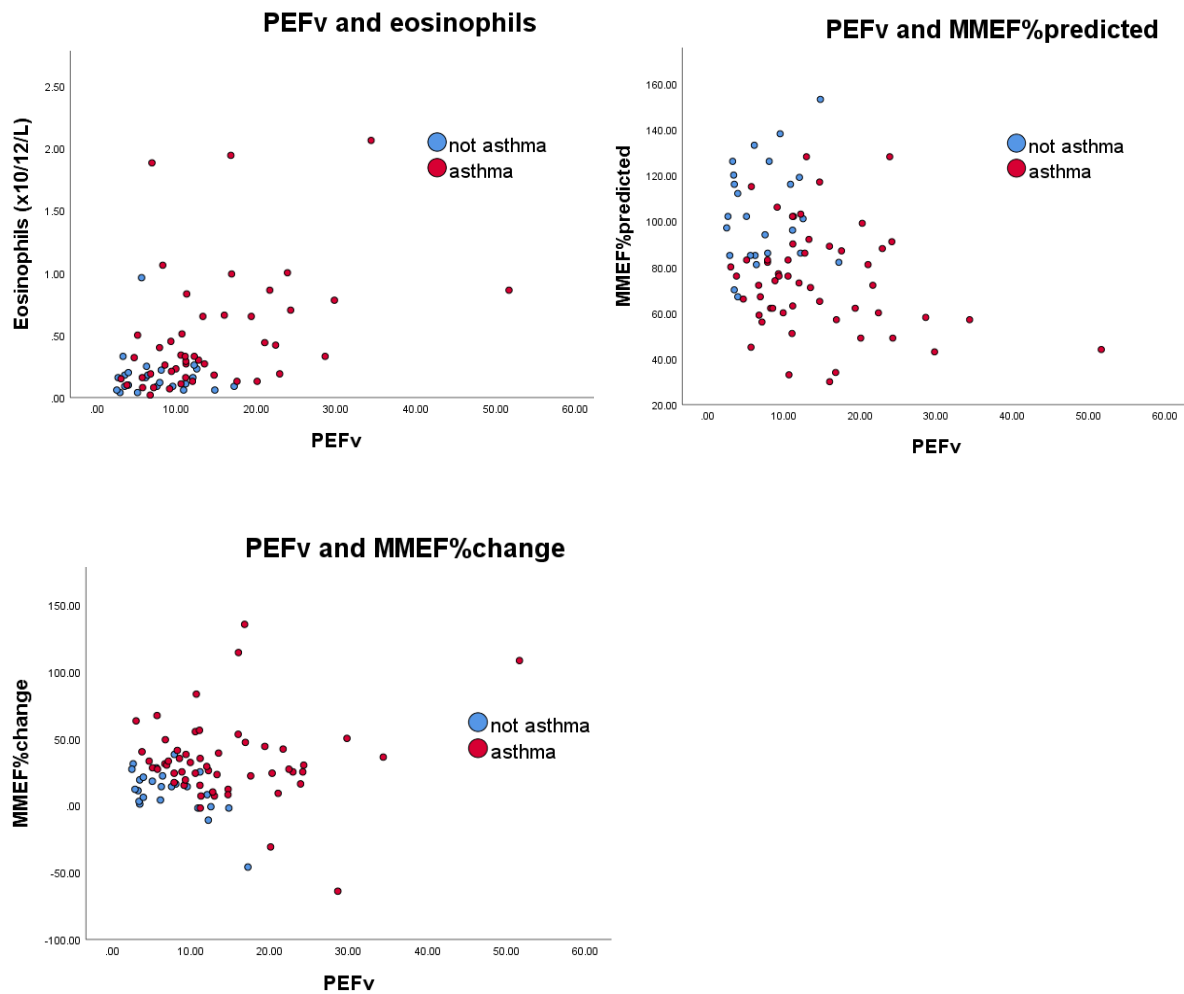
FeNO and MMEF%change
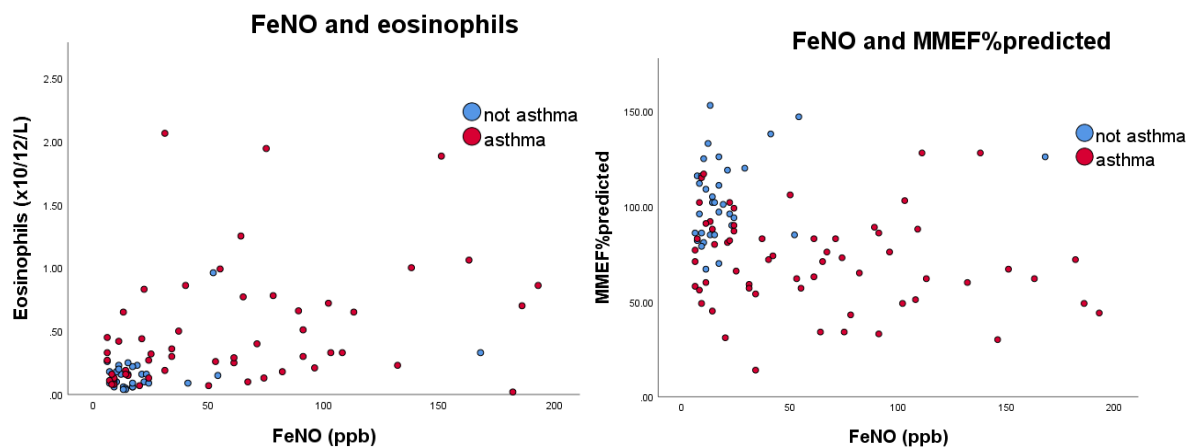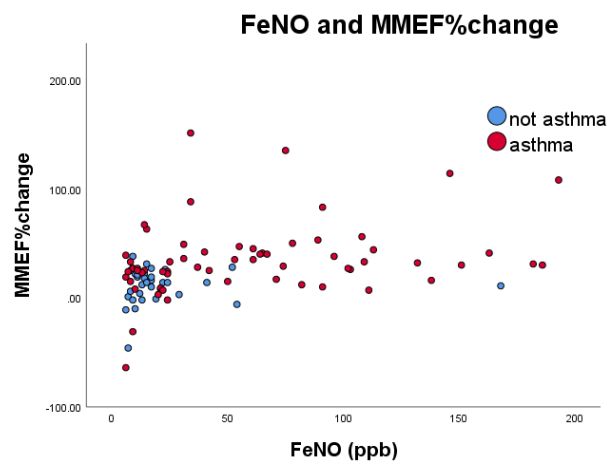
e) Comparison of wheeze with standard tests

FEV1/FVC and wheeze

FEV1%change and wheeze
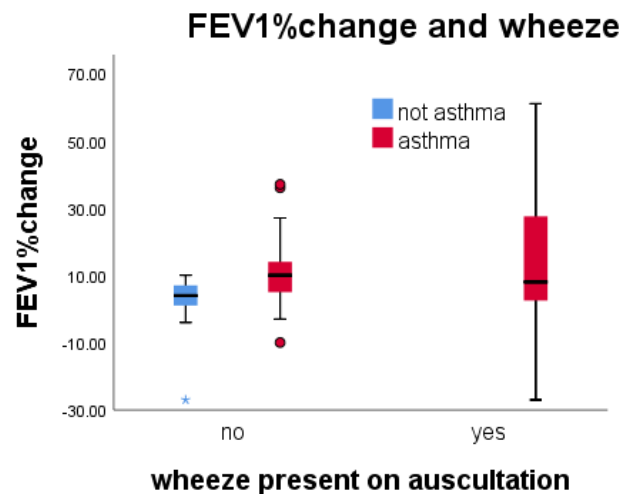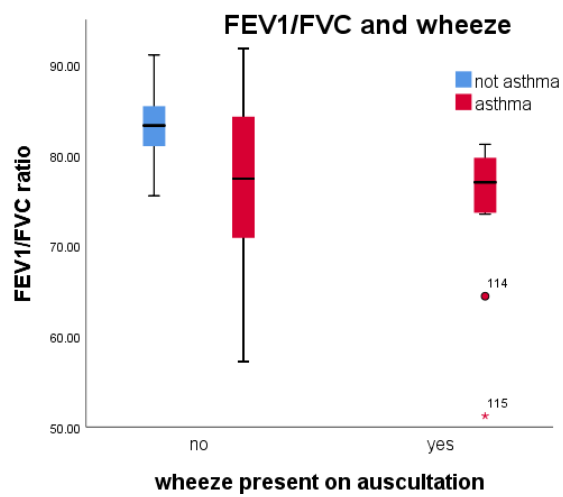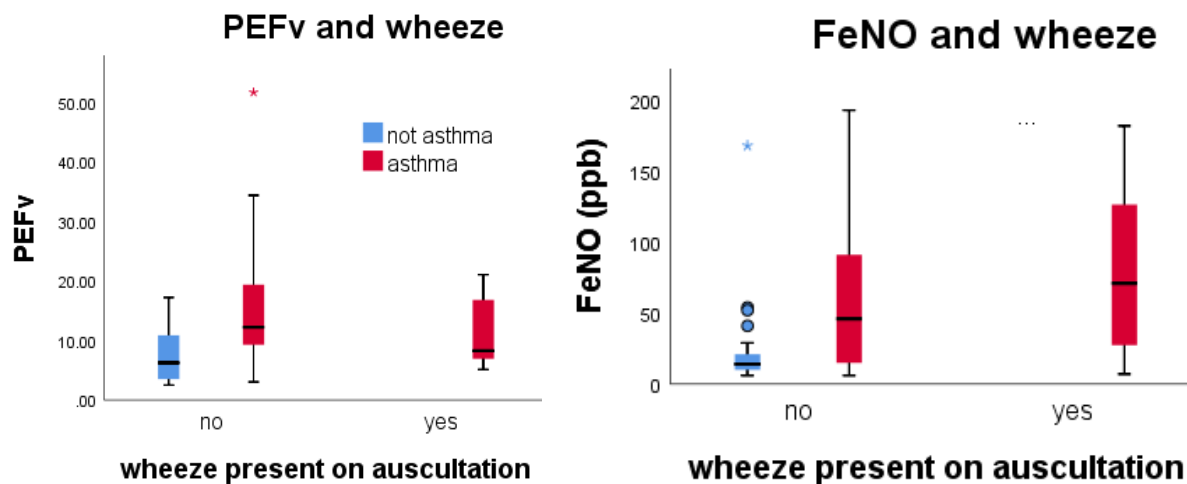
Our alternative measurements (i.e., eosinophils, MMEF%predicted, and MMEF%change) did not show strong correlation relationships with standard diagnostic tests. Therefore these tests could potentially be incorporated as additional diagnostic tests into an asthma diagnostic pathway if they are shown to be good predictors of asthma. We also looked at 'auscultated wheeze' as a diagnostic marker. Patients with auscultated wheeze were more likely to have lower FEV_1/FVC ratio, greater bronchodilator reversibility, more peak expiratory flow variability and higher FeNO when compared with 'not asthma' patients. Suggesting presence of an auscultated wheeze may be a useful diagnostic marker for asthma.

Correlations between the guideline recommended test of inflammation (i.e. FeNO) with other tests associated with airways inflammation

We explored the relationship between FeNO and other markers of allergy and inflammation. Scatter plots and box plots were generated to look for correlations between potential diagnostic tests that do not currently feature in the NG80 diagnostic algorithm (i.e., eosinophil level and skin prick testing), against FeNO in both 'asthma' and 'not asthma' groups (Figure 29).

Figure 29. FeNO and Eosinophils

FeNO and eosinophils (asthma vs not asthma)
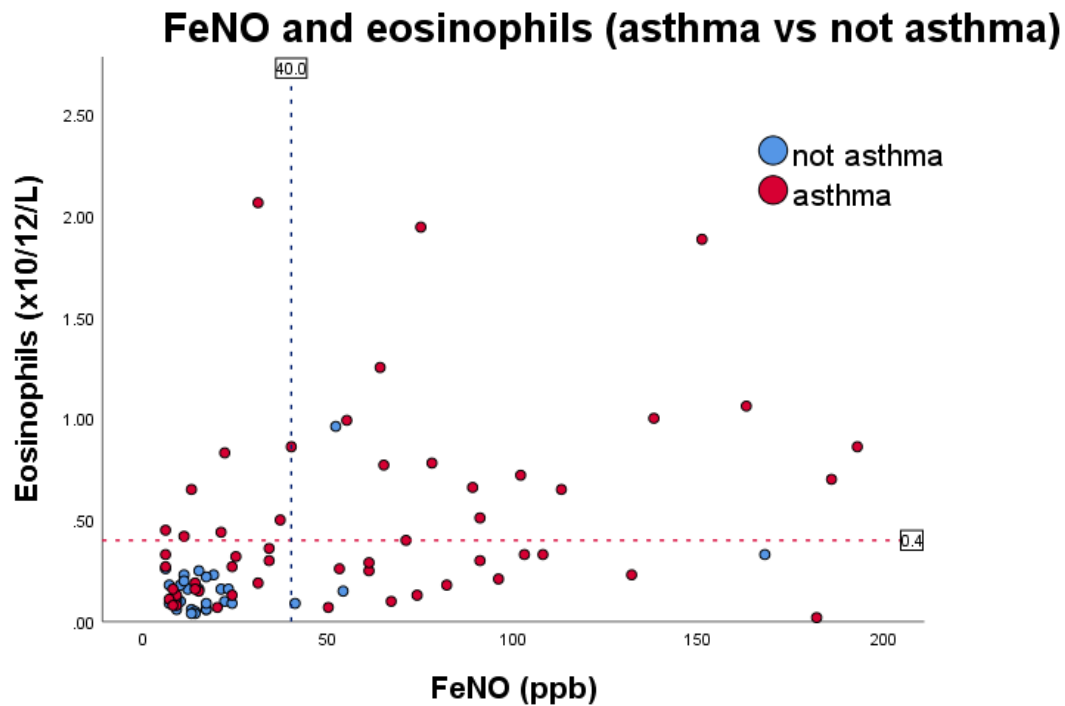
The scatter plots show in the 'not asthma' group both blood eosinophils and FeNO levels were predominantly low. High eosinophils were predominantly seen in asthma patients compared to not asthma patients. High FeNO levels were predominantly seen in asthma patients. However, the two tests do not correlate well together, therefore eosinophil levels could be used together with FeNO rather than to replace FeNO if they are shown to be a good predictor of asthma.

Figure 30. FeNO and atopic sensitisation

FeNO versus atopy
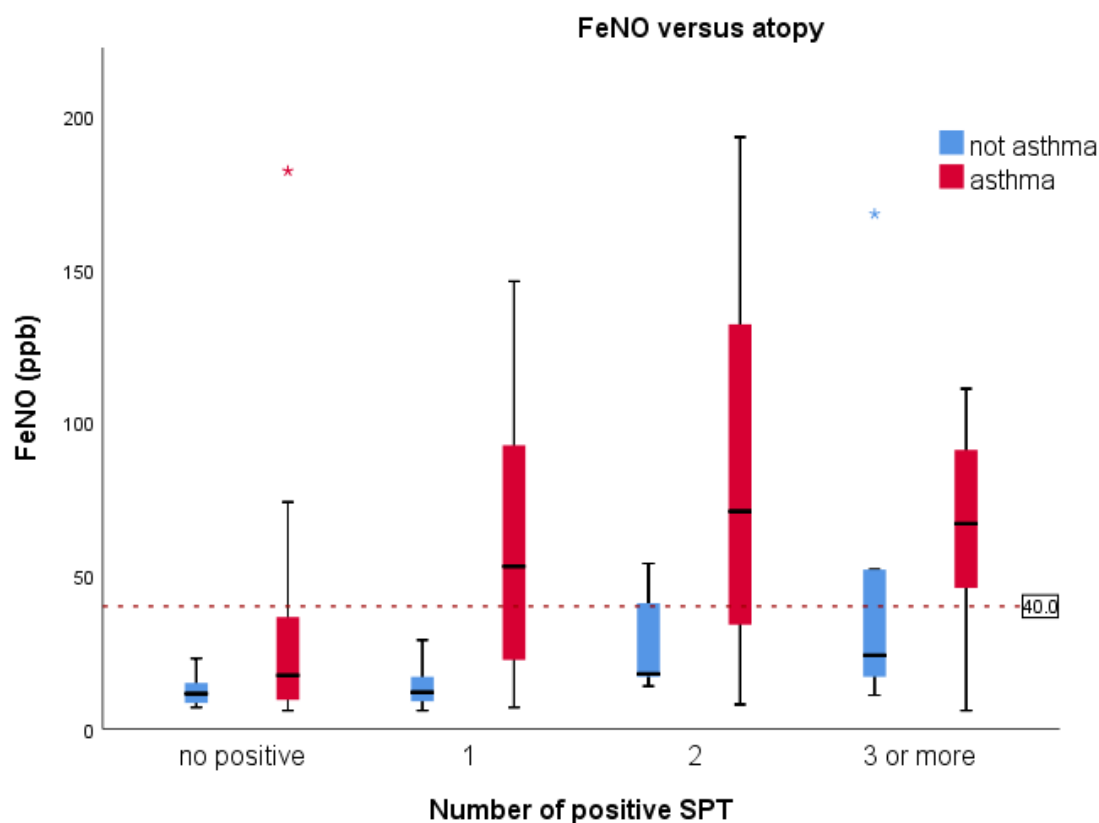
FeNO was also compared with skin prick testing (Figure 30). In the not asthma group, greater number of positive skin prick tests was associated with higher FeNO levels, however those with three or more positive skin prick tests still had a median FeNO of 24ppb (this is less than the NICE recommended cut-off for a positive test).(24) In the asthma group, median FeNO was greater than the NICE positive threshold for asthma in all patients that had at least one positive skin prick test. Where no allergic sensitisation was demonstrated, FeNO was more likely to be below the threshold for asthma diagnosis in both groups. We further explore the role of SPT in asthma diagnosis along with other tests (section 3.4.3 'predictors of asthma.')

3.4.1.6. Consort diagram of all patients included in final analysis

Figure 31. Consort diagram of the point at which patients were withdrawn ('asthma' and 'not asthma')

CV1 : n = 96

Withdrawn:
Subject Lost to follow up: n = 4
Clinical deterioration (started on ICS by GP): n = 3

Fast tracked due to pandemic:
CV2 cancelled move to CV3: n 1
CV2 cancelled move to CV4: n 3

CV2: n = 86

Withdrawn:
Adverse Event (Possible medication SE): n = 1

Withdrawn:
Subject Lost to follow up : n = 1

OV1: n = 47

Withdrawn:
Patient withdrew (patients decision) : n = 1

Withdrawn:
Subject Lost to follow up: n = 4
Patient withdrew (patients decision): n = 3

CV3: n = 59

Withdrawn:
Adverse Event (possible medication SE): n = 1

CV4: n = 78

Footnote: of the 96 patients (33 asthma and 63 not asthma) at CV1, 78 remained in the study through to the final visit CV4. 18 patients were withdrawn between the first and last visit. Not all patients in the study completed every visit. 86 of the 89 patients remaining in the study at CV2 attended. 59 of the 79 patients remaining in the study at CV3 attended. Not attending a visit was not an indication for withdrawal.

3.4.2. Accuracy of the NICE diagnostic algorithm (NG80) to predict asthma.

The NICE NG80 guideline proposes two algorithms for asthma diagnosis, one for children (5-16 years old) and one for adults (≥ 17 years old). We report upon the accuracy of each NG80 algorithm, we have separated our population into adults and children for the following analysis.

3.4.2.1. Subgroup analysis: adults ≥17yrs analysis

A subgroup analysis of the 63 adults (≥17years) was performed to test the performance of the adult NG80 algorithm. Of the 63 patients, 35 (55.6%) were coded EPOER asthma, 23 (36.5%) EPOER not asthma, 1 (1.6%) EPOER 'possible asthma,' and 4 (6.3%) EPOER 'insufficient evidence,' only patients (n= 58) with 'asthma' or 'not asthma' were used in the analysis, the remaining five patients are discussed above (section 3.4.1.3, *'patients excluded from the analysis,).*

Reason NICE algorithm not performed:

The algorithm could not be completed in 13/58 (22%) of the patients due to a missing test that prevented the patient to continue through the sequential algorithm (figure 32).

Figure 32. Reason NICE algorithm failed to be completed in adults
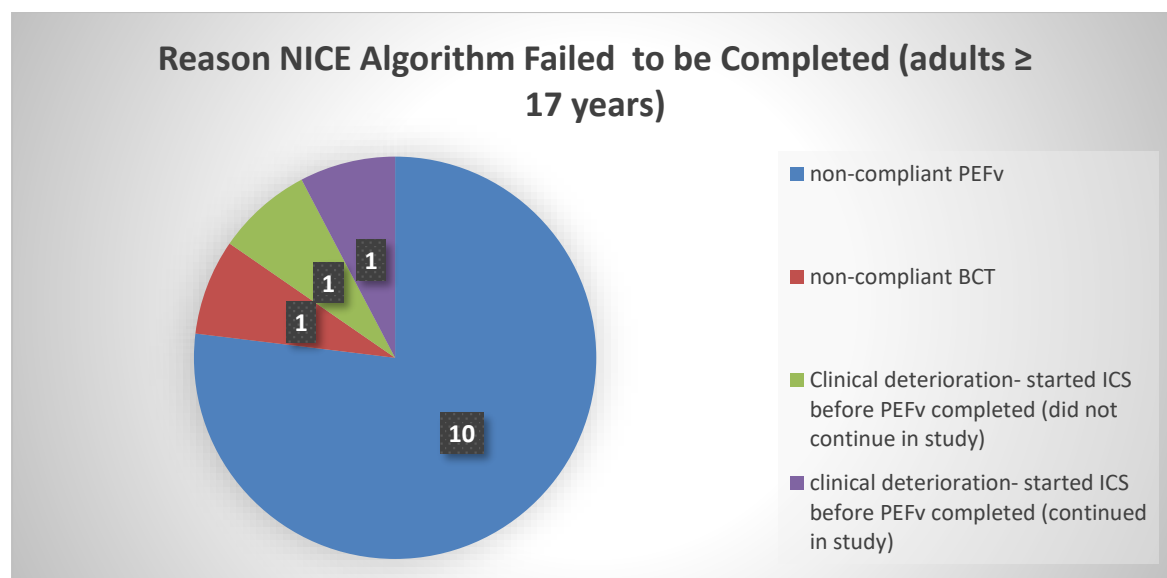
The most common reason that the algorithm was not able to be completed was non-compliance to complete the PEFv diary which occurred in 10 of the 13 (77%) of patients. The point at which the algorithm was terminated (resulting in more than a fifth of patients receiving no NG80 diagnostic outcome), is shown in figure 33. Clinical deterioration leading to early trial of ICS and non-compliance with a bronchial challenge test due to patient preference (anxiety) were the remaining reasons for unsuccessful completion of the algorithm.

Figure 33. The point at which the NICE algorithm terminated due to missing data in adults

Continuous line indicates patient flow through algorithm. Dotted line indicates the stage and reason the algorithm was terminated.
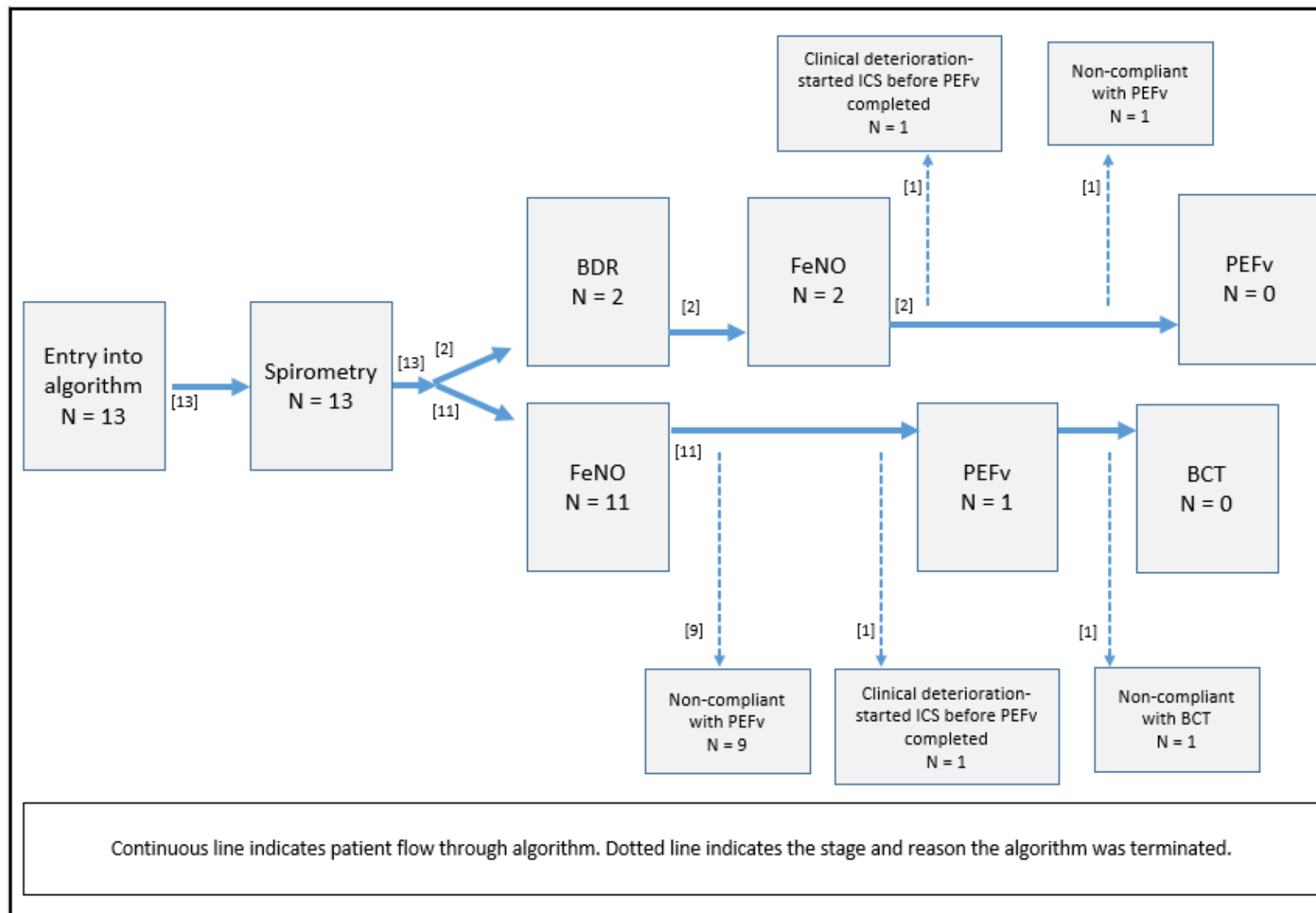
Overall performance of NG80 asthma with EPOER asthma in adults:

Of the remaining 45 patients that received a NG80 diagnosis, the algorithm was able to correctly diagnose EPOER confirmed 'asthma' or 'not asthma' in 27/45 (60%) of cases. However, the diagnosis was incorrect in a third of patients 15/45 (33.3%), of which EPOER confirmed asthma patients were coded as NG80 not asthma and therefore not commenced on treatment. Possible asthma was assigned in 3/45 (6.7%) of EPOER confirmed asthma patients; these patients would still receive a trial of treatment if following the NICE recommended guidelines (see table 26-27).

Table 26.   NG80 algorithm versus EPOER confirmed asthma in adults

EPOER Diagnosis				
		Not Asthma	Asthma	Total
NG80 Diagnosis	Not Asthma	17	15	32
	Asthma	0	10	10
	Possible Asthma	0	3	3
	Unable to complete NG80 (Missing tests)	6	7	13
	Total	23	35	58

Table 27.   Performance of the NG80 diagnostic algorithm

NG80 Outcome	Number	Percent
Correct Diagnosis i.e., 'NG80 Asthma' or 'NG80 Not Asthma'	27/45	60.0%
Incorrect Diagnosis i.e., 'NG80 Not Asthma'	15/45	33.3%
Incorrect Diagnosis i.e., 'NG80 Possible Asthma' (however patient put on appropriate treatment)	3/45	6.7%
Incorrect Diagnosis i.e., 'NG80 Possible Asthma' (however patient put on inappropriate treatment)	0/45	0.0%

Of the 35 patients with EPOER confirmed asthma, the NG80 was only able to definitively identify 10/35(28.6%) patients. The algorithm wrongly diagnosed 15/35(42.9%) as 'not asthma,' recommending an alternative diagnosis is sought or a specialist referral. Of the EPOER confirmed 'not asthma' patients, the NICE algorithm was able to correctly rule out asthma in 17/23(73.9%) of cases.

Performance of the NG80 to "rule in" asthma in adults:

When using the NICE diagnostic algorithm (NG80), one out of the three outcomes definitively "rules in" asthma, one is inconclusive and requires further clinical review before a diagnosis can be made, and one suggests 'not asthma' recommending alternate diagnosis or second opinion is sought. The performance of the NG80 to definitively "rule in" EPOER confirmed asthma had sensitivity 36%, specificity 100%, PPV 100% and NPV 49% (table 28).

Table 28. NG80 algorithm as a 'rule in' test

		EPOER Confirmed Asthma		Sn %	Sp %	PPV	NPV
		Positive	Negative				
NG80 Confirmed Asthma	Positive (i.e. asthma)	10	0	36	100	100	49
	Negative (i.e. not asthma/suspect asthma)	18	17				
Abbreviation: NICE diagnostic algorithm, NG80, Expert panel objective evidence review, EPOER, sensitivity, sn, specificity, sp, positive predictive value, PPV, negative predictive value, NPV							

Performance of the NG80 algorithm to "rule in" asthma (combining NG80 possible asthma with NG80 asthma; adults):

If patients with the NG80 outcome 'possible asthma' were to be included with the NG80 'asthma' patients; because both groups are treated as asthma with a trial of treatment, this improved the performance of the algorithm (sn 46%, sp 100%, PPV 100%, NPV 53%) (table 29).

Table 29. NG80 algorithm as a 'rule in' test (combining NG80 possible with asthma)

		EPOER Confirmed Asthma		Sn %	Sp %	PPV	NPV
		Positive	Negative				
NG80 Confirmed Asthma	Positive (i.e. asthma and possible asthma)	13	0	46	100	100	53
	Negative (i.e. not asthma)	15	17				
Abbreviation: NICE diagnostic algorithm, NG80, Expert panel objective evidence review, EPOER, sensitivity, sn, specificity, sp, positive predictive value, PPV, negative predictive value, NPV							

False negative cases

We discus all patients with false negative outcomes in detail (i.e., EPOER asthma with NG80 not asthma) below (section 3.4.2.3).

Pathways though the adult NG80 algorithm:

There are 18 diagnostic pathways through the adult NICE algorithm (section 3.3.1.2, table 14, and figure 22). We reviewed which pathways were most used and which pathways seemed to perform most well and least well (table 30). The small numbers of patients for each pathway are acknowledged in this exploratory analysis, more patients would be needed to draw firm conclusions.

Table 30. Performance of NICE (NG80) pathway in adults

		Performance of NG80 Algorithm in EPOER Asthma and not Asthma confirmed Patients				
		Correct diagnosis (True positive and True Negative)	Incorrect Diagnosis (False positive and false negative)	Inconclusive (NG80 'suspect asthma' - appropriated treated)	Inconclusive (NG80 'Suspect asthma' - inappropriately treated)	Total
NICE NG80 Algorithm pathway	**1** asthma	6	0	N/A	N/A	**6**
	3 possible asthma	N/A	N/A	1	0	**1**
	4 not asthma	0	2	N/A	N/A	**2**
	5 possible asthma	N/A	N/A	2	0	**2**
	9 not asthma	0	1	N/A	N/A	**1**
	11 asthma	4	0	N/A	N/A	**4**
	12 not asthma	2	2	N/A	N/A	**4**
	15 not asthma	15	10	N/A	N/A	**25**
Total		**27**	**15**	**3**	**0**	**45**
Of the 58 patients, the algorithm could not be completed in 13 patients due to missing tests						

The following pathways were never utilised in our cohort: 2, 6, 7, 8, 10, 13, 14, 16, 17, and 18. The most used pathway was 15, this pathway leads to a diagnosis of "not asthma" and included spirometry negative, FeNO negative, and PEFv negative. However, this pathway led to a missed diagnosis (i.e., false negative diagnosis) in 10/25 (40%) patients (see section 3.4.2.3 below, tables 39-40). The main reasons identified for the algorithm failing included borderline negative results and objective response on tests post steroids not being

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accounted for by the algorithm. This pathway did not utilise BCT, more than two fifths of patients in pathway 15 with a false negative diagnosis had positive bronchial challenge with methacholine.

Pathways 1 and 11 always resulted in a true positive result (i.e., NG80 asthma diagnosed in EPOER asthma patients. However, this only accounted for 10 patients. These paths require at least three positive tests (pathway 1) or confirmation with a positive BCT (pathway 11).

Pathways 4 and 9 also resulted in false negative outcome (i.e., NG80 not asthma in EPOER asthma patients) every time, however this only accounted for 3 patients. Pathway 4 leads to not asthma in patients with negative FeNO and PEFv despite positive FEV_1/FVC with BDR, Pathway 9 leads to not asthma in patients with negative FeNO and BDR despite positive FEV_1/FVC. On review of this one case (RAD032) he received EPOER diagnosis of asthma based on strong clinical history (felt high probability), plus positive eosinophils (>0.4 x10^9/L), positive PEFv (>20%), borderline and BCTmeth (PD$_{20}$ 0.248mg) with subjective improvement in symptoms following treatment.

Patients with an inconclusive NG80 outcome of "suspect asthma" are started on a trial of asthma treatment if following the NICE recommendations. Pathways 3 and 5 both lead to patients coded NG80 "possible asthma," on review the 3 patients that used these paths all had EPOER confirmed asthma.

3.4.2.2.   Subgroup analysis: children <17yrs analysis

A subgroup analysis of 52 children (<17years) was performed to analyse the performance of the NG80 paediatric algorithm. Of the 52 patients 28 (53.8%) were coded EPOER confirmed 'asthma,' 10 (19.2%) EPOER confirmed 'not asthma,' 3 (5.8%) EPOER 'possible asthma,' and 11 (21.2%) EPOER 'insufficient evidence.' Only patients (n =38) with 'asthma' or 'not asthma' were used in the analysis, the remaining fourteen patients are discussed in detail below (section 3.4.2.3, table 40).

Reason NICE algorithm not completed:

The algorithm could not be completed in 4/38 (11%) of the patients due to a missing test that prevented the patient to continue through the sequential algorithm (figure 34).

Figure 34. Reason NICE algorithm failed to be completed in children
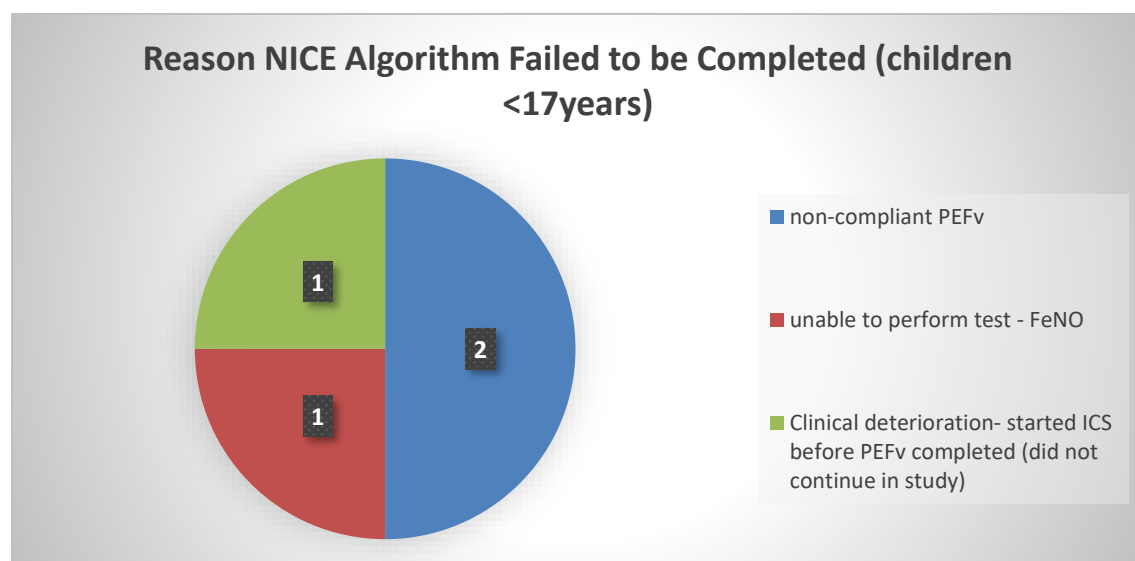
When also analysing the additional 14 patients with EPOER 'insufficient evidence' or 'possible asthma' the algorithm could not be performed in 7/14 (50%) due to; non-compliance with PEFv (n = 5), unable to perform FeNO (n = 1), and unable to perform PEFv in home setting (n = 1) were the reasons for failure to complete the NICE algorithm.

Overall performance of NG80 asthma and EPOER asthma:

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Of the remaining 34 patients that received a NG80 diagnosis, the algorithm was able to correctly diagnose EPOER confirmed 'asthma' or 'not asthma' in 17/34 (50%) of cases. However, the diagnosis was incorrect in 5/34 (15%) of cases, in which EPOER confirmed asthma patients were coded as NG80 not asthma and therefore not commenced on treatment. Possible asthma was assigned in 11/34 (32%) of EPOER confirmed asthma patients; these patients would still receive a trial of treatment if following the NICE recommended guidelines (see table 31-32).

Table 31. NG80 algorithm versus EPOER confirmed asthma in children

		EPOER Diagnosis		
		Not Asthma	Asthma	Total
NG80 Diagnosis	Not Asthma	7	5	12
	Asthma	0	10	10
	Possible Asthma	1	11	12
	Unable to complete NDA (Missing tests)	2	2	4
	Total	10	28	38

Table 32. Performance of the NG80 diagnostic algorithm in children

NG80 Outcome	Number	Percent
Correct Diagnosis i.e., 'NG80 Asthma' or 'NG80 Not Asthma'	17/34	50.0%
Incorrect Diagnosis i.e., 'NG80 Asthma' or 'NG80 Not Asthma'	5/34	14.7%
Incorrect Diagnosis i.e., 'NG80 Possible Asthma' (however patient put on appropriate treatment)	11/34	32.4%
Incorrect Diagnosis i.e., 'NG80 Possible Asthma' (however patient put on inappropriate treatment)	1/34	2.9%

Of the 28 patients with EPOER confirmed asthma, the NICE algorithm was only able to definitively identify 10/28(35.7%) patients. The algorithm wrongly diagnosed 5/28(17.9%) as 'not asthma,' recommending an alternative diagnosis is sought or a specialist referral. Of the EPOER confirmed 'not asthma' patients, the NICE algorithm was able to correctly rule out asthma in 7/10(70.0%) of cases.

Performance of the NG80 to "rule in" asthma in children:

When using the NICE diagnostic algorithm (NG80), one out of the three outcomes definitively "rules in" asthma, one is inconclusive and requires further clinical review before

a diagnosis can be made, and one suggests 'not asthma' and that an alternate diagnosis is to be sought. The performance of the NG80 to definitively "rule in" EPOER confirmed asthma had sensitivity 38%, specificity 100%, PPV 100% and NPV 33% (table 33).

Table 33.   NG80 algorithm as a 'rule in' test in children

		EPOER Confirmed Asthma		Sn %	Sp %	PPV	NPV
		Positive	Negative				
NG80 Confirmed Asthma	Positive (i.e. asthma)	10	0	38	100	100	33
	Negative (i.e. not asthma/possible asthma)	16	8				
Abbreviation: NICE diagnostic algorithm, NG80, Expert panel objective evidence review, EPOER, sensitivity, sn, specificity, sp, positive predictive value, PPV, negative predictive value, NPV							

Performance of the NG80 to "rule in" asthma (combining NG80 possible asthma with NG80 asthma) (children):

If patients with the NG80 outcome 'possible asthma' were to be included with the NG80 'asthma' patients; because both groups are treated as asthma with a trial of treatment, this improved the overall sensitivity of the algorithm but reduced its performance as a "rule in" asthma test (sn 81%, sp 88%, PPV 95%, NPV 58%) (table 34).

Table 34.   NG80 algorithm as a 'rule in' test in children (combining NG80 possible asthma with asthma)

		EPOER Confirmed Asthma		Sn %	Sp %	PPV	NPV
		Positive	Negative				
NDA Confirmed Asthma	Positive (i.e. asthma/ possible asthma)	21	1	81	88	95	58
	Negative (i.e. not asthma)	5	7				
Abbreviation: NICE diagnostic algorithm, NG80, Expert panel objective evidence review, EPOER, sensitivity, sn, specificity, sp, positive predictive value, PPV, negative predictive value, NPV							

False negative cases

We discus all patients with false negative outcomes (i.e., EPOER asthma with NG80 not asthma) in detail below (section 3.4.2.3).

Pathways though the Paediatric NICE Algorithm:

There are eight diagnostic pathways through the paediatric NICE algorithm, for the purpose of this thesis they have been labelled 19 to 26 (see table 14 and figure 23). We reviewed which pathways were most used and which pathways seemed to perform best and worst. The small numbers of patients for each pathway are acknowledged in this exploratory analysis, more patients would be needed to make assumptions.

Table 35. Performance of NG80 algorithm in EPOER asthma and not asthma confirmed patients

		Performance of NG80 Algorithm in EPOER Asthma and not Asthma confirmed Patients				
		Correct diagnosis (True positive and True Negative)	Incorrect Diagnosis (False positive and false negative)	Inconclusive (NG80 'Possible asthma' - appropriated treated)	Inconclusive (NG80 'Possible asthma' - inappropriately treated)	Total
NICE NG80 Algorithm Pathway	19 asthma	8	0	N/A	N/A	8
	21 possible asthma	N/A	N/A	1	0	1
	22 not asthma	0	2	N/A	N/A	2
	23 asthma	2	0	N/A	N/A	2
	24 possible asthma	N/A	N/A	6	1	7
	25 possible asthma	N/A	N/A	4	0	4
	26 not asthma	7	3	N/A	N/A	10
Total		17	5	11	1	34
Of the 38 patients, the algorithm could not be completed in 4 patients due to missing tests						

All pathways through the algorithm were used except for pathway 20 (spirometry positive, BDR negative, FeNO positive, PEFv positive) (table 35). The most used pathway was pathway 26; this pathway leads to a diagnosis of "not asthma" and included spirometry negative, FeNO negative, PEFv negative. This pathway resulted in the correct diagnosis 70% of the time. However, this pathway leads to a false negative diagnosis of 'not asthma' in 3/10 (30%). Of these 3 patients with EPOER confirmed asthma but NG80 Not asthma; RAD061,

RAD069, and RAD105, all cases were young children < 8 years with borderline tests (but not meeting NICE thresholds), all were atopic and had a positive BCT to Methacholine.

Pathways 19 and 23 always resulted in a true positive result (i.e., NG80 asthma diagnosed in EPOER-confirmed asthma patients (however this only accounted for 10 patients). Both paths require two positive tests.

Pathway 21 and 25 resulted in the NG80 diagnosis of possible asthma (n= 5), all cases were EPOER confirmed asthma.

3.4.2.3.   False negative cases (whole population)

Demographics and clinical characteristics for the twenty EPOER confirmed 'asthma' patients with NG80 outcome 'not asthma,' are described and compared with clinical characteristics of patients with confirmed EPOER 'asthma' and confirmed EPOER 'not asthma' (table 36-38). No false positive cases were identified.

Table 36.   False negative cases using the NG80 algorithms (comparison of demographics with asthma and not asthma groups in adults and children)

Patient Demographics	All Cases (N 115)	EPOER Asthma (N 63)	EPOER Not Asthma (N 33)	False Negative Algorithm Cases (i.e. EPOER asthma patients coded as NG80 'not asthma' (N 20)
Age, mean (SD) years	23.33 (15.57)	22.71 (15.38)	29.76 (16.09)	25.50 (14.17)
Adults, n (%)	63 (54.8)	35 (55.6)	23 (69.7)	15 (75.0)
Gender, n (%) females	64 (55.7)	33 (52.4)	22 (66.7)	15 (75.0)
Ethnicity, n (%) white	79 (68.7)	43 (68.3)	26 (78.8)	16 (80.0)
BMI, mean (SD) kg/m^2	24.59 (6.95)	24.23 (6.62)	26.59 (7.37)	26.66 (7.57)
Current or ex-smokers, n (%)	21 (18.3)	10 (15.9)	8 (24.2)	4 (20.0)
Pack years, median (IQR)	0.00 (0.00-0.00)	00.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.00)
Respiratory clinician suspect "high probability asthma), n (%)	56 (48.7)	41 (65.1)	7 (21.2)	10 (50.0)

Three-quarters of false negative patients were adults. The median BMI of this group was >25, which is clinically overweight, this was similar to the EPOER confirmed 'not asthma' group. Following the initial clinical consultation, half of the patients with a false negative NG80 outcome were categorised as 'high probability' of asthma following history and clinical examination, but before investigations.

Table 37. False negative cases using the NG80 algorithm (comparison of conventional tests with asthma and not asthma groups)

Conventional tests	All Cases (N 115)	EPOER Asthma (N 63)	EPOER Not Asthma (N 33)	Failed Algorithm Cases (i.e. EPOER asthma patients coded as NG80 'not asthma' (N 20)
FEV$_1$, mean (SD) L	2.71 (1.12) N = 114	2.64 (1.16)	3.07 (1.03)	2.88 (1.24)
FEV$_1$, mean (SD) %predicted	96.45 (15.95) N = 114	91.70 (16.81)	103.64 (13.41)	99.85 (15.47)
FVC, mean (SD) L	3.47 (1.50) N = 114	3.52 (1.58)	3.71 (1.30)	3.62 (1.59)
FVC, mean (SD) % predicted	103.55 (13.56) N = 114	102.64 (14.60)	104.70 (13.72)	107.25 (14.51)
FEV$_1$/FVC Ratio, mean (SD) %	79.69 (8.37) N = 114	76.52 (8.73)	83.37 (4.12)	80.01 (5.52)
BDR, median (IQR) %	7.00 (102.75-111.25) N = 114	9.00 (5.00-15.00)	4.00 (1.00-7.00)	5.00 (3.00-8.00)
FeNO, median (IQR) ppb	21.00 (11.00-63.25) N = 112	50.00 (17.50-93.50) N = 61	14.00 (9.50-21.50)	13.50 (8.25-24.00)
PEFv Calculation, median (IQR) %	10.56 (6.13-16.02) N = 91	11.64 (8.47-19.55) N = 54	6.24 (3.51-11.02) N = 25	11.17 (6.28-14.40)
PEFv Number Days >20%, median (IQR) days (data from returned PEF meters with minimum 4 days data)	3.0 (0.00-3.00) N = 91	2.00 (0.00-4.00) N = 54	0.00 (0.00-1.50) N = 25	2.00 (0.00-3.75)
Eos, median (IQR) x10^{9}cells/L	0.22 (0.12-0.45) N = 103	0.33 (0.18-0.72) N = 55	0.12 (0.09-0.20) N = 32	0.27 (0.13-0.43) N = 18
SPT (Number allergens sensitised), mean (SD)	1.48 (1.45) N = 113	1.76 (1.46)	1.09 (1.36)	1.55 (1.60)

BCTmeth PD$_{20}$, median (IQR) (N = 40 had <20% drop by final dose, therefore a censored PD$_{20}$ of 1.92mg was recorded)	0.54 (0.07-1.92) N = 84	0.116 (0.023-0.32) N = 47	1.92 (1.92-1.92) N 30	0.23 (0.07-0.53) N 19
Wheeze auscultated, n (%)	12 (10.4)	12 (19.0)	0 (0.0)	3 (15.0)

The groups average for spirometry (FEV$_1$, FVC, FEV$_1$/FVC ratio) and bronchodilator reversibility was similar to EPOER diagnosed 'asthma' and 'not asthma' groups. PEFv was similar to EPOER asthma group. However, FeNO was low and more comparable with the not asthma group. The false negative group were more sensitive to bronchial challenge testing with methacholine than the not asthma group.

Table 38. False negative cases using the NICE algorithm (comparison of conventional tests using dichotomised cut-offs, with asthma and not asthma groups)

Conventional tests (dichotomised cut-offs)	All Cases (N 115)	EPOER Asthma (N 63)	EPOER Not Asthma (N 33)	Failed Algorithm Cases (i.e. EPOER asthma patients coded as NG80 'not asthma') (N 20)
FEV$_1$/FVC <70%, n (%) positive	15 (13.2) N = 114	13 (20.6)	0 (0.0)	1 (5.0)
FEV$_1$/FVC <LLN, n (%) positive	26 (22.6) N = 114	24 (38.1)	0 (0.0)	5 (25.0)
FeNO, n (%) positive (adult: FeNO ≥40ppb, child: FeNO ≥35ppb)	39 (34.8) N = 112	33 (54.1) N = 61	4 (12.1)	2 (10.0)
BDR, n (%) positive (adult: ΔFEV1 ≥12% and 200ml, child ΔFEV$_1$ ≥12%)	27 (23.7) N = 114	26 (41.3)	0 (0.0)	3 (15.0)
PEFv >20%, n (%) positive	15 (16.5%) N = 91	13 (24.1) N = 54	0 (0.0)	3 (15.0)
PEFv(alt), n (%) positive	30 (33.0) N = 91	24 (44.4) N = 54	1 (4.0) N = 25	9 (45.0)
Eos >0.4x10^9/L, n (%) positive	28 (27.2) N = 103	23 (41.8) N = 55	1 (3.1) N = 32	5 (27.8) N = 18
Atopic (SPT ≥1 positive)	75 (65.2) N = 114	48 (76.2)	17 (51.5)	12 (60.0)
BCTmeth PD$_{20}$, n (%) positive (≤0.2mg threshold)	32 (38.1) N 84	30 (63.8) N = 47	1 (3.3) N = 30	9 (60%) N = 15

Using the conventional cut-offs for positive recommended by NICE. In the false negative group, one quarter had obstructed spirometry (FEV$_1$/FVC<LLN). Fifteen percent had significant BDR, and fifteen percent had positive PEFv using a standard recommended calculation. This increased to sixty percent when using the alternative PEFv criteria. Three out of five patients in this group that managed to complete BCTmeth had a positive challenge test.

Detailed exploration of False Negative Cases

Of the twenty patients, thirteen patients (65%) had at least one positive test from the five tests NICE guidelines recommend in asthma diagnosis (i.e., FEV$_1$/FVC, BDR, FeNO, PEFv, BCTmeth), this was in addition to their clinical referral for symptomatic physician suspected

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asthma. Of the fifteen adults with false negative NG80 diagnosis, eight adults had at least one positive test. Of the five children with false negative NG80 diagnosis, all five had a positive BCT (and some had other positive tests as well). Of note, BCT does not currently feature in the NG80 paediatric diagnostic algorithm.

The remaining seven patients (all adults, 17years+) had all negative test results using current NICE recommended thresholds for a positive result. The NG80 algorithm does not account for borderline results that are just below the threshold. The rationale for why these seven patients were diagnosed with EPOER-confirmed asthma is shown (table 39). Despite having negative NG80 algorithm tests, all seven patients had some objective evidence to support EPOER asthma diagnosis when considering the continuous data for NG80 tests (opposed to dichotomised cut-off thresholds) and when considering additional supporting evidence (e.g., clinical consultation, SPT, eosinophils, treatment response).

Table 39.   Patients with EPOER diagnosed asthma despite all baseline NICE recommended tests negative

Patient	Patient summary	Tests	Results		NICE NG80 path
			Pre ICS	Post ICS	
Patient 1 (RAD002) 44Female	Presented with variable symptoms of sob, nocturnal cough and wheeze. Previous diagnosis of 'childhood asthma'15% PEFv with 2days over 20% variability. Positive BCT at the last visit. Subjective improvement with ICS treatment.	ACQ5 FEV$_1$/FVC BDR FeNO PEFv PEFv (alt) Wheeze BCTmeth Eos	3.6 points 83.30 (LLN 70%) 5% 10 ppb 15% 2 Days >20% No PD$_{20}$ 0.53 (FEV1 26% Drop) Unsuccessful attempt at blood sampling	1.4 PD20 0.157	15
Patient 2 (RAD050) 23Female	Clinical consultation coded 'high possibility asthma' from clinical history. Evidence of 20% diurnal	ACQ5 FEV$_1$/FVC BDR FeNO PEFv	1.4 points 86.22 (LLN 76%) 3% 22ppb 11%	0 points 83.01 - 17 -	15

	variability on three days with high eosinophils. Plus subjective and objective improvement post treatment.	PEFv (alt) Wheeze BCTmeth Eos	3 Days >20% No FEV1 10% drop 0.83 x10^9/L	- - FEV1 5.5% drop 0.31 x10^9/L	
Patient 3 (RAD064) 29Male	Clinical consultation coded as 'high possibility asthma' from clinical history. GP reported wheeze. History of childhood asthma. Atopic. Borderline obstruction (based on LLN) for age, with 9% BDR and eosinophils 0.32x10^9/L.	ACQ5 FEV$_1$/FVC BDR FeNO PEFv PEFv (alt) Wheeze BCTmeth Eos	0.6 points 71.11 (*LLN 71%) 9% 25ppb 3% 0 days >20% No FEV1 3% drop 0.32 x10^9/L	1.6 points 76.65 - 21ppb - - - FEV1 2% drop 0.13 x10^9/L	15
Patient 4 (RAD074) 28Female	Clinical consultation coded as 'high possibility asthma' from clinical history. Variable cough, wheeze, chest-tightness. Diagnosis of childhood asthma. BCT not technically positive but objective improvement post ICS and subjective improvement in symptoms post ICS.	ACQ5 FEV$_1$/FVC BDR FeNO PEFv PEFv (alt) Wheeze BCTmeth Eos	0.8points 88.56 (LLN 74%) 4 % 9 ppb 6 % 0 Days >20% no PD$_{20}$ 0.516 (FEV1 28% drop) 0.08 x10^9/L	0 points FEV1 0% drop	15
Patient 5 (RAD075) 27Female	Presented with variable symptoms of sob, cough, chest-tightness and wheeze. Wheeze auscultated. BCT mild AHR (PD20 0.229) using ERS thresholds. Spirometry and BCT improved post ICS. Subjectively improved on ICS.	ACQ5 FEV$_1$/FVC BDR FeNO PEFv PEFv (alt) Wheeze BCTmeth Eos	2.2 points 78.93 (LLN is 74%) 7 % 7 ppb (repeat CV2 15ppb) 11% 4 Days >20% Present PD$_{20}$ 0.229 (FEV1 20% drop) 0.11 x10^9/L	1.2 82.41 PD20 1.92 (FEV1 14% drop)	15

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Patient	Patient summary	Tests	Results (Pre ICS)	Results (Post ICS)	NICE NG80 path
Patient 6 (RAD096) 25 Male	Presented with cough. Evidence of 11% PEFv, 3 days >20% diurnal variation. AHR (PD$_{20}$<0.4) and positive BCT mannitol.	ACQ5 FEV$_1$/FVC BDR FeNO PEFv PEFv (alt) Wheeze BCTmeth BCTmann Eos	0.2 points 88.62 (LLN 73%) 6 % 8ppb (14 at CV2) 11 % 3 Days >20% no PD$_{20}$ 0.318 (FEV1 38% drop) PD$_{15}$ 423 (FEV1 18% drop) 0.16 x10^9/L		15
Patient 7 (RAD103) 30Female	Clinical consultation coded as 'high possibility asthma' from clinical history. Wheeze auscultated. Positive FeNO (using CV2 data - not used for NG80 algorithm but if it was used the result would still be 'Not Asthma'), high eosinophils. Subjectively improved with treatment.	ACQ5 FEV$_1$/FVC BDR FeNO PEFv PEFv (alt) Wheeze BCTmeth Eos	1.6 points 80.54 (LLN 76%) 3% 37ppb (repeat at CV2 58ppb) 5% 0 days >20% Yes PD$_{20}$ >0.9 (FEV1 19% drop) 0.50 x10^9/L	0.6 points - - - - - - -	15

We also show detailed summary of the other thirteen patients (table 40).

Table 40. Patients with EPOER diagnosed asthma and NG80 not asthma with some baseline NICE recommended positive tests

Patient	Patient summary	Tests	Results		NICE NG80 path
			Pre ICS	Post ICS	
(RAD006) 47Female	Obstructive spirometry with bronchodilator reversibility and positive Methacholine challenge. Objective improvement post treatment.	ACQ5 FEV$_1$/FVC BDR FeNO PEFv PEFv (alt) Wheeze	0.8 points 68.99% (LLN 70%) 18% 14ppb 5.7%(13 days) 0 No	0 points 74% - 10 - - no	4

		BCTmeth	PD$_{20}$ 0.103 (FEV1 -26.8%)	0.331	
		Eos	0.16	0.14	
(RAD007) 6 male	Symptomatic with wheeze on auscultation. Obstructive spirometry. PEF variability. Raised eosinophils at final visit.	ACQ5 FEV$_1$/FVC BDR FeNO PEFv PEFv (alt) Wheeze BCTmeth Eos	1.4 points 77% (LLN is 80%) -17% 9ppb 20% 4 Yes 0.116 0.13	1 74% - 7 - - No - 0.48	22
(RAD010) 22 Female	Symptomatic with borderline obstruction (<LLN) and bronchodilator reversibility. Withdrawn due to developing widespread rash post salbutamol, unlikely to be related but patient withdrawn.	ACQ5 FEV$_1$/FVC BDR FeNO PEFv PEFv (alt) Wheeze BCTmeth Eos	4.2 points 75% (LLN 77%) 12.5% 6 ppb 13% 4 No - 0.27	- - - - - - - - -	4
(RAD011) 33Female	Previous symptoms of asthma in childhood and eczema. Represented over past year with self-reported wheeze & breathlessness. Positive FeNO. Positive BCTmeth.	ACQ5 FEV$_1$/FVC BDR FeNO PEFv PEFv (alt) Wheeze BCTmeth Eos	2 points 78.7% (LLN is 72%) -1% 24 ppb (repeat CV2 43) 11% 1 days >20% no PD$_{20}$ 0.048 0.27		15
(RAD027) 32 Male	Intermittent symptoms of wheeze, breathless and chest tightness well in-between. Two visits to A&E treated with nebulised salbutamol. High FeNO. Diagnosis with seasonal asthma.	ACQ5 FEV$_1$/FVC BDR FeNO PEFv PEFv (alt) Wheeze BCTmeth Eos	0 78% (LLN 72%) 5% 50 ppb 9% 0 No FEV1 -19% 0.07	0 80% - 19 - - - FEV1 -12% 0.006	12
(RAD032) 26 female	Symptomatic, clinician assessed as high possibility at consultation. Positive obstructive	ACQ5 FEV$_1$/FVC BDR	2.4 75% (LLN 77%) 8%	1.6 72% -	9

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	spirometry (<LLN), positive PEFv, and borderline negative challenge <0.4. Poor compliance with treatment so difficult to assess post ics results.	FeNO PEFv PEFv (alt) Wheeze BCTmeth Eos	11 ppb 22% (9 days) 7 days No PD$_{20}$ 0.248 0.42	11ppb - - No 0.235 -	
(RAD038) 25 female	Symptomatic with positive FeNO, borderline negative challenge (<0.4). Symptomatic and objective improvement post ics.	ACQ5 FEV$_1$/FVC BDR FeNO PEFv PEFv (alt) Wheeze BCTmeth Eos	1.4 points 81% (LLN 75%) 3% 91 ppb 13% 3 days No PD$_{20}$ 0.272 0.3	0.8 84% - 18 ppb - - - FEV1 -10% -	12
(RAD055) 7 Male	Symptomatic with PEF variability and positive BCT. Subjective and objective improvement with treatment	ACQ5 FEV$_1$/FVC BDR FeNO PEFv PEFv (alt) Wheeze BCTmeth Eos	2.4 points 78% (LLN 79%) -10% 6ppb 28% 5 days No PD$_{20}$ 0.018 0.33	1.2 78% - 9 ppb - - No PD20 0.098 -	22
(RAD061) 5 Female	Symptomatic, positive challenge with raised eosinophils. Subjective and objective improvements post treatment	ACQ5 FEV$_1$/FVC BDR FeNO PEFv PEFv (alt) Wheeze BCTmeth Eos	1.6 84% (LLN 82%) 4% 13 ppb 13% 2 no PD$_{20}$ 0.099 0.65	0 89% - 10 ppb - - No 0.304 0.99	26
(RAD067) 28 Female	Presented with self-reported cough, wheeze, and tight chest. Positive BCTmeth, borderline positive PEFv and obstructive spirometry. Symptom improved on ACQ after trial of treatment.	ACQ5 FEV$_1$/FVC BDR FeNO PEFv PEFv (alt) Wheeze BCTmeth Eos	0.8 points 76.5 (LLN 74%) 7% 24ppb 18% 3 days >20% no PD$_{20}$ 0.043 0.13	0 points PD$_{20}$.067 	15

		SPT	0/8		
(RAD069) 7 Female	Symptomatic, FeNO 30ppb, positive BCT. Subjective and objective improvement post treatment	ACQ5	0.6	0	26
		FEV$_1$/FVC	87% (LLN 80%)	85%	
		BDR	8%	-	
		FeNO	22ppb (CV2 30ppb)	5ppb	
		PEFv	8%	-	
		PEFv (alt)	1 day	-	
		Wheeze	No	-	
		BCTmeth	PD$_{20}$ 0.039	0.344	
		Eos	-	-	
(RAD102) 58Female	Cough and breathlessness, worse with exercise and at night. Eczema. Childhood asthma. Variable FEV1 between visits. Positive FeNO, BDR and BCTmeth.	ACQ5	2.2 points		15
		FEV$_1$/FVC	77% (LLN 68%)		
		BDR	12%		
		FeNO	15 ppb (51ppb at CV2)		
		PEFv	3%		
		PEFv (alt)	0 days >20%		
		Wheeze	no		
		BCTmeth	PD$_{20}$ 0.069		
		Eos	0.15		
		SPT	0/8		
(RAD105) 8	Symptomatic, Eos 0.45, positive challenge. Good objective responsive post treatment – unable to reassess due to start of COVID pandemic (remote telephone CV4)	ACQ5	0.6	0	26
		FEV$_1$/FVC	84% (LLN 81%)		
		BDR	5% (CV2 16ppb)		
		FeNO	6 ppb		
		PEFv	9%		
		PEFv (alt)	0 day		
		Wheeze	No		
		BCTmeth	PD$_{20}$ 0.185		
		Eos	0.45		

Two thirds of all adults (≥17 years) described above (tables 39-40) received a false negative diagnosis because of the same pathway through the NG80 pathway (pathway 15: not obstructed, normal FeNO but BDR and BCT not tested). The performance of individual NG80 pathways was described above (section 3.4.2.1, in adults, and section 3.4.2.1, in children).

3.4.3. Which tests, and what diagnostic combination of tests (algorithm), best predict EPOER confirmed asthma?

3.4.3.1.   Subgroup analysis: adults ≥17yrs analysis

Predictors of asthma (univariate analysis using continuous data in adults):

A univariate analysis was performed on all conventional asthma diagnostic tests available in the study to determine which tests best predict symptomatic 'asthma' patients from symptomatic 'not asthma' patients using continuous data (table 41).

Table 41.   Summary of potential asthma predictors in adults (≥17 years) (continuous variables)

	All Patient Group (N 58)	Patient Group Split into Sub Groups (N 97)		*P Value	AUROC
		Asthma (N 35)	Non Asthma (N 23)		
Spirometry					
FEV$_1$, mean (SD) L	3.28 (0.96)	3.29 (0.99)	3.25 (0.93)	0.882	0.451 (0.301-0.601)
FEV$_1$, mean (SD) %predicted	95.78 (17.24)	91.06 (17.50)	102.96 (14.41)	0.009	0.693 (0.557-0.828)
FVC, mean (SD) L	4.26 (1.25)	4.46 (1.29)	3.96 (1.15)	0.135	0.625 (0.479-0.771)
FVC, mean (SD) %predicted	102.98 (14.49)	102.71 (14.86)	103.39 (14.22)	0.864	0.504 (0.350-0.659)
FEV$_1$/FVC Ratio, mean (SD) %	**77.23 (8.43)**	**73.82 (8.98)**	**82.42 (3.69)**	**<0.001**	**0.815 (0.705-0.924)**
MMEF, mean (SD) L	**3.00 (1.12)**	**2.65 (1.10)**	**3.52 (0.96)**	**0.003**	**0.718 (0.588-0.848)**
MMEF, mean (SD) %predicted	**82.92 (31.83)**	**69.63 (24.14)**	**103.14 (31.87)**	**<0.001**	**0.870 (0.780-0.961)**
Bronchodilator Reversibility					
FEV$_1$, median (IQR) %change	**7.00 (3.00-12.00)**	**9.00 (6.00-15.00)**	**4.00 (1.00-7.00)**	**<0.001**	**0.838 (0.738-0.938)**
FVC, median (IQR) %change	2.00 (-0.25 – 4.00)	3.00 (1.00-5.00)	1.00 (-2.00- 2.00)	0.008	0.706 (0.572-0.839)
FEV$_1$/FVC, median (IQR) %change	**5.00 (2.00- 8.25)**	**7.00 (5.00-9.00)**	**3.00 (1.00-5.00)**	**<0.001**	**0.822 (0.712-0.932)**
MMEF, median (IQR) %change	**24.50 (10.00-38.25)**	**32.00 (22.00-44.00)**	**12.00 (-1.00-19.00)**	**<0.001**	**0.851 (0.755-0.947)**
Other					
FeNO, median (IQR) ppb *(from CV1 only)*	**24.00 (12.75-68.00)**	**61.00 (20.00-91.00)**	**15.00 (11.00-22.00)**	**0.001**	**0.754 (0.624-0.884)**
FeNO, median (IQR) % predicted	144.00 (93.75-411.75) N 46	285.50 (118.25-719.50) N 28	109.00 (79.75-141.00) N 18	0.006	0.741 (0.594-0.888)

PEFv Calculation, median (IQR) %	**9.72 (5.11 – 12.09)** **N 46**	**10.68 (6.73-13.13)** N 29	6.13 (3.36-11.60) N 17	0.033	0.690 (0.524-0.855)
PEFv Number Days >20%, median (IQR) days (data from returned PEF meters with minimum 4 days data)	1.00 (0.00-2.00) N 46	1.00 (0.00-3.00) N 29	0.00 (0.00-2.00) N 17	0.085	0.644 (0.486-0.802)
BCTmeth PD20, median (IQR), mg	**1.92 (0.08-1.92)** N= 45	**0.10 (0.02-0.52)** N =25	1.92 (1.92-1.92) N =20	<0.001	0.900 (0.802-0.998)
BCTmeth DRR, median (IQR)	**17.71 (6.67122.08)** N= 45	**119.58 (26.45-590.00)** N = 25	6.67 (4.47-13.75) N=20	<0.001	0.940 (0.863-1.00)
Eos, median (IQR) x10^{9}cells/L	**0.18 (0.10-0.33)** N =56	**0.28 (0.15-0.55)** N =34	0.10 (0.06-0.17) N =22	<0.001	0.799 (0.686-0.913)
SPT (Number allergens sensitised), mean (SD)	2.00 (0.00-2.25)	2.00 (0.00-3.00)	1.00 (0.00-2.00)	0.126	0.623 (0.474-0.771)
ACQ points, mean (SD)	1.67 (1.17)	1.75 (1.23)	1.56 (1.07)	0.544	0.536 (0.385-0.687)
Auscultated wheeze, n (%)	**7 (12.1)**	**7 (20.0)**	**0 (0.0)**	**0.035*I*	**n/a**

P values refer to the difference between the 'asthma' and 'not asthma' groups, t-test for normally-distributed data; Mann Whitney U for non-normally distributed data, chi squared (or Fisher's exact test[I]) for categorical data.

Abbreviation: BMI, Body mass index, FEV1, Forced Expiratory Volume in one second, FVC, Forced vital capacity, BDR, Bronchodilator reversibility, FeNO, Fractional exhaled nitric oxide, PEFv, Peak expiratory flow variability, Eos, Eosinophil levels, SPT, skin prick test, BCTmeth, Methacholine Bronchial challenge test.

Objective tests in asthma diagnosis (adults):

The univariate analysis showed Spirometry (FEV1/FVC ratio, MMEF, MMEF%predicted), BDR (FEV1%change, FEV1/FVC%change, MMEF%change), FeNO, PEFv, BCTmeth, Eosinophils, and auscultated wheeze, were all significantly different between the two groups and therefore have the potential to be used in asthma diagnosis.

Box Plots demonstrating the Predictors of Asthma in adults:

Boxplots demonstrating all variables that were showed to be significant predictors of asthma are displayed (figure 35). In addition, common recommended cut-offs for a positive test are displayed where applicable.

Figure 35. Predictors of asthma in adults (boxplots)

a) Spirometry:

b) Bronchodilator Reversibility:

c) Other Tests:

FeNO CV1 (adults)

FeNO % predicted CV1 (adults)

PEFv (adults)
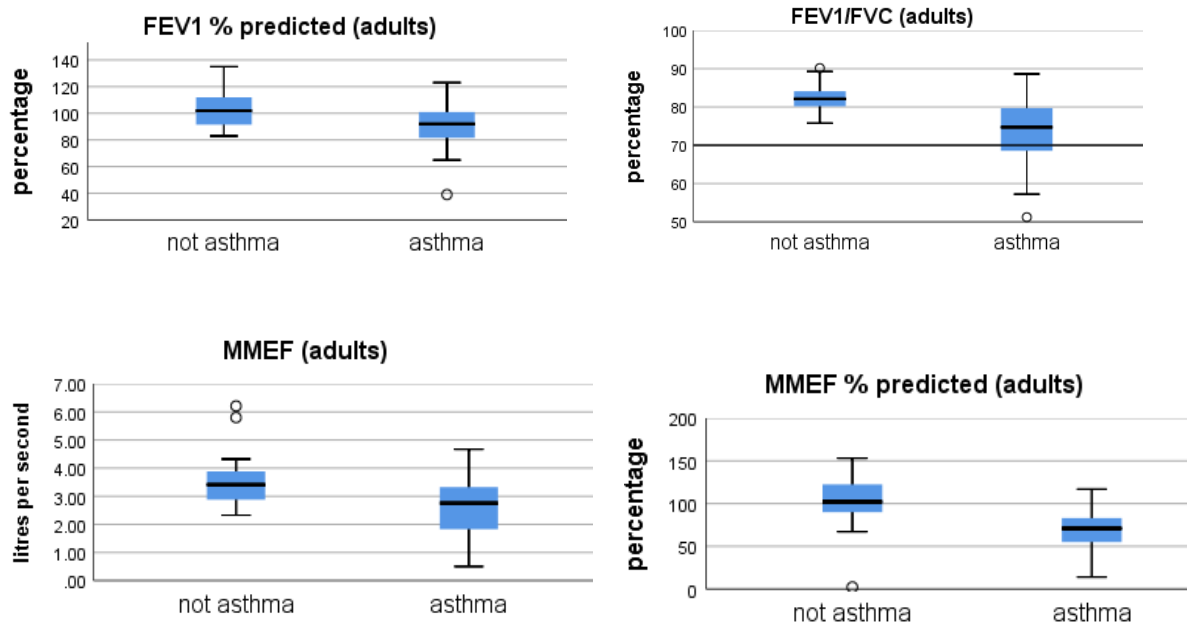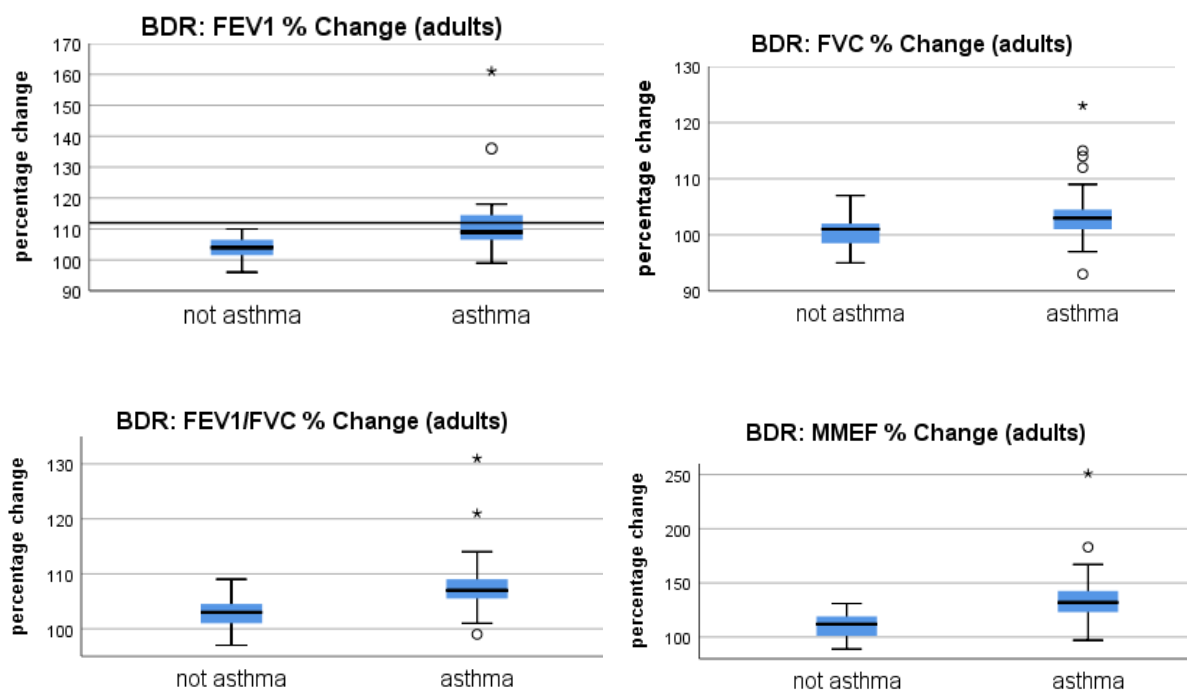
Eos (adults)

Of the boxplots shown, previously established cut-offs for a positive test in asthma are available for FEV_1/FVC ratio, BDR (FEV_1%change), FeNO and PEFv. All these plots (with the exception of FeNO) demonstrate that all 'not asthma' patients fall below the positive threshold cut off. This indicates that these tests are highly specific for asthma. However, the plots also show that patients in the 'asthma' group fall below the positive cut-off reflecting the lower sensitivity of the test. Of the three tests, FEV_1/FVC ratio appeared to perform least well using the commonly applied threshold (<70%), illustrated by the median and upper and lower quartile lines all falling on the wrong side of the positive threshold line. A more detailed look at the dichotomised data using established thresholds is shown later in this chapter (table 42).

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ROC curves: predictors of asthma in adults

ROC curves are shown for all tests which showed moderate AUC (≥0.7) (figure 36).

Figure 36. ROC curves showing predictors of asthma in adults

a) Baseline spirometry
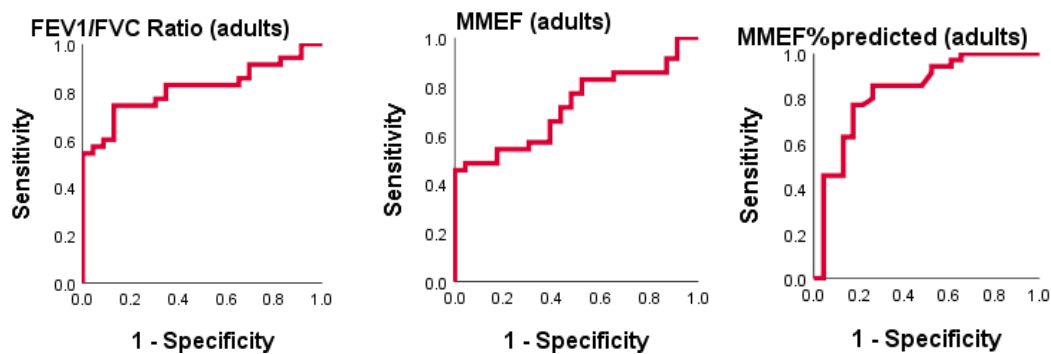
b) Bronchodilator reversibility
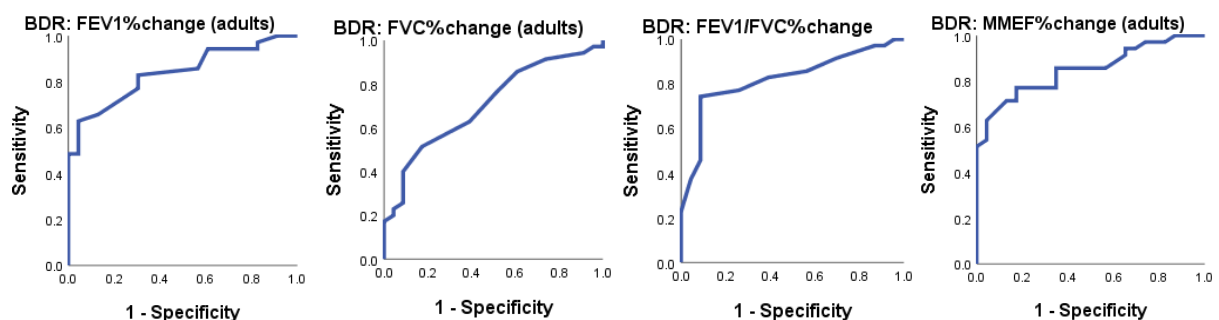
c) Other tests
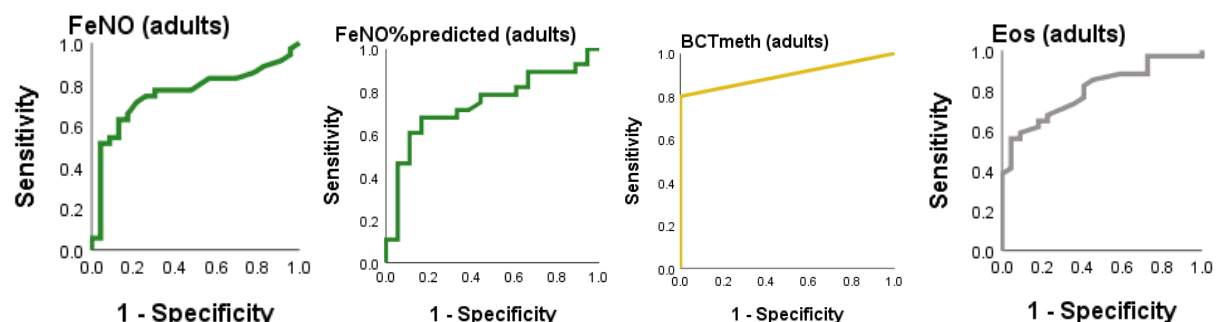
ROC curves demonstrate all significant predictors of asthma (P<0.05) in the univariate analysis, those with AUROC >0.7 are illustrated. Tests that are known to correlate (section 3.4.1.5) are grouped together by colour. Our results: using univariate analysis and area under curve, show that the best predictors of asthma from each group are MMEF%pred, MMEF%change, FeNO, PEFv, BCTmeth, and Eos. Of interest, the spirometry measurement

MMEF outperformed the conventional measurement FEV$_1$/FVC ratio, 'FeNO %predicted' had no advantage over using standard FeNO, and eosinophil level performed well despite that this test is not currently included in the NG80 diagnostic algorithms (see table 41 above).

Predictors of asthma (univariate analysis using dichotomised data in adults):

A univariate analysis was performed on tests with dichotomised data using established cut-offs (where available) to determine the ability of each test to predict symptomatic 'asthma' patients from symptomatic 'not asthma' patients (table 42). Sensitivity, specificity, PPV, and NPV was computed for each test.

Table 42. Summary of potential asthma predictors (dichotomised data in adults)

Objective tests	Patient Group Split into Sub Groups (N 58)			Sensitivity	Specificity	PPV	NPV	Asthma detected number (%) positive
	Asthma (N 35)	Non Asthma (N 23)	P value					
FEV$_1$/FVC <70%, n (%) positive	11 (31.4)	0 (0.0)	↑0.02	31.4	100.0	100.0	48.9	11 (31)
FEV$_1$/FVC <LLN, n (%) positive	13 (37.1)	0 (0.0)	↑0.01	37.1	100.0	100.0	51.1	13 (37)
BDR ≥12% and 200ml, n (%) positive	16 (45.7)	0 (0.0)	↑ <0.001	45.7	100.0	100.0	54.8	16 (46)
FeNO ≥40ppb, n (%) positive	19 (54.3)	3 (13.0)	↑0.002	54.3	87.0	86.4	55.5	19 (54)
Eos >0.4x10^9/L, n (%) positive	11 (32.4) N 34	1 (4.3) N 22	↑0.004	32.4	100.0	100.0	48.9	11 (32)
PEFv >20%, n (%) positive	3 (10.3) N 29	0 (0.0) N 17	↑0.286	10.3	100.0	100.0	39.5	3 (10)
PEFv(alt), n (%) positive (≥3 days 20%diurnal variability)	10 (34.5) N 29	0 (0.0) N 17	↑0.005	34.5	100.0	100.0	47.2	10 (34)
Wheeze auscultated, n (%) positive	7 (20.0)	0 (0.0)	↑0.035	20.0	100.0	100.0	45.1	7 (20)
BCTmeth PD$_{20}$ ≤0.2mg, n (%) positive	14 (56.0) 25	0 (0.0) N 20	↑ <0.001	56.0	100.0	100.0	64.5	14 (56)
P values refer to the difference between the 'asthma' and 'not asthma' groups, t-test for normally-distributed data; Mann Whitney U for non-normally distributed data, chi squared or 1Fisher's exact test for categorical data.								

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All dichotomised tests (with the exception PEFv) were significantly associated with EPOER asthma, sensitivities and NPV tended to be lower (sensitivity range 31% -56%, NPV range 45%-65%) than specificities and PPVs (specificity range 87% - 100%, PPV range 86%-100%). All tests except FeNO had specificity 100%, these tests could therefore be used together in a 'rule-in' asthma multi-variable model.

Exploratory analysis: "Rule in" asthma algorithm in adults (≥17 years)

Using predictors of asthma from the univariate analysis and/or NG80 recommended tests, we looked at candidate multivariable algorithms that may be able to confidently "rule-in' asthma and compared this to current performance of the NICE algorithm to rule-in asthma (table 43). In our algorithms we included PEFv using the currently recommended NG80 calculation (although this wasn't a significant predictor of asthma in our cohort) and we also substitute the alternative PEFv measurement (PEFv(alt)) to compare performance of each. We also compare performance of FEV_1/FVC ratio within the algorithm using <70 and <LLN.

Table 43. Multivariate algorithms (asthma vs not asthma: adults)

	N	Sensitivity % (95% CI)	Specificity % (95% CI)	NPV % (95% CI)	PPV % (95% CI)	Asthma Detected Number (%) positive	Number (%) patients requiring further investigation due to asthma not 'ruled in'
Algorithms:							
All tests:							
A. One of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv, Wheeze, BCTmeth	38	87 (66-97)	87 (60-98)	81 (54-96)	91 (71-99)	20 (87)	18 (47)
B. Two of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv, Wheeze, BCTmeth	38	61 (39-80)	100 (78-100)	62 (41-81)	100 (77-100)	14 (61)	24 (63)
C. Three of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv, Wheeze, BCTmeth	38	43 (23-66)	100 (78-100)	54 (34-72)	100 (69-100)	10 (43)	28 (74)
Without FeNO							
D. One of: FEV1/FVC <70%, BDR, Eos, PEFv>20%, Wheeze, BCTmeth	38	78 (56-93)	100 (78-100)	75 (51-91)	100 (81-100)	18 (78)	20 (53)

E. Two of: FEV1/FVC <70%, BDR, Eos, PEFv>20%, Wheeze, BCTmeth	38	78 (56-93)	100 (78-100)	75 (51-91)	100 (81-100)	14 (61)	24 (63)
Without BCTmeth							
F. One of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv>20%, Wheeze	44	82 (63-94)	88 (62-98)	74 (49-91)	92 (74-99)	23 (82)	21 (48)
G. Two of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv>20%, Wheeze	44	57 (37-76)	100 (79-100)	57 (37-76)	100 (79-100)	16 (57)	28 (64)
Without wheeze							
H. One of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv>20%, BCTmeth	38	83 (61-95)	87 (60-98)	76 (50-93)	90 (70-99)	19 (83)	19 (50)
I. Two of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv>20%, BCTmeth	38	57 (34-77)	100 (78-100)	60 (39-79)	100 (75-100)	13 (57)	25 (66)
Without FeNO and BCTmeth (most accessible in primary care)							
J. One of: FEV1/FVC <70%, BDR, Eos, PEFv>20%, Wheeze	44	75 (55-89)	100 (79-100)	70 (47-87)	100 (84-100)	21 (75)	23 (52)
K. Two of: FEV1/FVC <70%, BDR, Eos, PEFv>20%, Wheeze	44	36 (19-56)	100 (79-100)	47 (30-65)	100 (69-100)	10 (36)	34 (77)
All - substitute in PEFv(alt)							

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L. One of: FEV1/FVC <LLN, BDR, FeNO, Eos, PEFv(alt), Wheeze, BCTmeth	38	91 (72-99)	87 (60-98)	87 (60-98)	91 (72-99)	21 (91)	17 (45)
M. Two of: FEV1/FVC <LLN, BDR, FeNO, Eos, PEFv(alt), Wheeze, BCTmeth	38	78 (56-93)	100 (78-100)	75 (51-91)	100 (81-100)	18 (78)	20 (53)
N. Three of: FEV1/FVC <LLN, BDR, FeNO, Eos, PEFv(alt), Wheeze, BCTmeth	38	52 (31-73)	100 (78-100)	58 (37-77)	100 (74-100)	12 (52)	26 (68)
Without FeNO – substitute in PEFv(alt)							
O. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, BCTmeth	38	87 (66-97)	100 (78-100)	83 (59-96)	100 (83-100)	20 (87)	18 (47)
P. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, BCTmeth	38	74 (52-90)	100 (78-100)	71 (48-89)	100 (80-100)	17 (74)	21 (55)
Without BCTmeth – substitute in PEFv(alt)							
Q. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, FeNO	44	89 (72-98)	88 (62-98)	82 (57-96)	93 (76-99)	25 (89)	19 (43)
R. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, FeNO	44	75 (55-89)	100 (79-100)	70 (47-87)	100 (84-100)	21 (75)	23 (52)
S. Three of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, FeNO	44	32 (16-52)	100 (79-100)	46 (29-63)	100 (66-100)	9 (32)	35 (80)

Without wheeze – substitute in PEFv(alt)							
T. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), FeNO, BCTmeth	38	91 (72-99)	87 (60-98)	87 (60-98)	91 (72-99)	21 (91)	17 (45)
U. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), FeNO, BCTmeth	38	70 (47-87)	100 (78-100)	68 (45-86)	100 (79-100)	16 (70)	22 (58)
V. Three of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), FeNO, BCTmeth	38	48 (27-69)	100 (78-100)	56 (35-75)	100 (72-100)	11 (48)	27 (71)
Without FeNO and BCTmeth (most available to primary care) – substitute in PEFv(alt)							
W. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze	44	86 (67-96)	100 (79-100)	80 (56-94)	100 (86-100)	24 (86)	20 (45)
X. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze	44	50 (31-69)	100 (79-100)	53 (34-72)	100 (77-100)	14 (50)	30 (68)
Y. Three of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze	44	18 (6-37)	100 (79-100)	41 (26-58)	100 (48-100)	5 (18)	39 (89)
Existing national guidelines							
NICE algorithm	45	36 (19-56)	100 (80-100)	49 (31-66)	100 (69-100)	10 (36)	35 (78)

Abbreviation: Eos, Eosinophil levels, PEFv, Peak expiratory flow variability, PEFv(alt), Peak expiratory flow variability alternative, FeNO, Fractional exhaled nitric oxide, FEV$_1$, Forced Expiratory Volume in one second, FVC, Forced vital capacity, BDR, Bronchodilator reversibility, BCTmeth, Methacholine Bronchial challenge test, Wh, wheeze.

<u>Best "Rule in" algorithms:</u>

The best algorithms to 'rule in' asthma in adults are shown for i) use in primary care (i.e., we did not include FeNO or BCT because these may not be accessible to primary care), and ii) best overall. Only one positive test was required to confirm asthma in all algorithms. In adults we found that using the recommended cut-offs: FEV_1/FVC ratio <LLN and PEFv(alt) >20%, performed better than using <70% and PEFv(standard).

Best in primary care (i.e., no FeNO, no BCTmeth)

    Algorithm W. One of: FEV1/FVC <LLN, BDR, Eos, PEFv (alternative), Wheeze (sn 86, sp 100%)

Best overall:

    Algorithm O. One of: FEV1/FVC <LLN, BDR, Eos, PEFv (alternative), Wheeze, BCTmeth (sn 87, sp 100)

Of the proposed algorithms, the two algorithms highlighted in blue performed best as "rule-in" algorithms with specificity 100% and sensitivity ranging 86-87%. Of these algorithms, Algorithm W would be most accessible in primary care. These outperformed the current NICE algorithm as a 'rule in' asthma pathway. However, the remaining 45% of patients completing this pathway (asthma patients (n= 4) and non-asthma patients (n= 16)) that did not fulfil the algorithm criteria therefore did not receive the diagnosis asthma, would still require further review to confirm or exclude asthma. This is still a smaller proportion compared to 78% of patients that would still require further work up if using the NG80 algorithm.

The performance of our proposed algorithm in adults was further improved by adding in BCTmeth (Algorithm O). This increased sensitivity marginally (87%) without reducing specificity (100%). Algorithm O could confidently rule-in asthma in 20 (87%) asthma patients. The remaining 53% of patients completing this pathway (asthma patients (n= 3) and non-asthma patients (n= 15)) that did not fill the algorithm criteria and therefore did

not receive the diagnosis asthma, would still require further review to confirm or exclude asthma. In our proposed algorithms, any one positive feature is enough to diagnose asthma.

3.4.3.2. Subgroup analysis: children <17yrs analysis

Predictors of asthma (univariate analysis using continuous data in children):

A univariate analysis was performed on all conventional asthma diagnostic tests available in the study to determine which tests best predict symptomatic 'asthma' patients from symptomatic 'not asthma' patients using continuous data (Table 44).

Table 44. Summary of potential asthma predictors in children(<17 years) (continuous variables)

	All Patient Group (N 38)	Patient Group Split into Sub Groups (N 38)		*P Value	AUROC (95CI)
		Asthma (N 28)	Non Asthma (N 10)		
Spirometry					
≠ FEV$_1$, mean (SD) L	2.04 (0.95)	1.82 (0.78)	2.64 (1.16)	0.018	0.713 (0.522-0.903)
FEV$_1$, mean (SD) %predicted	95.84 (15.94)	92.50 (16.18)	105.20 (11.31)	0.029	0.759 (0.595-0.923)
≠ FVC, mean (SD) L	2.54 (1.21)	2.33 (1.03)	3.13 (1.51)	0.072	0.654 (0.450-0.858)
FVC, mean (SD) %predicted	103.90 (14.09)	102.54 (14.54)	107.70 (12.67)	0.327	0.598 (0.397-0.800)
FEV$_1$/FVC Ratio, mean (SD) %	81.40 (7.03)	79.91 (7.25)	85.57 (4.40)	0.027	0.718 (0.547-0.889)
≠ MMEF, mean (SD) L	2.05 (1.02)	1.76 (0.86)	2.85 (1.04)	0.003	0.807 (0.647-0.967)
MMEF, mean (SD) %predicted	79.29 (24.94)	73.04 (24.66)	96.80 (16.43)	0.008	0.782 (0.638-0.927)
Bronchodilator Reversibility					
FEV$_1$, median (IQR) %change	5.50 (2.75-13.00)	7.50 (3.25-15.50)	4.00 (-0.25-7.00)	0.056	0.704 (0.537-0.870)
FVC, median (IQR) %change	1.50 (-1.25- 5.00)	2.00 (-1.00- 5.75)	1.00 (-2.25-2.00)	0.125	0.668 (0.496-0.840)
FEV$_1$/FVC, median (IQR) %change	6.00 (3.00-8.25)	6.00 (3.00-12.75)	3.50 (0.50-6.00)	0.031	0.730 (0.563-0.898)
MMEF, median (IQR) %change	23.00 (15.50-39.00)	28.00 (20.00-51.50)	19.00 (4.75-26.50)	0.034	0.729 (0.559-0.898)
Other					
FeNO, median (IQR) ppb *(from CV1 only)*	27.50 (13.00-83.75) N = 30	54.00 (22.50-107.50) N = 20	12.00 (7.75-18.50)	0.001	0.807 (0.710-0.990)

PEFv Calculation, median (IQR) %	13.30 (7.88-20.67) N = 33	16.02 (9.92-22.31) N = 25	6.32 (4.38-8.03) N = 8	<0.001	0.895 (0.750-1.000)
PEFv Number Days >20%, median (IQR) days (data from returned PEF meters with minimum 4 days data)	2.00 (0.50-4.00) N = 33	3.00 (2.00-5.00) N = 25	0.00 (0.00-0.75) N = 8	0.001	0.865 (0.698-1.000)
BCTmeth PD$_{20}$, median (IQR) mg	0.19 (0.04-1.92) N = 32	0.13 (0.03-0.20) N =22	1.92 (1.55-1.92)	0.001	0.859 (0.706-1.000)
BCTmeth DRR, median (IQR)	91.94 (17.85-415.42) N = 32	120.50 (85.05-482.92) N= 22	16.75 (9.06-28.04)	0.003	0.827 (0.654-1.00)
Eos, median (IQR) x10^{9}cells/L	0.33 (0.18-0.86) N = 31	0.45 (0.28-0.93) N = 21	0.17 (0.10-0.23)	0.002	0.838 (0.676-1.000)
SPT (Number allergens sensitised), mean (SD)	1.47 (1.52)	1.71 (1.54)	0.80 (1.32)	0.103	0.795 (0.508-0.903)
ACQ points, mean (SD)	1.19 (1.08) N = 37	1.28 (1.02) N = 27	0.94 (1.24)	0.400	0.648 (0.423-0.873)
Auscultated wheeze, n (%)	5 (13.2)	5 (17.9)	0 (0.0)	0.298ⱡ	n/a

*P values refer to the difference between the 'asthma' and 'not asthma' groups, t-test for normally-distributed data; Mann Whitney U for non-normally distributed data, chi squared or Fisher's exact test for categorical data.

≠ interpret with caution because children in our cohort were not age and height matched across the two groups (percent predicted values are more appropriate in our cohort)

Abbreviation: BMI, Body mass index, FEV1, Forced Expiratory Volume in one second, FVC, Forced vital capacity, BDR, Bronchodilator reversibility, FeNO, Fractional exhaled nitric oxide, PEFv, Peak expiratory flow variability, Eos, Eosinophil levels, SPT, skin prick test, BCTmeth, Methacholine Bronchial challenge test.

Objective tests in asthma diagnosis (children):

The univariate analysis showed Spirometry (FEV1, FEV1%predicted, FEV1/FVC ratio, MMEF, MMEF%predicted), BDR (FEV1/FVC%change, MMEF%change), FeNO, PEFv, BCTmeth, and Eosinophils, were all significantly different between the two groups and therefore have the potential to be used in asthma diagnosis. We show that approximately one in five children with asthma had wheeze on auscultation compared to no children in the symptomatic not asthma group. In this exploratory analysis with a small sample size, this was not statistically significant.

Box Plots demonstrating the predictors of asthma in children:

Boxplots demonstrating all variables that were showed to be significant predictors of asthma are displayed (figure 37).

Figure 37. Predictors of asthma in children (boxplots)

a) Spirometry:
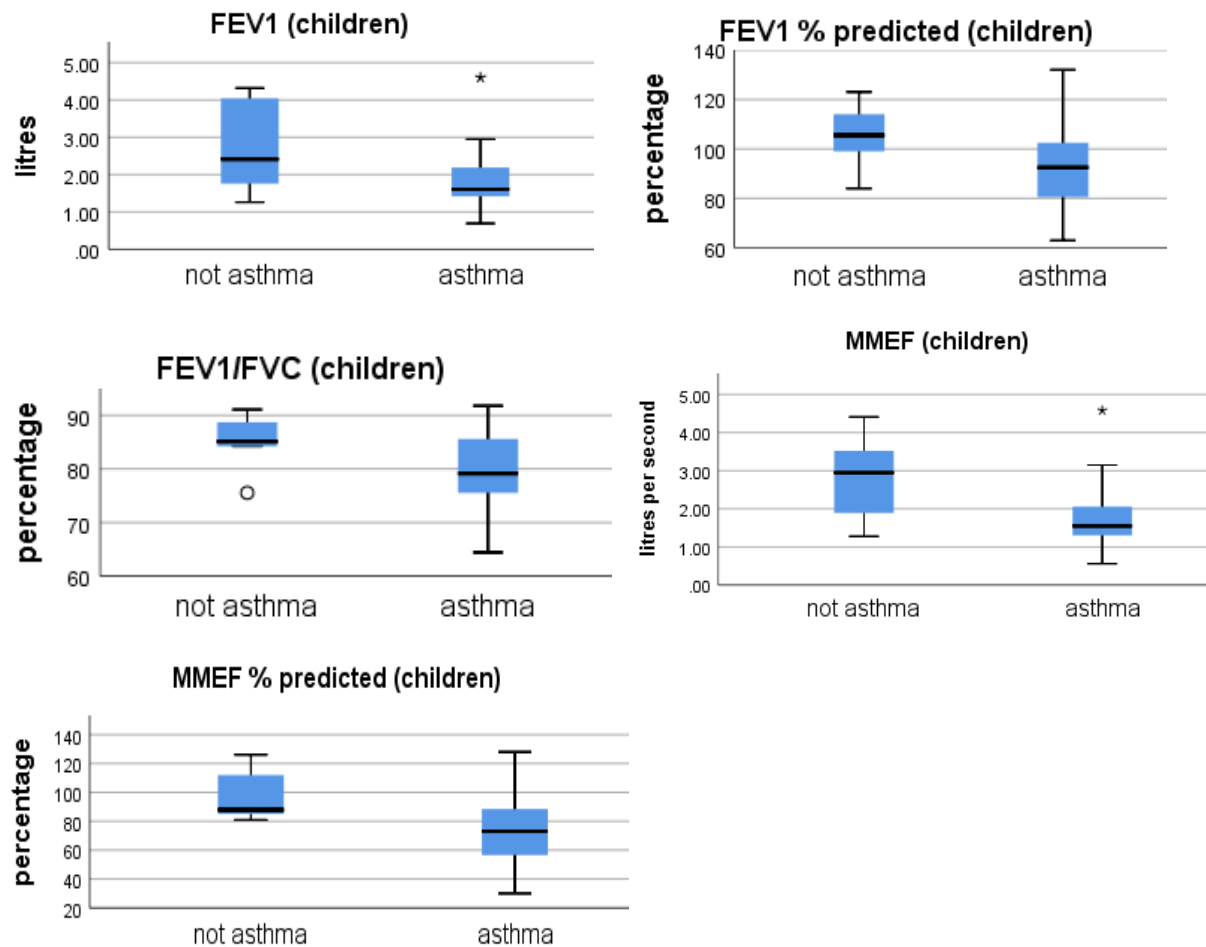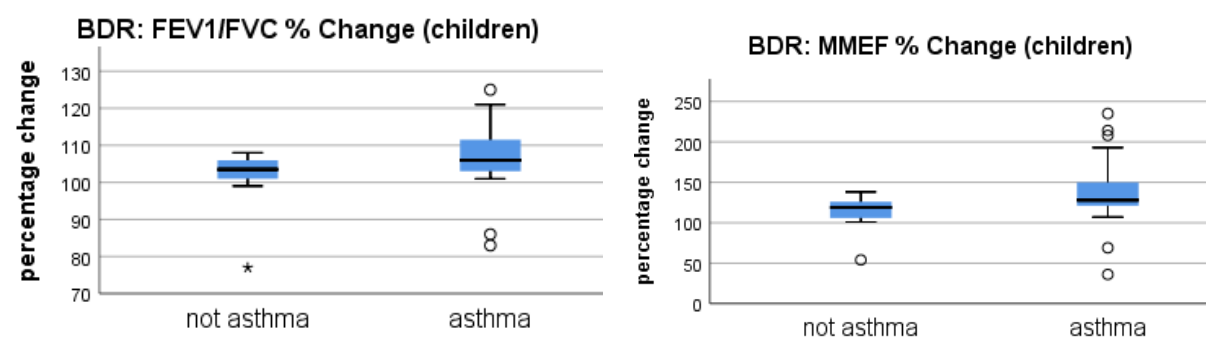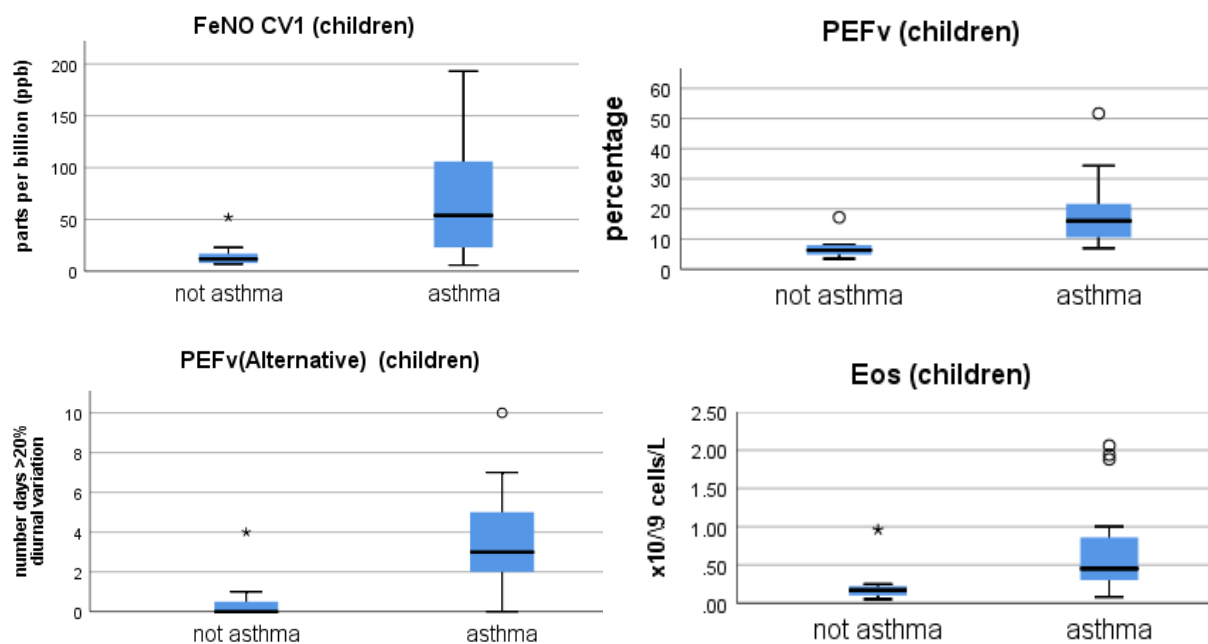
b) Bronchodilator reversibility:

c) Other tests:

FeNO CV1 (children)

PEFv (children)

PEFv(Alternative) (children)

Eos (children)

Of the boxplots shown, previously established cut-offs for a positive test in asthma are available for FEV_1/FVC ratio, FeNO and PEFv. All these boxplots (with the exception of FeNO) demonstrate that all 'not asthma' patients fall below the positive threshold cut off. This indicates that these tests are highly specific for asthma. However, the plots also show that patients in the 'asthma' group fall below the positive cut-off reflecting the lower sensitivity of the test. A more detailed look at the dichotomised data using established thresholds is shown later in this chapter (table 45).

ROC curves: predictors of asthma in children (<17 yrs)

ROC curves are shown for all tests which showed moderate AUC (≥0.7) (figure 38).

Figure 38. ROC curves showing predictors of asthma in children

a) Spirometry

b) Bronchodilator reversibility

c) Other tests

Eoc (children) (ROC curve, y-axis: Sensitivity, x-axis: 1 - Specificity)
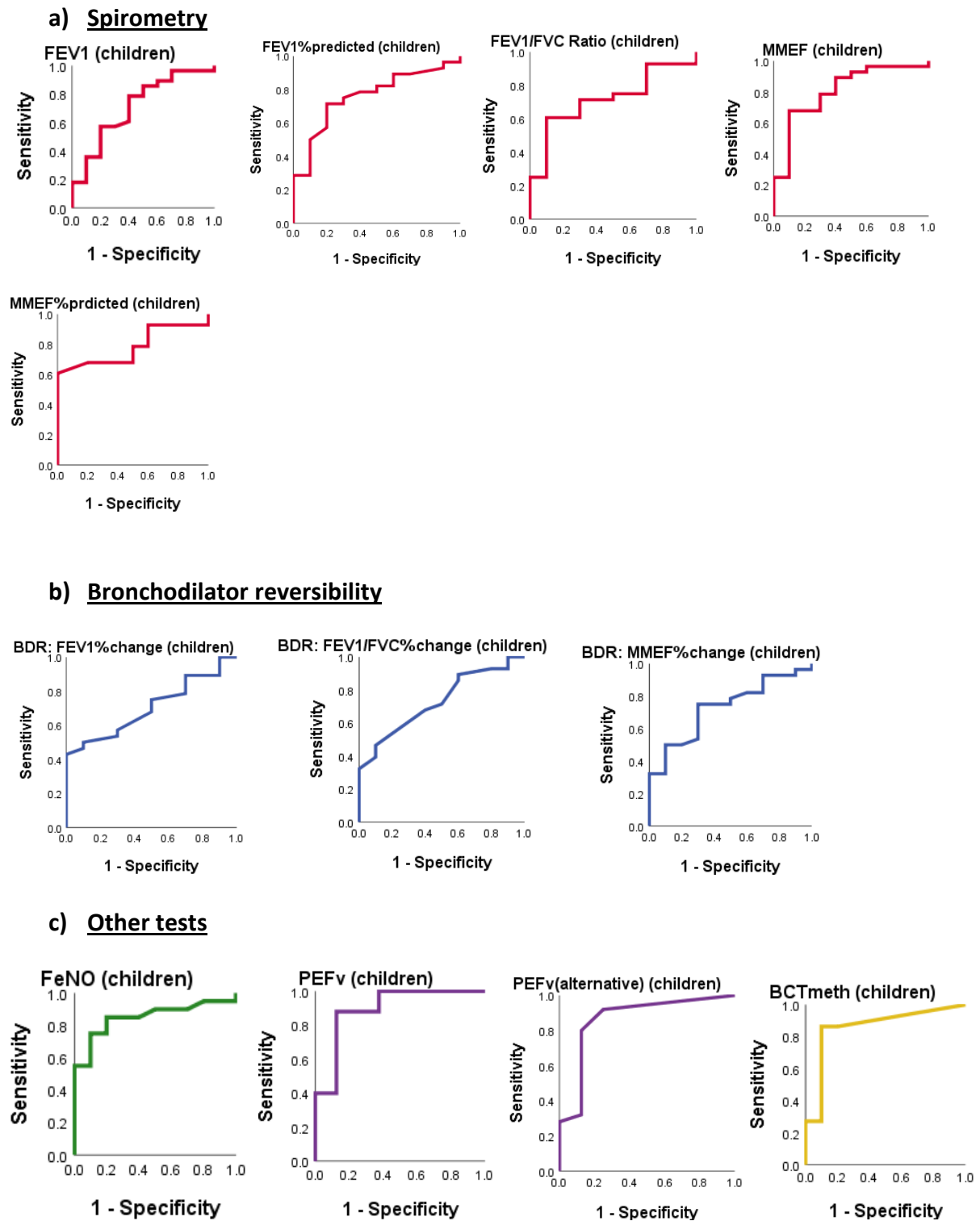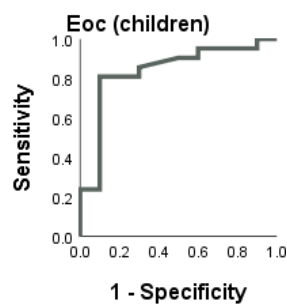
Predictors of asthma (univariate analysis using dichotomous data in children):

A univariate analysis was performed on tests with dichotomised data using established cut-offs (where available) to determine the ability of each test to predict symptomatic 'asthma' patients from symptomatic 'not asthma' patients (table 45). Sensitivity, specificity, PPV, and NPV was computed for each test.

Table 45.   Summary of potential asthma predictors (dichotomised data) in children

Objective tests	Patient Group Split into Sub Groups (N 38)		P value	Sensitivity	Specificity	PPV	NPV	Asthma detected number (%) positive
	Asthma (N 28)	Non Asthma (N 10)						
FEV$_1$/FVC <70%, n (%) positive	2 (7.1)	0 (0.0)	1.000	7.1	100.0	100.0	27.8	2 (7)
FEV$_1$/FVC <LLN, n (%) positive	11 (39.3)	0 (0.0)	0.037	39.3	100.0	100.0	37.0	11 (39)
BDR ≥12%, n (%) positive	10 (35.7)	0 (0.0)	0.038	35.7	100.0	100.0	35.7	10 (36)
FeNO ≥35ppb, n (%) positive	12 (60%) N = 20	1 (10.0)	0.017	60.0	90.0	92.3	52.9	12 (60)
Eos >0.4x10^9/L, n (%) positive	12 (57.1) N = 21	1 (10.0)	0.020	57.1	90.0	92.3	50.0	12 (57)
PEFv >20%, n (%) positive	10 (40.0) N = 25	0 (0.0) N = 8	0.071	40.0	100.0	100.0	34.8	10 (40)
PEFv(alt), n (%) positive (≥3 days 20%diurnal variability)	14 (56.0) N = 25	1 (12.5) N = 8	0.046	56.0	87.5	93.3	38.9	14 (56)
Wheeze auscultated, n (%) positive	5 (17.9)	0 (0.0)	0.298	17.9	100.0	100.0	30.3	5 (18)
BCTmeth PD$_{20}$ ≤0.2mg, n (%) positive	16 (72.7) N = 22	1 (10.0)	0.002	72.7	90.0	94.1	60.0	16 (73)
P values refer to the difference between the 'asthma' and 'not asthma' groups, t-test for normally-distributed data; Mann Whitney U for non-normally distributed data, chi squared or †Fisher's exact test for categorical data.								

All dichotomised tests (with the exception PEFv and FEV$_1$/FVC <70) were significantly associated with EPOER asthma, sensitivities and NPV tended to be lower (sensitivity range 36% -73%, NPV range 34%-60%) than specificities and PPVs (specificity range 87% - 100%, PPV range 92%-100%). Three tests (FEV$_1$/FVC ratio, BDR, and PEFv) had specificity 100%, these tests could therefore be used together in a 'rule-in' asthma multi-variable model. Auscultated wheeze was also very specific for asthma, although this didn't reach statistical significance.

Exploratory Analysis: "Rule in" Asthma Algorithm in Children (<17 years)

Using predictors of asthma from the univariate analysis and/or NG80 recommended tests, we looked at candidate multivariable algorithms that may be able to confidently "rule-in' asthma and compared this to current performance of the NICE algorithm to rule-in asthma (table 46). In our algorithms we included PEFv using the currently recommended NG80 calculation (although this wasn't a significant predictor of asthma in our cohort), and we also substitute the alternative PEFv measurement (PEFv(alt)) to compare performance of each. We also compare performance of FEV$_1$/FVC ratio within the algorithm using <70 and <LLN. In addition, we considered auscultated wheeze in our algorithm, this was very specific in our cohort, although sensitivity was low and it did not reach statistical significance on its own.

Table 46.   Multivariate algorithms (asthma vs not asthma: children)

	N	Sensitivity % (95% CI)	Specificity % (95% CI)	NPV % (95% CI)	PPV % (95% CI)	Asthma Detected Number (%) positive	Number (%) patients requiring further investigation due to asthma not 'ruled in'
Algorithms:							
All tests:							
A. One of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv, Wheeze, BCTmeth	17	100 (66-100)	75 (35-97)	100 (54-100)	82 (48-98)	9 (100)	8 (47)
B. Two of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv, Wheeze, BCTmeth	17	89 (52-100)	88 (47-100)	88 (47-100)	89 (52-100)	8 (89)	9 (53)
C. Three of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv, Wheeze, BCTmeth	17	44 (14-79)	100 (63-100)	62 (32-86)	100 (40-100)	4 (44)	13 (76)
Without FeNO							
D. One of: FEV1/FVC <70%, BDR, Eos, PEFv>20%, Wheeze, BCTmeth	23	100 (78-100)	75 (35-97)	100 (54-100)	88 (64-99)	15 (100)	8 (35)
E. Two of: FEV1/FVC <70%, BDR, Eos, PEFv>20%, Wheeze, BCTmeth	23	73 (45-92)	100 (63-100)	67 (35-90)	100 (72-100)	11 (73)	12 (52)
Without BCTmeth							
F. One of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv>20%, Wheeze	21	100 (75-100)	88 (47-100)	100 (59-100)	93 (66-100)	13 (100)	8 (38)

Criterion	N						
G. Two of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv>20%, Wheeze	21	54 (24-81)	88 (47-100)	54 (25-81)	88 (47-100)	7 (54)	14 (67)
G. Three of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv>20%, Wheeze	21	46 (19-75)	100 (63-100)	53 (27-79)	100 (54-100)	6 (46)	15 (71)
Without wheeze							
H. One of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv>20%, BCTmeth	17	100 (66-100)	75 (35-97)	100 (54-100)	82 (48-98)	9 (100)	8 (47)
I. Two of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv>20%, BCTmeth	17	89 (52-100)	88 (47-100)	88 (47-100)	89 (52-100)	8 (89)	9 (53)
I. Two of: FEV1/FVC <70%, BDR, FeNO, Eos, PEFv>20%, BCTmeth	17	33 (7-70)	100 (63-100)	57 (29-82)	100 (29-100)	3 (33)	14 (82)
Without FeNO and BCTmeth (most accessible in primary care)							
J. One of: FEV1/FVC <70%, BDR, Eos, PEFv>20%, Wheeze	27	84 (60-97)	88 (47-100)	70 (35-93)	94 (71-100)	16 (84)	11 (41)
K. Two of: FEV1/FVC <70%, BDR, Eos, PEFv>20%, Wheeze	27	47 (24-71)	100 (63-100)	44 (22-69)	100 (66-100)	9 (47)	18 (67)
All - substitute in PEFv(alt)							
L. One of: FEV1/FVC <LLN, BDR, FeNO, Eos, PEFv(alt), Wheeze, BCTmeth	17	100 (66-100)	62 (24-91)	100 (48-100)	75 (43-95)	9 (100)	8 (47)

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M. Two of: FEV1/FVC <LLN, BDR, FeNO, Eos, PEFv(alt), Wheeze, BCTmeth	17	90 (55-100)	100 (59-100)	88 (47-100)	100 (66-100)	9 (90)	8 (47)
Without FeNO – substitute in PEFv(alt)							
O. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, BCTmeth	23	100 (78-100)	62 (24-91)	100 (48-100)	83 (59-96)	15 (100)	8 (35)
P. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, BCTmeth	23	87 (60-98)	100 (63-100)	80 (44-97)	100 (75-100)	13 (87)	10 (43)
Without BCTmeth – substitute in PEFv(alt)							
Q. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, FeNO	21	100 (75-100)	75 (35-97)	100 (54-100)	87 (60-98)	13 (100)	8 (38)
R. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, FeNO	21	69 (39-91)	88 (47-100)	64 (31-89)	90 (55-100)	9 (69)	12 (57)
S. Three of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, FeNO	21	62 (32-86)	100 (63-100)	62 (32-86)	100 (63-100)	8 (62)	13 (62)
Without wheeze – substitute in PEFv(alt)							
T. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), FeNO, BCTmeth	17	100 (66-100)	62 (24-91)	100 (48-100)	75 (43-95)	9 (100)	8 (47)
U. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), FeNO, BCTmeth	17	100 (66-100)	88 (47-100)	100 (59-100)	90 (55-100)	9 (100)	8 (47)

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V. Three of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), FeNO, BCTmeth	17	44 (14-79)	100 (63-100)	62 (32-86)	100 (40-100)	4 (44)	13 (76)
Without FeNO and BCTmeth (most available to primary care) – substitute in PEFv(alt)							
W. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze	27	89 (67-99)	75 (35-97)	75 (35-97)	89 (67-99)	17 (89)	10 (37)
X. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze	27	68 (43-87)	100 (63-100)	57 (29-82)	100 (75-100)	13 (68)	14 (52)
Existing national guidelines							
NICE algorithm	34	38 (20-59)	100 (63-100)	33 (16-55)	100 (69-100)	10 (38)	24 (71)
Abbreviation: Eos, Eosinophil levels, PEFv, Peak expiratory flow variability, PEFv(alt), Peak expiratory flow variability alternative, FeNO, Fractional exhaled nitric oxide, FEV$_1$, Forced Expiratory Volume in one second, FVC, Forced vital capacity, BDR, Bronchodilator reversibility, BCTmeth, Methacholine Bronchial challenge test, Wh, wheeze.							

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Best "Rule in" algorithms in children:

The best algorithm to 'rule in' asthma in children using tests available to primary care, was algorithm X. This algorithm requires any two positive tests from: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), and wheeze (sensitivity 68%, specificity 100%). Our best algorithm in children is slightly more sensitive than our proposed 'best' adult algorithm (sensitivity 90%, specificity 100%), however it requires at two positive tests to diagnose asthma. Like adults, addition of BCTmeth (Algorithm P) improved performance of the primary care algorithm (sensitivity 87%) without reducing specificity. Interesting, in children, the addition of FeNO to the latter algorithm further optimised performance (Algorithm M, sensitivity 90%, specificity 100%).

Best in Primary Care (i.e., no FeNO, no BCTmeth)

> **Algorithm X.** Two of: FEV1/FVC <LLN, BDR, Eos, PEFv(alternative), Wheeze (Sn 68%[43-87], Sp 100%[63-100])

Best overall:

> **Algorithm P.** Two of: FEV1/FVC <LLN, BDR, Eos, PEFv (alternative), Wheeze, BCTmeth (sn 87, sp 100)
> **Algorithm M.** Two of: FEV1/FVC <LLN, BDR, FeNO, Eos, PEFv (alternative), Wheeze, BCTmeth (sn 90, sp 100)

Algorithm X outperformed the current NICE algorithm as a 'rule in' asthma pathway and doesn't require use of FeNO, (a test not always available in primary care). Using this proposed 'best' primary care algorithm, 52% of children put through the algorithm would still require further review to confirm or exclude asthma. This is better than the NG80 paediatric algorithm in whom 71% still required further review. In our 'best overall' algorithm in children (Algorithm M), 47% of children not diagnosed through the algorithm would still need a clinical review to confirm or exclude asthma.

3.4.3.3. Exploratory analysis using MMEF:

In the univariate analysis to determine best predictors of asthma we found that the spirometry measure MMEF%predicted (p<0.001, AUC 0.845) outperformed the more conventional FEV_1/FVC ratio (p<0.001, AUC 0.749), and the BDR measure MMEF%change (p<0.001, AUC 0.845) outperformed the more conventional FEV1%change (p<0.001, AUC 0.778). We investigate if these markers could be used an alternative.

Using binary logistic regression, we show decreasing MMEF%predicted OR[95%CI] 0.939 [0.906-0.974]), p= 0.01, was associated with increased likelihood of asthma after adjusting for FEV1/FEV ratio 1.007 [0.892-1.135], p= 0.915 (included in the model). We also show greater MMEF%change (1.040 [0.998-1.083]), p= 0.060, was associated with increased likelihood of asthma after adjusting for FEV1%change 1.035 [0.923-1.161]), p= 0.558.

Best cut-off thresholds for MMEF%predicted and MMEF%change:

For each of the identified diagnostic markers (MMEF%predicted and MMEF%change) cut-points were calculated for 100% specificity to select optimal thresholds for diagnosing asthma (sensitivity+specificity-1). Children and adults were examined separately similar to other NICE recommended tests (e.g., FeNO, BDR) which use different cut-off thresholds for positive in these two populations (table 47).

Table 47. MMEF 'best cut-point' for a rule in test

Adults		AUROC	Cut-point	Sn	sp	NPV	PPV	N (%) positive asthma detected	Cases; Asthma /not asthma
Spirometry	MMEF%pred	0.83	<66	43 (26-61)	96 (78-100)	52(36-68)	94 (70-100)	15 (43)	35 /23
BDR	MMEF%change	0.85	>132	49 (31-66)	100 (85-100)	56 (40-72)	100 (80-100)	17 (49)	35 /23
Children									
Spirometry	MMEF%pred	0.74	<77	57 (37-76)	100 (69-100)	45 (24-68)	100 (79-100)	16 (57)	28 10
BDR	MMEF%change	0.73	>142	29 (13-49)	100 (69-100)	33 (17-53)	100 (63-100)	8 (29)	28 /10

Substituting conventional spirometry measurements (FEV₁/FVC ratio and FEV₁%change) with novel spirometry measurements (MMEF%predicted and MMEF%change) respectively):

Using the cut-offs MMEF%predicted (adults <66%, children <77%) and BDR: MMEF%change (adults >35%, children >42%) as a marker for asthma we looked to see if substituting this for FEV_1/FVC ratio and BDR: FEV_1%change respectively, optimised the performance of our proposed best algorithms in adults and children (table 48).

Table 48. Multivariate algorithms with MMEF (asthma vs not asthma)

	N	Sensitivity % (95% CI)	Specificity % (95% CI)	NPV % (95% CI)	PPV % (95% CI)	Asthma detected number (%) positive	Number (%) patients requiring further investigation due to asthma not 'ruled in'
Adults only							
W. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze	44	86 (67-96)	100 (79-100)	80 (56-94)	100 (86-100)	24 (86)	20 (45)
W*. One of: MMEF%pred, MMEF%change, Eos, PEFv(alt), Wheeze	44	89 (72-98)	94 (70-100)	83 (59-96)	96 (80-100)	25 (89)	18 (41)
O. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, BCTmeth	38	87 (66-97)	100 (78-100)	83 (59-96)	100 (83-100)	20 (87)	19 (49)
O*. One of: MMEF%pred, MMEF%change, Eos, PEFv>20% or alt, Wheeze, BCTmeth	38	91 (72-99)	93 (68-100)	88 (62-98)	95 (77-100)	21 (91)	16 (42)
Children only							
X. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze	27	68 (43-87)	100 (63-100)	57 (29-82)	100 (75-100)	13 (68)	14 (52)
X*. Two of: MMEF%pred, MMEF%change, Eos, PEFv(alt), Wheeze	27	74 (49-91)	100 (63-100)	62 (32-86)	100 (77-100)	14 (74)	13 (48)

M. Two of: FEV1/FVC <LLN, BDR, FeNO, Eos, PEFv(alt), Wheeze, BCTmeth	17	90 (55-100)	100 (59-100)	88 (47-100)	100 (66-100)	9 (90)	8 (47)
M*. Two of: MMEF%pred, MMEF%change, FeNO, Eos, PEFv(alt), Wheeze, BCTmeth	17	89 (52-100)	88 (47-100)	88 (47-100)	89 (52-100)	8 (89)	9 (53)

In adults (≥17years) the addition of MMEF (MMEF%predicted and MMEF%change) improved sensitivity of our 'rule-in' algorithms however reduced specificity and therefore compromised the 'rule in' algorithm. In children, our best algorithm for primary care (i.e., Algorithm X) was optimised by the addition of MMEF%predicted and MMEF%change (sensitivity 74% versus 68%, specificity 100%). The addition of the MMEF markers resulted in three-quarter of asthma patients receiving correct diagnosis, compared to two thirds using original algorithm. However, it didn't further optimise the performance of our 'overall' best algorithm.

3.5. Discussion

Asthma diagnosis using standard tests

We propose a new approach to asthma diagnosis in adults and children. Our diagnostic algorithm is designed to 'rule in' asthma and is appropriate for symptomatic patients (at least one of, breathlessness, wheeze, cough, and/or tight chest) with clinician suspected asthma. In adults (≥17years), 'any one positive' of: wheeze on auscultation, FEV_1/FVC <LLN, BDR (FEV_1%change) ≥12% and 200ml, PEFv>20% (alternative), Eos >0.4x10^9/l, BCTmeth≤0.2mg, and in children (5-16 years), 'any two positive' of: wheeze on auscultation, FEV_1/FVC <LLN, BDR (FEV_1%change) ≥12%, PEFv>20% (alternative), Eos >0.4x10^9/l, BCTmeth≤0.2mg, confidently rules in asthma (sensitivity 87% and 90% respectively, specificity 100%). Investigations can be performed sequentially and in any order. This novel approach outperformed the current NICE NG80 guideline (sensitivity 36% in adults and 38% in paediatric) when used to 'rule in' asthma.

3.5.1. Performance of the NICE diagnostic algorithm (NG80) in asthma

Asthma Guidelines in England(38) recommend that if a clinician suspects asthma (unless there is a clinical urgency to commence immediate treatment), a diagnostic algorithm (NG80) should be followed to demonstrate objective evidence of asthma before starting treatment. The guideline was produced because of concerns over misdiagnosis of asthma.(5, 7) Until now, the performance of the algorithm has not been tested for accuracy.

3.5.1.1. Application of the NICE algorithm (NG80) in adults (≥17 years)

Feasibility of recommended tests:

In this study, the NICE algorithm could not be performed on one in six adults due to poor compliance with PEFv monitoring. Compliance with all other tests in the algorithm was good. In addition, technically acceptable measurements for all NICE (NG80) recommended

tests was achieved in everyone. NICE guidelines recommend mean PEF variability is calculated over two to three weeks. Using this recommendation, only one in nine patients successfully completed all fourteen days monitoring despite active encouragement in the research setting and use of a digital peak flow meter, meaning participants did not need to write down results. Compliance with PEF monitoring has been shown to be as little as nine percent when used in asthma monitoring.(174) The alternative approach (PEFv(alt)), using daily variability >20%) on three or more days(98) was considered "acceptable" in the 'NICE (NG80) full report (section, 'recommendations and link to evidence.')(24) In our study a minimum three days diurnal measurements was achieved over the two week monitoring period in four out of five participants. However, we acknowledge less days monitoring could result in an increase in false negative test results. In clinical practice, the feasibility of the algorithm may be further compromised by lack of access to tests (i.e., FeNO and BCT) and this is often a problem in primary care. According to a survey of over 600 general practices conducted in 2018, less than two percent had direct in-house access to FeNO, and only one in six had access through referral.(175) All pathways through the adult algorithm require FeNO, therefore lack of access would directly impact feasibility to perform the algorithm. NICE acknowledge that additional infrastructure and training is needed and recommend that in the interim primary care should "implement what they can of the new guidelines using currently available approaches to diagnosis." In their own "primary care implementation feasibility project" only seventeen practices were included in the project (all self-referred), none of whom had prior experience in using FENO. FeNO was provided to each practice free of charge during the feasibility assessment (NICE NG80 Appendix Q).(24) NICE encourage formulating specialist community asthma hubs in order improve efficiency and reduce costs.

Performance of the NICE algorithm

NG80 algorithm correctly identified patients as 'asthma' or 'not asthma' (e.g., consider alternate diagnosis/second opinion) in three out of five patients. However, less than one third of EPOER confirmed asthma patients were identified using the algorithm. The NG80 algorithm recommended investigating for an alternate diagnosis or referral for a second opinion in a third of patients with EPOER 'asthma.' Of concern, these symptomatic asthma

patients would not be commenced on inhaled corticosteroids, and the asthma diagnosis could either be dismissed or delayed awaiting secondary opinion. This could result in harm. These results indicate that by attempting to reduce the over-diagnosis of asthma, the NG80 algorithm may result in under-diagnosis of asthma or delayed diagnosis whilst a second opinion is sought. The latter has the potential for more serious consequences.

On review, all adults with the NG80 'possible asthma' (i.e., suspect asthma and review diagnosis after treatment) were EPOER confirmed 'asthma.' NICE recommend(38) that all patients categorised as NG80 'possible asthma' require review of diagnosis at six to ten weeks of treatment, through repeating spirometry and other objective measures. This could unnecessarily use up primary care time and resources in patients that could be coded as asthma from the initial algorithm.

Using the NICE algorithm as a "rule in" test for asthma diagnosis

The NICE algorithm was tested as a 'rule in asthma' algorithm. Our aim was to investigate if this would improve the performance of the algorithm. To achieve a 'rule in' algorithm, only patients receiving the outcome NG80 'asthma' (i.e., 'diagnose with asthma') would have asthma "ruled in." The other two outcomes do not confirm a diagnosis of asthma and therefore do not 'rule in' the diagnosis. Used in this way the algorithm performed with high specificity (sensitivity 36%, specificity 100%, PPV 100% and NPV 49%) demonstrating that that the NG80 is a good rule in algorithm. However, in two thirds of patients the diagnosis would remain uncertain. In attempt to optimise the NG80 algorithm further, in adults, the NG80 'asthma' and NG80 'possible asthma' (i.e., "suspect asthma and review diagnosis after treatment") outcomes were combined. This resulted in a more sensitive 'rule in' algorithm whilst maintaining its specificity (sensitivity 46%, specificity 100%, PPV 100% and NPV 53%). We called this algorithm the 'Simplified NICE diagnostic algorithm to "rule in" asthma (alternative)' (figure 39). Our data suggests that making this change in the current NICE diagnostic algorithm in adults could improve its performance. However, this has not been formally tested and/or validated.

Figure 39. Suggested simplified NG80 algorithm to "rule-in" asthma (alternative) (17+years)
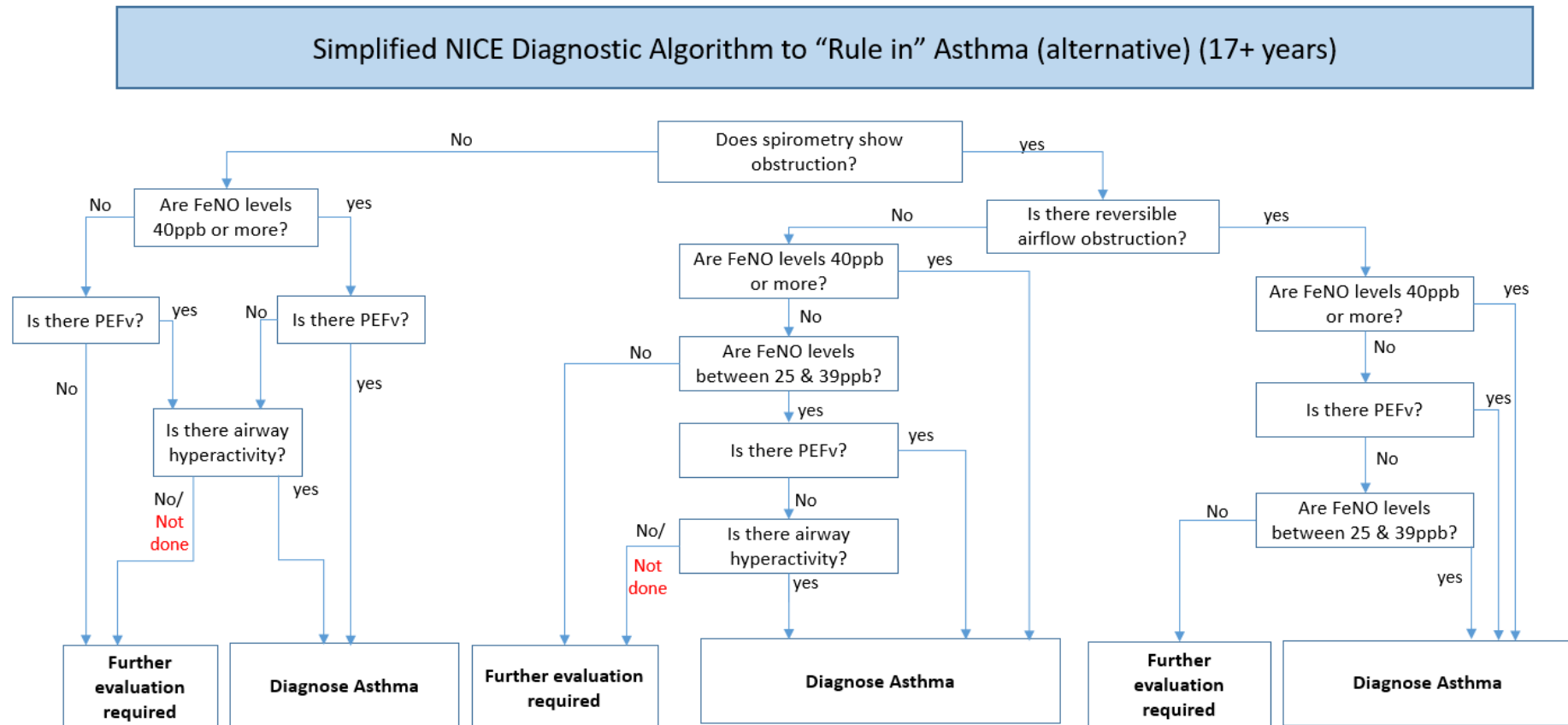
In the suggested 'simplified NG80 algorithm to "rule-in" asthma (alternative),' we speculate that patients in the NG80 'not asthma' (i.e., consider alternative diagnoses/referral for second opinion) category, could be categorized to "further evaluation required." We propose that this could reduce the risk of false negative diagnosis by removing the recommendation 'consider alternative diagnosis.' We suggest that this new wording may encourage GPs or clinicians to further investigate these patients in order to follow recommended guidance. However, this adjusted algorithm has not been tested, before any such changes are considered further collaboration with primary care would be essential before deciding how to improve the NG80 algorithm.

Performance of individual pathways in the NICE algorithm

Understanding the performance of each pathway through the NICE algorithm could guide future asthma diagnosis recommendations. Over half of all potential routes through the NICE algorithm were never utilised in our cohort. False negative diagnosis (i.e., patients with EPOER asthma who did not receive the NG80 outcome 'diagnose with asthma' or 'suspect asthma') was a problem in pathway fifteen (table 14), the most frequently used pathway. In this pathway two out of five patients with EPOER asthma received the outcome "consider alternate diagnosis or referral for a second opinion," this could result in misdiagnosis or treatment delays. The pathway comprises of three negative tests: spirometry, FeNO and PEFv, using dichotomised cut offs for positive. Whilst we show these cut-offs are specific for asthma; 100%, 87%, and 100% specificity respectively, they are not sensitive; 37%, 54%, 10% sensitivity respectively, in our cohort.

Pathway four (table 14) also resulted in false negative diagnosis in all patients; although this pathway was only utilised twice, patients presented with symptoms suggestive of asthma and had obstructive spirometry with bronchodilator reversibility. The NG80 outcome does not recommend starting inhaled corticosteroids in these patients at this stage. Whilst the NG80 algorithm is clearly able to rule in asthma with confidence, we have concerns over the outcome "consider alternate diagnosis or referral for a second opinion," Our results suggest a considerable amount of patients with this outcome will also have asthma, we recommend that this should be made clearer to GPs and clinicians using the NG80 algorithm. We suggest

that these patients would benefit from a clinical consultation with their general practitioner or specialist for review of tests and consideration of further testing so that a diagnosis of asthma is not missed. Of interest, in our new proposed 'rule-in asthma' algorithm (table 43, further discussed below), our data shows that symptomatic adults with obstructed reversible airways disease could be diagnosed with asthma without requiring further tests.

These tests were very specific in our population (i.e., non-smoker or <10 pack year). This algorithm has not yet been tested and would need validation.

The use of rigid dichotomised (positive or negative) test outcomes used in a sequential order, means borderline test results could be overlooked or some tests would not be completed (some pathways do not include BDR or BCT). We speculate that review of a patients' individual test results may help further triage patients with borderline results into the 'asthma' group or 'not asthma' group. For example, patients with all negative tests and no borderline results may then be deemed unlikely asthma by their general practitioner and alternate diagnosis sought or referred for specialist opinion. However, patients presenting with high clinical suspicion, some positive tests, and/or some borderline negative tests, could perhaps be treated as asthma and re-evaluated post treatment trial. Those who remain uncertain after this stage would still require referral to a specialist for further opinion. This approach has not been tested, further studies are required on this group of patients (i.e., patients who do not have asthma 'ruled-in' by the NG80 algorithm), in order to optimise the best approach to confirm or refute a diagnosis of asthma.

We note that over half of our patients with negative NG80 tests following pathway fifteen, had a positive BCTmeth (not included in pathway 15) and/or other potential markers of asthma (such as high eosinophils, auscultated wheeze neither of which is in the NG80 algorithm) in addition to borderline test results. Whilst BCTmeth was the most sensitive and specific test for confirming asthma in adults, the weight applied to a negative BCTmeth test in the NG80 algorithm could lead to potential treatment delays or misdiagnosis. In the NG80 algorithm, a positive BCT test confirms asthma, however a negative BCT leads to the recommendation "consider alternate diagnosis or refer for second opinion." We found a quarter of our adults (all EPOER asthma) that required BCTmeth to complete the NG80

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algorithm, received a "false negative" outcome as a direct result of a negative BCTmeth. In our cohort almost one in two adults with EPOER asthma had negative BCTmeth. Contoli et al (2010) has previously raised false negatives as a concern in BCTmeth and asthma diagnosis.(176) Our results suggest too much weight is applied to this test as a negative predictor for asthma in the NG80, and that perhaps BCTmeth should not automatically result in the outcome "consider alternate diagnosis or refer for a second opinion." It may be that current thresholds for all the NG80 recommended tests are too extreme, further research into optimising thresholds for all NICE recommended tests to 'rule-in' asthma is needed. Current thresholds are specific but not sensitive, adjusting thresholds for positive may enable better sensitivity whilst maintaining specificity.

3.5.1.2. Application of the NICE algorithm (NG80) in Children (<17 years)

Feasibility of recommended tests:

The NICE algorithm could not be performed in two out of five children. Like adults, compliance with complete PEFv was the main reason for algorithm failure. Due to the poor compliance with this test, we suggest its use in a sequential algorithm may not be the best approach. Six out of eight pathways in the paediatric NICE algorithm require PEFv measurement, failure to obtain a result (due to poor compliance) prevents the algorithm progressing. In addition to poor compliance, other studies show fabricating diaries can be a problem.(177) Interpretation of manual PEFv results should be completed with caution. The use of smart PEF meters (as used in this cohort of patients), has been recommended in children to improve accuracy and compliance.(177) Technically acceptable spirometry measurements were completed in all children. FeNO was achieved in four out of five children, all children over nine years completed FeNO at the first visit. This may not be replicable in primary care due to technician inexperience or time restraints. In our children, performance improved further if children were offered a second chance with further coaching at a subsequent visit. Only two children; both aged 5 years, failed to complete FeNO when given this opportunity. Seven of the eight pathways through the paediatric NICE

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algorithm require FeNO. We have concerns that reduced access to this test in primary care would further impact on feasibility of implementing the algorithm.

BCTmeth is not recommended in the NG80 paediatric algorithm, we question why this test is excluded from NG80 algorithm. We found seven out of eight children were able to perform BCTmeth. All children over eight years completed this test. In those children under eight years that didn't complete the test, reduced attention span leading to concerns over inconsistent spirometry was the reason this test was not completed. Of all conventional asthma tests, BCTmeth was the most sensitive (sensitivity 73%) at predicting 'asthma' from 'not asthma.' The European Respiratory Society (ERS) have published recent guidelines for asthma diagnosis in children (2021).(178) The guideline recommend use of BCTmeth where first line objective tests (spirometry, BDR, and FeNO) don't confirm diagnosis. Whilst all recommended diagnostic tests were achievable in our study for the majority, the practicality of completing FeNO and BCTmeth in primary care may be less feasible due to access.

Performance of the NICE algorithm

NG80 algorithm correctly identified patients as 'asthma' or 'not asthma' in one in two children, this is worse than the performance of the adult NG80 algorithm. One in seven children with EPOER asthma, received NG80 algorithm outcome "consider alternative diagnosis and referral for specialist assessment," and may therefore not be established on treatment they required, or use of the algorithm could lead to a delay in starting treatment. Only one in three children with asthma received a definitive diagnosis using NG80. Where the algorithm outcome was 'possible asthma' (i.e., suspect asthma and review after treatment), one child out of twelve would have been commenced on inhaled corticosteroids despite being EPOER 'not asthma.' In summary, our data shows that children given the diagnosis of asthma using the NG80 algorithm (i.e., "diagnose with asthma") can be confidently diagnosed with asthma. Most children with the outcome "suspect asthma and review diagnosis after treatment" do have asthma, however many of those in the remaining category ("consider alternate diagnosis and referral for specialist assessment") also have asthma. More research is needed to investigate this group of patients to investigate how to accurately confirm or refute asthma in this cohort.

Using the NICE algorithm as a "rule in" test for asthma diagnosis in children

Like our adult algorithm, we tested if the paediatric NG80 algorithm could be used as a 'rule in' algorithm. The paediatric algorithm has a similar sensitivity to our adult algorithm, confidently ruling in asthma with sensitivity 38%. When we attempted to optimise the algorithm; combining the NG80 algorithm outcome 'possible asthma,' with 'asthma,' the sensitivity improved greatly (81%). However, it didn't perform as well as a 'rule in' algorithm due to a compromise in specificity (88%). Therefore, a purest 'rule in' algorithm would use the former NG80 'simplified 'rule in' asthma' algorithm in children (figure 40).

Figure 40. Simplified NG80 diagnostic algorithm to rule in asthma in children

Simplified NICE Diagnostic Algorithm to "Rule-in" Asthma (5-16years)
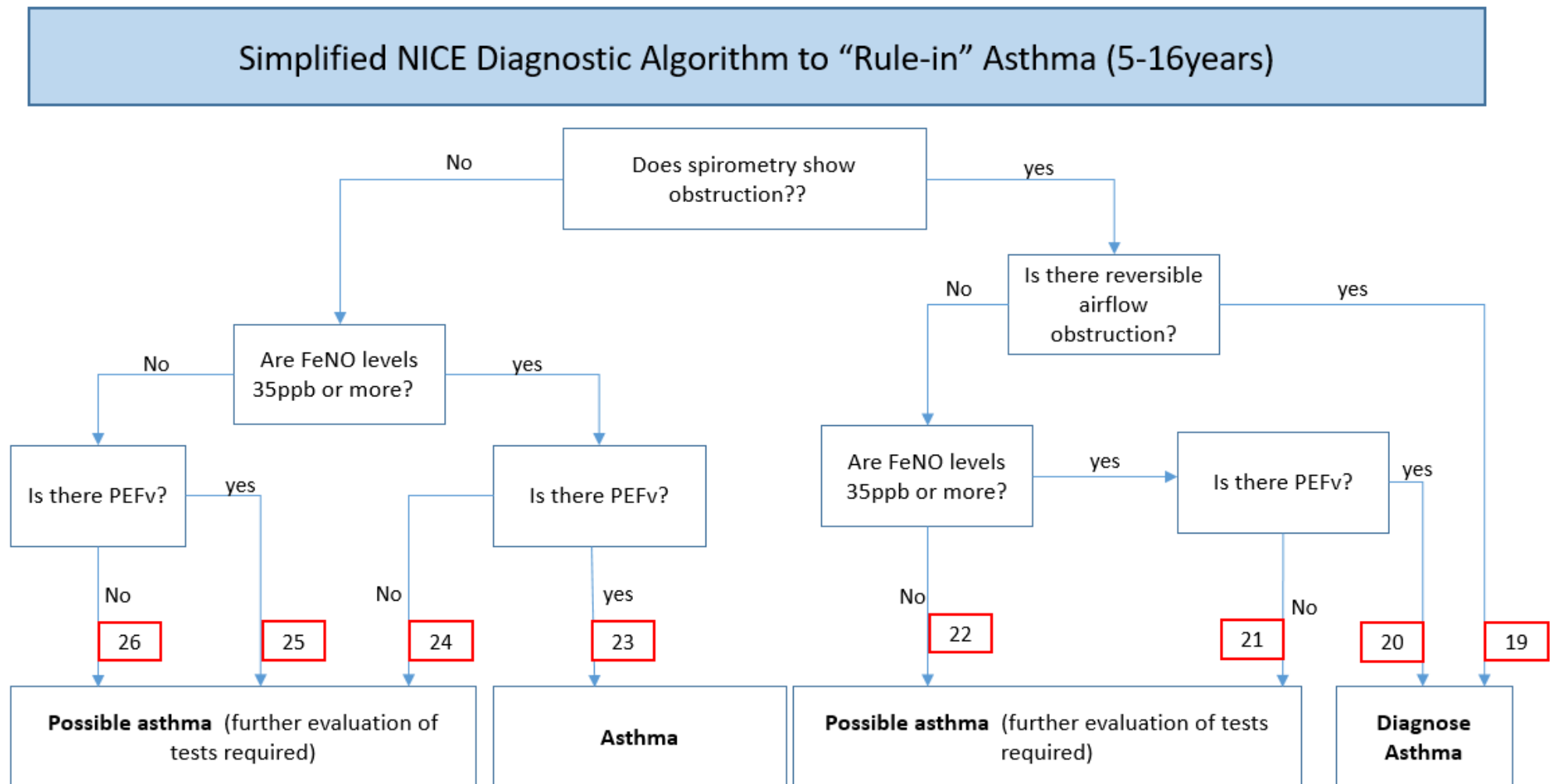
Using this proposed 'rule in' algorithm, a confident diagnosis of asthma could be made, however two out of three children presenting with symptoms of asthma would still be coded 'possible asthma' and still require further clinical assessment with the test results or a trial of treatment.

Performance of individual pathways in the paediatric NICE algorithm

We tested performance of individual pathways in the paediatric NICE algorithm. The most used pathway (pathway twenty-six), in which all three tests were negative (spirometry, FeNO, and PEFv), failed to detect asthma in three out of ten children. This would result in either misdiagnosis or treatment delay whilst waiting specialist assessment. If individual test results (opposed to the dichotomised positive/negative results), were considered, perhaps missed- or delayed- diagnosis and treatment could potentially be avoided by further triaging symptomatic children with some borderline or positive results into 'asthma' or 'possible asthma' groups. This has not been tested. However, we have identified that the current NG80 cut-offs for a positive test are set high, resulting in tests that have poor sensitivity (39%, 60%, and 40% respectively) but are very specific for asthma. Further research looking at optimising the cut-offs for sensitivity whilst maintaining specificity is needed if we are to further optimise the algorithm in children.

We show that all of our EPOER asthma children that were classified "consider alternate diagnosis and referral for specialist assessment" had a positive BCTmeth. We speculate that addition of this test may improve the performance of the paediatric algorithm. The most recent asthma diagnostic guideline produced by the ERS (2021) incorporate BCTmeth into their paediatric asthma diagnostic algorithm. The new ERS guideline uses similar combination of tests to the NG80 (albeit in a different order) but use less extreme thresholds for positive (FEV1/FVC <LLN or <80 if former not available, FeNO ≥25ppb, PEFv ≥12%). Cut-offs were based upon review of the sparse pre-existing literature with, three, four, and one identified study respectively. Validation of these cut-off thresholds in children, and consideration of alternative cut-offs are required to explore the best cut-off for each test in children to diagnose (i.e., 'rule-in') asthma with high specificity.

The most concerning pathway in the paediatric NG80 algorithm is pathway twenty-two. In this pathway, children with obstructive spirometry are not commenced on treatment due to negative BDR and FeNO. In our cohort, we identified two children in this category, both who had EPOER asthma and would have therefore received treatment delay following NG80 recommendations to "refer for specialist assessment.' Of our three children with obstructed spirometry with negative bronchodilator reversibility, all three had PEFv and a positive BCTmeth. Whilst the pathway correctly identified that these children require a specialist review, we speculate adding PEFv (or BCTmeth if available and not contra-indicated) may improve performance of this pathway without the need for specialist referral.

Half of the pathways in the paediatric NG80 algorithm do not take BDR into account. This is based upon the fact that FEV_1/FVC ratio is the first test to be completed and if this test is negative BDR is not recommended. In our cohort only 7% of asthmatic children had FEV_1/FVC ratio <70, increasing to 39% when using <LLN. However, one in eight children with EPOER asthma had positive BDR ≥12% despite negative obstructive spirometry. If the FEV_1/FVC ration of <70% is used instead of <LLN, fewer children with obstruction were identified and this would have led to one in three patients with positive bronchodilator reversibility and 'negative' spirometry not having BDR assessed within the NG80 algorithm. In our cohort, BDR independent of obstructed spirometry, performed well as a rule in diagnostic test for asthma (specificity 100%). This is consistent with other literature in children.(179) However, to our knowledge we are the first study to directly address the diagnostic accuracy of BDR testing in steroid naïve symptomatic children.

We appreciate our patient numbers are small when separating the cohort into adults and children and reviewing individual algorithm pathways. Further validation studies would be essential to inform on the best approach to optimise the NG80 algorithm.

3.5.2. Which tests best predict asthma using standard asthma tests?

After identifying the above concerns with the NG80 algorithm, we tested which variables best predicted EPOER asthma. In adults, univariate analysis revealed seven tests (spirometry [FEV_1/FVC ratio, MMEF, MMEF%pred], BDR [FEV_1%change, FEV_1/FVC%change], FeNO, BCTmeth, PEFv(alt), blood eosinophil count, and auscultated wheeze) that were predictors for asthma. In children univariate analysis revealed six tests (spirometry [FEV_1%predicted, FEV_1/FVC ratio, MMEF, MMEF%pred], BDR [FEV_1/FVC%change, MMEF%change], FeNO, BCTmeth, PEFv (standard/alt), and blood eosinophil count) that were predictors for asthma. In our univariate analysis in children BDR using FEV_1/FVC ratio and MMEF were better predictors of asthma when compared with FEV_1. We also show blood eosinophil levels (not currently used in the NG80 algorithm) were a predictor of asthma in both adults and children. Wheeze was very specific for asthma in both adults and children, however only reached statistical significance in adults, we speculate this is likely due to small proportion of patients with auscultated wheeze in the paediatric group.

Of the NG80 tests (using NICE defined thresholds) all tests except PEFv were significant predictors for asthma in our univariate analysis using dichotomized data. However, PEFv (alt) was a clinically significant predictor. This method accepts diurnal variation >20% on three or more days as a positive test.(98) The authors report this method in symptomatic adults, it has not been tested in children. This method performed better than the standard calculation in both adults and children, however, it was more effective as a 'rule-in' test in adults compared to children (specificity 100% versus 88%). In adults, PEFv(alt) detected one in three asthma cases, opposed to the standard PEFv which detected one in ten asthma cases. This alternative method is also easier to calculate and is likely more practical for use in primary care.

In children FEV_1/FVC ratio using <LLN was a significant predictor, but <70 was not significant in this cohort. Using FEV_1/FVC ratio with the cut-off threshold of '<LLN' opposed to '<70% improved sensitivity of this test five-fold in children and doubles the sensitivity of this test in

adults, without compromising specificity. Our data suggests that LLN should replace the generic <70% threshold. This recommendation is supported by the ERS guideline for asthma diagnosis in children.(178)

In adults, BCTmeth and FeNO had the greatest sensitivity (56% and 54% respectively), however FeNO had lower specificity than other tests which would compromise its use as a rule-in asthma test. In children BCTmeth, Eosinophils and FeNO had the greatest sensitivity (73%, 57%, and 60% respectively), however all had lower specificity when compared to other NG80 recommended tests.

Clinical history alone was not a good tool to select asthma (tables 19-20). Features in the clinical history such as number of symptoms (from wheeze, breathlessness, tight-chest, and cough), reported triggers (e.g., temperature change, exercise, aerosol exposure), co-morbidities (e.g., eczema, hay fever), family history of asthma or allergy, and environmental exposures (e.g., damp living conditions, pets), did not differentiate between the two symptomatic groups. This contrasts guidelines from NICE, BTS and GINA, (37, 38, 71) who all recommend taking some of these factors into account when taking clinical history in patients who present with possible asthma. GINA (2020)(71)recommend a history of more than one of the quartet of symptoms (e.g. breathless, chest tightness, cough, and wheeze) and specific symptom triggers (e.g. exercise and cold air) are features of asthma. We demonstrate that there is no significant difference between our symptomatic 'asthma' and symptomatic 'not asthma' patients in these features. BTS and NICE(37, 38) both recommend taking into account personal or family history of atopic disorder, however this did not predict asthma from 'not asthma' in our study. The only clinical information that demonstrated significant difference between the two groups in our cohort was self-reported anxiety in 'not asthma' patients. Our data suggests features such as 'multiple symptoms from the asthma quartet,' symptom triggers (i.e. "cold air" "exercise"), and personal or family history of atopy, are prevalent in both asthma and symptomatic patients without asthma and did not help distinguish these two groups. Our findings are in keeping with the latest ERS asthma guideline(178) in children that recommend against diagnosing asthma based upon symptoms alone. This information may still be useful in primary care

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because perhaps the absence of these symptoms may be helpful in reducing suspicion of asthma, further studies are required to investigate the role of these variables to 'rule out asthma.'

3.5.2.1.   MMEF in asthma diagnosis (exploratory work)

In addition to considering conventional measurements of established asthma diagnostic tests, we also explored maximal mid expiratory flow (MMEF) pre and post bronchodilation to test if this measurement performed better than standard spirometry-based tests (i.e., FEV_1/FVC ratio and BDR (FEV_1%change)). We demonstrated that the spirometry-based measurement MMEF; a marker of mid-to-small airways disease, was able to predict 'asthma' patients from 'not asthma' patients. This measure performed similar to the current established measurement; FEV_1/FVC ratio ($p<0.001$, AUC 0.765 [0.670-0.860] versus $p<0.001$, 0.749[0.653-0.845]), however if we used 'MMEF%predicted' this outperformed the FEV_1/FVC ratio OR (95%CI) 0.939 [0.906-0.974], p 0.01. This finding is supported by a systematic review of small airways tests in asthma diagnosis that has previously demonstrated MMEF as a predictor of asthma.(180) The authors include five studies assessing MMEF%predicted and show that this marker was lower in asthma in all studies and that it performed better than FEV_1%predicted. The studies only report on MMEF in asthma compared to healthy controls, our study is the first study to our knowledge to report diagnostic ability of MMEF%predicted compared to FEV_1/FVC ratio in symptomatic steroid naïve patients' with and without asthma.

MMEF was also a better predictor of asthma when used as a marker for bronchodilator reversibility (BDR). The conventional test for BDR is FEV1%change, we show that MMEF%change outperformed the conventional measure in our cohort of patients ($p<0.001$, AUC 0.866[0.723-0.893] versus $p<0.001$, 0.779[0.689-0.868]). A greater MMEF%change OR [95%CI] 1.040[0.998-1.083], p=0.06, was more strongly associated with likelihood of asthma after adjusting for FEV1%change.

Using AUC, we identified the optimal cut-off point for a 'rule in asthma' test by maximising specificity (100%specificity) with best sensitivity to define cut-off thresholds for MMEF%predicted and MMEF%change. We looked at adults and children separately in order to optimise our model.

In adults we found MMEF%predicted <66% was the best cut point (Sensitivity 43(26-61), specificity 96(78-100),[AUC 0.83]) and BDR: MMEF%change>32% (Sensitivity 49(31-66), specificity 100(85-100),[AUC0.85]). In children we found MMEF%predicted <77% was the best cut point (Sensitivity 57(37-76), specificity 100(69-100),[AUC0.74]) and BDR: MMEF%change>42% (Sensitivity 29(13-49), specificity 100(69-100),[AUC0.73]). This is an exploratory analysis using small numbers, it would need formal validation. However, these results highlight potential ways moving forwards to optimise asthma diagnosis. Using these measurements didn't further improve our proposed 'rule in' asthma algorithms.

3.5.3. What diagnostic combination (algorithm) of tests best predicts asthma?

3.5.3.1.   What is the best algorithm to diagnose asthma in adults?

Using only tests with high specificity, we developed multivariate algorithms that confidently "rule-in" asthma. Our best performing algorithm for primary care (i.e., excluding BCT and FeNO, as these are not widely available) comprises clinical examination and up to four additional objective tests. Any one positive; from auscultated wheeze, FEV1/FVC <LLN, BDR >12% and 200mls, PEFv[alternative] >20%, and eos >0.4x10^9/L, confirms asthma (figure 41). This algorithm confidently detects six out of seven asthma patients. This performs better than standard NG80 algorithm when used as a 'rule-in' algorithm (i.e., in those with outcome 'diagnose with asthma' the diagnosis is ruled-in), sensitivity 36%. In our new recommended model; similar to the NG80 algorithm, tests could be done sequentially. However, the order of testing is not rigid, testing sequence is flexible to accommodate the

preferences of the clinician and/or the patient. An example: in needle-phobic patients or patients with memory problems, blood eosinophils or PEFv respectively may be performed after the other tests. Once a test is positive, no further tests are required for diagnosis. This is the first diagnostic algorithm that does not enforce a specific order of testing. In addition to NICE, more recent diagnostic guidelines for asthma diagnosis in children produced by the ERS(178) also recommend a specified sequence of testing. Six out of eleven pathways require FeNO, and three out of eleven pathways require BCT to complete the algorithm. We suggest our algorithm may be more practical in clinical practice because lack of access to a test or patient inability to perform a test would not prevent completion of other tests within the algorithm that may lead to a diagnosis.

The addition of the test BCTmeth marginally improved the performance of our recommended asthma diagnostic algorithm (sensitivity 87%, specificity 100%) (figure 41). The addition of BCTmeth could be performed after other tests that are easier to access in primary care. A specialised community asthma hub could perhaps bridge the gap between primary care and secondary care and enable a more thorough assessment of patients in the community. National guidelines (NICE 2017)(38) recommend Asthma diagnostic hubs, however these are yet to be established in many areas. Diagnostic hubs may have a better chance of receiving funding for tests such as FeNO and BCT because one hub would serve multiple GP practices. Neither of our recommended models in adults incorporate FeNO. The addition of FeNO reduced specificity of our algorithms when applied to adults. Both algorithms performed best when LLN (opposed to <70%) was used in spirometry, and when PEFv (alternative) was used opposed to PEFv (standard). More studies are required to validate the performance of the latter.

Figure 41. Diagnostic algorithm "rule asthma in" (adults ≥17)

Diagnostic Algorithm "Rule Asthma in" (Adults ≥17)

Clinical suspicion asthma AND at least one symptom (breathless, wheeze, cough, tight chest)

↓ Yes

*Any one test positive:
1. Wheeze auscultated
2. FEV1/FVC: <LLN
3. BDR: ≥12% and 200ml
4. PEFv(alternative): >20%
5. Eosinophils: >0.4x10$_9$/L

* Tests can be performed in any order, once a positive test is detected no further tests required for diagnosis.

Yes →

Diagnose asthma

↓ No

Refer for BCTmeth (if available)
Is *BCTmeth positive?
(*PD20≤0.2mg or PC20≤8mg/ml)

Yes →

↓ No / Not available

Asthma has not been 'ruled in.' Review all test results and consider; trial of treatment (if positive and borderline positive tests), alternate diagnosis (if all negative and no borderline results), referral to specialist.

3.5.3.2.   What is the best algorithm to diagnose asthma in children?

Our best performing algorithm for primary care (excluding BCT and FeNO), comprises clinical examination and up to four additional objective tests. Any two positive; from auscultated wheeze, FEV1/FVC <LLN%, BDR ≥12%, PEFv[alt] >20%, and eos >0.4x10$_9$/L, confirms asthma. This algorithm confidently detects two thirds of asthma patients that are put through the algorithm. This performs comparable to the NG80 algorithm when it is used as a 'rule-in' algorithm (sensitivity 67%). However, in our recommended model for primary care, we do not include FeNO, a test not easily accessible in primary care.(175) Therefore this algorithm may be more feasible to complete than the current recommended NG80 algorithm. Tests in the algorithm can be completed sequentially and, in any order, once any two positive tests are confirmed there is no need to complete further testing. The requirement for two at least two positive objective tests is in keeping with other paediatric asthma diagnostic guidelines.(24, 178)

In children, addition of the BCTmeth (PD20 ≤0.2mg) and FeNO (≥ 35 ppb) improved the performance of our recommended asthma diagnostic model (sensitivity 90%, specificity

100%) (Figure 42). In our paediatric algorithm, BCTmeth and FeNO could be performed after other tests that are easier to access in primary care. The second model would be well placed in a diagnostic hub due to it featuring more specialist tests.

Figure 42. Diagnostic algorithm to "rule asthma in" (Children <17)
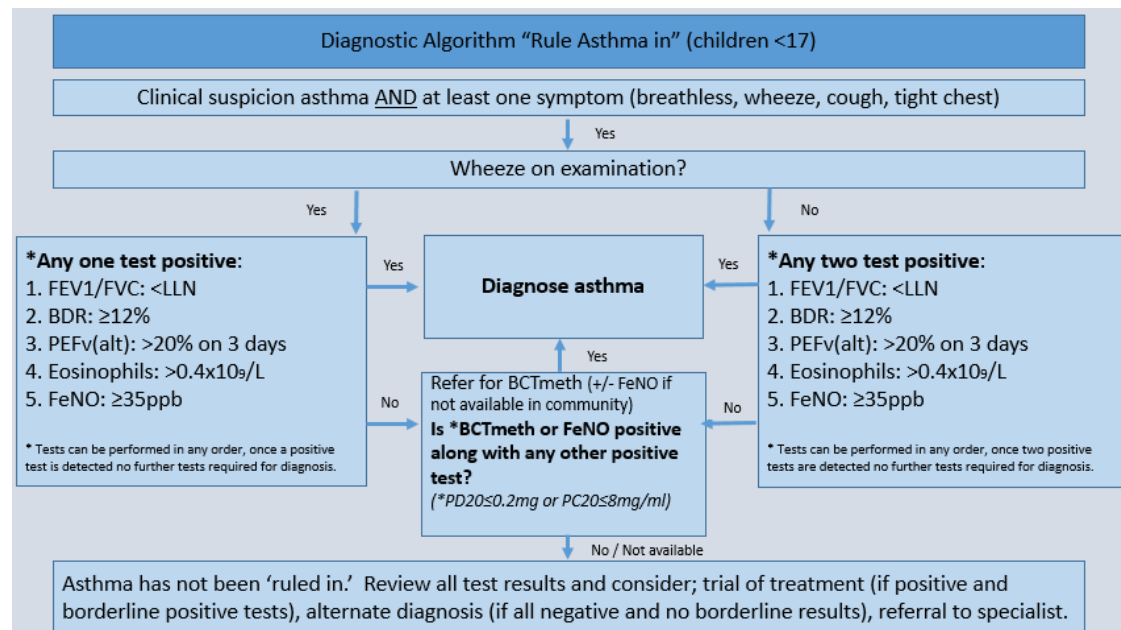
3.5.4. Strengths and weaknesses of our proposed algorithm

Using our best 'rule in' algorithms for adults and children, whilst most asthma patients were correctly identified (87% and 90% respectively), we acknowledge that the remaining asthma cases and the not asthma patients (accounting for around half of presenting patients), would still require a clinical review to confirm or exclude the diagnosis. This still performs better than the NICE algorithm when used to 'rule in' asthma. In patients not diagnosed with asthma through our algorithm, we recommend review of tests completed (to consider actual test values opposed to dichotomised cut-offs) in the remaining patients. In patients with one positive test and borderline tests, a trial of treatment and/or referral for specialist opinion may be appropriate. In patients with all tests negative and no borderline results, consideration of alternate diagnosis and/or referral for specialist opinion may be

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appropriate. However, we have not tested the best approach in these patients. Further studies are required to explore how best to confirm or refute diagnosis in these patients.

Advantages of the 'rule-in' asthma algorithm is the flexibility of order for investigations. Tests can be organised in any order; this differs from the current NICE (NG80) algorithm, allowing clinicians to start with the most accessible, economical, or practical test for their patient. Once a test is positive, no further tests would be required, and asthma would be "ruled-in."

3.5.5. Strengths and weaknesses of the study

This is the first study to assess the best approach to asthma diagnosis (using all common tests available in the NICE guidelines) in a steroid naïve population of symptomatic adults and children. Asthma diagnosis was made using expert panel who had access to clinical history, and objective tests pre and post treatment. We acknowledge this is an exploratory analysis using small patient numbers. Our patients did not have other significant co-morbidity and are non-smokers or have less than ten pack year smoking history. The results must not be extrapolated beyond this population. Validation studies are required to confirm the findings.

3.5.6. Future Work

These are important findings that contribute new knowledge on asthma diagnosis. We highlight potential concerns in using the current NG80 diagnostic algorithm, suggest alterations to improve the performance of the NG80 algorithm in the short term, and explore potential diagnostic algorithms that may have a role in asthma diagnosis to replace current national recommendations. However, these results need to be validated before recommendations can be made. In addition, we have shown alternative spirometry measures (MMEF%predicted and MMEF%change) have promise in asthma diagnosis when compared to current measurements. These need further exploration, and findings validated. The work presented in this thesis is an interim analysis of an ongoing asthma diagnostics

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study. We plan to attempt to validate our findings with a larger data set once the study has completed recruitment. From this interim analysis, we identify that current thresholds for a positive tests across all NG80 recommended tests perform with poor sensitivity. All tests perform as good 'rule-in' asthma tests due to high specificity, however further work investigating more optimal thresholds for asthma diagnosis that improve sensitivity whilst maintaining specificity is needed.

4. Asthma diagnosis using Airways Oscillometry

4.1. Introduction

Airways Oscillometry (AO) is a novel test that enables assessment of airways mechanics by measuring components of airways impedance (resistance and reactance). Sound waves of multiple frequencies are projected into the lungs during simple tidal breathing, relationship between pressure and flow are measured at each frequency and information is obtained on in-phase and out-phase components (i.e., resistance and reactance respectively) (See Section 1.2.6 for more details). Lower frequency sound waves (i.e. 5Hz) penetrate further representing lower airways. An Oscillogram is produced following the test, this gives information on different components of the airways (see section 1.2.6, figure 9). Baseline measurements are commonly reported; Resistance at 5Hz (R5), resistance at 20Hz (R20), and reactance at 5Hz (X5). Using these measurements, other key measurements can be derived to provide further information on resistance and reactance measures (i.e. Resistance at 5Hz minus Resistance at 20Hz (R5-R20), resonant frequency (Fres), area of reactance (AX), reactance at 5Hz in inspiratory phase of tidal breath (X5in) and expiratory phase of tidal breath (X5ex)).

In people with asthma, Airways Oscillometry has been shown to differentiate between severe and mild disease, (155) it is also shown to detect asthmatic lungs from healthy controls(62, 156) (refer to literature review Section 1.3.1.4) However, there is limited research that looks at the ability of AO to diagnose asthma in a cohort of symptomatic patients (i.e. the group on which the test would be used in if AO was performed in clinical practice).

4.2. Aim

We set out to determine if the novel test 'Airways Oscillometry' could be used in clinical practice as a diagnostic test to predict asthma in symptomatic patients. The aim of this analysis was to i) investigate repeatability of the novel test airways oscillometry (AO) in healthy control volunteers, ii) investigate whether repeatability is affected in patients with respiratory symptoms and a diagnosis of 'asthma' or 'not asthma,' iii) investigate whether patient demographics affect AO measurements, iv) investigate the predictive power of AO to diagnose asthma from a cohort of symptomatic adults, v) Explore the relationship between AO and conventional asthma diagnostic tests in adults.

4.3. Methods

Patients (5-70 years) with clinician suspected asthma (symptoms of cough, wheeze, chest tightness, and/or breathlessness) referred from primary or secondary care, plus asymptomatic healthy controls (5-70 years) with no underlying co-morbidity, recruited into the RADicA research clinic were included (see methods, chapter 2).

To determine the repeatability of AO we used data from the 'asymptomatic healthy control' group (n= 65) of the healthy volunteer arm of the RADicA study. At visit one, participants underwent a structured consultation (clinical history and physical examination), before completing AO, standard diagnostic tests (FEV$_1$/FVC ratio, BDR, FeNO), skin prick testing, and blood eosinophils. AO was performed pre- and post- bronchodilation with 400mcg salbutamol. In addition, baseline AO measurements were analysed from visit two. Results for all standard tests were recorded from visit one, however if data from visit one was missing, for the missing test(s) data was used from visit two. We also analysed data from both the 'symptomatic asthma' group (n= 63) and 'symptomatic not asthma' group (n= 33) of the patient arm of RADicA. In addition to the tests described above, the symptomatic patient group also completed PEFv and BCTmeth. After completing all baseline tests the symptomatic group received inhaled corticosteroids (see section 2.2.2.4 for details) for six to eight weeks, after which diagnostic tests were repeated. Participants were classified as asthma, not asthma, possible asthma, or insufficient evidence, during an expert panel

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objective evidence review (EPOER, section 2.6.1). Patients with the outcome 'Possible asthma' (n= 4), or 'insufficient evidence' (n= 15), were excluded from the analysis. Details on patients excluded from the analysis is described earlier (section 3.4.1.3).

To determine AO as a predictor for asthma in adults (17-70 years) we used data from the above three groups but excluded children. A summary table of patients' demographics and clinical characteristics to be presented is displayed below (table 49).

Table 49.   Demographics and clinical characteristics

Baseline characteristics	
Demographics	age, gender, ethnicity, BMI, smoking status, pack years
Airways oscillometry variables (continuous data)	
AO	R5, R5%pred, R20, R5-R20, X5, X5in, X5ex, AX, Fres, TV
AO post bronchodilator Reversibility	R5%change, R20%change, X5%change, X5ex%change, X5in%change, AX%change, Fres%change, T_V%change
Conventional tests (continuous data)	
Spirometry	FEV_1, FEV_1%pred, FVC, FVC%pred, FEV_1/FVC
BDR	FEV_1%change
FeNO	FeNO level
Blood	Eos
Abbreviations: Body Mass Index, BMI, Airways Oscillometry, AO, Resistance at 5Hz, R5, Resistance at 20Hz, R20, Reactance at 5Hz, X5, expiration, ex, inspiration, in, Reactance area, AX, Resonant frequency, Fres, Tidal volume, T_V, Forced expiratory volume in 1 second, FEV_1, Forced vital capacity, FVC, Bronchodilator reversibility, BDR, Fractional exhaled nitric oxide, FeNO, Eosinophils, Eos	

AO was performed using the commercially available TremoFlo C-100 (Airwave Oscillometry System AOS). The device was calibrated daily with standard adult test load (paediatric test load was used in children <14years) (see methods, section 2.3.10).

4.3.1. **Statistical analysis plan**

i) Determine the repeatability of 'Airways Oscillometry' in healthy volunteers, ii) Explore repeatability in symptomatic patients, iii) Determine if baseline AO measurements are affected by patient demographics.

The following analyses were performed:

- Baseline AO measurements are reported for each of the three groups (healthy control, asthma, symptomatic not asthma) using one way ANOVA for normally distributed data. If there were significant differences between one or more groups, post hoc analysis using the Tukey test was performed. Kruskal-Wallis one way ANOVA on non-normally distributed data and post hoc analysis by pairwise comparison (values adjusted using Bonferroni correction for multiple tests). Chi-square test was performed for categorical data.

- Scatter plots were produced to illustrate associations between standard AO measurements across the two visits in asymptomatic healthy volunteers. Correlation coefficients using Spearman Rank correlation were reported to determine relationships between each measurement between visits.

- Repeatability of AO measurements in the 'asymptomatic healthy control' group were then reported by computing 'difference between visits' and 'mean of two visits.' A one sample T Test was performed on the 'difference between visits.' When no significant difference was detected (i.e., p value >0.05) Bland Altman plots were generated to illustrate the correlation between visits. Where a significant difference was noted between two visits, no further analysis was required on the data and the measurement was assumed to show poor repeatability. Limits of agreement were reported and adjusted to report mean difference between visits. Linear regression co-efficient of the 'difference' and 'mean' were computed, when the value is >0.05 'no bias' will be assumed.

- We computed intra-class correlation coefficients (ICC) with standard error of measurement (SEM) to determine agreement between two visits to show the reliability of each repeated measurement.

- Minimal detectable change (MDC); the minimal thresholds beyond the random measurement error with 95% confidence interval, was reported along with internal consistency (Cronbach's' alpha), values greater than 0.7 will be considered good.

- Effect of confounding variables on repeatability of AO measurements in healthy controls were reported.

- The repeatability analyses were then performed on the 'symptomatic asthma' group and 'symptomatic not asthma' group to compare AO measurements in patients with respiratory symptoms with the healthy control group.

- Correlations of AO measurements against age, gender, BMI, and height, for airways resistance (R5) and airways reactance (X5) were illustrated using scatter plots and box plots.

iv) Determine the predictive power of 'Airways Oscillometry' (AO) to predict EPOER confirmed asthma in adults (Exploratory analysis)

To determine the predictive power of AO to predict asthma, we used AO data collected from visit one in symptomatic patients. Adults (≥ 17 years) from the patient arm of the RADicA study, i) 'symptomatic asthma' and ii) 'symptomatic not asthma' were investigated. This is the population that the test would be used on in clinical practice. In addition, we compare each group in the patient arm of RADicA, with the 'asymptomatic healthy control' group.

The following analyses were performed:

- Patient demographics, and clinical characteristics were reported for all three groups.

- Analysis of all AO measurements to determine predictors of asthma from 'symptomatic not asthma,' 'symptomatic asthma,' and 'asymptomatic healthy

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controls,' was performed using the following: one way ANOVA for normally distributed data. If there was significant difference between one or more groups, post hoc analysis using the Tukey test was performed. Kruskal-Wallis one way ANOVA on non-normally distributed data and post hoc analysis by pairwise comparison (values adjusted using Bonferroni correction for multiple tests). Chi-square test was performed for categorical data.

- We used linear regression to examine effects of potential confounders on AO measurements. ANCOVA was used to adjust for any confounders identified. We report 95% confidence intervals and p-values from the F-test with two degrees of freedom. Where relevant post-hoc pairwise comparisons were performed with Benjamini-Hochberg adjustment for multiple testing.

- Predictors of asthma from all symptomatic patients were further explored using Youdens Index to illustrate 'cut-points' for detecting asthma. Sensitivity and specificity were reported. We also use cut points for AO to 'rule in' asthma by selecting thresholds based upon a 100% specificity.

- We added our significant AO measurements (using defined cut-offs for positive) into previously defined multivariate algorithms for asthma diagnosis (see chapter 3) in order to explore how this affected performance of our proposed algorithm.

- We repeated this analysis, however this time we used AO to replace spirometry-based tests in order to explore the role of AO when spirometry-based procedures are not available.

v) Determine if 'Airways Oscillometry' measurements correlate with current tests used in the NICE diagnostic algorithm (NG80). Could Airways Oscillometry replace pre-existing tests within the algorithm? (Exploratory analysis in adults)

The following analyses were performed in the patient group: 'symptomatic asthma' and 'symptomatic not asthma.'

- Using all AO measurements that were shown to discriminate 'asthma' from 'not asthma,' correlation coefficients using Spearman Rank correlation were reported to determine relationships between each measurement and standard diagnostic tests.

- Further analysis was performed to assess ability of each of the selected AO measurements to determine patients with positive standard asthma tests (from NICE algorithm) using established cut-offs (e.g., FEV_1/FVC ratio <70%, FEV_1/FVC ratio < LLN, FeNO ≥40ppb, PEFv >20%, BCTmeth PD_{20} ≤0.2mg.)

- Scatter dot correlation plots of each AO measurement that was shown to discriminate 'asthma' from 'not asthma' were correlated with standard asthma tests from the NICE algorithm: FEV_1/FVC, BDR, FeNO, PEFv, BCTmeth, using continuous data.

4.4. Results

4.4.1. Determine the repeatability of 'Airways Oscillometry'

4.4.1.1. Baseline demographics

Baseline demographics of the 'asymptomatic healthy control' group, the 'symptomatic asthma' group, and the 'symptomatic not asthma' group are presented in table 50.

Table 50. Patient demographics

Patient Demographics	Asymptomatic healthy (N 65)	Symptomatic Asthma (N 63)	Symptomatic Not Asthma (N 33)	P value	Post Hoc Tests p Value
Age, mean (SD) years	28.51 (13.71)	22.71 (15.38)	29.76 (16.09)	0.035	*0.074 ⱡ0.074 ≠0.918
Gender, n (%) females	37 (56.9)	33 (52.4)	22 (66.7)	0.405	n/a
Ethnicity, n (%) white	60 (92.0)	43 (68.3)	26 (78.8)	**0.003**	*0.276 ⱡ**0.001** ≠ 0.054
Height, median (IQR)	166.00 (161.60-173.05)	160.00 (135.80-171.80)	160.60 (152.00.-168.90)	**0.016**	1.00 ⱡ**0.021** ≠ 0.134
BMI, mean (SD) kg/m^2	24.02 (5.59)	24.23 (6.62)	26.59 (7.37)	0.143	n/a
Current or ex-smokers, n (%)	15 (23.1)	10 (15.9)	8 (24.2)	0.503	n/a
Pack years, median (IQR)	0.00 (0.00-0.00) N 63	00.00 (0.00-0.00)	0.00 (0.00-0.00)	0.617	n/a
Days between visits, median (IQR)	17.00 (9.00-34.00)	22.00 (15.25-27.5)	21.5 (14.00-28.00)	0.366	n/a

*P value, difference between ' asthma' and 'not asthma' groups, ⱡP value, difference between 'asthma' and 'healthy control,' ≠P value, difference between 'not asthma' and 'healthy controls'

Mean (SD) was recorded if whole population (i.e. all three groups combined) was normally distributed (skewness and kurtosis between -1/+1), otherwise median (IQR) was reported. One way ANOVA for normally distributed data, Post hoc analysis (Tukey) was performed if significant difference between one or more groups. Kruskal-Wallis one way ANOVA for non-normally distributed data, Post hoc analysis by pairwise comparison (values adjusted by the Bonferroni correction for multiple tests). Chi-square test for categorical data.

Patient demographics for age, gender, BMI, and smoking history were comparable between groups. All groups were predominantly white ethnic background, white ethnicity was significantly higher in the 'healthy' group (90%), compared with the 'asthma' group (68%) (p

0.001). Height was significantly greater in the healthy control group compared to asthma group. This is because a smaller proportion of children were present in the healthy control group (25% versus 44% respectively). When we separated adults (≥17years) from children (5-16 years) there was no difference between groups.

4.4.1.2.   Repeatability of Airways Oscillometry measurements in asymptomatic healthy control group

Scatter Plots

All patients in the healthy control arm of the RADicA study performed Airways Oscillometry (AO) at visit one and visit two. Baseline measurements were plotted for visit one and visit two for each patient (figure 43).

Figure 43. Scatter plots of visit one and two in healthy control group
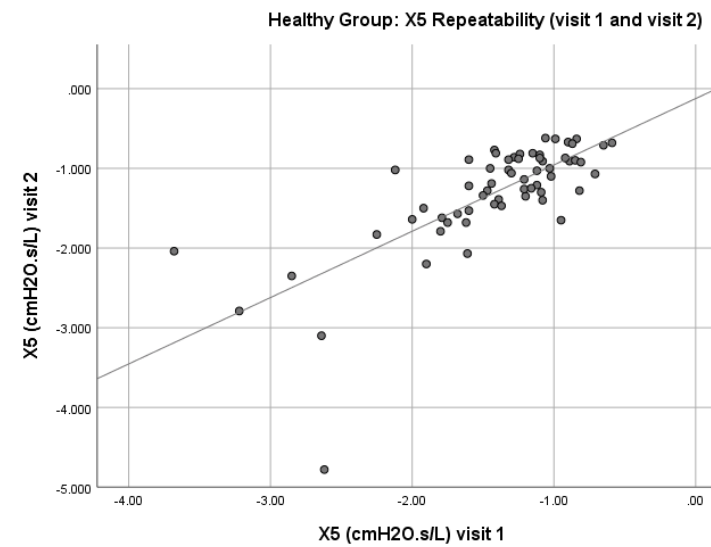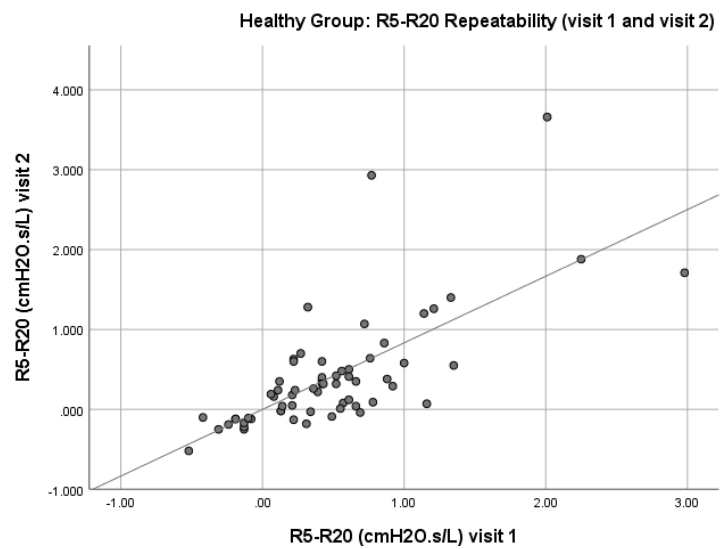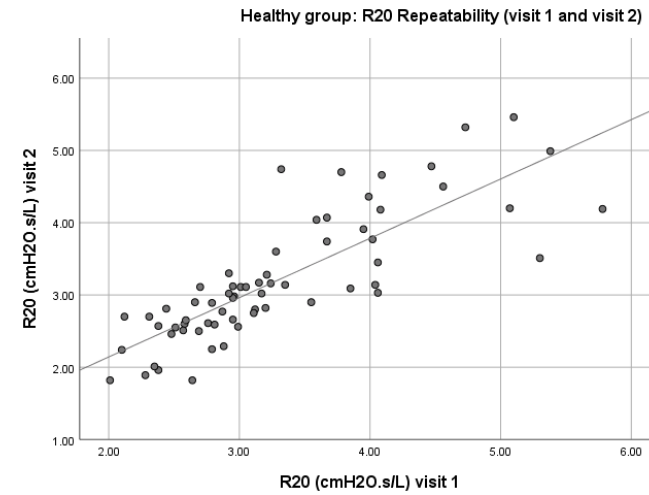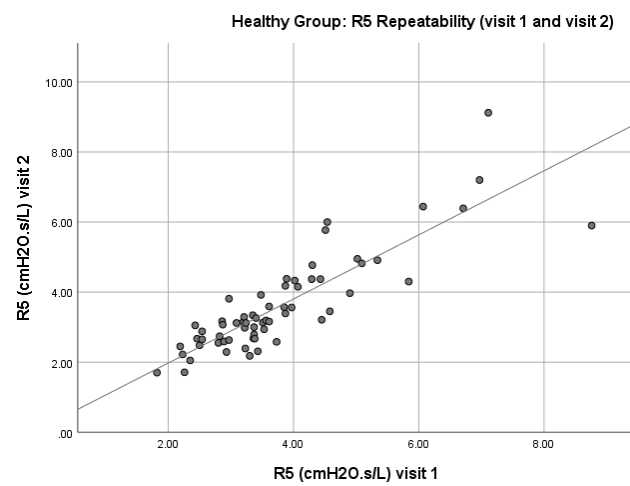
Healthy Group: AX Repeatability (visit 1 and visit 2)
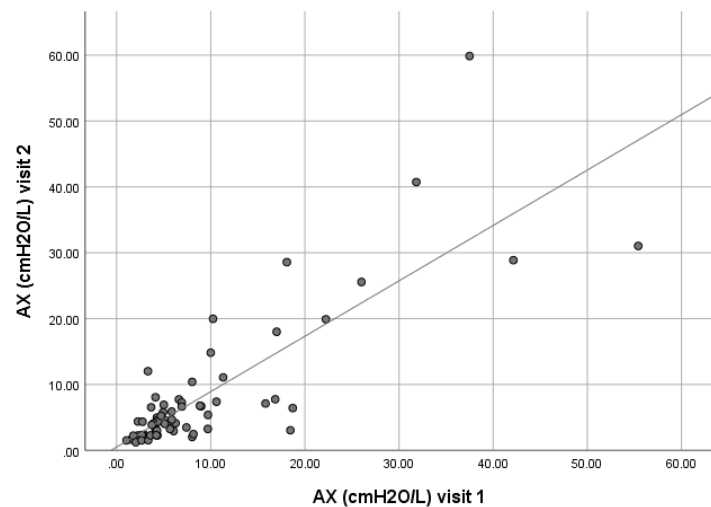
Healthy Group: Fres Repeatability (visit 1 and visit 2)
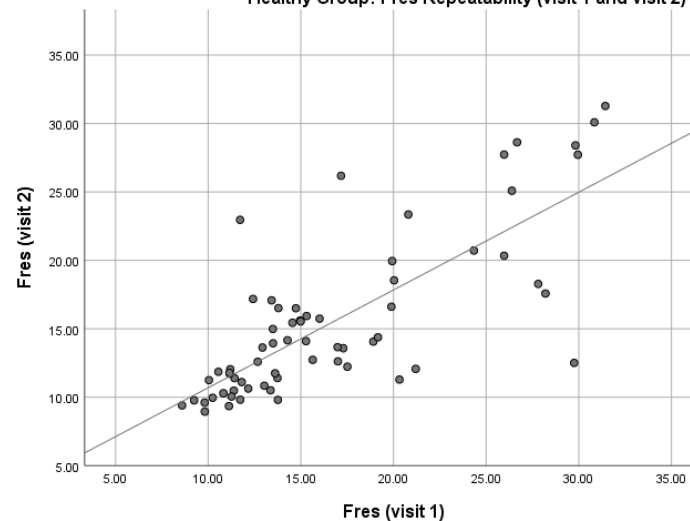
Healthy Group: X5.in Repeatability (visit 1 and visit 2)
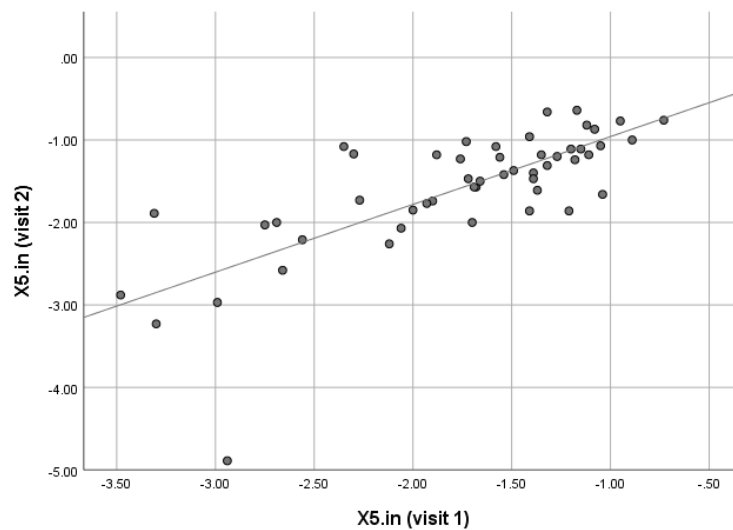
Healthy Group: X5.ex Repeatability (visit 1 and visit 2)
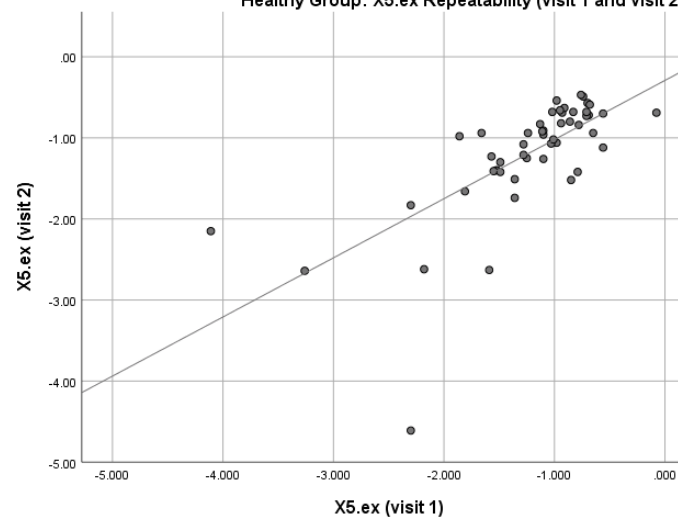
The scatter plots visually demonstrate the relationship between each AO variables across the two visits. At the extremes of measurements (i.e., positive in resistance measurements (R5, R20), and negative in reactance measurements (X5)) there was greater variation between visits. For example, those with greater airways resistance (R5) or reactance (X5) appeared to show lower repeatability.

We generated correlation coefficients using Spearman Rank correlation to determine the strength of relationship between each measurement between visits (table 51).

Table 51.   Spearman rank correlation coefficients between visits in the healthy group

Variable	Correlation coefficient (95%CI)	*P value
R5	0.850 (0.73, 0.91)	<0.001
R20	0.871 (0.8, 0.91)	<0.001
R5-R20	0.705 (0.52, 0.83)	<0.001
X5	0.695 (0.52, 0.82)	<0.001
AX	0.727 (0.57, 0.85)	<0.001
Fres	0.770 (0.62, 0.88)	<0.001
X5in	0.720 (0.51, 0.85)	<0.001
X5ex	0.737 (0.56, 0.85)	<0.001
*correlation is significant at the 0.01 level		

All measurements showed statistically significant Spearman rank-order correlation (p <0.01). All measurements show a strong correlation coefficient (> 0.60).

Bland Altman Plots

Bland Altman plots were then created for each Airways Oscillometry measure in the 'asymptomatic healthy' group.

Table 52.   Repeatability analysis in healthy control group using test-retest reliability

Variable	≠n	Difference between visits, mean (SD)	P-value*	Limits of agreement	1.96*SD	Regression coefficient (95% CI)	P-value*	ICC (95%CI)	SEM	MDC	Cronbach's alpha (95%CI)
R5	63	0.18 (0.71)	0.329	(-1.22, 1.571)	1.40	-0.064 (-0.203, 0.076)	0.547	0.855 (0.771 , 0.909)	0.512	1.420	0.925 (0.887 , 0.956)
R20	65	0.09 (0.52)	0.688	(-0.937, 1.117)	1.03	-0.004 (-0.164, 0.155)	0.858	0.815 (0.715 , 0.883)	0.372	1.032	0.9 (0.837 , 0.946)
R5-R20	63	0.08 (0.52)	0.688	(-0.93, 1.095)	1.01	-0.191 (-0.401, 0.019)	0.334	0.697 (0.545 , 0.804)	0.366	1.014	0.823 (0.689 , 0.906)
X5	62	-0.12 (0.48)	0.329	(-1.041, 0.811)	0.93	-0.141 (-0.341, 0.059)	0.493	0.721 (0.577 , 0.822)	0.340	0.943	0.845 (0.77 , 0.922)
AX	65	0.97 (6.13)	0.688	(-11.043, 12.978)	12.01	-0.026 (-0.184, 0.131)	0.834	0.82 (0.721 , 0.886)	4.335	12.016	0.902 (0.847 , 0.934)
Fres	65	1.21 (4.13)	0.149	(-6.893, 9.315)	8.10	0.087 (-0.094, 0.267)	0.547	0.755 (0.628 , 0.843)	3.016	8.359	0.87 (0.753 , 0.941)
X5in	51	-0.18 (0.52)	0.138	(-1.18, 0.827)	1.00	-0.107 (-0.324, 0.11)	0.547	0.724 (0.563 , 0.832)	0.378	1.047	0.854 (0.767 , 0.927)
X5ex	51	-0.10 (0.69)	0.688	(-1.132, 1.043)	1.09	-0.068 (-0.315, 0.178)	0.745	0.691 (0.516 , 0.811)	0.388	1.076	0.815 (0.741 , 0.904)

*ANOVA or Kruskal-Wallis test with adjustment for multiple testing
≠ n varies due to technical issue with Tremoflow software resulting in lost data, if both visits not available patient data was excluded for repeatability analysis
Abbreviations; ICC, intra-class correlation coefficient (from one-way ANOVA), SEM, standard error of measurement (SD*sqrt(1-ICC)), MDC, minimal detectable change (1.98*sqrt(2)*SEM)

In our healthy control group there was no significant difference between the mean AO measurements between visits (table 52). Repeatability was assessed by investigating 'agreement'. Limits of agreement present the 95% confidence interval around the mean difference between visits. These were non-significant indicating good agreement in all measurements. Reliability was tested through investigating the ability of each measurement to differentiate between patients at different visits, intra-class correlation (ICC) was greater than 0.6 for all measurements indicating good to excellent reliability. We calculated the minimal detectable change (MDC) for all measurements, indicating the minimal magnitude of change that can be put down to random measurement error, beyond which change is more likely to be real. We also show internal consistency using Cronbach's alpha in all measurements. Bland Altman plots display mean difference between visits and display 95% confidence intervals (figure 44).

Figure 44. Bland Altman plots for repeatable measurements in asymptomatic healthy group (continuous line shows mean difference between visits, dotted line shows limits of agreement)
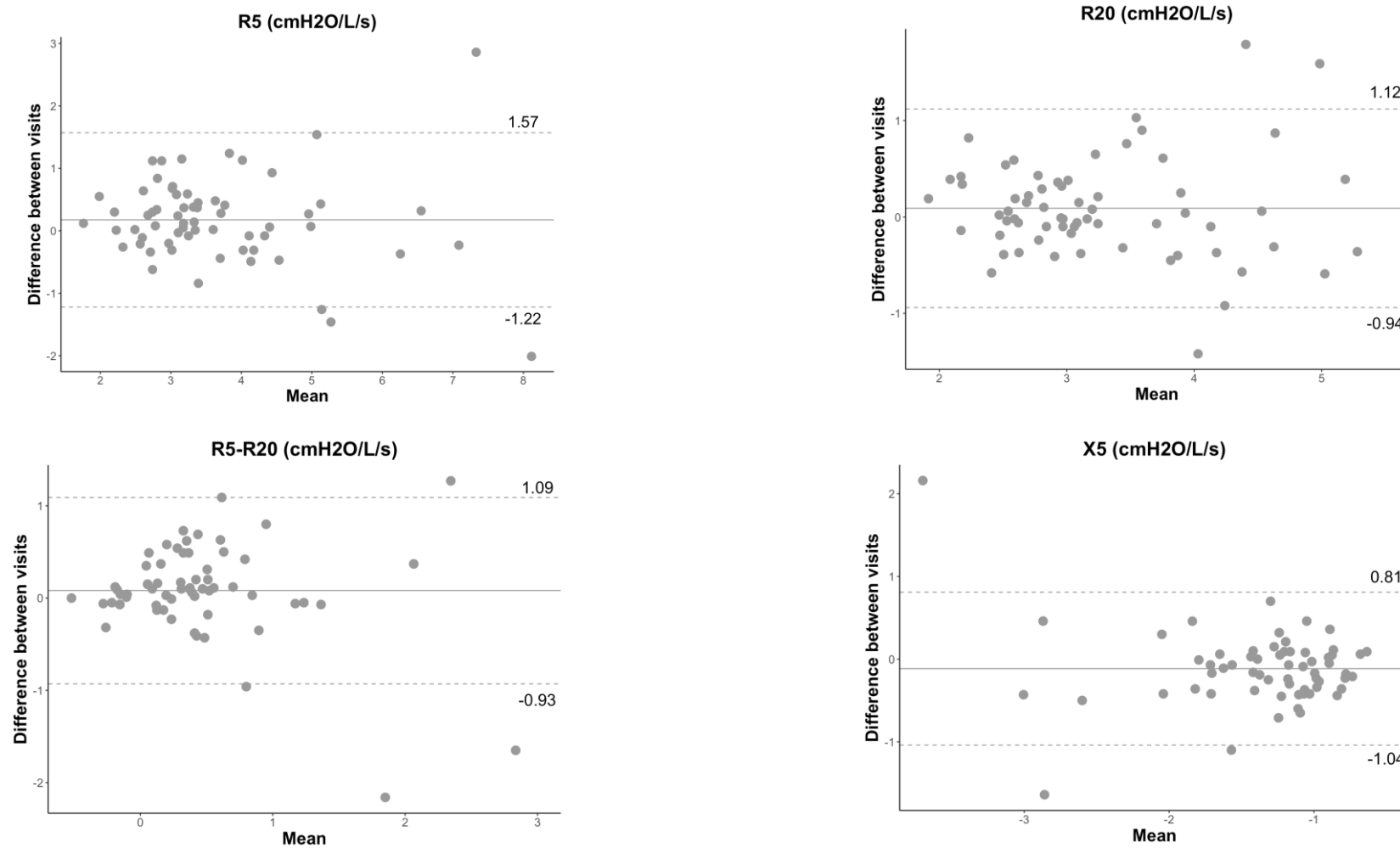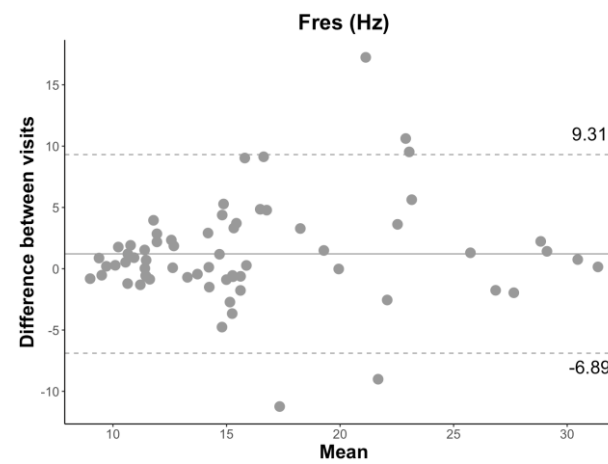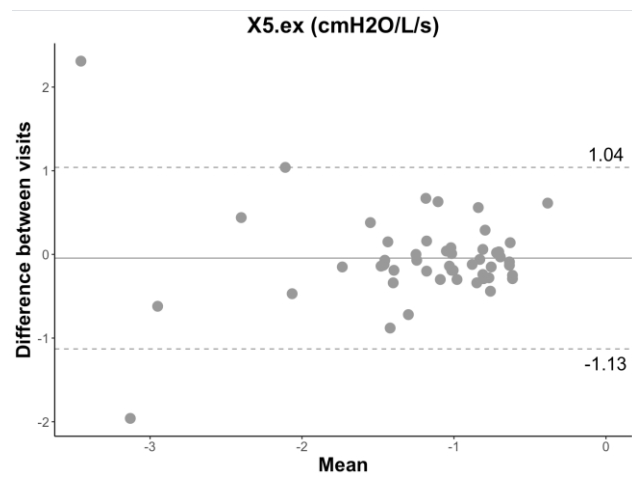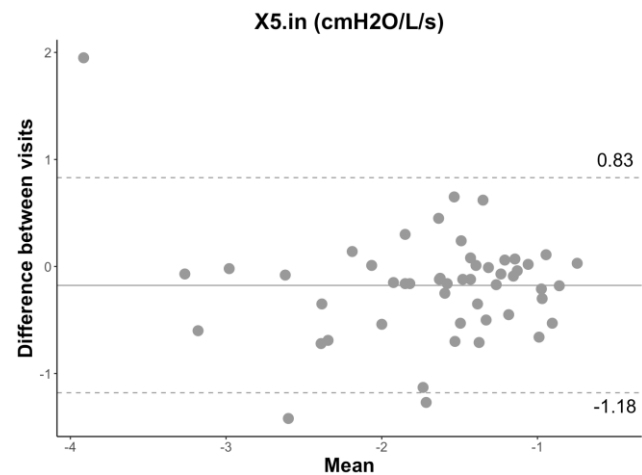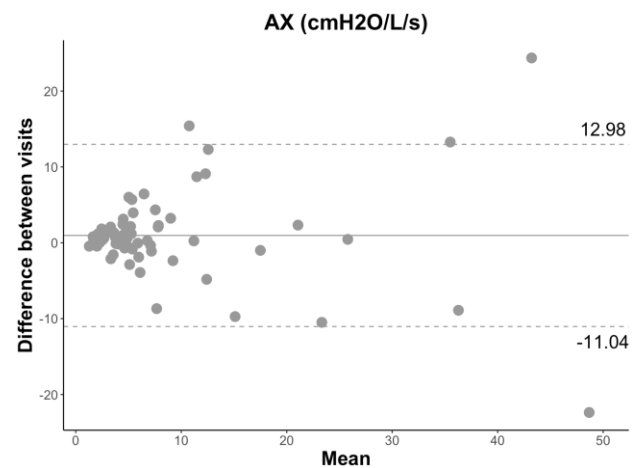
BA plots illustrate agreement between visits for all AO measurements

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4.4.1.3. Airways Oscillometry in healthy control group compared with symptomatic patient groups

After showing that AO measurements are repeatable. We then wanted to test to see if the repeatability was sustained in symptomatic patients with asthma, and symptomatic patients with a diagnosis of asthma excluded. We computed correlation coefficients; for 'asthma' and 'not asthma' patients, using Spearman Rank correlation to determine the strength of relationship between AO measurements at visit one and visit two (table 53).

Table 53. Spearman rank correlation coefficients between visits in the symptomatic groups

Variable	Symptomatic 'asthma'		Symptomatic 'not asthma'	
	Correlation coefficient (95%CI)	*P value	Correlation coefficient (95%CI)	*P value
R5	0.924 (0.88, 0.98)	<0.001	0.959 (0.88, 0.96)	<0.001
R20	0.905 (0.62, 0.94)	<0.001	0.849 (0.79, 0.94)	<0.001
R5-R20	0.850 (0.56, 0.94)	<0.001	0.815 (0.7, 0.9)	<0.001
X5	0.874 (0.81, 0.96)	<0.001	0.920 (0.86, 0.95)	<0.001
AX	0.924 (0.85, 0.98)	<0.001	0.945 (0.86, 0.95)	<0.001
Fres	0.873 (0.81, 0.96)	<0.001	0.922 (0.76, 0.92)	<0.001
X5in	0.932 (0.71, 0.94)	<0.001	0.856 (0.88, 0.96)	<0.001
X5ex	0.899 (0.77, 0.96)	<0.001	0.895 (0.82, 0.94)	<0.001
*correlation is significant at the 0.01 level				

All measurements showed statistically significant Spearman rank-order correlation (p <0.01). All measurements show a strong correlation coefficient (> 0.60).

BA Plots:

Bland Altman plots were then created for each Airways Oscillometry measure comparing all three groups (e.g., healthy control, symptomatic 'asthma,' symptomatic 'not asthma.') We looked to see if repeatability was lost in symptomatic patients (table 54).

Table 54. Repeatability analysis in all groups using test-retest reliability

Variable	n	Difference between visits, mean (SD)	P-value*	Limits of agreement (95%CI)	1.96*SD	Regression coefficient (95% CI)	P-value*	ICC (95%CI)	SEM	MDC	Cronbach's alpha (95%CI)
R5											
Healthy	63	0.18 (0.71)	0.329	(-1.22, 1.571)	1.4	-0.064 (-0.203, 0.076)	0.547	0.855 (0.771 , 0.909)	0.512	1.420	0.925 (0.887 , 0.956)
Asthma	55	-0.095 (1.19)	1	(-2.429 , 2.239)	2.33	-0.026 (-0.142 , 0.089)	0.74	0.912 (0.855 , 0.948)	0.835	2.314	0.954 (0.924 , 0.978)
Not asthma	30	0.185 (0.54)	0.482	(-0.883 , 1.253)	1.07	0.103 (0.004 , 0.201)	0.076	0.957 (0.913 , 0.979)	0.398	1.103	0.98 (0.949 , 0.988)
R20											
Healthy	65	0.09 (0.52)	0.688	(-0.937, 1.117)	1.03	-0.004 (-0.164, 0.155)	0.858	0.815 (0.715 , 0.883)	0.372	1.032	0.9 (0.837 , 0.946)
Asthma	55	-0.081 (1.02)	1	(-2.081 , 1.918)	2	-0.109 (-0.267 , 0.048)	0.27	0.839 (0.739 , 0.903)	0.714	1.979	0.911 (0.827 , 0.959)
Not asthma	30	0.068 (0.51)	0.954	(-0.94 , 1.075)	1.01	-0.072 (-0.225 , 0.081)	0.393	0.917 (0.835 , 0.96)	0.358	0.993	0.956 (0.839 , 0.987)
R5-R20											
Healthy	63	0.08 (0.52)	0.688	(-0.93, 1.095)	1.01	-0.191 (-0.401, 0.019)	0.334	0.697 (0.545 , 0.804)	0.366	1.014	0.823 (0.689 , 0.906)
Asthma	55	-0.012 (0.79)	1	(-1.567 , 1.543)	1.56	0.104 (-0.057 , 0.264)	0.271	0.835 (0.734 , 0.9)	0.555	1.537	0.909 (0.843 , 0.947)
Not asthma	30	0.13 (0.57)	0.828	(-0.985 , 1.246)	1.12	0.338 (0.11 , 0.565)	**0.013**	0.777 (0.586 , 0.887)	0.404	1.119	0.877 (0.803 , 0.922)
X5											
Healthy	62	-0.12 (0.48)	0.329	(-1.041, 0.811)	0.93	-0.141 (-0.341, 0.059)	0.493	0.721 (0.577 , 0.822)	0.340	0.943	0.845 (0.77 , 0.922)
Asthma	55	-0.127 (0.89)	1	(-1.88 , 1.626)	1.75	0.292 (0.135 , 0.449)	**0.003**	0.807 (0.692 , 0.883)	0.631	1.749	0.894 (0.846 , 0.949)
Not asthma	30	-0.201 (0.52)	0.374	(-1.22 , 0.818)	1.02	0.349 (0.181 , 0.518)	**0.001**	0.832 (0.679 , 0.916)	0.386	1.069	0.918 (0.815 , 0.962)
AX											
Healthy	65	0.97 (6.13)	0.688	(-11.043, 12.978)	12.01	-0.026 (-0.184, 0.131)	0.834	0.82 (0.721 , 0.886)	4.335	12.016	0.902 (0.847 , 0.934)
Asthma	55	-0.05 (-0.51 , 0.26)**	0.067**	(-19.68 , 22.48)**	21.08**	-0.196 (-0.363 , -0.029)	0.058	0.811 (0.697 , 0.885)	12.744	35.324	0.894 (0.853 , 0.967)
Not asthma	30	2.576 (6.62)	0.374	(-10.399 , 15.551)	12.97	0.373 (0.247 , 0.499)	**<0.001**	0.867 (0.741 , 0.934)	4.909	13.608	0.936 (0.872 , 0.969)
Fres											
Healthy	65	1.21 (4.13)	0.149	(-6.893, 9.315)	8.1	0.087 (-0.094, 0.267)	0.547	0.755 (0.628 , 0.843)	3.016	8.359	0.87 (0.753 , 0.941)
Asthma	55	-1.118 (4.36)	0.56	(-9.655 , 7.418)	8.54	0.022 (-0.135 , 0.18)	0.783	0.835 (0.734 , 0.9)	3.140	8.704	0.914 (0.85 , 0.958)

	N	Mean (SD)		CI				ICC	SEM	MDC	ICC
Not asthma	30	0.87 (2.93)	0.575	(-4.877 , 6.618)	5.75	0.148 (-0.034 , 0.331)	0.158	0.869 (0.746 , 0.935)	2.117	5.868	0.934 (0.884 , 0.968)
X5in											
Healthy	51	-0.18 (0.52)	0.138	(-1.18, 0.827)	1	-0.107 (-0.324, 0.11)	0.547	0.724 (0.563 , 0.832)	0.378	1.047	0.854 (0.767 , 0.927)
Asthma	55	-0.114 (0.62)	1	(-1.331 , 1.103)	1.22	0.199 (0.087 , 0.311)	**0.003**	0.898 (0.831 , 0.939)	0.440	1.219	0.947 (0.92 , 0.969)
Not asthma	30	-0.144 (0.61)	0.828	(-1.341 , 1.053)	1.2	0.374 (0.171 , 0.577)	**0.003**	0.797 (0.62 , 0.898)	0.434	1.203	0.89 (0.747 , 0.949)
X5ex											
Healthy	51	-0.10 (0.69)	0.688	(-1.132, 1.043)	1.09	-0.068 (-0.315, 0.178)	0.745	0.691 (0.516 , 0.811)	0.388	1.076	0.815 (0.741 , 0.904)
Asthma	55	-0.183 (1.01)	1	(-2.156 , 1.789)	1.97	0.354 (0.205 , 0.503)	**<0.001**	0.803 (0.685 , 0.88)	0.714	1.979	0.892 (0.849 , 0.957)
Not asthma	30	-0.168 (0.49)	0.482	(-1.125 , 0.788)	0.96	0.328 (0.168 , 0.488)	**0.001**	0.852 (0.715 , 0.926)	0.357	0.989	0.926 (0.834 , 0.959)
*ANOVA or Kruskal-Wallis test with adjustment for multiple testing, **Non-parametric approach where extreme outliers present											
Abbreviations; ICC, intra-class correlation coefficient (from one-way ANOVA), SEM, standard error of measurement (SD*sqrt(1-ICC)), MDC, minimal detectable change (1.98*sqrt(2)*SEM)											

When comparing symptomatic patients to healthy controls we show mean difference between visits remains non-significant in all measurements (table 63 above). However, regression coefficients are statistically significant in symptomatic patients for the following measurements: R5-R20, X5, AX, X5in, and X5ex. In these measurements the limits of agreement have wider 95% confidence intervals in symptomatic patients. In addition, standard error of measurement (SEM) is greater in patients with asthma suggesting greater variation in repeated measurements in patients with asthma. Minimal detectable change is also greater in asthma when compared to healthy controls which also suggests greater variability in asthma patients. We show good internal consistency (Cronbach's alpha >0.7), in all measurements in all groups. BA plots illustrate differences across the three groups by highlighting mean difference and limits of agreement for each group (figure 45).

Figure 45. BA Plots to show mean difference between visits between all three groups (continuous line shows mean difference between visits, dotted line shows limits of agreement)

R20 (cmH2O/L/s)

R5-R20 (cmH2O/L/s)

X5 (cmH2O/L/s)

X5.in (cmH2O/L/s)

X5.ex (cmH2O/L/s)

AX (cmH2O/L/s)

Fres (Hz)
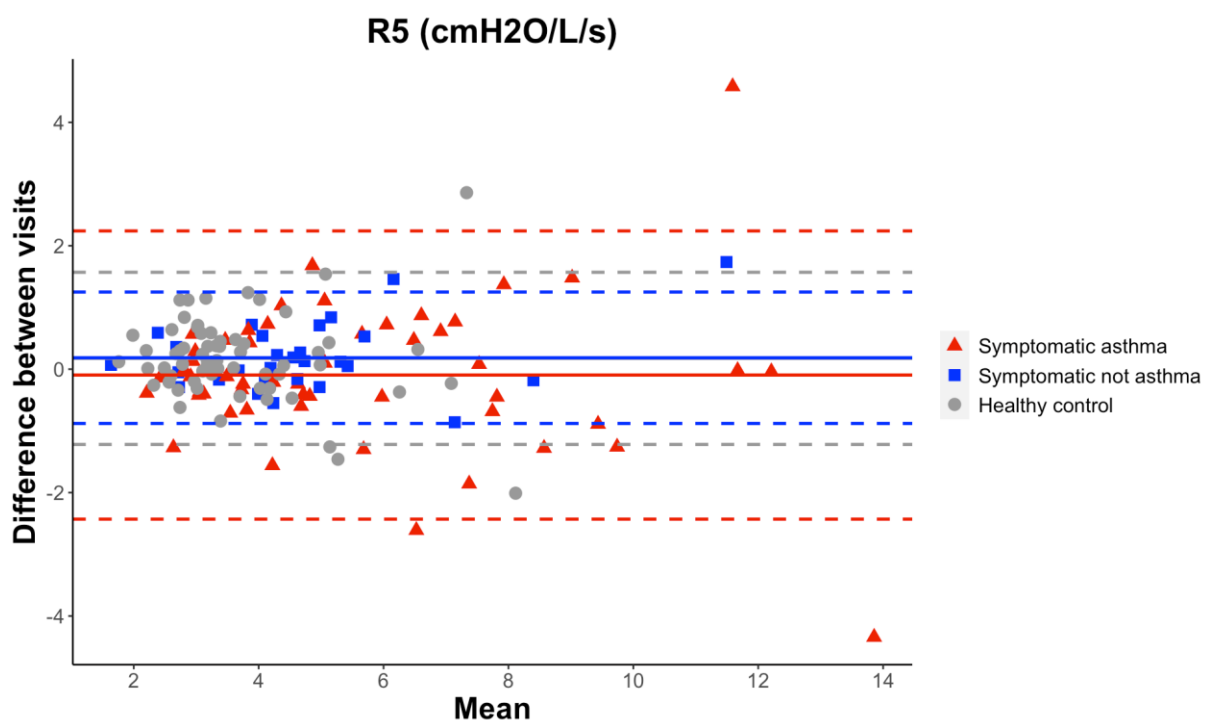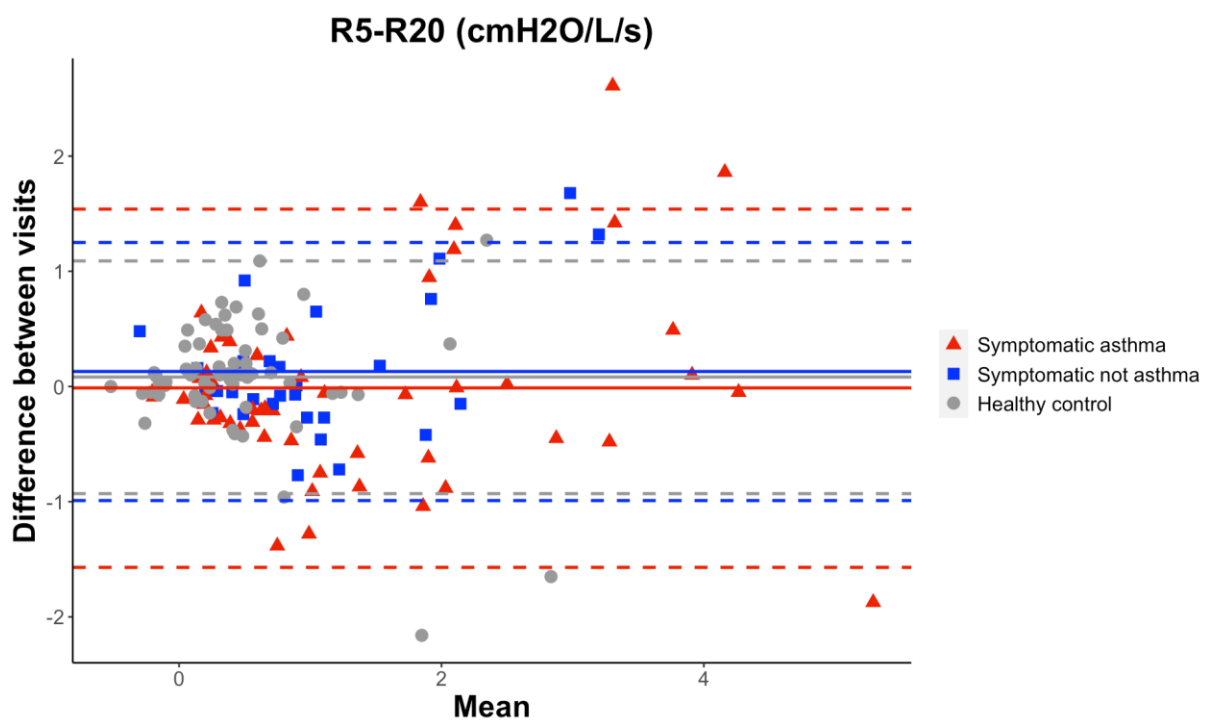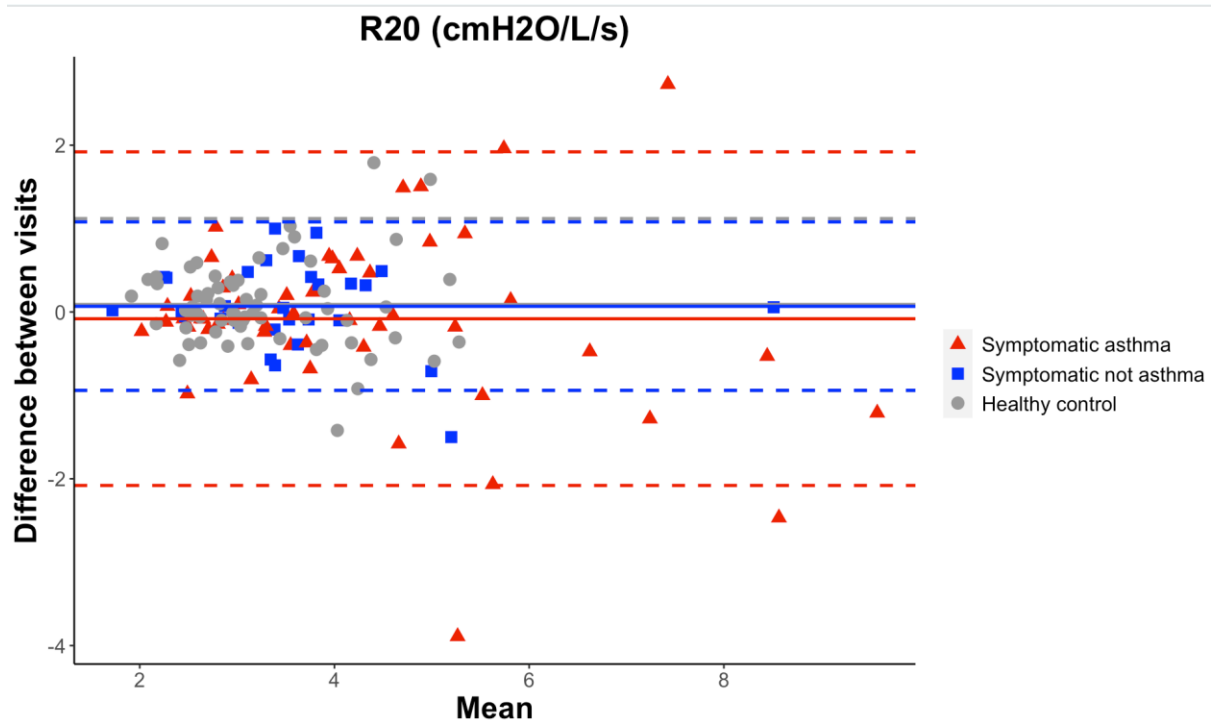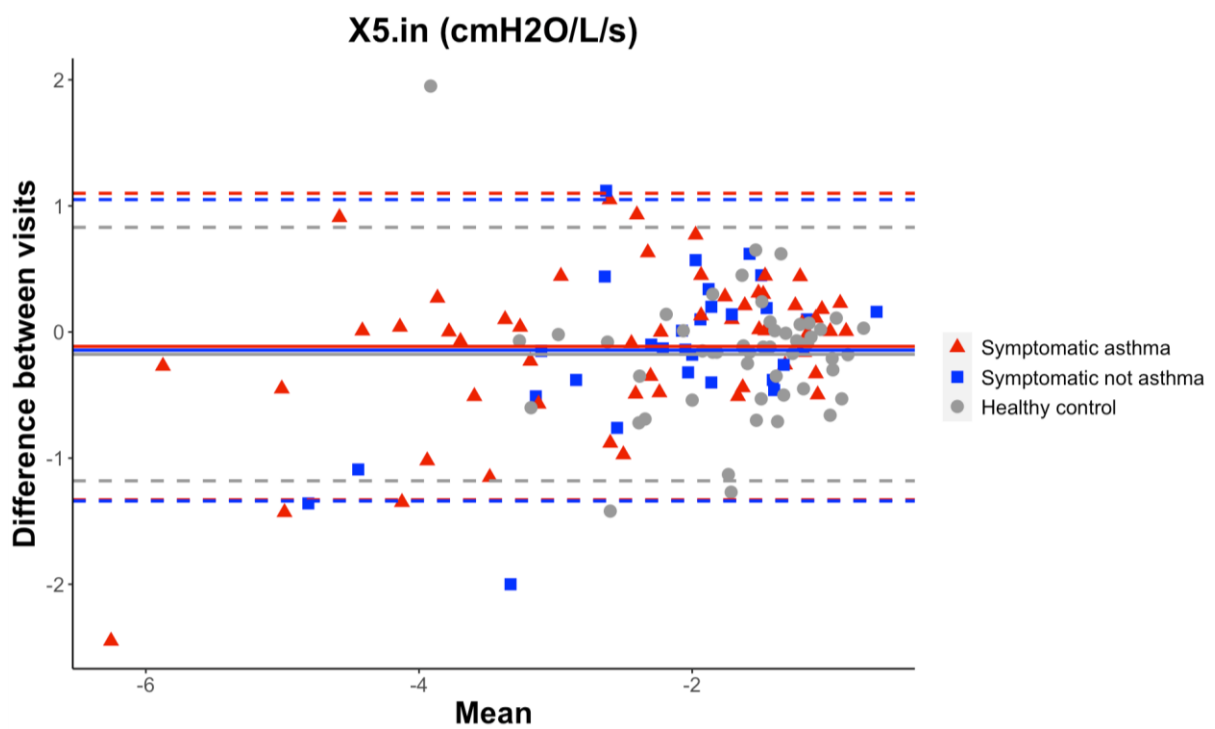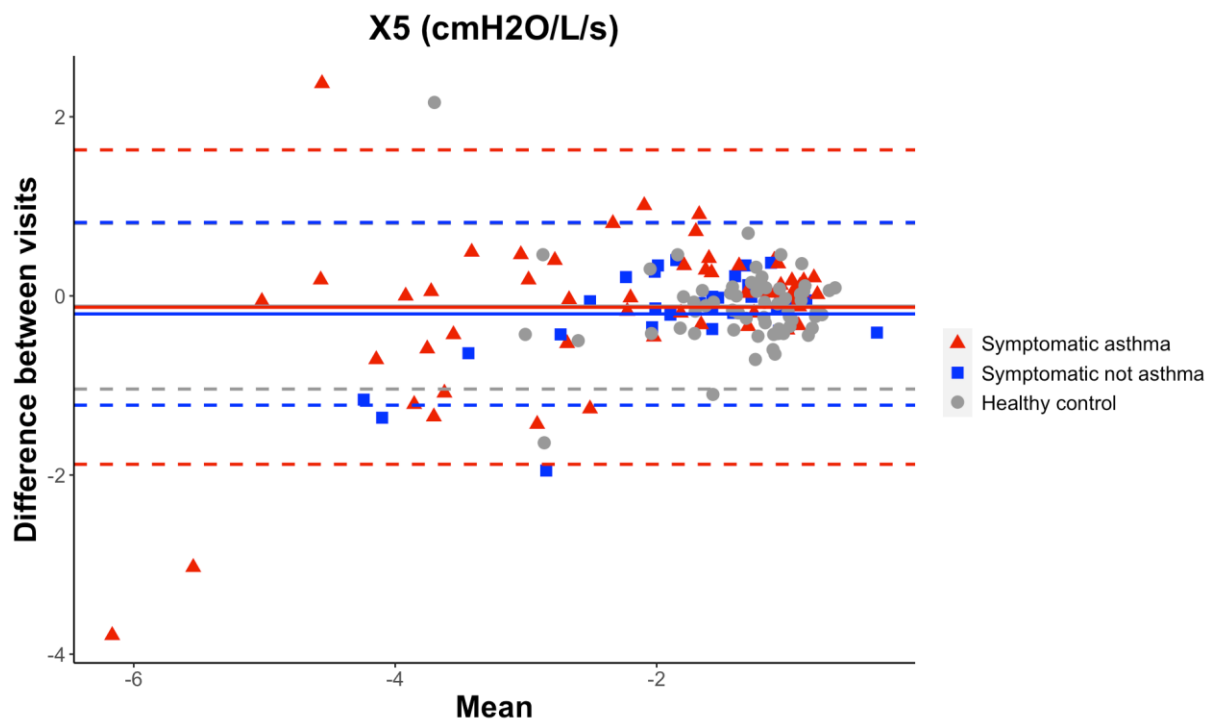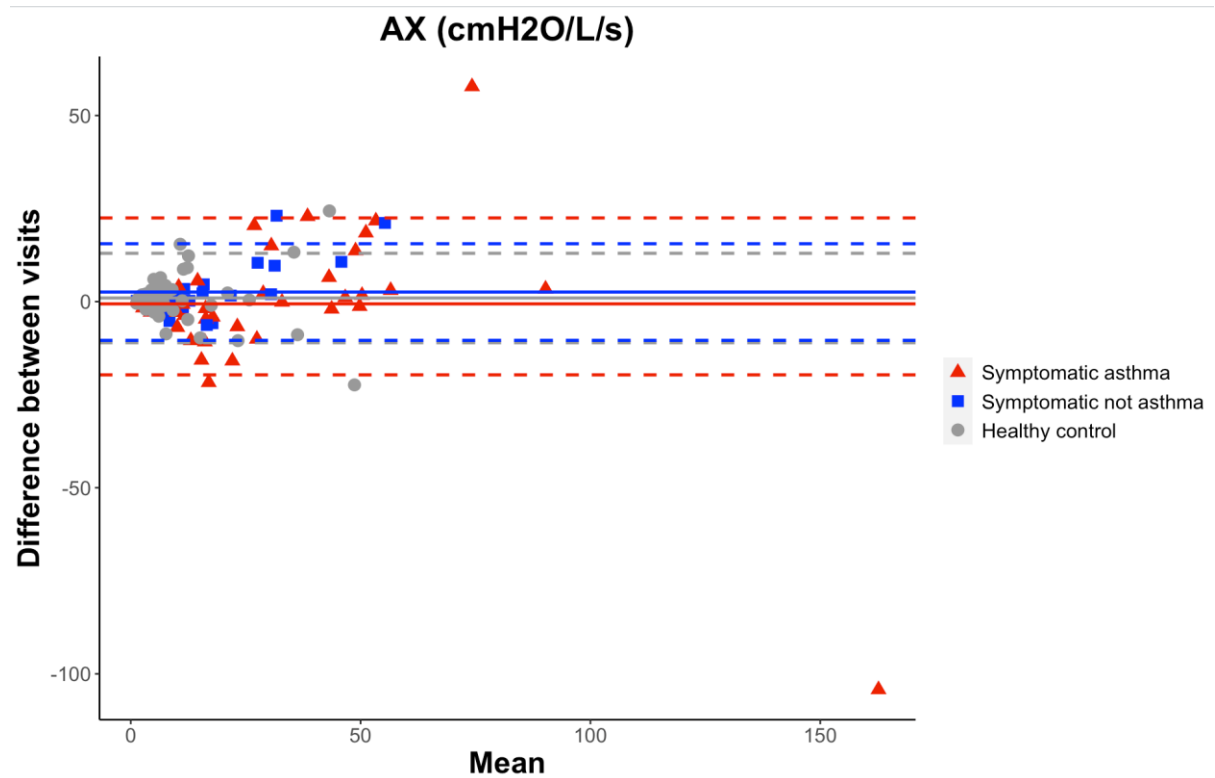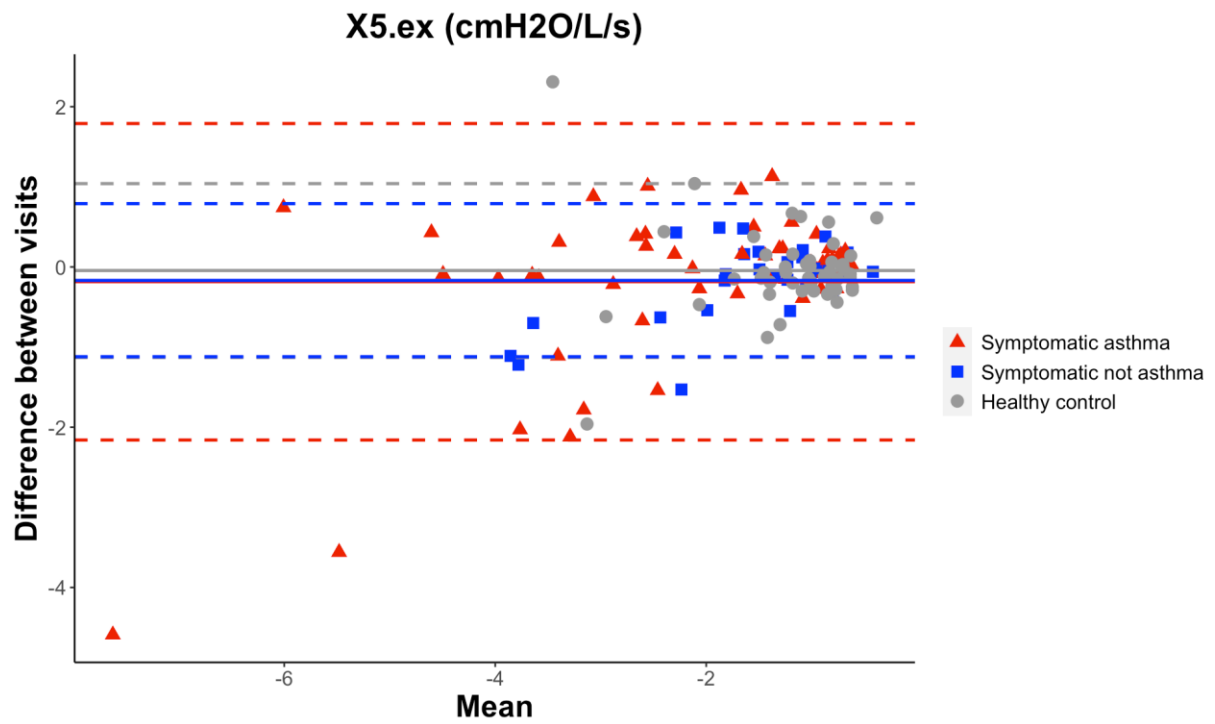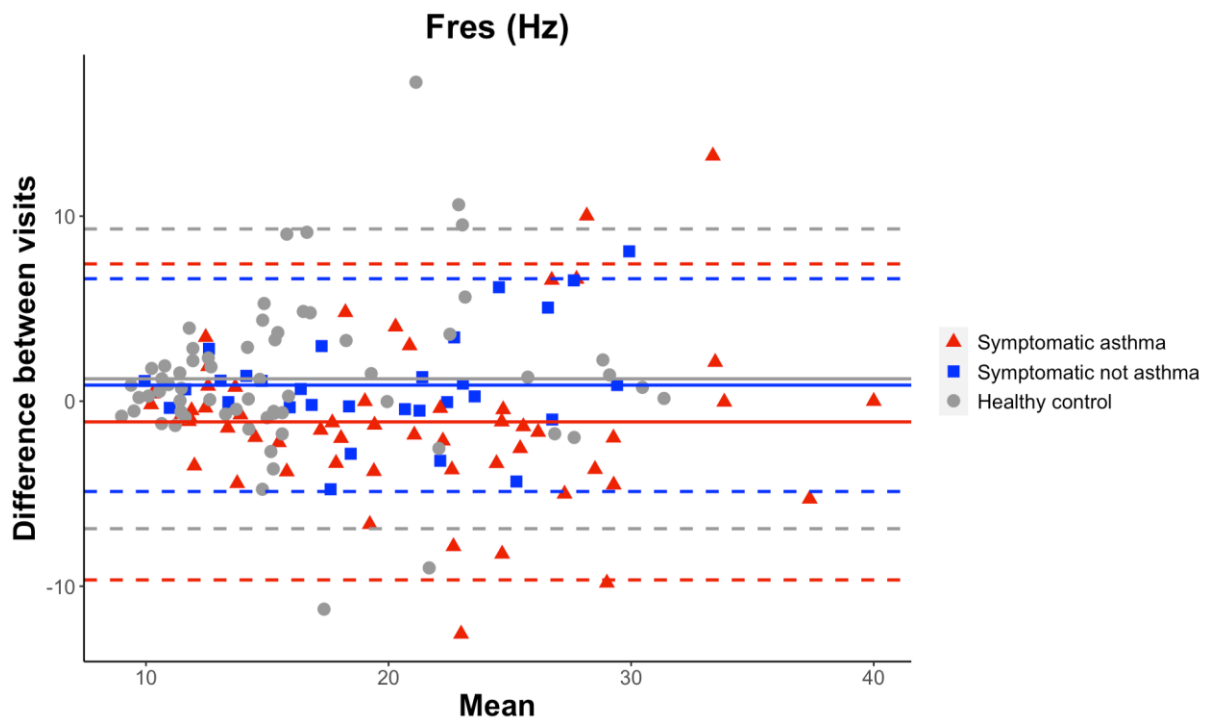
The Bland Altman plots confirm repeatability in all groups. Average difference between visits is close to zero for all AO measurements in all three groups. Limits of agreement for the average difference is wider in the asthma groups when compared to the other two groups. In addition, more spread in the mean difference is seen in those with more positive resistance and more negative reactance measurements.

Using Minimal Detectable Change (MDC) to identify asthma patients

We explored whether patients with asthma were more likely to have greater variability in AO results between visits (table 55) than the reported minimal detectable change (MDC) previously identified in healthy controls (see section 4.4.1.2).

Table 55.   Using AO MDC to detect asthma from not asthma

AO Variable	Group	Number (%) patients with variability greater than MDC
R5	Asthma	3 (5.5)
	Not asthma	2 (6.7)
	Healthy	2 (3.3)
R20	Asthma	4 (7.3)
	Not asthma	0 (0)
	Healthy	2 (3.1)
R5-R20	Asthma	6 (10.9)

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	Not asthma	3 (10.0)
	Healthy	2 (3.1)
X5	Asthma	2 (3.6)
	Not asthma	0 (0)
	Healthy	1 (1.6)
AX	Asthma	7 (12.7)
	Not asthma	2 (6.7)
	Healthy	4 (6.5)
Fres	Asthma	2 (3.6)
	Not asthma	0 (0)
	Healthy	5 (7.7)
X5in	Asthma	1 (1.8)
	Not asthma	1 (3.3)
	Healthy	1 (2.0)
X5ex	Asthma	1 (1.82)
	Not asthma	0 (0.0)
	Healthy	1 (2.0)

Repeatability was not significantly different between groups. Few patients were identified with a mean difference between two visits that was greater than the MDC computed for healthy controls. Whilst more patients in symptomatic groups did fall above the MDC defined by our healthy controls, the numbers were few and they were similar in the asthma and not asthma groups. In our cohort this would not be a clinically useful measurement for diagnosing asthma.

4.4.2. Is Airways Oscillometry affected by patient demographics (descriptive analysis)

Using the whole population (asymptomatic healthy, symptomatic asthma, and symptomatic not asthma) (n = 161). We explore the relationship between baseline AO measurements, resistance (R5, R20) and reactance (X5), and potential co-variables. Derivatives of baseline measurements (i.e., R5-R20, X5in, X5ex, Fres, and AX) were not included because they correlated with their baseline AO measures (Figure 46).

Figure 46. Correlation of airways resistance and reactance measurements

i)	Resistance measures:
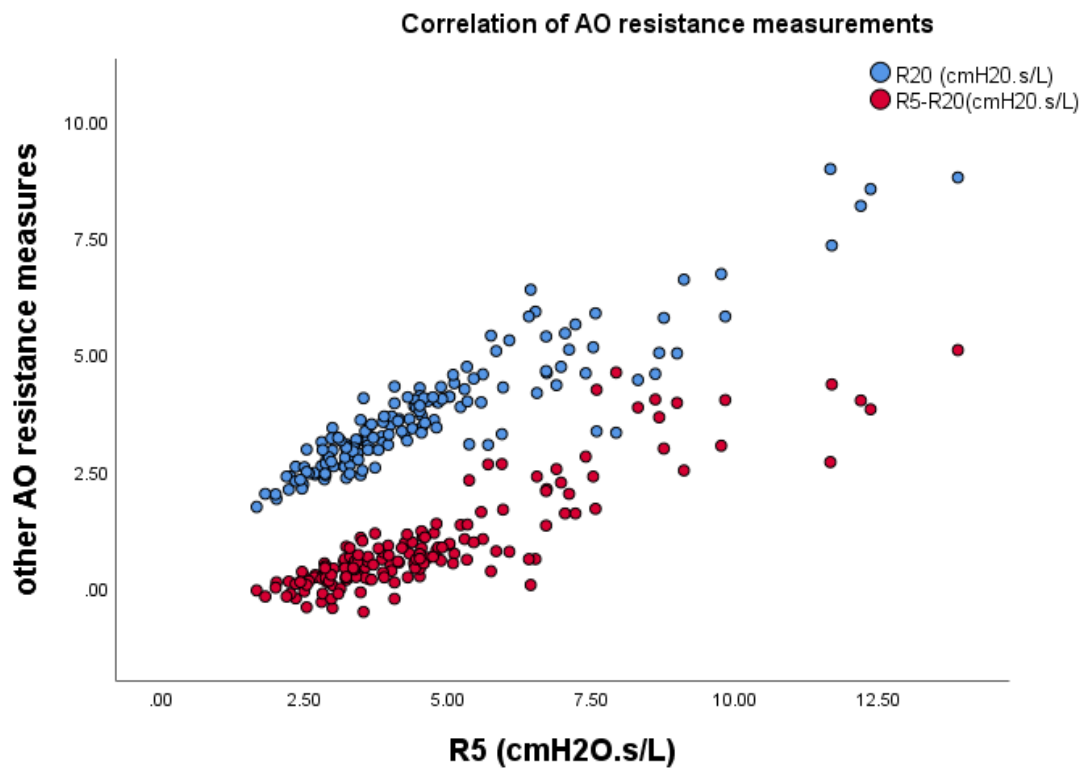
Reactance measures:
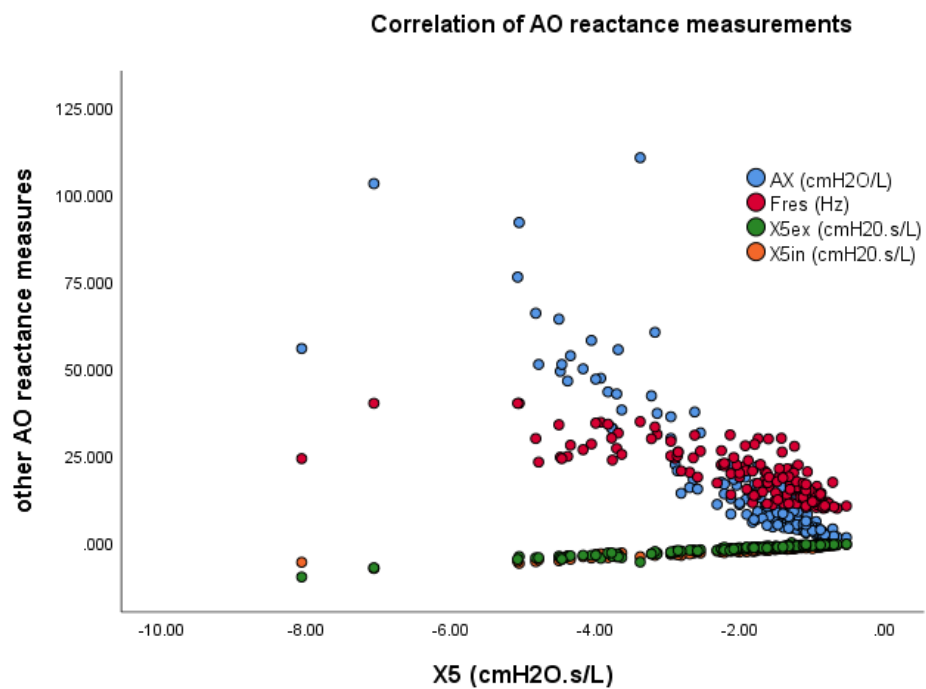
4.4.2.1. Correlations of AO measurements with age, height, gender, Body Mass Index (BMI)

Correlation to Age:

We look at the effect of age on AO markers (figure 47, Table 56) in all patients (n = 161)

Figure 47. Effect of age on airways oscillometry
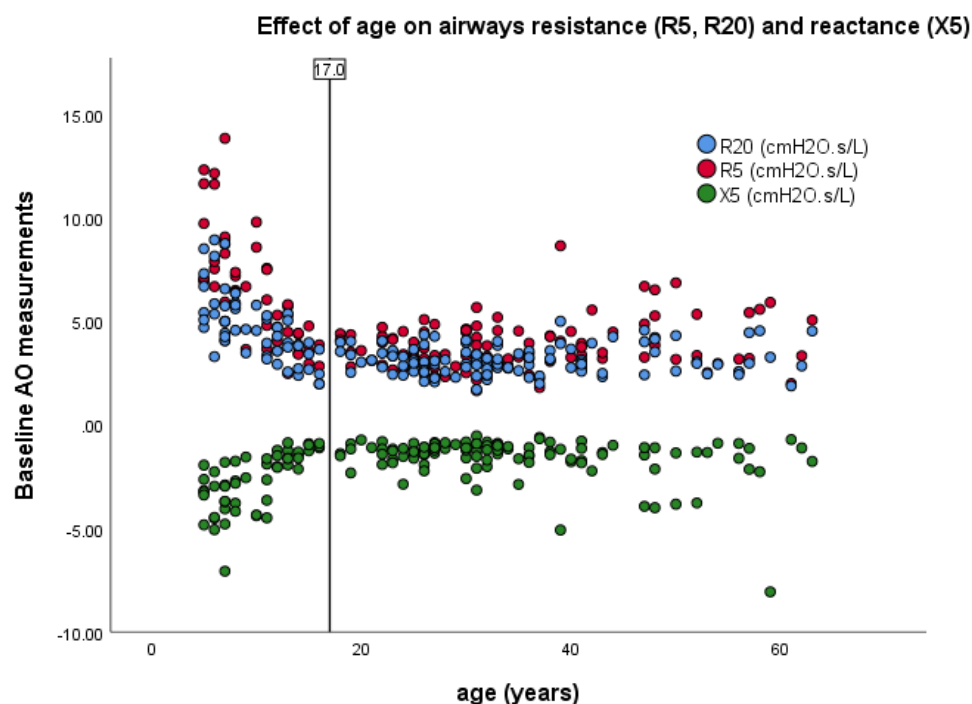
Using the cut off for adults (≥17 years) defined in the NICE diagnostic algorithm, there was a significant difference between adults and children for baseline AO measurements (p <0.001). Resistance and reactance measurements are greater in children, this would be expected due to smaller immature lungs. Whilst age may directly impact AO measurements, we need to look at the influence of height.

Table 56. Effect of age on Airways Oscillometry

measurement	Age		P value
Baseline AO measures	Adults (17+ years)	Children (5-16years)	
R5, median (IQR), cmH2O.s/L	3.43 (2.93-4.51)	6.24 (4.42-7.85)	<0.001
R20, median (IQR), cmH2O.s/L	3.07 (2.62-3.61)	4.53 (3.57-5.67)	<0.001
X5, median (IQR), cmH2O.s/L	-1.28 (-1.68- -0.99)	-2.39 (-3.70- -1.55)	<0.001

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Both measures of impedance (resistance and reactance) show statistically significant age-related bias.

Correlation to height:

We look at the effect of height on AO measurements in all patients (n = 161) (figure 48)

Figure 48. Effect of height on Airways Oscillometry
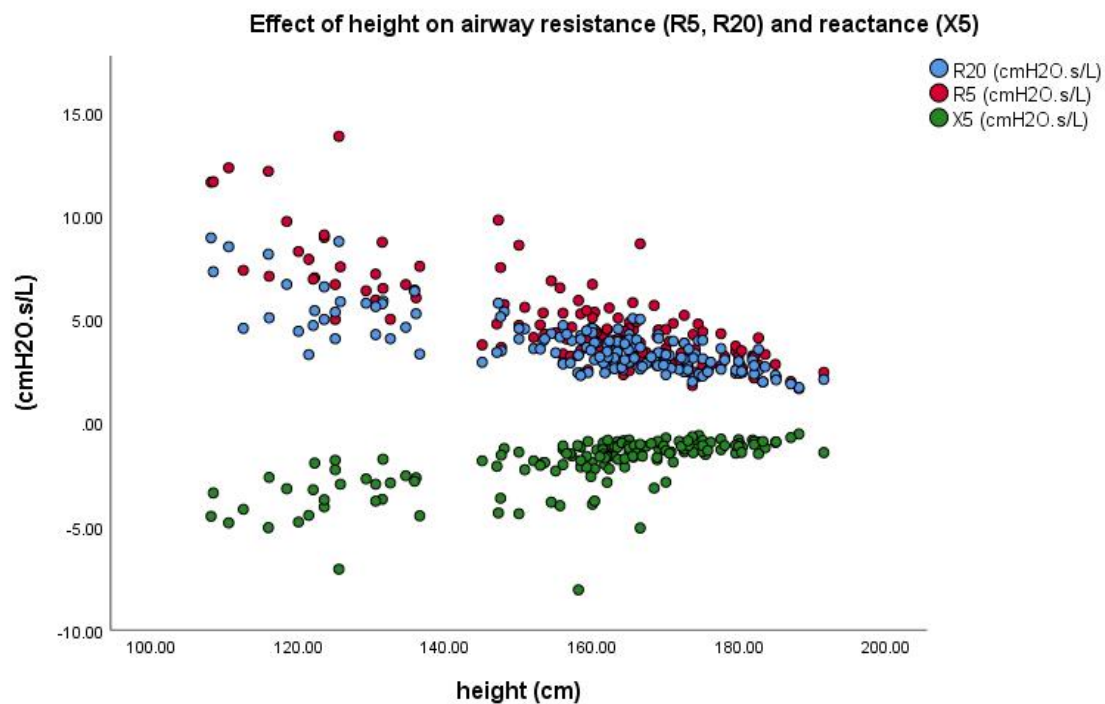
Shorter stature appeared to be associated with greater variability in resistance and reactance. We investigated to see if short stature may be less stable due to a greater proportion of children. We produced scatter plots with adults (≥17years) and children (<17years) separately (figure 49).

Figure 49. Effect of height on AO in i) adults ii) children

i)

Effect of height on airway resistance (R5, R20) and reactance (X5) (adults only)

ii)

Effect of height on airway resistance (R5, R20) and reactance (X5) (children only)
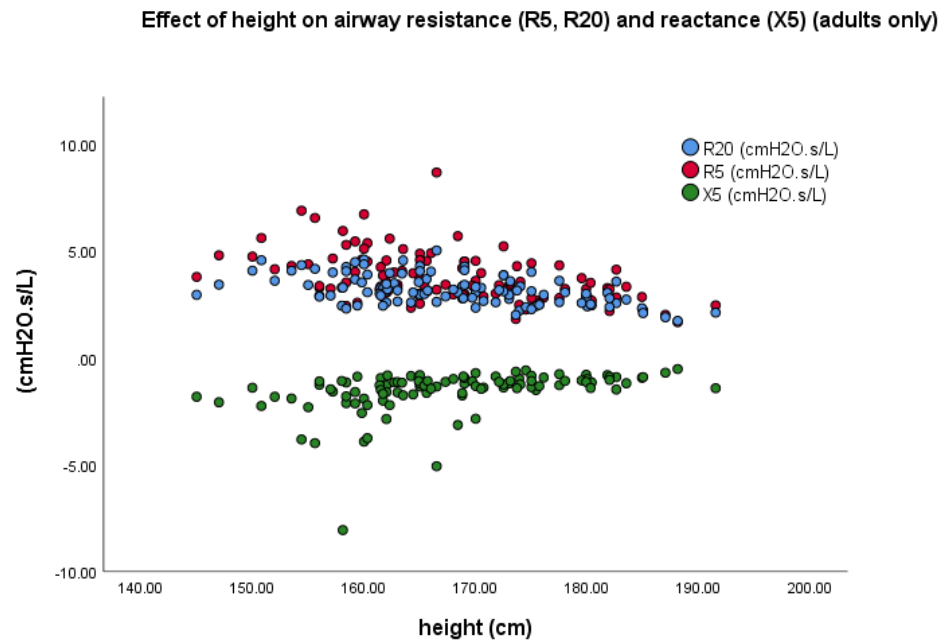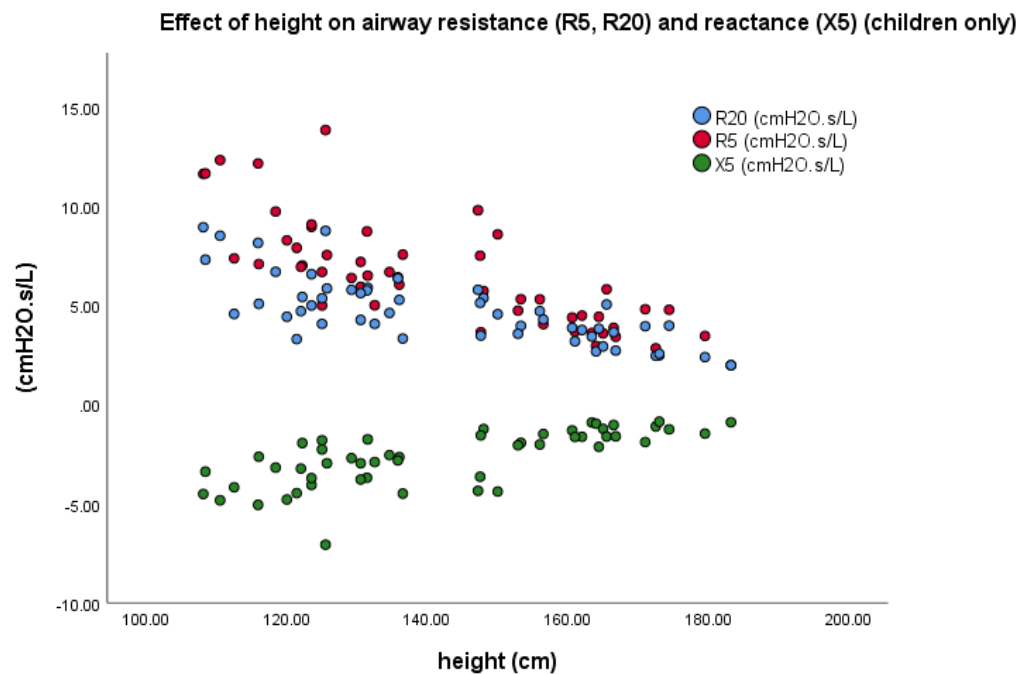
The scatter plots show short stature is associated with younger age. Height appears to an influential variable in resistance and reactance in the lower airways. Short stature is also associated with greater variability in results.

Correlation to Gender:

A simple bar chart showing effect of gender on airways resistance and reactance in all patients (n = 161) (figure 50)

Figure 50. Effect of gender on AO
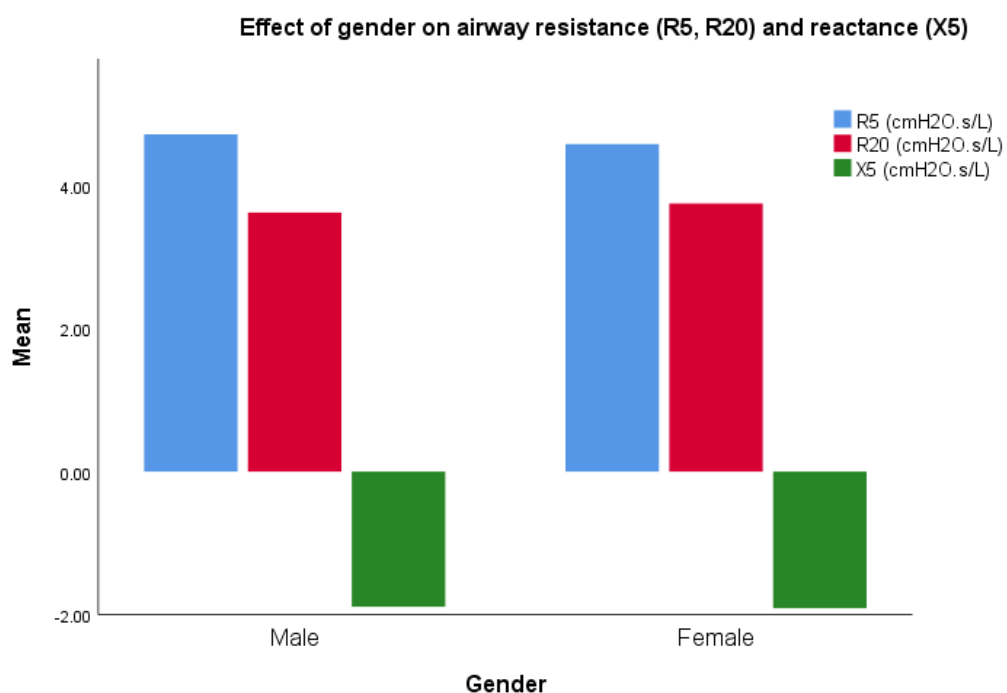
There was no significant difference between males and females for lower airways resistance (R5) (p 0.102) or reactance (X5) (p 0.125). Upper airways resistance was higher in females when compared to males (Table 57). This may be due to the 'height effect,' females were shorter than males, median[IQR] 172.50cm [149.00-179.70] versus 161cm (155.15-165.55)).

Table 57.   Effect of gender on Airways Oscillometry

	Gender		P value
Baseline AO measures	**Male**	**Female**	
R5, median (IQR), cmH2O.s/L	3.49 (2.85-5.93)	4.20 (3.37-5.29)	0.102
R20, median (IQR), cmH20.s/L	3.06 (2.48-4.44)	3.52 (2.97-4.23)	**0.015**
X5, median (IQR), cmH2O.s/L	-1.29 (-2.61- -0.99)	-1.59 (-2.13- -1.15)	0.125

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Correlation to BMI:

Scatter plot showing effect of BMI on airways resistance and reactance in all patients (n = 161) (figure 51)

Figure 51. Effect of BMI on AO
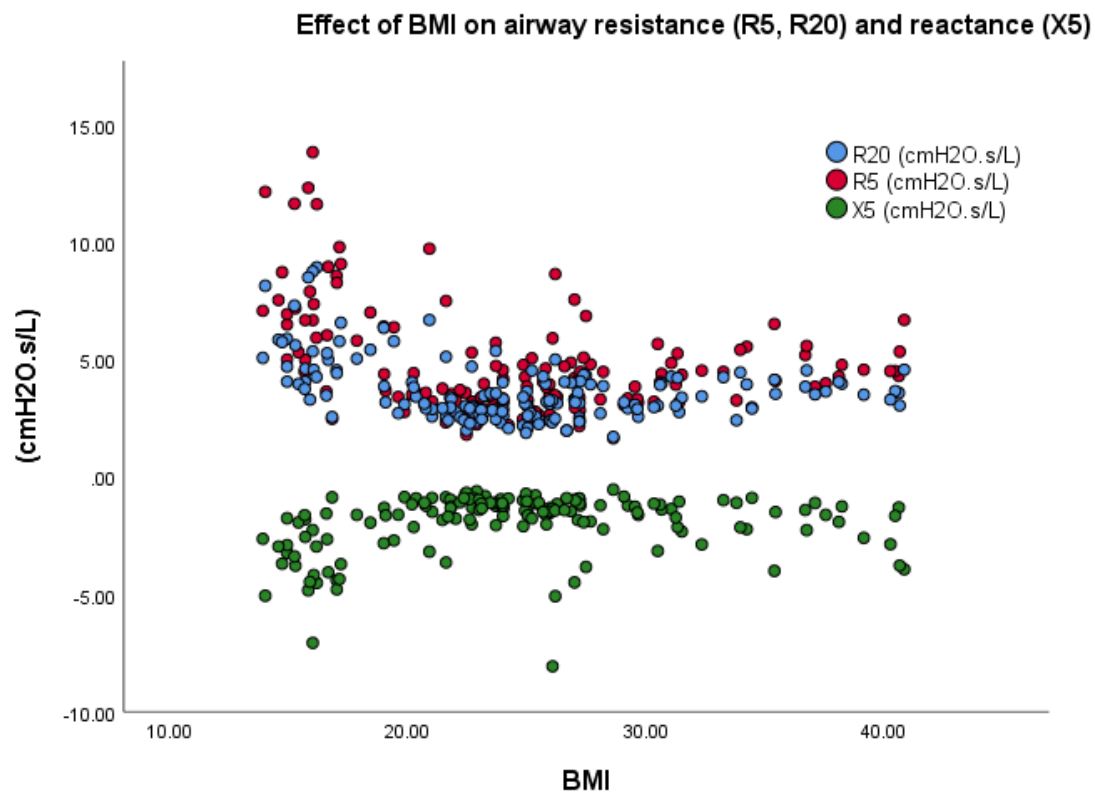
Lower extremes of BMI appeared to be less stable. We investigated to see if lower BMI may be less stable because most patients with low BMI were children. We therefore produced scatter plots with adults (≥17years) and children (<17years) plotted separately (Figure 52).

Figure 52. Effect of BMI on AO in i) adults ii) children

i)

Effect of BMI on airway resistance (R5, R20) and reactance (X5) (adults only)

ii)

Effect of BMI on airway resistance (R5, R20) and reactance (X5) (children only)
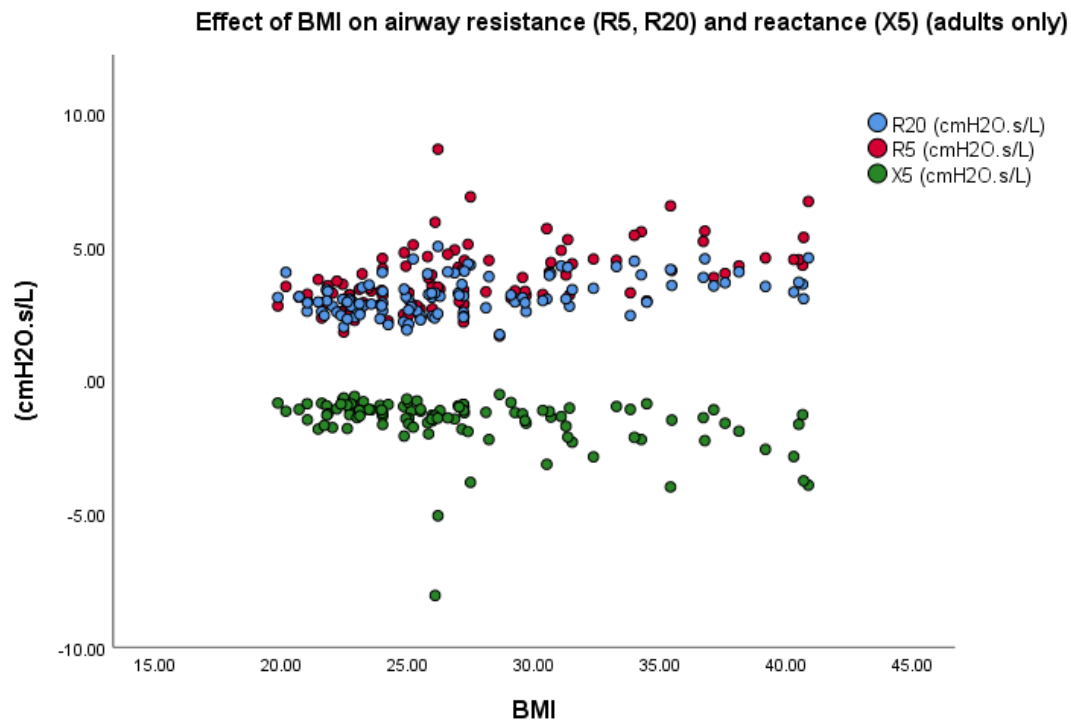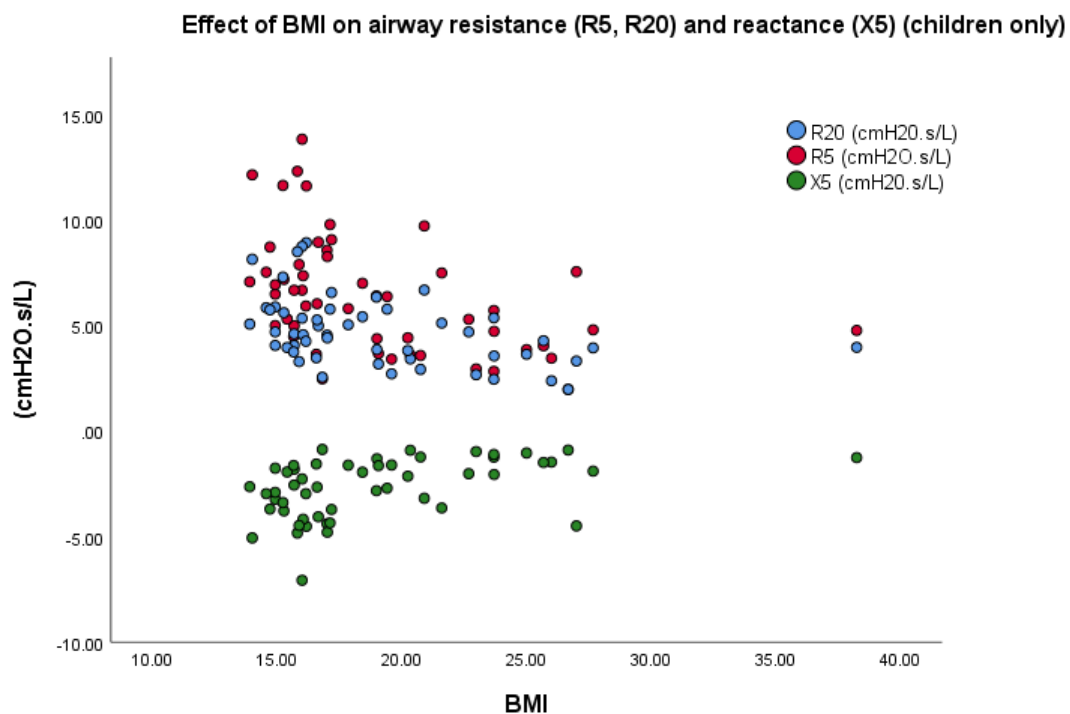
It is likely that lower BMI is associated with younger age. It is most likely that the age effect (and associated short stature) is the main cause of poor correlation in children.

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The above descriptive analysis of confounding variables affecting AO measurements indicate that both age and height are important variables to consider because they may directly impact AO results. In our subsequent analysis 'Power of AO to predict asthma,' we investigate only adults. We will analyse the raw AO data (including %predicted values from pre-existing equations adjusting for co-variables), we will then perform multivariable linear regression to investigate the effects of confounders in our adult cohort and we will adjust data to account for any confounding variables (see section 4.4.3.2).

4.4.3. Determine the power of 'Airways Oscillometry' to predict asthma (Exploratory analysis in adults)

4.4.3.1. Airways Oscillometry to predict asthma in symptomatic adults

The following analysis was performed on adults (≥17 years). Univariate analysis of AO measurements was computed to determine which AO variables could predict 'asthma' from 'not asthma' in all symptomatic adult (≥17years) patients. We also included the healthy control group in the analysis to explore differences between healthy controls and the symptomatic groups. First, we look at patient demographics of each group (Table 58).

Table 58. Patient demographics in adults (≥17 years)

	All cases (N = 107)	Asymptomatic Healthy (N = 49)	Symptomatic Asthma (N = 35)	Symptomatic Non Asthma (N = 23)	P Value	Post Hoc Tests *P Value ⇈P value ≠ p value
Demographics						
Age, mean (SD) years	34.87 (11.27)	34.2 (10.4)	33.57 (12.24)	38.13 (2.42)	0.285	n/a
Gender, n (%) females	67 (62.6)	29 (59.2)	20 (57.1)	18 (78.3)	0.212	n/a
Ethnicity, n (%) white	90 (84.1)	44 (89.8)	28 (80)	18 (78.3)	0.330	n/a
Height, mean (SD)	167.79 (9.55)	170.00 (8.01)	168.54 (10.66)	163.66 (10.05)	0.060	n/a
BMI, mean (SD) kg/m^2	27.41 (5.32)	26.00 (4.68)	28.40 (5.17)	28.91 (6.25)	0.038	* 0.932 ⇈0.098 ≠ 0.075
Current or ex-smokers, n (%)	33 (30.8)	15 (30.6)	10 (28.6)	8 (34.8)	0.881	n/a

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Pack years, median (IQR)	0.0 (0.00 - 0.85) N = 105	0.0.00 (0.00-0.05) N = 47	0.00 (0.00-0.10)	0.00 (0.00-0.10)	0.839	n/a

*P value, difference between ' asthma' and 'not asthma' groups, ƗP value, difference between 'asthma' and 'healthy control,' ≠P value, difference between 'not asthma' and 'healthy controls'

Mean (SD) was recorded if whole population (i.e. all three groups combined) was normally distributed (skewness and kurtosis between -1/+1), otherwise median (IQR) was reported. One way ANOVA for normally distributed data, Post hoc analysis (Tukey) was performed if significant difference between one or more groups. Kruskal-Wallis one way ANOVA for non-normally distributed data, Post hoc analysis by pairwise comparison (values adjusted by the Bonferroni correction for multiple tests). Chi-square test for categorical data.

Abbreviations;

No significant differences in baseline demographics was found between the three groups.

We present the clinical characteristics of the three groups (table 59).

Table 59.   Clinical characteristics of all three groups

	All cases (N = 107)	Asymptomatic Healthy (N = 49)	Symptomatic Asthma (N = 35)	Symptomatic Non Asthma (N = 23)	P Value	Post Hoc Tests *P Value ꜛP value ≠ p value
Conventional Asthma Tests						
Spirometry						
FEV_1, mean (SD) L	3.44 (0.87)	3.64 (0.72)	3.29 (0.99)	3.25 (0.93)	0.096	n/a
FEV_1, median (IQR) %predicted	97.00 (89.00-109.00)	98.00 (90.00-111.50)	92.00 (81.00-102.00)	102.00 (90.00-112.00)	0.010	* **0.042** ꜛ**0.019** ≠ 1.00
FVC, mean (SD) L	4.42 (1.12)	4.62 (0.91)	4.46 (1.29)	3.96 (1.15)	0.064	n/a
FVC, mean (SD) % predicted	104.69 (13.91)	106.71 (13.05)	102.71 (14.86)	103.39 (14.22)	0.382	n/a
FEV_1/FVC Ratio, median (IQR) %	79.80 (73.51-83.30)	80.00 (76.07-83.88)	74.71 (68.13-80.54)	82.13 (79.88-84.27)	<0.001	* **<0.001** ꜛ**0.012** ≠ 0.182
Bronchodilator Reversibility						
FEV_1, median (IQR) %change	5.00 (3.00-9.00)	4.00 (2.00-6.00)	9.00 (6.00 – 15.00)	4.00 (1.00-7.00)	<0.001	* **<0.001** ꜛ**<0.001** ≠ 1.00
Other Tests						
FeNO, median (IQR) ppb	21.00 (12.00-40.25)	18.00 (12.00-25.75)	61.00 (20.00-91.00)	15.00 (11.00-22.00)	0.001	* **0.001** ꜛ**0.001** ≠ 1.00
Eos, median (IQR) $x10^9$cells/L	0.15 (0.09-0.26)	0.13 (0.09-0.21)	0.28 (0.15-0.55) N 34	0.10 (0.06-0.17) N 22	<0.001	* **0.001** ꜛ**0.001** ≠ 1.00

*P value, difference between ' asthma' and 'not asthma' groups, ꜛP value, difference between 'asthma' and 'healthy control,' ≠P value, difference between 'not asthma' and 'healthy controls'

Mean (SD) was recorded if whole population (i.e. all three groups combined) was normally distributed (skewness and kurtosis between -1/+1), otherwise median (IQR) was reported. One way ANOVA for normally distributed data, Post hoc analysis (Tukey) was performed if significant difference between one or more groups. Kruskal-Wallis one way ANOVA for non-normally distributed data, Post hoc analysis by pairwise comparison (values adjusted by the Bonferroni correction for multiple tests). Chi-square test for categorical data.

The asthma group had significantly lower FEV_1/FVC ratio, and higher bronchodilator reversibility with higher FeNO and blood eosinophils, when compared to 'not asthma' and 'healthy control' groups. There was no significant difference between symptomatic 'not asthma' and 'healthy control' groups.

Univariate analysis of AO measurements was computed to determine which AO variables could predict between the three groups. Where a significant difference was shown, post hoc analysis demonstrated which groupings were significantly different.

We then present AO measurements across the three groups (table 60)

Table 60. Summary of potential asthma predictors using Airways Oscillometry in adults (≥17 years)

	All cases (N = 107)	Asymptomatic Healthy (N = 49)	Symptomatic Asthma (N = 35)	Symptomatic Non Asthma (N = 23)	P Value	Post Hoc Tests *P Value ↑P value ≠ p value
Baseline Oscillometry						
R5, median (IQR) cmH20.s/L	3.43 (2.93-4.51)	3.36 (2.87- 3.86)	4.13 (3.01-4.88)	4.00 (2.99 – 4.80)	0.021	* 1.00 ↑0.051 ≠ 0.088
R5, median (IQR) %pred	114.00 (96.00-130.00) N = 104	108.90 (92.84-125.28) N = 48	119.00 (106.50-138.25) N = 34	109.60 (87.50-130.50) N = 22	0.080	n/a
R20, mean (SD) cmH20.s/L	3.18 (0.69)	3.02 (0.59)	3.35 (0.78)	3.24 (0.70)	0.094	n/a
R20, median (SD) %pred	97.31 (90.88-112.00) N = 104	97.07 (89.32-111.55) N = 48	102.50 (93.00-114.03) N = 34	96.00 (78.00-112.00) N = 22	0.162	n/a
R5-R20, median (IQR) cmH20.s/L	0.43 (0.19-0.85)	0.34 (0.10 – 0.57)	0.54 (0.24-1.04)	0.84 (0.27-0.97)	0.004	* 1.00 **↑0.031** **≠ 0.012**
X5, median (IQR) cmH20.s/L	-1.28 (-1.68 - -0.99)	-1.21 (-1.45- -1.05)	-1.30 (-2.25- -0.97)	-1.58 (-2.08- -1.08)	0.172	n/a
X5in, median (IQR) cmH20.s/L	-1.60 (-2.10- -1.22) N = 97	-1.49 (-1.76- -1.18) N = 39	-1.51 (-2.49- -1.16)	-1.89 (-2.35 - -1.27)	0.196	n/a
X5ex, median (IQR) cmH20.s/L	-1.01 (-1.48 - -0.77) N = 97	-0.98 (-1.28- -0.76) N = 39	-0.98 (-2.14- -0.76)	-1.17 (-1.56—0.86)	0.459	n/a
AX, median (IQR) cmH20/L.	5.82 (3.75-9.71)	4.71 (3.68-7.17)	6.15 (3.53-13.97)	8.76 (5.06-13.40)	0.026	* 0.996 ↑0.244 **≠ 0.032**
AX, median (IQR) %pred	224.00 (158.75-286.60) N = 104	198.10 (145.25-267.78) N = 48	228.50 (173.50-396.00) N = 34	235.00 (128.23-310.50) N = 22	0.262	n/a
Fres, median (IQR) Hz (where Fres does not cross Y axis value of 40Hz was assigned)	14.96 (12.16-20.03)	13.61 (11.32-16.99)	16.42 (12.97-22.30)	17.02 (13.63-22.04)	0.011	* 1.00 ↑0.058 **≠ 0.028**
Fres, median (IQR) %pred	128.00 (109.00-155.00)	120.70 (104.70-148.10)	136.00 (117.00-178.50)	134.00 (113.50-152.75)	0.105	n/a

	N = 103	N = 48	N = 33	N = 22		
Tv, median (IQR) L	0.87 (0.69-1.15)	0.90 (0.70-1.21)	0.82 (0.69-0.94)	0.95 (0.66-1.14)	0.189	n/a
AO %change post bronchodilator reversibility						
R5, mean (SD) %change	-18.55 (13.38)	-17.02 (-26.50- -7.50)	-20.89 (13.03)	-18.25 (14.65)	0.427	n/a
R20, median (IQR) %change	-16.00 (-21 - -8.00)	-15.00 (-21.00 - -6.50)	-16.00 (-23 - -11)	-15.00 (-28.00- -10.00)	0.561	n/a
R5-R20, median (IQR) %change	-43.00 (-73.00- 7.00)	-42.00 (-72.00- 12.00)	-54.00 (-88.00- -8.00)	-26.00 (-69.00- 19.00)	0.519	n/a
X5, mean (SD) %change	17.93 (24.08)	12.67 (27.13)	27.31 (17.73)	14.83 (22.13)	0.017	* 0.120 ⇑ **0.015** ≠ 0.929
X5in, median (IQR) %change	27.00 (8.50-35.50) N = 97	22.00 (13.00-35.00) N 39	31.00 (16.00 – 37.00)	16.00 (3.00-32.00)	0.096	n/a
X5ex, mean (SD) %change	22.40 (25.44) N = 97	22.46 (23.00) N = 39	29.89 (20.72)	10.91 (31.94)	0.019	* **0.014** ⇑ 0.403 ≠ 0.181
AX, mean (SD) %change	-32.48 (29.37)	-30.53 (30.54)	-41.58 (23.98)	-22.98 (31.64)	0.051	**n/a**
Fres, median (IQR) %change	-14.00 (-26.00- -5.00)	-11.00 (-25.50- -0.50)	-21.00 (-28.00- -10.00)	-9.00 (-16.00 - -2.00)	0.019	* **0.041** ⇑ 0.054 ≠ 1.00
TV, median (IQR) %change	0.00 (-17.00-15.00)	-5.00 (-23.50- 9.0)	10.00 (-11.00-21.00)	-3.00 (-17.00-15.00)	0.077	n/a

*P value, difference between ' asthma' and 'not asthma' groups, ⇑P value, difference between 'asthma' and 'healthy control,' ≠P value, difference between 'not asthma' and 'healthy controls'

Mean (SD) was recorded if whole population (i.e. all three groups combined) was normally distributed (skewness and kurtosis between -1/+1), otherwise median (IQR) was reported. One way ANOVA for normally distributed data, Post hoc analysis (Tukey) was performed if significant difference between one or more groups. Kruskal-Wallis one way ANOVA for non-normally distributed data, Post hoc analysis by pairwise comparison (values adjusted by the Bonferroni correction for multiple tests). Chi-square test for categorical data.

None of the baseline AO measurements were significantly different between symptomatic 'asthma' patients and symptomatic patients with the diagnosis of asthma excluded. Small airways resistance was significantly greater in asthma when compared to the healthy control group. However, this finding was lost when using the percent predicted outcomes. AO reversibility post bronchodilation with salbutamol was the only measure that differentiated the 'asthma' and 'not asthma' patients. Two measures; X5ex%change and Fres%change, post bronchodilation showed improvement in airways reactance.

Box plots demonstrating AO measurements in all three groups:

Boxplots demonstrating distributions for each AO measurement across the three groups are displayed for measurements that showed significant difference between groups (figure 53).

Figure 53. Boxplots to show distribution of AO variables in asthma, not asthma and healthy volunteers groups

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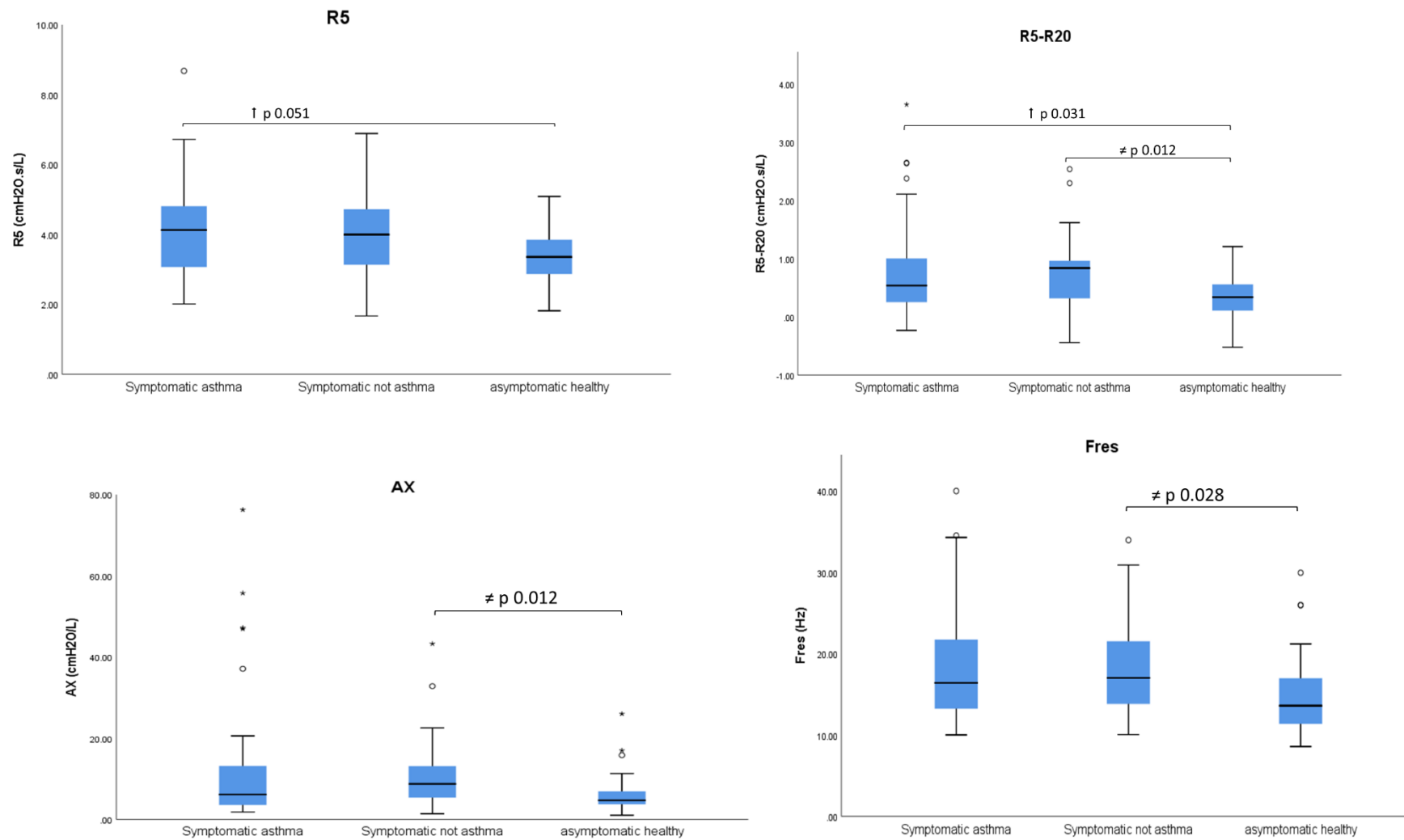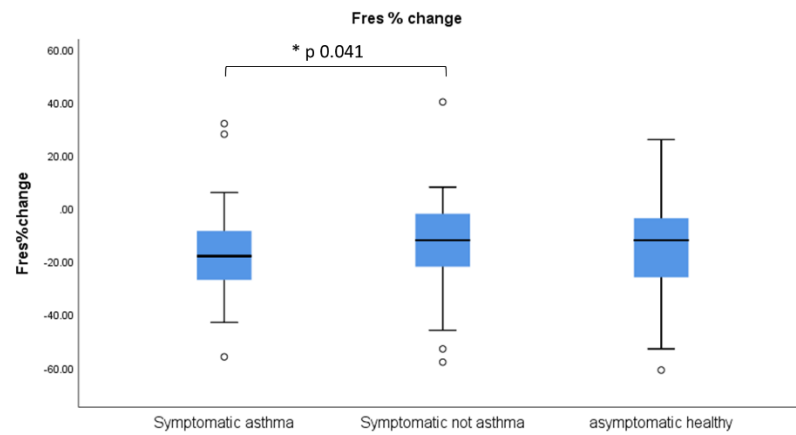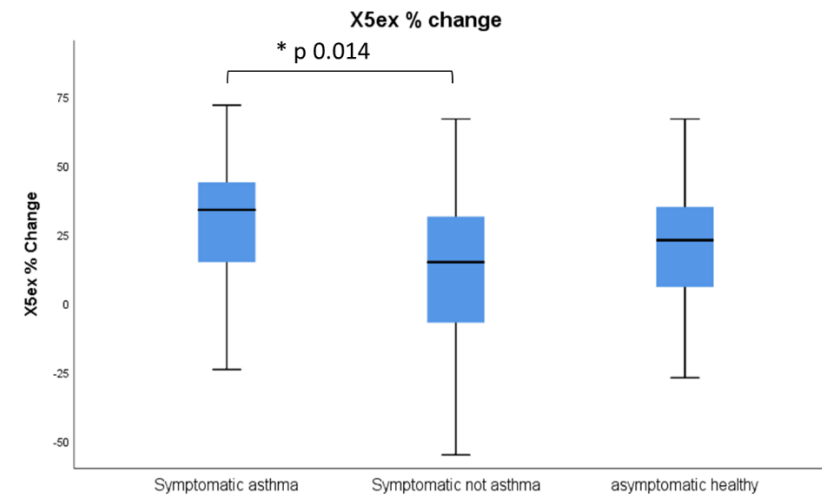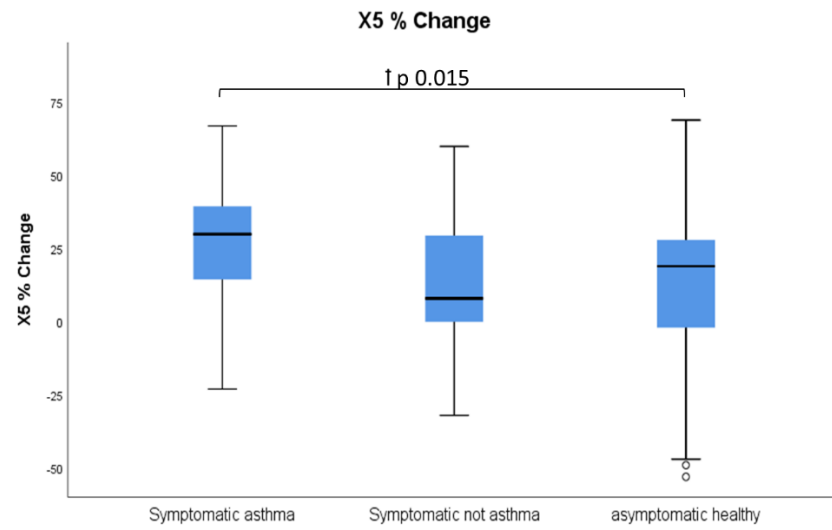
## 4.4.3.2.	Adjusting baseline AO measurements for Height

Following our analysis on 'confounding variables affecting airways oscillometry, 'multivariable linear regression was used to examine the effects of the potential confounders on the AO measures. Age, height, and smoking status were considered, but only height was shown to be significantly independently associated with the AO measures; R5, R20, R5-R20, and X5in, at the 5% significance level. As height was shown to have a linear relationship with the AO measures, ANCOVA methods could be used to adjust for this confounding effect, with height added to the models as a covariate. The height-adjusted group means and 95% confidence intervals are reported, as well as the p-values from the F-test with two degrees of freedom, and post-hoc pairwise comparisons, where necessary, with Benjamini-Hochberg adjustment for multiple testing (Table 61).

**Table 61.	ANCOVA adjusting for height**

		Adults>17yrs		
	Estimated marginal means (EMM) (95%CI)		****adjusted p**	**post-hoc**
R5			**0.018**	ⱡ<0.002
Healthy	3.43 (3.16 , 3.71)			
Asthma	4.19 (3.86 , 4.51)			
Not asthma	3.78 (3.37 , 4.2)			
R20			0.069	
Healthy	3.08 (2.91 , 3.24)			
Asthma	3.37 (3.18 , 3.57)			
Not asthma	3.08 (2.84 , 3.33)			
R5-R20			**0.018**	ⱡ<0.004
Healthy	0.36 (0.18 , 0.54)			
Asthma	0.81 (0.6 , 1.02)			
Not asthma	0.7 (0.43 , 0.97)			
X5			**0.043**	ⱡ<0.018
Healthy	-1.33 (-1.58 , -1.07)			
Asthma	-1.88 (-2.18 , -1.58)			
Not asthma	-1.47 (-1.85 , -1.09)			
X5in			0.088	
Healthy	-1.64 (-1.88 , -1.4)			
Asthma	-2.03 (-2.28 , -1.77)			
Not asthma	-1.77 (-2.09 , -1.45)			

X5ex		**0.045**	**ĭ<0.035**
Healthy	-1.1 (-1.46 , -0.74)		
Asthma	-1.77 (-2.14 , -1.39)		
Not asthma	-1.19 (-1.66 , -0.72)		
AX		**0.043**	**ĭ<0.011**
Healthy	6.59 (3.47 , 9.71)		
Asthma	13.78 (10.11 , 17.46)		
Not asthma	9.8 (5.17 , 14.42)		
Fres		**0.043**	**ĭ<0.029**
Healthy	15.04 (13.4 , 16.69)		
Asthma	18.42 (16.48 , 20.36)		
Not asthma	17.71 (15.27 , 20.15)		
Tv		0.088	
Healthy	1.01 (0.91 , 1.12)		
Asthma	0.84 (0.72 , 0.96)		
Not asthma	1.01 (0.85 , 1.16)		

*P value, difference between ' asthma' and 'not asthma' groups, ĭP value, difference between 'asthma' and 'healthy control,' ≠P value, difference between 'not asthma' and 'healthy controls'
**adjusted p, p values adjusted for multiple testing

After adjusting for height there was still no baseline measurement that was able to predict 'asthma' from 'not asthma' patients. Symptomatic asthma patients showed significantly different baseline measurements of resistance (R5, R5-R20) and reactance (X5, X5ex, AX, Fres) compared to healthy controls (p <0.05).

4.4.3.3.   Summary of Airways Oscillometry Measurements that are able to discriminate between each group in adults (after adjusting airways oscillometry measurements for height)

The following table summarises significant predictors of AO after adjusting for height (table 62).

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Table 62. Significant AO predictors between the three groups

Asthma Vs Not Asthma	Asthma Vs Healthy	Symptomatic not asthma Vs Healthy
Baseline Reactance measurements: None	**Reactance measurements:** R5 R5-R20	**Reactance measurements:** None
Baseline Resistance measurements: None	**Resistance measurements:** X5 AX Fres X5ex	**Resistance measurements:** none
Bronchodilator reversibility measurements: X5ex%change Fres %change	**Bronchodilator reversibility measurements:** X5%change	**Bronchodilator reversibility measurements:** None
Standard asthma tests to predict between groups: FEV1%predicted FEV1/FVC Ratio BDR (FEV1%change) FeNO Eos	**Standard asthma tests to predict between groups:** FEV1%predicted FEV1/FVC ratio BDR (FEV1%change) FeNO Eos	**Standard asthma tests to predict between groups:** None

Baseline AO measurements did not predict 'asthma' from 'symptomatic not asthma' patients. Post bronchodilator improvements in lower airways reactance measurements (X5ex%change and Fres%change) did differentiate the two symptomatic groups. When compared to healthy controls, symptomatic 'not asthma' patients did not show statistically significant difference in any AO measurements. When comparing the three groups with conventional tests, we show obstructed reversible airways was a key predictor between asthma and the other groups. No significant reversibility was shown in our 'asymptomatic not asthma' group.

4.4.4. Exploring cut-points for significant predictors of asthma

4.4.4.1. X5ex%change and Fres%change to diagnose asthma from not asthma

We investigate if X5ex%change and Fres%change; which both show significant differences between symptomatic groups, have a cut-point to predict asthma from not asthma. We

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investigate using Youdens Index, and we also investigate using a defined specificity of 100% to see if we can use AO as a 'rule in' test (Table 63-65) similar to above analysis in 'asthma diagnosis using standard tests' (see Chapter 3) .

Table 63.   Cut points for AO BDR measurements in asthma diagnosis

variable	Criteria	Cut point	Sensitivity	Specificity	Accuracy	AUC
X5ex%change	Youden	26	68.6	69.6	69.0	0.684
	Sp =100	72	2.9	100	41.4	
Fres%change	Youden	-19	61.8	78.3	68.4	0.696
	Sp =100	-30	20.6	91.3	49.1	

Table 64.

Table 65.   X5ex%change to predict EPOER asthma

		Asthma	Not asthma	Total
X5ex%change	Positive (>25)	24	7	31
	Negative (≤25)	11	16	27
	Total	35	23	58
X5ex%change	Positive (>71)	1	0	1
	Negative (≤71)	34	23	57
	Total	35	23	58

Table 66.   Fres%change to predict EPOER asthma

		Asthma	Not asthma	Total
Fres%change	Positive (<-18)	21	4	25
	Negative (≥-18)	14	19	33
	Total	35	23	58
Fres%change	Positive (<-29)	8	2	10
	Negative (≥-29)	27	21	48
	Total	35	23	58

4.4.5. AO predictors versus conventional tests

We compare AO predictors of asthma (using defined thresholds above) with standard dichotomised asthma tests (table 66).

Table 67.   Conventional tests versus AO

	N	Sensitivity % (95% CI)	Specificity % (95% CI)	NPV % (95% CI)	PPV % (95% CI)	Asthma Detected Number (%) positive
Conventional tests:						
FEV1/FVC <LLN	58	37 (21-55)	100 (85-100)	51 (36-66)	100 (75-100)	13 (37)
FEV1/FVC <70	58	31 (17-49)	100 (85-100)	49 (34-64)	100 (72-100)	11 (31)
BDR: FEV1%change ≥12% and 200ml (≥12 in children)	58	46 (29-63)	100 (85-100)	55 (39-70)	100 (79-100)	16 (46)
FeNO (≥40ppb adults, ≥35ppb children <17years)	58	54 (38-71)	87 (66-97)	56 (38-72)	86 (65-97)	19 (54)
PEFv >20%	46	10 (2-27)	100 (80-100)	40 (25-56)	100 (29-100)	3 (10)
PEFv(alt) >3Days>20%	46	34 (18-54)	100 (80-100)	47 (30-65)	100 (69-100)	10 (34)
Eos >0.4x10^9/L	56	32 (17-51)	100 (85-100)	49 (34-64)	100 (72-100)	11 (32)
BCTmeth PD20 ≤0.2mg	45	56 (35-76)	100 (83-100)	65 (45-81)	100 (77-100)	14 (56)
Wheeze auscultated	58	20 (8-37)	100 (85-100)	45 (31-60)	100 (59-100)	7 (20)
AO tests:						
X5ex%change >25 (youden)	58	69 (51-83)	70 (47-87)	59 (39-78)	77 (59-90)	24 (69)
X5ex%change >71 (sp = 100) spec	58	3 (0-15)	100 (85-100)	40 (28-54)	100 (3-100)	1 (3)
Fres%change <-18 (youden)	57	59 (41-75)	83 (61-95)	58 (39-75)	83 (63-95)	21 (60)
Fres%change <-29 (sp =100)	57	21 (9-38)	91 (72-99)	44 (29-59)	78 (40-97)	7 (21)

When comparing Airways Oscillometry Bronchodilator reversibility (AO BDR) using 'youdens index' or '100% specificity' defined thresholds with conventional asthma tests, we show that conventional tests used in current guidelines(38) outperform AO BDR for use as a 'rule in' test.

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4.4.6. Can Airways Oscillometry improve the asthma diagnostic pathway

4.4.6.1.   AO and the NICE (NG80) algorithm

We looked at performance of the NICE diagnostic algorithm (NG80) to rule in EPOER asthma (i.e., patients receiving NG80 outcome 'diagnose with asthma') (see chapter 3, section 3.4.2.1, table 28), and then added in Airways Oscillometry using the measurements we have shown to be best at predicting asthma from non-asthma above. We look to see if the addition of AO improves performance of NG80 to rule in asthma (Table 67).

Table 68.   NG80 and AO to diagnose (rule in) asthma

	N	Sensitivity % (95% CI)	Specificity % (95% CI)	NPV % (95% CI)	PPV % (95% CI)	Asthma Detected Number (%) positive
NICE (NG80) algorithm	42	40 (21-61)	100 (80-100)	53 (35-71)	100 (69-100)	10 (40)
One of: NICE (NG80), X5ex%change>25	42	84 (64-95)	71 (44-90)	75 (48-93)	81 (61-93)	21 (84)
One of: NICE (NG80), X5ex%change>71	42	40 (21-61)	100 (80-100)	53 (35-71)	100 (69-100)	10 (40)
One of: NICE (NG80), Fres%change<-18	42	76 (55-91)	76 (50-93)	68 (43-87)	83 (61-95)	19 (76)
One of: NICE (NG80), Fres%change<-29	42	56 (35-76)	88 (64-99)	58 (37-77)	88 (62-98)	14 (56)

N refers to the number of patients that completed both NICE NG80 algorithm <u>and</u> AO BDR.

The addition of AO using youdens index threshold improves the sensitivity of the model (40% and 84% respectively), however compromises specificity (100% versus 71%). However, when using the 'specificity=100' thresholds to 'rule in' asthma, the addition of AO did not improve the NG80 algorithm.

4.4.6.2.   AO and multivariate algorithms (primary care)

We explored how we could use Airways Oscillometry with our recommended diagnostic algorithms from 'asthma diagnosis using standard tests' (section 3.4.3.1, Table 43). We

used AO BDR as an additional test, and also as a replacement test for spirometry (i.e., in the event spirometry-based tests were contra-indicated) (tables 68 and 69).

Table 69. **Best multivariate algorithm (for primary care) with addition of Airways Oscillometry (asthma versus not asthma)**

	N	Sensitivity % (95% CI)	Specificity % (95% CI)	NPV % (95% CI)	PPV % (95% CI)	Asthma Detected Number (%) positive
Proposed best asthma algorithm for primary care						
W. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze	44	86 (67-96)	100 (79-100)	80 (56-94)	100 (86-100)	24 (86)
AO as additional test to Asthma Algorithm						
W*. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, X5ex%change>25	44	93 (76-99)	69 (41-89)	85 (55-98)	84 (66-95)	26 (93)
W*. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, X5ex%change>25	44	71 (51-87)	100 (79-100)	67 (45-84)	100 (83-100)	20 (71)
W*. Three of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, X5ex%change>25	44	39 (22-59)	100 (79-100)	48 (31-66)	100 (72-100)	11 (39)
W#. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, X5ex%change>71	44	86 (67-96)	100 (79-100)	80 (56-94)	100 (86-100)	24 (86)
W**. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, Fres%change< -18	43	93 (76-99)	75 (48-93)	86 (57-98)	86 (68-96)	25 (93)
W**. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, Fres%change< -18	43	67 (46-83)	100 (79-100)	64 (43-82)	100 (81-100)	18 (67)
W**. Three of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, Fres%change< -18	43	37 (19-58)	100 (79-100)	48 (31-66)	100 (69-100)	10 (37)
W##. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, Fres%change< -29	43	89 (71-98)	88 (62-98)	82 (57-96)	92 (75-99)	24 (89)

W##. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, Fres%change< -29	43	57 (35-75)	100 (79-100)	57 (37-76)	100 (78-100)	15 (56)

The addition of AO BDR using youdens index or 'specificity=100' cut point did not improve the performance of our current 'rule in' asthma algorithm.

Table 70. Best multivariate algorithm (for primary care) with AO replacing spirometry tests (asthma versus not asthma)

	N	Sensitivity	Specificity	NPV	PPV	Asthma Detected
		% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	Number (%) positive
Proposed best asthma algorithm for primary care						
W. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze	44	86 (67-96)	100 (79-100)	80 (56-94)	100 (86-100)	24 (86)
Wi. One of: Eos, PEFv(alt), Wheeze (without spirometry-based tests)	44	57 (37-76)	100 (79-100)	57 (37-76)	100 (79-100)	16 (57)
AO replacing spirometry						
W*. One of: X5ex%change>25, Eos, PEFv(alt), Wheeze	44	82 (63-94)	69 (41-89)	69 (41-89)	82 (63-94)	23 (82)
W*. Two of: X5ex%change>25, Eos, PEFv(alt), Wheeze	44	46 (28-66)	100 (79-100)	52 (33-70)	100 (75-100)	13 (46)
W*. Three of: X5ex%change>25, Eos, PEFv(alt), Wheeze	44	21 (8-41)	100 (79-100)	42 (26-59)	100 (54-100)	6 (21)
W#. One of: X5ex%change>71, Eos, PEFv(alt), Wheeze	44	57 (37-76)	100 (79-100)	57 (37-76)	100 (79-100)	16 (57)
W**. One of: Fres%change< -18, Eos, PEFv(alt), Wheeze	43	78 (58-91)	75 (48-93)	67 (41-87)	84 (64-95)	21 (78)
W**. Two of: Fres%change< -18, Eos, PEFv(alt), Wheeze	43	44 (25-65)	100 (79-100)	52 (33-70)	100 (74-100)	12 (44)
W**. Three of: Fres%change< -18, Eos, PEFv(alt), Wheeze	43	19 (6-38)	100 (79-100)	42 (26-59)	100 (48-100)	5 (19)
W##. One of: Fres%change< -29, Eos, PEFv(alt), Wheeze	43	70 (50-96)	88 (62-98)	64 (41-83)	90 (70-99)	19 (70)
W##. Two of: Fres%change< -29, Eos, PEFv(alt), Wheeze	43	33 (17-54)	100 (79-100)	47 (30-65)	100 (66-100)	9 (33)
Wi*. One of: Eos, Wheeze, X5ex%change>71	56	47 (30-65)	100 (85-100)	55 (38-71)	100 (79-100)	16 (47)

We explored whether AO BDR may have a role to 'rule in' asthma in the event of spirometry-based tests being contraindicated. AO BDR did not optimise the performance of our proposed algorithm in this setting. In the event of all AGPs including home based tests (PEFv) not being available, we show X5ex%change combined with blood eosinophils and auscultated wheeze detected 47% asthma patients.

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4.4.6.3. AO and multivariate algorithms (primary and secondary care)

We explored multivariate algorithms (table 70), using AO BDR with our best overall diagnostic algorithm described in 'asthma diagnosis using standard tests' (section 3.4.3.1, table 43).

Table 71. Best multivariate algorithm (overall) with addition of Airways Oscillometry (asthma versus not asthma)

	N	Sensitivity	Specificity	NPV	PPV	Asthma Detected
		% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	Number (%) positive
Proposed best asthma algorithm overall						
O One of: FEV1/FVC <70% or <LLN, BDR, Eos, PEFv>20% or alt, Wheeze, BCTmeth	38	87 (66-97)	100 (78-100)	83 (59-96)	100 (83-100)	20 (87)
AO as additional test to Asthma Algorithm						
O*. One of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, X5ex%change>25, BCTmeth	38	96 (78-100)	67 (38-88)	91 (59-100)	81 (62-94)	22 (96)
O*. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, X5ex%change>25, BCTmeth	38	78 (56-93)	100 (78-100)	75 (51-91)	100 (81-100)	18 (78)
O*. Three of: FEV1/FVC <LLN, BDR, Eos, PEFv(alt), Wheeze, X5ex%change>25, BCTmeth	38	57 (34-77)	100 (78-100)	60 (39-79)	100 (75-100)	13 (57)
O#. One of: FEV1/FVC <LLN, BDR, Eos, PEFv (alt), Wheeze, X5ex%change>71, BCTmeth	38	87 (66-97)	100 (78-100)	83 (59-96)	100 (83-100)	20 (87)
O#. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv (alt), Wheeze, X5ex%change>71, BCTmeth	38	74 (52-90)	100 (78-100)	71 (48-89)	100 (80-100)	17 (74)
O#. Three of: FEV1/FVC <LLN, BDR, Eos, PEFv (alt), Wheeze, X5ex%change>71, BCTmeth	38	26 (10-48)	100 (78-100)	47 (29-65)	100 (54-100)	6 (26)
O**. One of: FEV1/FVC <LLN, BDR, Eos, PEFv (alt), Wheeze, Fres%change< -18, BCTmeth	37	95 (77-100)	73 (45-92)	92 (62-100)	84 (64-95)	21 (95)

O**. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv (alt), Wheeze, Fres%change< -18, BCTmeth	37	77 (55-92)	100 (78-100)	75 (51-91)	100 (80-100)	17 (77)
O**. Three of: FEV1/FVC <LLN, BDR, Eos, PEFv (alt), Wheeze, Fres%change< -18, BCTmeth	37	45 (24-68)	100 (78-100)	56 (35-75)	100 (69-100)	10 (45)
O##. One of: FEV1/FVC <LLN, BDR, Eos, PEFv (alt), Wheeze, Fres%change< -29, BCTmeth	37	91 (71-99)	87 (60-98)	87 (60-98)	91 (71-99)	20 (91)
O##. Two of: FEV1/FVC <LLN, BDR, Eos, PEFv (alt), Wheeze, Fres%change< -29, BCTmeth	37	77 (55-92)	100 (78-100)	75 (51-91)	100 (80-100)	17 (77)
O##. Three of: FEV1/FVC <LLN, BDR, Eos, PEFv (alt), Wheeze, Fres%change< -29, BCTmeth	37	32 (14-55)	100 (78-100)	50 (31-69)	100 (59-100)	7 (32)

We show that addition of AO BDR into our proposed 'best overall' algorithm did not improve the performance of our algorithm.

4.4.7. Determine if 'Airways Oscillometry' measurements correlate with current tests used in the NICE Diagnostic Algorithm (NG80): Could Airways Oscillometry improve the algorithm or replace pre-existing tests within the Algorithm? (Exploratory analysis in Adults)

4.4.7.1. AO baseline measurements with NICE diagnostic tests in symptomatic patients

We computed Spearman's Rank correlation to look for associations between the baseline airways oscillometry (AO) measurements for resistance (R5, R20, R5-R20) and reactance (X5, Fres, AX) with NICE recommended asthma diagnostic tests (table 71). We also compared the AO BDR predictors of asthma (X5%change and Fres%change) with NICE recommended tests.

Table 72.   Associations between baseline AO and NICE recommended tests

Variable	Correlation coefficient (95%CI)	*P value
R5		
FEV$_1$/FVC ratio	-0.077 (-0.339, 0.219)	0.567
BDR (FEV$_1$%change)	0.113 (-0.206, 0.382)	0.399
PEFv	0.082 (-0.213, 0.358)	0.586
FeNO	-0.174 (-0.408, 0.096)	0.192
R5%pred		
FEV$_1$/FVC ratio	**-0.337 (-0.578, -0.073)**	**0.011**
BDR (FEV$_1$%change)	**0.444 (0.193, 0.652)**	**0.001**
PEFv	0.083 (-0.223, 0.344)	0.593
FeNO	-0.052 (-0.328, 0.208)	0.702
R20		
FEV$_1$/FVC ratio	0.018 (-0.238, 0.283)	0.896
BDR (FEV$_1$%change)	0.069 (-0.225, 0.346)	0.609
PEFv	0.046 (-0.226, 0.333)	0.761
FeNO	-0.225 (-0.476, 0.058)	0.089
R20%pred		
FEV$_1$/FVC ratio	-0.192 (-0.431, 0.087)	0.156
BDR (FEV$_1$%change)	**0.282 (0.014, 0.507)**	**0.035**
PEFv	0.033 (-0.291, 0.340)	0.832
FeNO	-0.050 (-0.332, 0.229)	0.712
R5-R20		
FEV$_1$/FVC ratio	-0.090 (-0.344, 0.221)	0.503
BDR (FEV$_1$%change)	0.097 (-0.191, 0.390)	0.468

PEFv	0.093 (-0.203, 0.387)	0.539
FeNO	-0.111 (-0.369, 0.157)	0.405
X5		
FEV$_1$/FVC ratio	0.033 (-0.265, 0.314)	0.804
BDR (FEV$_1$%change)	-0.141 (-0.405, 0.145)	0.292
PEFv	-0.103 (-0.389, 0.213)	0.498
FeNO	0.094 (-0.176, 0.353)	0.482
AX		
FEV$_1$/FVC ratio	-0.084 (-0.360, 0.209)	0.529
BDR (FEV$_1$%change)	0.115 (-0.167, 0.372)	0.389
PEFv	0.117 (-0.171, 0.387)	0.439
FeNO	-0.105 (-0.371, 0.147)	0.431
AX%pred		
FEV$_1$/FVC ratio	**-0.306 (-0.556, -0.023)**	**0.022**
BDR (FEV$_1$%change)	**0.431 (0.177, 0.656)**	**0.001**
PEFv	0.164 (-0.129, 0.465)	0.288
FeNO	-0.014 (-0.304, 0.260)	0.921
Fres		
FEV$_1$/FVC ratio	-0.148 (-0.438, 0.133)	0.268
BDR (FEV$_1$%change)	0.138 (-0.142, 0.393)	0.301
PEFv	0.195 (-0.078, 0.458)	0.193
FeNO	-0.071 (-0.341, 0.184)	0.596
Fres%pred		
FEV$_1$/FVC ratio	0.087 (-0.488, 0.054)	0.087
BDR (FEV$_1$%change)	0.234 (-0.042, 0.495)	0.085
PEFv	0.234 (-0.053, 0.496)	0.126
FeNO	-0.088 (-0.352, 0.214)	0.522
X5ex%change		
FEV$_1$/FVC ratio	**-0.316 (-0.530, -0.068)**	**0.016**
BDR (FEV$_1$%change)	0.254 (-0.043, 0.514)	0.055
PEFv	**0.298 (0.008, 0.133)**	**0.045**
FeNO	0.085 (-0.164, 0.346)	0.524
Fres%change		
FEV$_1$/FVC ratio	**0.359 (0.089, 0.588)**	**0.006**
BDR (FEV$_1$%change)	**-0.335 (-0.585, -0.073)**	**0.010**
PEFv	**-0.377 (-0.587, -0.121)**	**0.010**
FeNO	-0.073 (-0.340, 0.170)	0.584
*correlation is significant at the 0.05 level		

The NICE recommended test FEV$_1$/FVC ratio showed statistically significant Spearman rank-order correlation with R5%predicted, AX%predicted, X5ex%change, and Fres%change (p <0.05). BDR showed correlation with R5%predicted, AX%predicted, and Fres%change (p

<0.05). PEFv showed correlation with X5ex%change and Fres%change (p <0.05). However, for all significant association's correlation was weak (<0.6).

4.4.7.2. Comparing AO predictors of asthma (X5ex%change and Fres%change) with conventional recommended asthma diagnostic tests in symptomatic patients

X5%change and Fres%change

AO BDR measurements (X5ex%change and Fres%change) that were shown to be predictors of asthma (table 69) also demonstrated weak significant relationship with current asthma tests (FEV1/FVC ratio, BDR, and PEFv) (spearman rank <0.6, p <0.05). We further explore the relationships between our AO predictors of asthma and NICE recommended tests using scatter plot diagrams (Figure 54). We highlighted NICE recommended thresholds for positive for the standard tests, and youdens cut offs for the novel AO BDR tests.

Figure 54. Relationships between AO predictors of asthma and conventional tests

Relationship between FEV1/FVC ratio and Fres%change

Relationship between FEV1%change and X5ex%change

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Relationship between FEV1%change and Fres%change

Relationship between FeNO and X5ex%change

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Relationship between FeNO and Fres%change

Relationship between PEFv and X5ex%change

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Relationship between PEFv and Fres%change
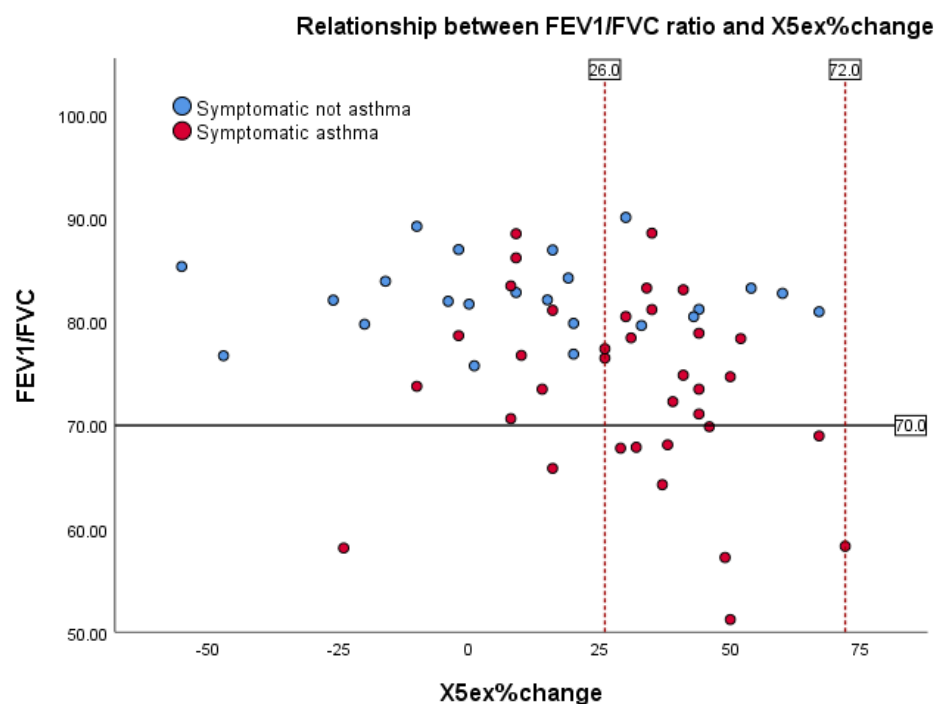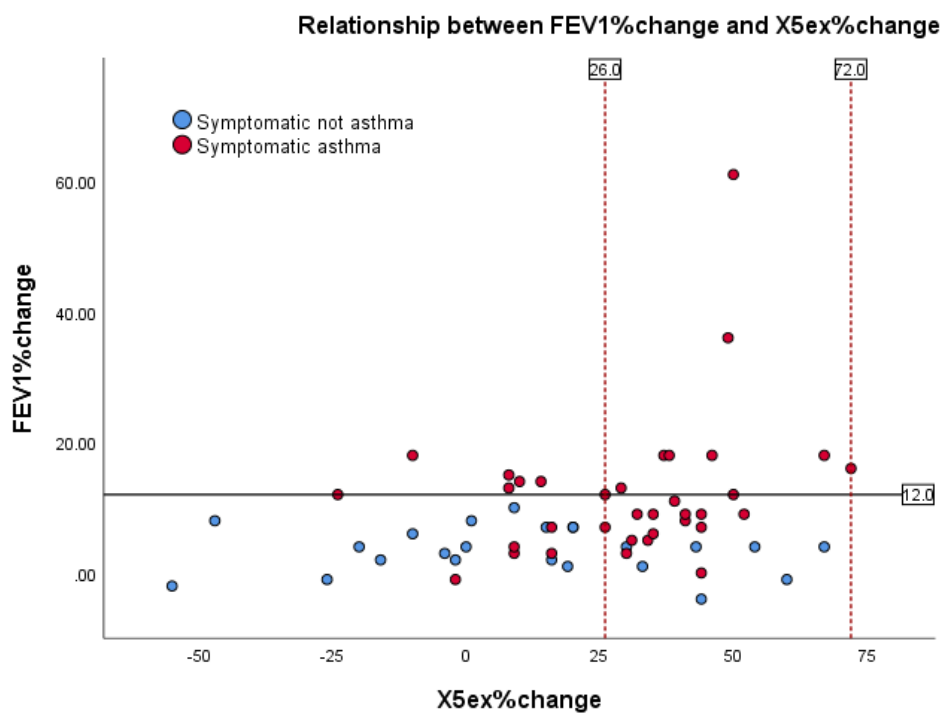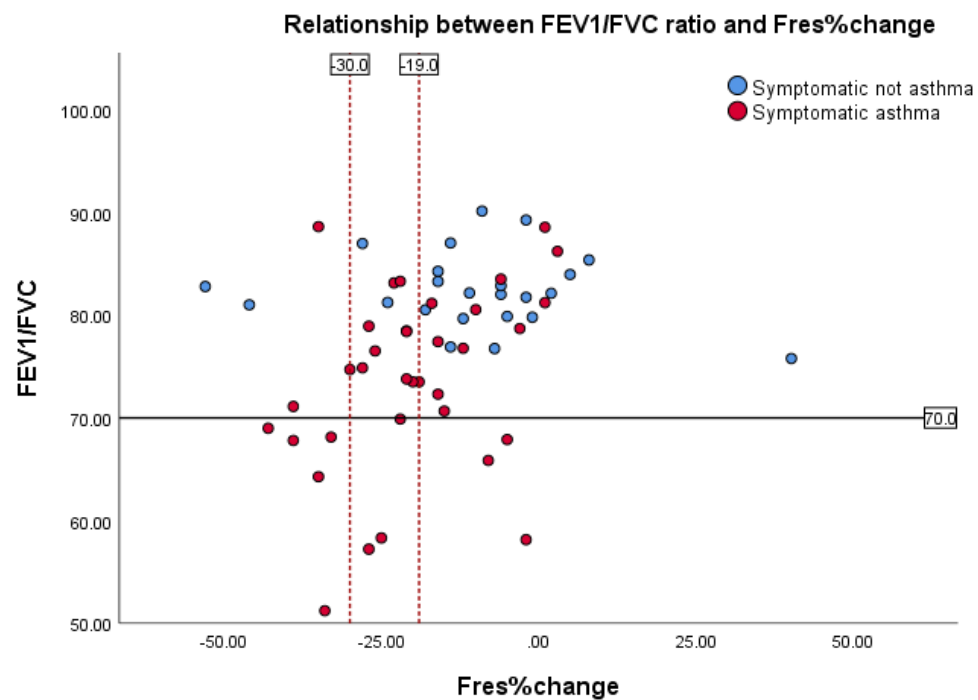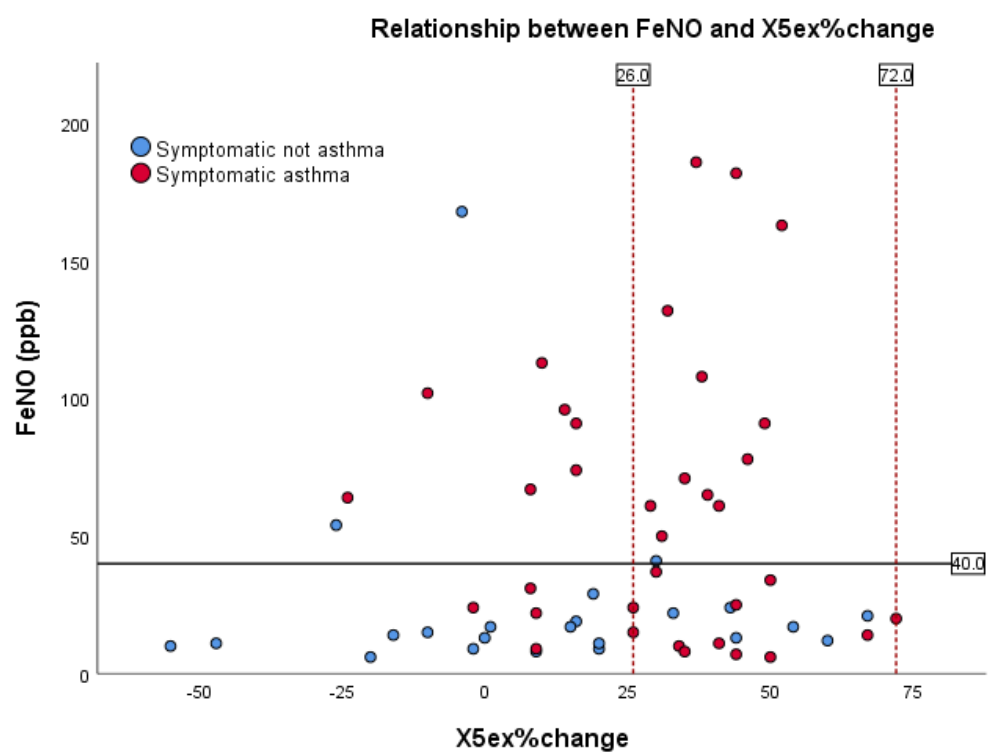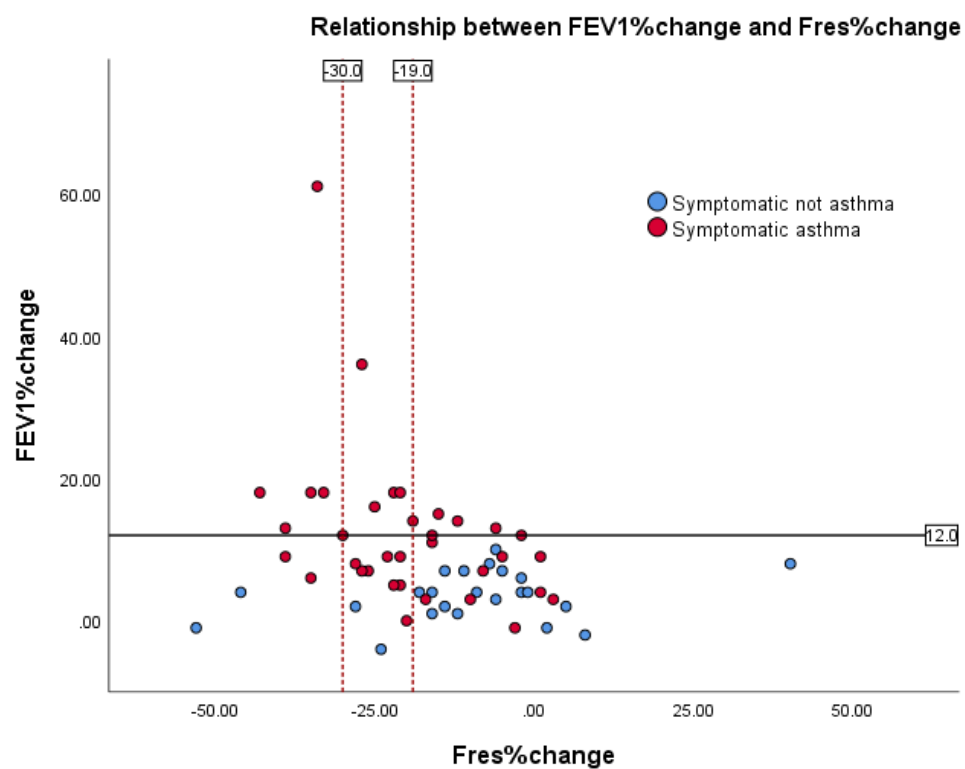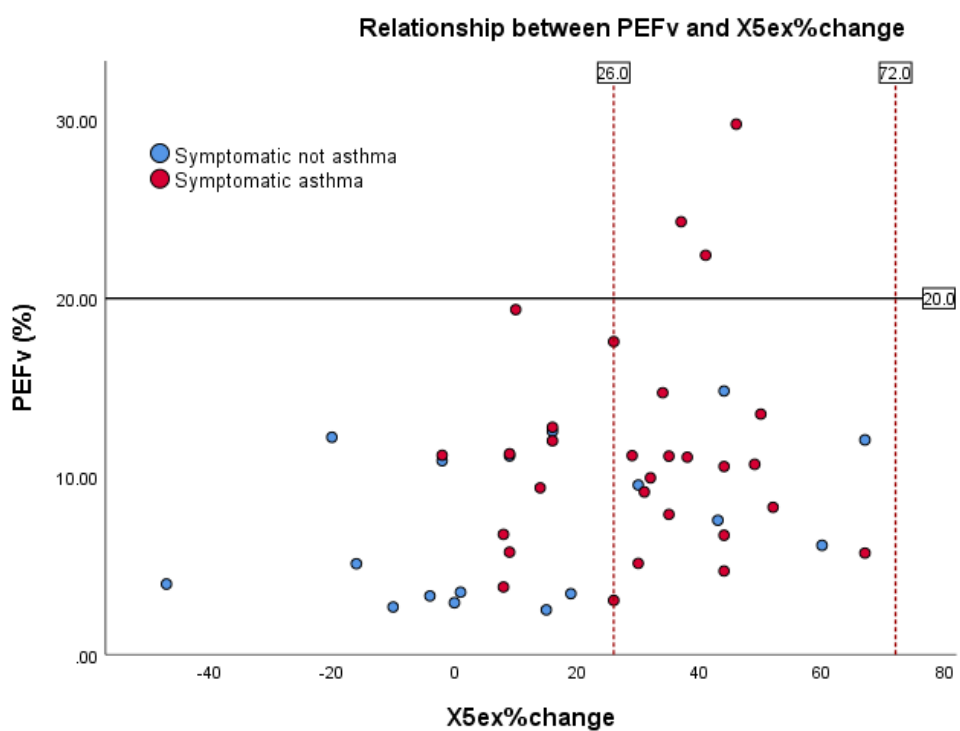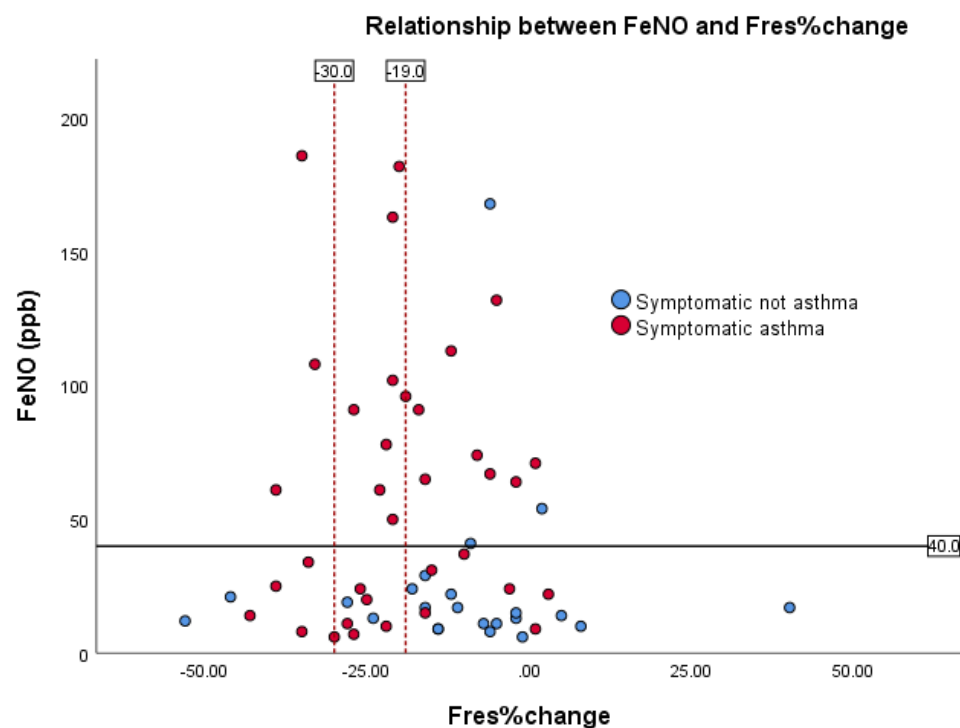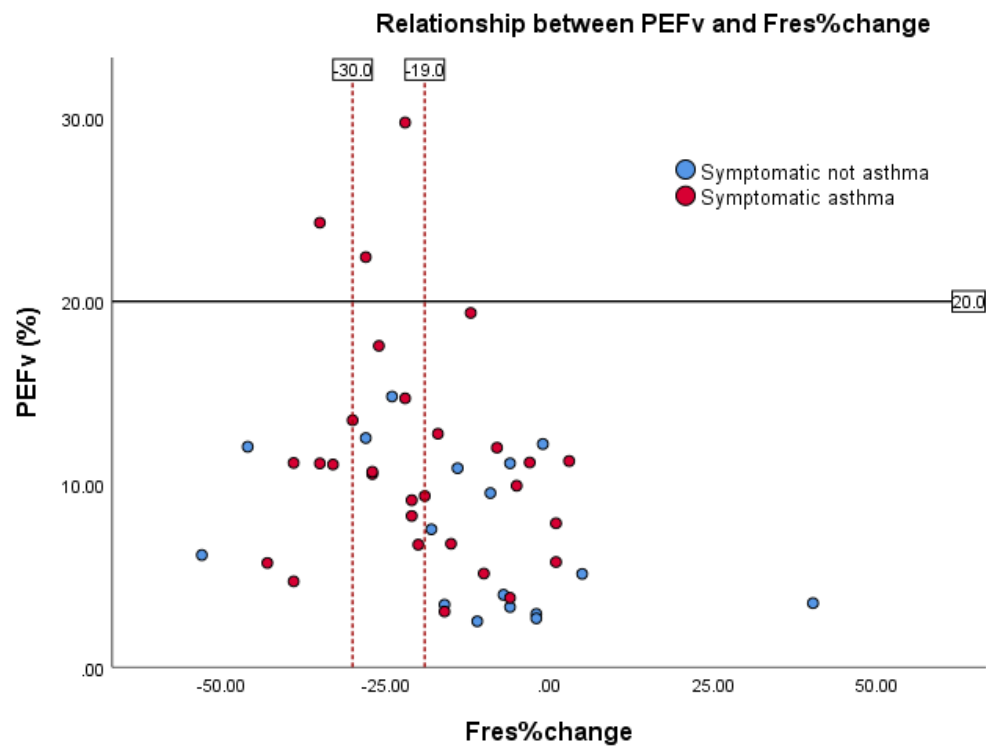
If AO were to become a test used in clinical practice, based upon the above correlation coefficients and scatter plots (table 71, figure 54) we show AO would be better used in addition to current tests and not as a replacement for current tests.

AO Predictors of asthma (i.e. X5ex%change and Fres%change) and NICE diagnostic tests (dichotomised data) in symptomatic patients

We performed univariate analysis to look for correlations between standard asthma tests used in asthma diagnosis and our AO measurements (X5ex%change and Fres%change) that were shown to be potential predictors of asthma previously (table 72).

Table 73. AO measurements compared to dichotomised standard asthma tests

	Obstructed Airways: FEV1/FVC <70		
	Positive (N11)	Negative (N 47)	P value
AO Bronchodilator reversibility			
X5ex, median IQR) %change	38.00 (29.00- 50.00)	20.00 (1.00- 41.00)	**0.028**
Fres, median (IQR) %change	-27.00 (-35.00- -8.00)	-15.00 (-22.00- -5.00)	**0.029**
	Obstructed Airways: FEV1/FVC <LLN		
	Positive (N 13)	Negative (N 45)	
X5ex, median IQR) %change	41.00 (30.50- 50.00)	19.00 (0.50- 37.00)	**0.011**
Fres, median (IQR) %change	-28.00 (-34.50- -18.50)	-14.00 (-21.00- -4.00)	**0.004**
	BDR: FEV1%Change >12% and 200mls		
	Positive (N 16)	Negative (N 42)	
X5ex, mean, (SD) %change	29.38 (26.85)	19.69 (27.12)	0.228
Fres, median (IQR) %change	-23.50 (-33.75- -15.25)	-13.00 (-22.25- -2.75)	**0.011**
	FeNO ≥40ppb		
	Positive (N 22)	Negative (N 36)	
X5ex, mean, (SD) %change	22.86 (22.74)	22.06 (29.84)	0.914
Fres, median (IQR) %change	-18.00 (-22.25- -6.00)	-15.50 (-27.75- -3.50)	0.936
	PEFv >20%		
	Positive (N 3)	Negative (N 43)	
X5ex, median IQR) %change	41.00 (39.00 – 43.50)	26.00 (8.50- 40.50)	0.125
Fres, median (IQR) %change	-28.00 (-31.50- -25.00)	-16.00 (-26.50- -6.00)	0.103
	BCTmeth ≤0.2mg		
	Positive (N 14)	Negative (N 31)	
X5ex, mean, (SD) %change	29.86 (19.80)	16.77 (29.46)	0.138
Fres, median (IQR) %change	-17.50 (-23.75- -5.75)	-14.00 (-27.00- -2.00)	0.677
* P values refer to the difference between the positive and negative test outcomes groups, t-test for normally-distributed data; Mann Whitney U for non-normally distributed data			

Greater AO BDR (using X5ex%change and Fres%change) was seen in patients with obstructive airways disease and greater Fres%change in reversible airways disease using FEV1%change.

Of our AO predictors of asthma, both measurements are post bronchodilation reactance measures. It is likely that both measurements are all looking at the same pathophysiology. We performed a simple scatter plot to assess correlation between these two measurements (figure 55).

Figure 55. Correlation between AO BDR predictors of asthma
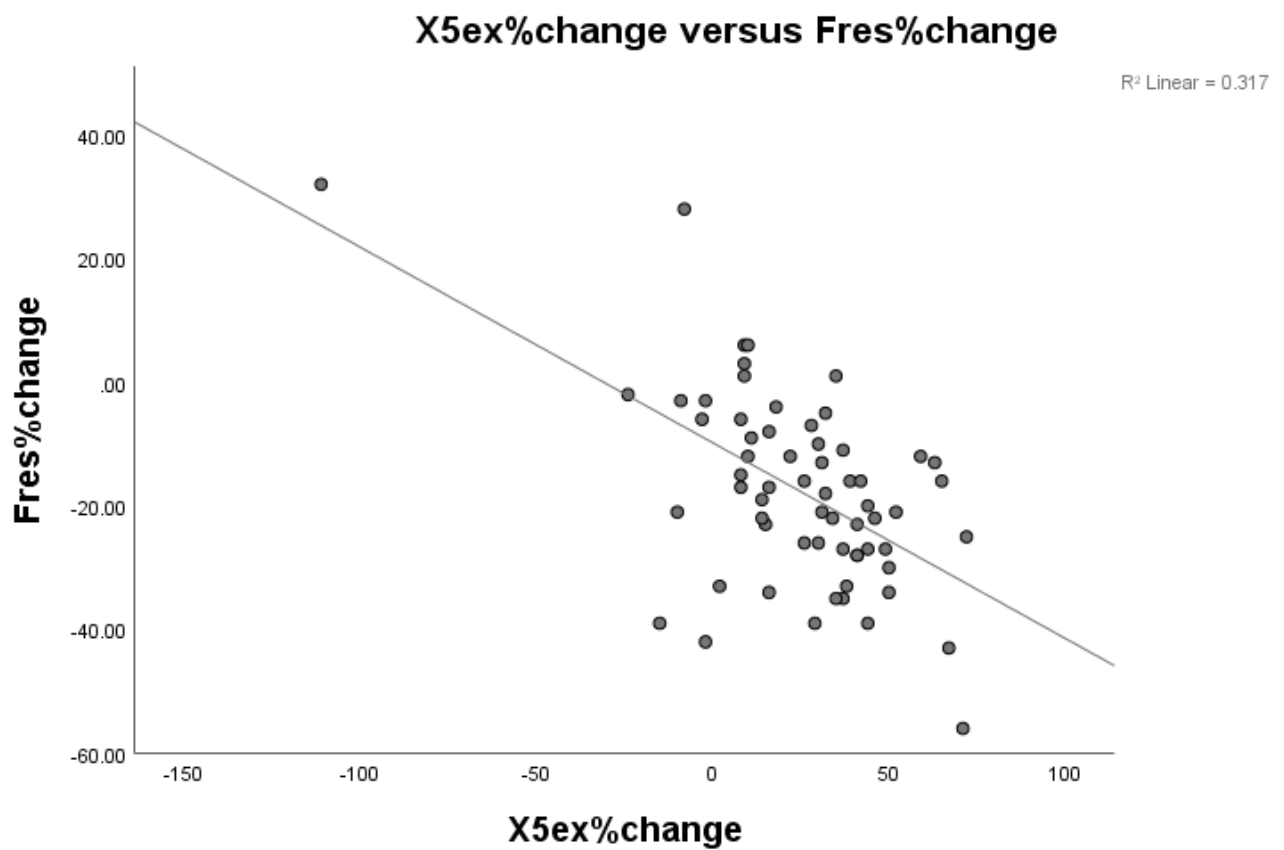
Spearman's rank correlation indicates good correlation between these two measurements (-0.630, p <0.001). If considering AO BDR to replace existing NICE recommended test, only one of these measurements would be required.

4.5. Discussion

We have tested the role of AO in asthma diagnosis by collecting measurements in symptomatic adults and children and comparing findings in those who did receive a diagnosis of asthma with those who did not, (as well as a control group of healthy volunteers). To our knowledge, this is the first study that has directly compared measurement taken in subjects who have recently reported symptoms in keeping with asthma to their GP, but do not yet have a diagnosis, and importantly, who are not on asthma treatment.

We assessed reliability and repeatability of AO in a healthy asymptomatic population. We demonstrate good repeatability for all resistance (R5, R20, R5-20) and reactance (X5, AX, Fres, X5in, X5ex) measurements. Following this, in adults, we explored which AO measures have the potential to predict asthma from a group of symptomatic patients. We show no baseline AO measurements predicted between symptomatic asthma and symptomatic not asthma patients. This remained the case after adjusting for height which was a significant confounding variable in the study. Airways Oscillometry Bronchodilator Reversibility (AO BDR) reactance measurements (X5in%change or Fres%change) were significantly different between symptomatic groups. These measurements show potential for use in asthma diagnosis. We explore cut-off values for AO BDR measurements using Youdens index (X5in%change and Fres%change of >25% and <-18% respectively). Using these cut-off values AO BDR performed with higher sensitivity (range 60-69%) than conventional asthma tests (sensitivity range 20-56%), however specificity is lower (range 77-84%) compared with NICE recommended thresholds for conventional tests (range 86-100%).(24) We also explored cut-off values for AO BDR to 'rule-in' asthma using 'specificity=100' where possible (X5ex%change and Fres%change of >71% and >-29% respectively). However, the addition of AO BDR using these thresholds did not further optimise the performance of our proposed multivariate algorithms from chapter three. Finally, we look at the concordance between AO BDR and current tests used in asthma diagnostic guidelines (e.g., FEV_1/FVC, FEV_1%change, PEFv, and FeNO). We show poor correlation using Spearman's rank correlation, suggesting AO could be used in addition to, rather than to replace, current asthma diagnostic tests.

4.5.1. Is Airways Oscillometry a reliable test with good repeatability?

Repeatability in healthy controls

We show AO is repeatable in healthy subjects. AO was shown to be a reliable test with good intra-class correlation (ICC >0.69) and internal consistency (Cronbach's alpha >0.8). This supports previously reported findings of between-visit agreement of AO measurements reported in young children (181). Our population includes adults and children (5-69 years).

Repeatability in patients with respiratory symptoms

Using AO measurements; all of which had good repeatability in our healthy group, we demonstrate that patients who presented with respiratory symptoms (i.e., cough, breathlessness, wheeze, chest tightness) and a diagnosis of asthma, also had repeatable AO measurements (mean difference between visits was not significant in any measurement, and all measurements had excellent intra-class correlation coefficients (ICC >0.75)). Despite this, Bland Altman plots comparing healthy controls and symptomatic patients, visually demonstrated greater spread in mean difference between visits in asthma compared with healthy controls for all baseline measures (R5, R20, X5) and the following AO derivatives (R5-20, X5in, X5ex, AX). Wider limits of agreement between visits were shown when compared with healthy controls (see table 54; results section). We also observed, between visit variance appears more marked (see Bland-Altman plots in figure 45) at the extremes of measurements, and these were more frequently observed in the asthma group. We speculate that greater variability observed in the asthma group may reflect variable airways pathology characteristically described in patients with asthma. Whilst between-visit variability was not significant in our patient group, it is possible that patients with more severe airways disease (i.e., moderate/severe presentation of asthma) may have more between-visit variance, it is important to investigate this group before concluding between visit variance in AO measurements is not a useful tool in asthma diagnosis. Gonem et al (2014)(155) try to answer this question in a small study of eighteen adults with moderate-severe asthma. The authors concluded AO measurements are reproducible over time in asthma patients, however all patients were treated with inhaled corticosteroids. This is not

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representative of the population in whom AO would be used upon if used as a diagnostic tool in asthma. Harkness et al (2021) explore within-session variability in AO measurements and show variability is greater in disease compared to a healthy control group. However, coefficient of variation (CoV) ≤10% was still achieved in the disease group and the authors don't show CoV can distinguish between disease (i.e., COPD and asthma). Further studies with larger sample size are still needed to answer this question in a steroid naïve population of symptomatic patients with suspected asthma.

In our study we derived 'minimal detectable change' (MDC) values for all baseline AO measurements from our healthy control group (see table 52). We investigated if between-visit change greater than the MDC could be used to identify asthma patients. We show that this value is unlikely to be useful in clinical practice because it represents such a small proportion of patients (see table 55).

4.5.2. Co-variables affecting AO measurements

Effect of age, height, gender, and BMI on airways oscillometry was explored. Younger age was associated with greater airways resistance and reactance (figure 47). This 'age effect' started to stabilise after approximately seventeen years of age. We speculate that this 'age effect' in children relates to the size of developing airways in children's lungs (i.e., smaller airways surface area in immature lungs may lead to higher resistance and reactance). Using the cut-off for adults (≥17 years) used in NICE asthma guidelines, we demonstrated a significant difference in adults and children for baseline AO measurements (resistance, R5 and R20, and reactance, X5). Height was also associated with AO, those with shorter stature showed greater resistance (demonstrated with more positive resistance values) and greater reactance (demonstrated with more negative reactance values). Height has frequently been reported as a significant confounder for AO measurements in the literature (182, 183). It is likely that the 'age effect' noted in children is secondary to height. Several studies exploring reference equations for AO also demonstrate height to be the most influential cofounding variable (168, 184). In our study, gender and BMI did not appear to impact baseline AO

measurements. Based on the findings of this study we show that if implementing AO into clinical practice of asthma diagnosis, any thresholds for positive would need to use age-appropriate cut-offs (i.e., grouping children <17yrs, and adults ≥17years) for positive or alternatively use predictive equations that account for age and/or height.

At present predicted values are readily available within the Tremflow C-100 software (version 1.0) for the following variables: R5, R20, AX, and Fres. Normative data is derived from two studies in adults; Oostveen et al (2013)(168) and Brown et al (2010)(185), and two studies in children; Calogero et al (2013)(170) and Nowowiejska et al (2008)(171). All studies used a predominantly Caucasian cohort. Calogero et al,(170) report cut offs for normal bronchodilator reversibility in children (Fres%change >-47 and X6%change >74), they do not look at intra-breath thresholds. Oostveen et al,(168) explore bronchodilator response in healthy adults for R5 and X5, and more recently Jetmalani et al (2021)(186) published normal limits for bronchodilator response in adults over forty years old (R6, R6ins, X6, and X6ins). However, we have found no normative data available for change with bronchodilator reversibility for X5exp%change or Fres%change in adults (the two measurements that best predicted symptomatic asthma from symptomatic not asthma in this population).

4.5.3. Can 'Airways Oscillometry' predict asthma in adults?

Individual AO measurements in asthma diagnosis

We demonstrate that AO BDR (recorded as %change post salbutamol) has potential as a diagnostic test in asthma diagnosis in adults. Significant improvements were observed in reactance markers (X5ex%change, and Fres%change), when comparing patients with symptomatic 'asthma' to patients with respiratory symptoms that were not due to asthma (p 0.014 and p 0.041 respectively). AO BDR is easier for patients to perform than the current recommended spirometry-based tests because it requires simple tidal breathing opposed to more technical forced expiratory manoeuvres. This may be an advantage over pre-existing asthma diagnostic tests. At present, much of the research on AO in asthma diagnosis has

been in paediatric cohorts, it is likely that this is because AO is easy to perform and has been identified as a possible alternative test to spirometry in children.(187)

AO reactance measurements have previously been linked to the monitoring of asthma control in adults and children.(188-191) Respiratory system reactance is shown to be an independent predictor of asthma control.(188) A recent study has also investigated reliability of AO to identify patients with bronchodilator reversibility.(192) The authors show AO resistance measurements (R5, R20, R5-20) were a reliable measure of bronchodilator reversibility and suggest the test correlates with conventional bronchodilator reversibility (FEV_1%change). However, the authors were unable to show that AO resistance measurements could differentiate between asthma and other respiratory disease (i.e., symptomatic 'not asthma' patients).

When we tested AO measurements in 'asthma' versus 'symptomatic not asthma,' resistance variables could not differentiate between these two symptomatic groups in our cohort. After adjusting for confounding variables, we show that the AO resistance markers (R5, R5-R20) and reactance markers (X5, X5ex, AX, and Fres) were able to predict symptomatic asthma from healthy controls ($p < 0.05$). However, this finding is not clinically useful because this is not the population in which clinicians would need to interpret the test.

Several other studies show AO BDR is a good indicator of asthma control.(189, 190) Kuo et al(189) show both resistance measurements (R5%change) and reactance measurements (X5%change, AX%change, and Fres%change) are able to predict poor asthma control in adults with established diagnosis of asthma. The authors report X5%change >14 could predict poor asthma control (sensitivity 84%, specificity 61%, AUC 0.76). It is not clear if these known asthma patients were already established on asthma treatment, this may explain the lower thresholds used compared to our suggested thresholds in a steroid naïve population. The authors do not report intra-breath measurements (X5in and X5ex) for comparison. Two recent studies report on intra-breath analysis of AO measurements (193, 194). Sol et al (2019)(194) investigate intra-breath measurements in children. They show that intra-breath measurements are a sensitive diagnostic marker of asthma in children,

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however they only compare with a healthy control group. Chiabai et al (2021)(193) investigate intra-breath AO measurements in severe asthma patients, the authors conclude intra-breath technique is a more sensitive measure of asthma control than standard AO measurements.

In our study, airways resistance measures (e.g., R5, R20, and R5-20) pre- and post-bronchodilation medication did not allow us to differentiate between our symptomatic 'asthma' and 'not asthma' patients. Neither did AO measurements for airways reactance (X5, X5in, X5ex, AX, Fres). This is in contrast to several studies reporting the use of baseline AO resistance measurements to detect asthma. Williamson et al report significant difference in baseline R5 and R5-20 in asthma patients, however once again they compare asthma patients with healthy volunteers,(195) similarly Gonem et al report differences in R5 and R20 comparing moderate and severe asthma.(155) In both studies, baseline spirometry was more obstructed (despite patients receiving asthma treatments) when compared to patients referred into RADicA. We speculate that patients referred into RADicA had more mild asthma (e.g., less severe airways obstruction) and therefore baseline AO measurements may be less effective at detecting between our asthma, symptomatic not asthma, and healthy control groups. Neither of the above studies compare the specific population that present to primary care for diagnosis (i.e., steroid naïve symptomatic patients with 'asthma' or 'not asthma'), which may explain the difference in findings.

Reactance (X5%predicted) has previously been linked with asthma diagnosis(196), when compared to to healthy controls, but this study did not test whether X5%predicted could differentiate between symptomatic 'asthma' and 'not asthma,' an important factor when considering the usefulness of AO in clinical practice. More recently, Bhatawadekar et al (2019)(197) use multi-branch airway-tree modelling to demonstrate that in asthma, elastance (a component of respiratory reactance), was more sensitive to bronchodilation than resistance. The authors suggest that the lower airways measurement 'reactance' is superior to resistance in detecting airway response to bronchodilation. This supports the findings of our study.

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In our study we test the ability of AO to differentiate asthma patients from 'not asthma' patients in a symptomatic adult population. Using the two measurements (X5ex%change and Fres%change) that were shown in the univariate analysis to be significant predictors for asthma, we used Youdens index to compute potential thresholds for these measurements in asthma diagnosis (>25% and <-18% respectively). Using these thresholds we show AO BDR reactance measurements (X5ex%change and Fres%change), were sensitive for asthma diagnosis (sensitivity 69% and 59% respectively). However, when using 'X5ex%change,' seven out of twenty-three patients without asthma had a positive test. When using 'Fres%change,' four out of twenty-three patients without asthma had a positive test. Therefore, the test wasn't specific for asthma (specificity 70% and 83% respectively) at this threshold. Of note, the diagnostic tests currently recommended in the NICE (NG80) algorithm performed with poorer sensitivity (Range 10 – 56%) but higher specificity (87-100%), making them more accurate rule-in tests at the thresholds recommended. Therefore we tested thresholds for AO which were comparably specific, to investigate the role of AO BDR measurements as a 'rule-in' asthma test (i.e., cut-points based upon specificity=100% where possible). Used in this way AO BDR sensitivity was hugely compromised (3-21%).

Which AO BDR measurement is best?

Of the AO BDR predictors of asthma (X5ex%change and Fres%change), both a measure of reactance, we show good correlation between the two measurements using Spearman rank correlation coefficients (-0.630, p <0.001). Therefore, only one of these measurements would be needed. X5ex%change, a measure of reactance in expiration phase, was more sensitive using the Youden's index threshold for a positive test (sensitivity 69% versus 59%). In addition, the advantage of using X5ex%change is that a raw value is always available. If using Fres, a raw value isn't always generated by the AO software, some patients have a measurement beyond the limit of detection in this test (i.e., reactance line does not intercept 'y' axis) (see figure 9), in these patients a 'greater than' arbitrary value is assigned. Therefore, Fres%change may be less useful in clinical practice in a small number of cases because it will misrepresent percentage change.

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4.5.4. Could 'Airways Oscillometry' replace current tests in asthma diagnostic guidelines?

We show that in our cohort, using Spearman rank correlation, FEV_1/FVC ratio showed weak correlation with AO BDR using X5ex%change (-0.36 (-0.530—0.068), p 0.016) and Fres%change (0.359 (0.089, 0.588), 0.006). Similarly, a weak correlation was seen with standard BDR and Fres%change (-0.335 (-0.585- -0.073), p 0.01). Our results would suggest AO BDR could be used alongside pre-existing tests opposed to as an alternative.

Jorden et al (2021) reports a small case series and review of the literature concluding AO could be used as an alternative to conventional lung function testing in order to support asthma diagnosis when standard LFT are not available.(198) Park et al (2019)(192) demonstrate obstructed spirometry and bronchodilator reversibility is correlated with AO measurements (e.g., R5, R20, R5-20, X5, and AX), however the authors conclude AO should be a complement to spirometry opposed to alternative. Patients in RADicA were less obstructed than in this study, this may explain why the authors show better correlation between AO and standard asthma tests. A large retrospective study of 592 children also sought to answer this question in BDR, the authors report low concordance between standard BDR and AO BDR using R5%change and X5%change, suggesting AO BDR is better used in addition to spirometry based BDR.(199)

4.5.5. Limitations

We acknowledge the small numbers of subjects in our study; validation studies are needed to confirm our results. Whilst several studies report the usefulness of AO BDR in asthma, more studies are needed specifically in diagnosis of asthma in adults presenting with 'asthma-like' symptoms. Children, smokers (>10 pack years), and patients with significant pre-existing co-morbidities, were excluded from our study. Results should not be extrapolated to these populations.

4.5.6. Conclusions

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We show airways oscillometry is a reliable and repeatable test. Whilst acknowledging the limitations imposed by our sample size, AO BDR (X5ex%change, or Fres%change) was the only measurement able to differentiate between symptomatic 'asthma' and 'not asthma,' and therefore, potentially clinically useful in asthma diagnosis. Whilst AO BDR measurements did not improve the current performance of the NICE diagnostic algorithm for asthma, it did improve overall sensitivity of detecting patients with asthma (using Youden's index). However, this was less useful as a 'rule in' asthma test (specificity range 70-78%). AO BDR correlates weakly with standard asthma tests in our cohort, we speculate AO could potentially be used in addition to current tests, opposed to an alternative to current tests. Our results need validation in a similar population of steroid naïve adults presenting with asthma-type symptoms.

5. Asthma diagnosis during a pandemic

5.1. Introduction

The recent SARS-CoV-2 pandemic brought about the question 'Can we diagnose asthma when aerosol generating procedures are not available?' Current asthma diagnostic guidelines require procedures with aerosol generating potential to guide decision-making. Restricted access to Aerosol Generating Procedures (AGP) poses significant challenges in primary care and resource-poor countries, this has been further amplified during the COVID-19 pandemic. We investigate the best diagnostic test or best diagnostic combination of tests (algorithm) to predict asthma when AGPs are not available. We compare our proposed diagnostic algorithm (without AGPs) to current standard UK (NICE) and International (GINA) asthma guidelines.

This analysis, entitled "Diagnosing asthma with and without aerosol-generating procedures" (published 2021)(2) was completed as part of a reactive piece of research during the SARS-CoV-2 pandemic.

5.2. Aim

We sought to determine the best investigations and diagnostic algorithm to predict expert panel objective evidence review (EPOER) asthma in adults (≥17 years) when Aerosol Generating Procedures (AGPs) are not available. We review how the proposed non AGP algorithm compares to current standard UK (NICE) and international (GINA) guidelines.

5.3. Methods

Adults (16-70 years) with clinician-suspected asthma (symptoms of cough, wheeze, chest tightness and/or breathlessness) referred from primary care to the Rapid Access Diagnostics in Asthma (RADicA) research clinic were included (see methods, chapter 2). Only patients with the EPOER diagnosis 'asthma' or 'not asthma' were included in this analysis (see EPOER

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diagnostic criteria, section 2.6.1), those without definitive diagnosis (insufficient evidence or possible asthma) are not included but are described in detail (section 3.4.1.3). Participants underwent a structured clinical history and physical examination before asthma diagnostic tests were performed over two visits (see section 2.2.2 for details). Participants were then commenced on ICS (Flixotide Diskus, 250mcg twice daily) for six to eight weeks after which time the diagnostic tests were repeated. Participants were classed as Asthma or not asthma during an expert panel objective evidence review (EPOER; see section 2.6.1), and by following NICE and GINA diagnostic algorithms (see section 5.3.2 and 5.3.3 below).

For this analysis the following tests are recorded and grouped as, i) 'In clinic Aerosol Generating Procedures' (AGPs), or ii) 'non aerosol generating procedures (Non AGPs) and home Peak expiratory flow' (table 73).

Table 74. Classification of tests (AGP versus non-AGP and home testing)

Category	Test	Variables recorded
In clinic AGPs		
	Spirometry	FEV_1, FVC, FEV_1/FVC, FEV_1%pred, FVC%pred
	BDR	FEV_1%change
	BCTmeth	PD_{20} (positive/negative)
Non AGPs and home PEF		
	FeNO	FeNO (ppb),
	PEFv	Mean daily amplitude percentage mean: [(PEFhighest – PEFlowest) % PEFmean]x100
	PEFv(alt)	Days >20%
	SPT	Positive/negative
	Auscultated wheeze	Positive/negative

5.3.1. Statistical analysis

5.3.1.1. Determine the best diagnostic test or group of tests (algorithm) to predict asthma when Aerosol Generating Procedure (AGPs) tests are not available (Exploratory work in adults)

The following analysis was performed:

- Descriptive analysis
  - A table displaying patient demographics is presented.
  - Consort diagram of 'population selection,' a diagram of selected patients' attendance to clinic visits, and a diagram of clinical outcomes is also presented.
  - A table summarising characteristic of patients whom have EPOER confirmed asthma without any positive spirometry will be displayed.

- Univariate analysis was performed on each of the above tests to look at which tests could best discriminate between EPOER asthma and not asthma. For normally distributed continuous data, comparison for differences between the two groups; 'asthma,' and 'not asthma,' was analysed using an independent samples T Test. Where data was non-normally distributed, comparison for differences between the groups was analysed using the Mann-Whitney U Test. Tests with dichotomised data were analysed using Chi-squared analysis; or Fishers Exact tests for groups with less than five patients. A 'p value' of <0.05 was recorded as significant.
- Area under curve was reported for all predictors of asthma from univariate analysis. Receiver operating characteristic curves (ROC curves) was presented for both non AGP and AGP tests.
- Box plots displaying commonly used cut offs were computed for each test with continuous data that was shown to be significant in the univariate analysis.

- Exploratory analysis is performed on PEFv in order to consider alternative thresholds for positive that have been suggested in the literature.(45, 46, 98)

    i)   PEFv (amplitude%highest, cut-off >10%, >15%, and >20%)

- Exploratory analysis is performed on Skin prick testing to assess diagnostic performance of this test in asthma diagnosis.

- Candidate multivariate algorithms are considered in order to identify a diagnostic model that could "rule in" asthma using only non-AGP tests. The sensitivity, specificity, positive and negative predictive values (PPV and NPV) based on the cut off values of each model for confidently diagnosing the disease were calculated. Analyses was be performed using SPSS 25 (IBM, New York, USA) and R version 3.6.

5.3.1.2.   How does the proposed algorithm for diagnosing asthma without AGPs compare to current standard UK (NICE) and International (GINA) asthma guidelines?

- The current performance of the NG80 as a "rule in" test for asthma was reported through sensitivity, specificity, AUC, positive and negative predictive values, this was compared to our non-AGPs algorithm.

- Similarly we determined the performance of the GINA asthma diagnostic pathway.

- Discriminative ability of both algorithms was described using the area under the receiver operating characteristic curve (AUROCC). The sensitivity, specificity, positive and negative predictive values (PPV and NPV) based on the cut off values of each model for confidently diagnosing the disease were calculated.

5.3.2. Assigning NICE (NG80) asthma diagnosis

The full method for assigning NICE NG80 diagnosis is described in section 3.3.1.2. Following the NICE diagnostic algorithm for objective tests in adults(38) (figure 56), all patients with the outcome 'Diagnose with Asthma' when following the sequential algorithm using the recommended cut-offs [e.g., spirometry (FEV$_1$ <70% or LLN), BDR (FEV$_1$ ≥12% and 200ml), FeNO (≥40ppb), PEFv (>20%), bronchial challenge test (PD$_{20}$ ≤0.2mg, equivalent of PC$_{20}$ ≤8mg/ml)] were recorded as NICE-defined asthma.

Figure 56. Diagnostic guideline (Algorithm C) objective tests for asthma aged 17 and over and the defined positive test threshold. Redrawn from NICE 2017(24)
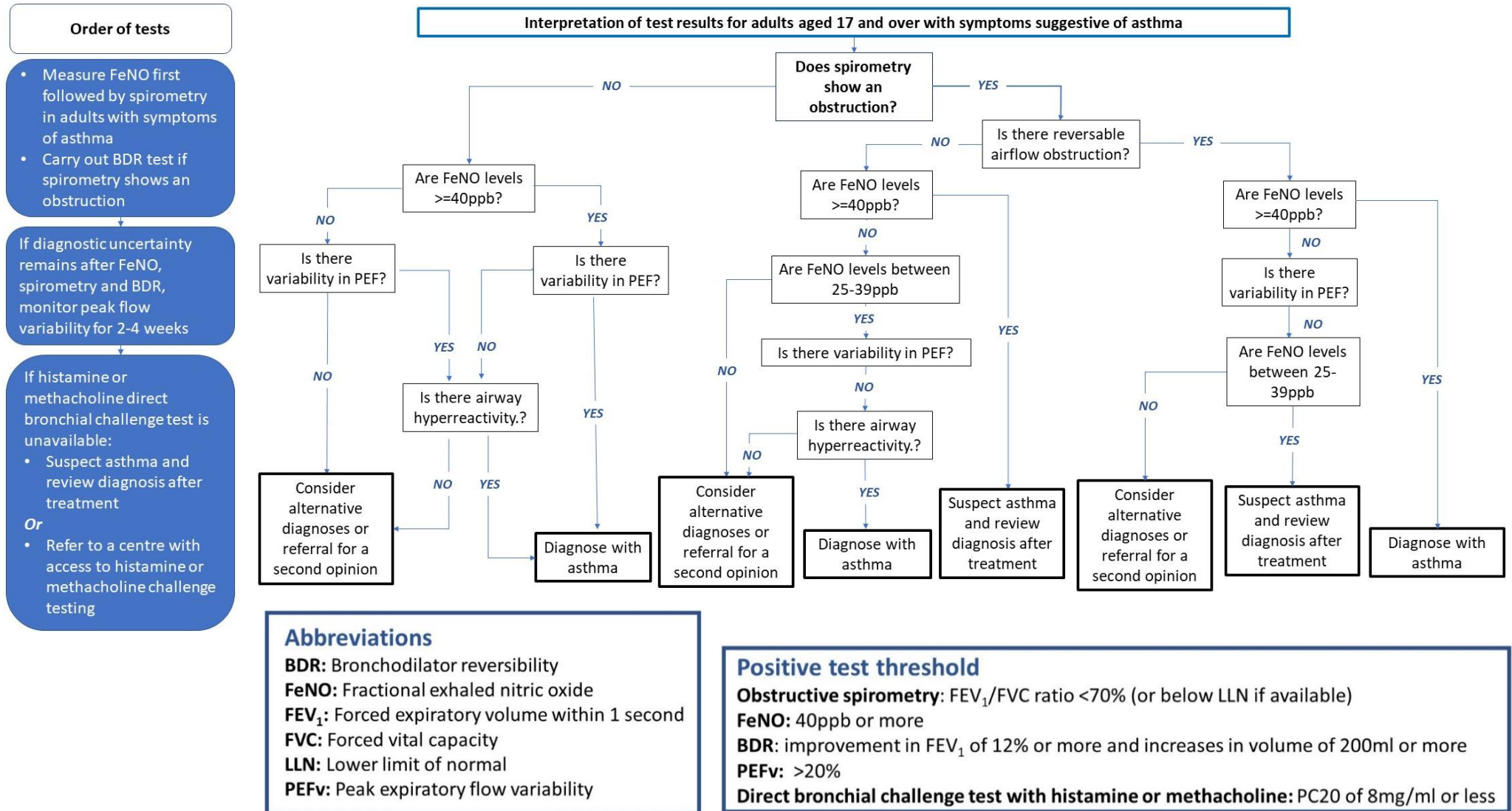
5.3.3. Assigning GINA asthma diagnosis

Following the GINA diagnostic pathway(71) (figure 57) patients with obstructed spirometry [i.e. reduced FEV_1/FVC ratio (<LLN, at any visit) AND evidence of variability in airflow obstruction [i.e., at least one of the following tests being positive: 1) BDR with FEV_1 reversibility >12% and 200ml following 400mcg inhaled salbutamol], 2) at least PEFv >10%, *3)* increase in lung function after >4 weeks anti-inflammatory treatment, defined as FEV_1>12% and 200ml, 4) positive bronchial challenge test (FEV_1 ≥20% fall following methacholine with PD_{20} ≤0.2mg and/or positive mannitol challenge with a ≥15% fall in FEV_1), *or 5)* excessive lung function variation (FEV_1≥12% and 200ml) between pre-treatment visits] were recorded as GINA-defined asthma.

Figure 57. GINA diagnostic algorithm. (Redrawn from GINA 2020(71))
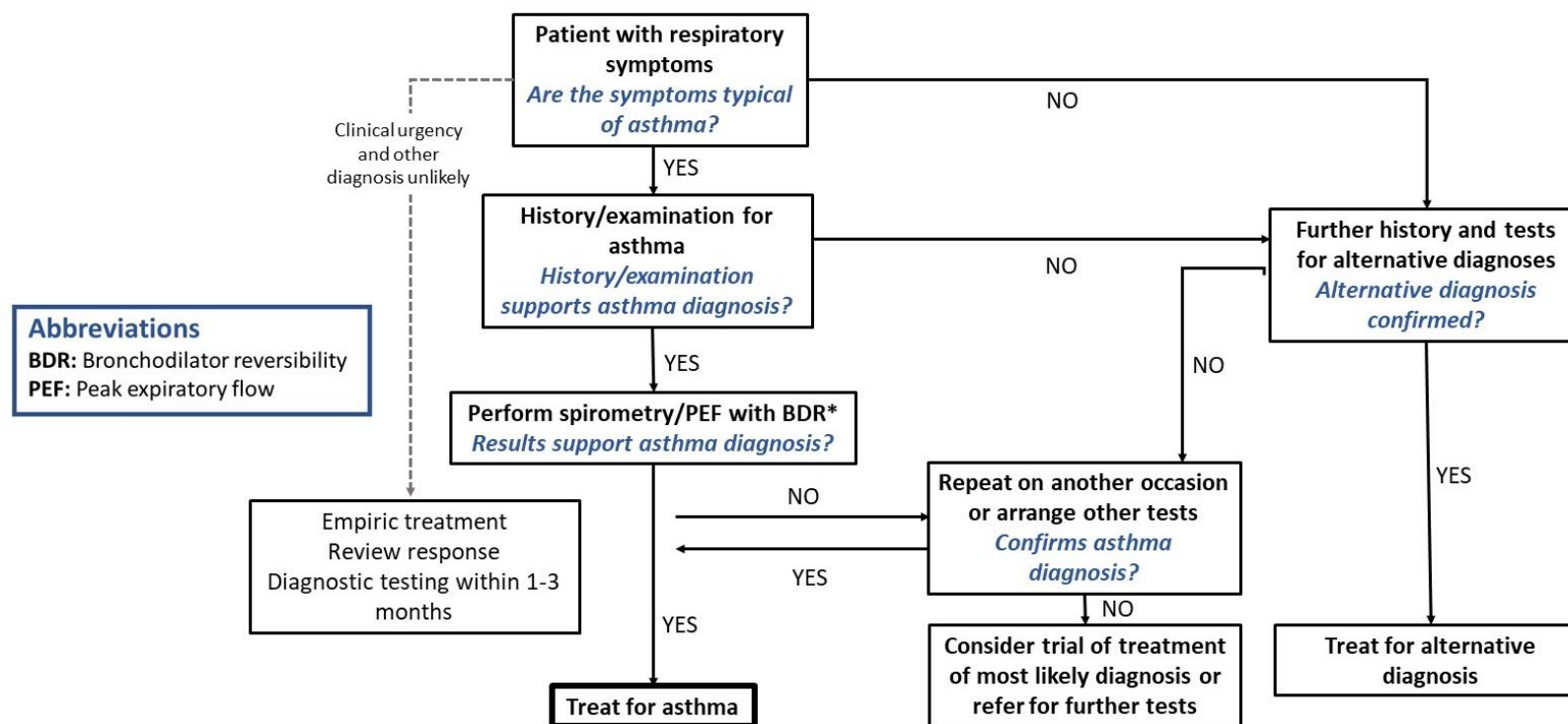
*Refer to Box1-2 on GINA Full Report 2020 (69)
Interpretation in the current thesis:
Asthma diagnosis require both obstructive spirometry with FEV1/FVC ratio < lower limit of normal AND evidence of variability with one of the following tests being positive:
- BDR (>12% and 200ml change in FEV1 post bronchodilator)
- PEF variability >10%
- Bronchial challenge test (including methacholine challenge positive)
- Significant increase in lung function after 4 weeks of anti-inflammatory treatment
- Excessive variation in lung function between visits (FVE1 of >12% and >200ml between visits)

5.4. Results

Determine the best diagnostic test or group of tests (algorithm) to predict asthma when Aerosol Generating Potentials (AGPs) tests are not available (Exploratory work in adults)

5.4.1. Population selection and clinical characteristics

Of 117 patients recruited into the study, 65 were adults [defined as ≥16 years and <70 years, mean (SD) age: 34.8 (12.2) years, 36.9% male]. Five patients (7.7%) with EPOER diagnosis of 'possible asthma' or 'insufficient evidence' were excluded from the analysis, leaving 60 adults with a definitive diagnostic outcome ("asthma" confirmed or refuted). Of these 36 (60%) had asthma and 24 (40%) did not have asthma. The number of patients attending each clinical visit are summarised (figure 58 and 59).

Figure 58. Selected population

Referred: N = 196

Withdrawn:
-Screen Fail: N = 26
-Subject lost to follow up: N = 26

Enrolled (passed screening): N = 144

Withdrawn:
-Declined study (subject decision): N = 16
-Recruitment Paused (COVID pandemic): N = 11

CV1 Consent: N = 117

Withdrawn:
-Withdrawn as per Protocol (alternate diagnosis more likely at initial consultation): N = 1
-Not Eligible (>10 pack year discovered at initial consultation): N = 1

Started study N = 115

Adults (≥16 years and < 70 years)
N = 65

Children (<16 years)
N = 50

Unable to confirm diagnosis
N = 5

Confirmed Diagnosis
N = 60
(Included in analysis

Excluded

Excluded

Asthma
N = 36

Not asthma
N = 24

Figure 59. a) Number of patients attending each clinic visit   b) Characteristics of EPOER diagnosed asthma
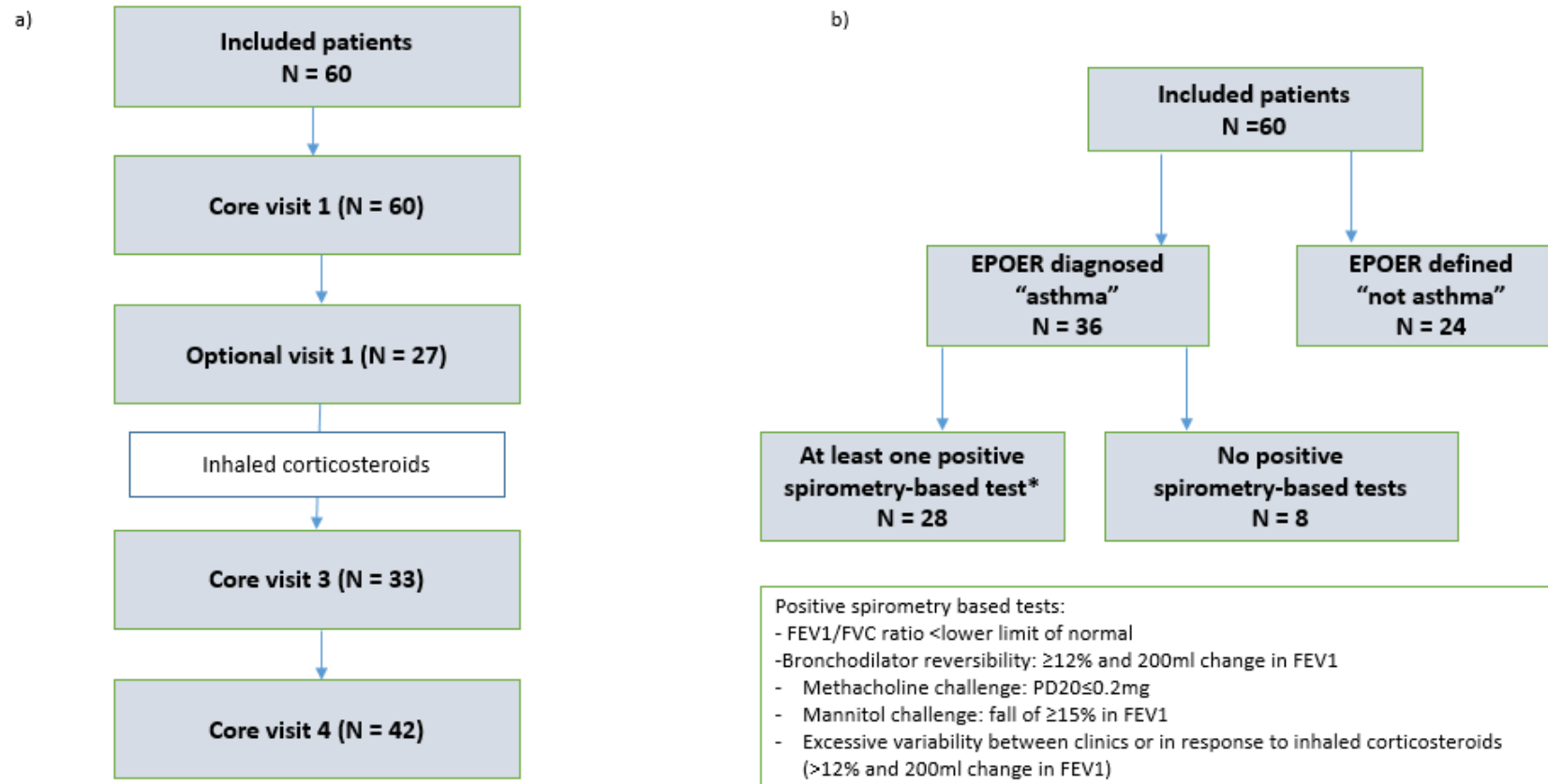
Table 74 shows the demographic details of the population as a whole and those with and without asthma.

Table 75. Patient demographics

	All cases N = 60	Asthma N = 36	Not asthma N =24	P value
Clinical and demographic features:				
Age, mean (SD) years	34.7 (12.3)	33.1 (12.4)	37.2 (12.0)	0.206
Age range (years)	16-61	16-61	16-57	n/a
Gender, n (%) females	38 (63.3%)	20 (55.6%)	18 (75.0%)	0.126
Current or ex-smokers, n (%)	18 (30.0%)	10 (27.8%)	8 (33.3%)	0.645
Pack years, median (IQR)	2.5(1-4.3)	2.5 (1-3.3)	2.7 (1-5.8)	0.628
BMI, mean (SD) kg/m^2	28.5 (5.5)	28.4 (5.1)	28.7 (6.2)	0.822
Duration symptoms, median (IQR) years	3.00 (1.00-7.00)	2.00 (0.60-9.75)	3.50 (2.00-7.00)	0.560

*Normally distributed (mean, SD) reported, Not normally distributed (median, IQR) reported. Categorical data (number, %) * P values refer to the difference between the 'asthma' and 'not asthma' groups. T-test for normally-distributed data; Mann Whitney U for non-normally distributed data, chi squared (or Fisher's exact test [1]) for categorical data. N = 'x' refers to the number of patients with data (only used when data set incomplete) Abbreviation, BMI, body mass index*

There was no significant difference in the demographic characteristics of those with and without asthma.

Table 75 shows the results of the AGP and non-AGP tests measured at the first baseline visit, in the whole population and in asthma and non-asthma individuals.

Table 76. Patient clinical characteristics

	All cases N = 60	Asthma N = 36	Not asthma N =24	P value
Clinical and physiological data:				
Aerosol generating procedures (AGPs)				
FEV_1, mean (SD) L	3.32 (0.96)	3.33 (1.00)	3.30 (0.94)	0.906
FEV_1, mean (SD) %predicted	96.2 (17.1)	91.7 (17.7)	103.0 (14.1)	0.011
FVC, mean (SD) L	4.30 (1.24)	4.49 (1.28)	4.01 (1.15)	0.143
FVC, mean (SD) % predicted	103.3 (14.4)	103.1 (14.8)	103.5 (13.9)	0.928
FEV_1/FVC Ratio, mean (SD) %	77.5 (8.4)	74.1 (9.0)	82.5 (3.6)	<0.001
FEV_1/FVC Ratio, n (%) <LLN	13 (21.7)	13 (36.1)	0 (0.0)	ⱡ0.001
FEV1/FVC Ratio, n (%) <70 or LLN (Criteria: NICE guideline, England)	14 (23.3)	14 (38.9)	0 (0.0)	ⱡ0.002
BDR, median (IQR) %	7.0 (3.3 – 12.0)	9.0 (5.3-14.8)	4.0 (1.3 – 7.0)	<0.001
BDR, n (%) FEV_1 ≥12% and 200mls	16 (26.7)	16 (44.4)	0 (0.0)	<0.001
BCTmeth, n (%) PD_{20} ≤0.2mg (equivalent of PC_{20} ≤8mg/ml)	15 (31.9%) N = 47	15 (57.7%) N = 26	0 (0.0%) N = 21	<0.001
Positive mannitol challenge (>15% fall in FEV_1), n(%)	10 (37.0%) N=27	8 (57.1%) N=14	2 (15.4%) N=13	ⱡ0.031
Non Aerosol generating procedures (Non AGPs)				
FeNO, median (IQR) ppb	24 (13 – 70)	61 (21 – 95)	16 (11 – 23)	0.001
FeNO, n (%) ≥ 40ppb	23 (38.3)	20 (55.6)	3 (12.5)	0.001
PEFv, median (IQR) %	9.9 (5.1 – 12.2)	10.9 (6.7-13.0)	6.1 (3.4-11.6)	0.027

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	N = 47	N = 30	N = 17	
PEFv, n (%) mean >10% (Criteria: GINA guideline)	23 (48.9)	17 (56.7)	6 (35.3)	0.159
	N = 47	N = 30	N = 17	
PEFv, n (%) mean >20% (Criteria: NICE guideline, England)	3 (6.4)	3 (10.0)	0 (0.0)	0.292
	N = 47	N = 30	N = 17	
PEFv alternative, n (%) at least 3 days of >20% diurnal variability	10 (21.3)	10 (33.3)	0 (0.0)	0.008
	N = 47	N = 30	N = 17	
Eos, median (IQR) x10^{9}cells/L	0.18 (0.10 -0.33)	0.29 (0.15- 0.51)	0.10 (0.06-0.16)	<0.001
	N = 58	N = 35	N = 23	
Eos, n (%) >0.4x10^9 cells/L	11 (19.0)	11 (31.4)	0 (0.0)	0.002
Eos, n (%) >0.3x10^9 cells/L	18 (31.0%)	17 (48.6%)	1 (4.3%)	<0.001
Sensitised, n (%) ≥1 SPT allergen positive	40 (66.7%)	27 (75.0%)	13 (54.2%)	0.094
Auscultated wheeze, n (%)	7 (11.7%)	7 (19.4%)	0 (0.0%)	0.035

Normally distributed (mean, SD) reported, Not normally distributed (median, IQR) reported. Categorical data (number, %) * P values refer to the difference between the 'asthma' and 'not asthma' groups. T-test for normally-distributed data; Mann Whitney U for non-normally distributed data, chi squared (or Fisher's exact test [1]) for categorical data. N = 'x' refers to the number of patients with data (only used when data set incomplete)

Abbreviations: BMI, body mass index; FEV$_1$, forced expiratory volume in one second; FVC, forced vital capacity; BDR, bronchodilator reversibility; BCTmeth, methacholine bronchial challenge test; FeNO, fractional exhaled nitric oxide; PEFv, peak expiratory flow variability; Eos, eosinophil levels; SPT, skin prick test.

For spirometry-based tests, patients with asthma had significantly lower FEV$_1$ % predicted and FEV$_1$/FVC ratio, with increased reversibility to bronchodilators and increased bronchial hyper responsiveness on bronchial challenge test. For non-spirometry-based tests, patients with asthma had a higher FeNO, and higher eosinophils and were more likely to have audible wheeze.

The majority (n=28) of EPOER-defined "asthma" had at least one positive spirometry-based test (FEV$_1$/FVC ratio<LLN; ≥12% reversibility to bronchodilators, positive bronchial challenge test). Eight patients were diagnosed with EPOER confirmed asthma despite no positive spirometry-based tests. These patients are described in more detail in the table below (see table 76). All patients had either i) at least one pre-treatment NICE recommended positive test (4/8) ii) at least two borderline positive tests (3/8) iii) at least one borderline test plus one objective evidence of improvement post treatment (4/8) iv) objective improvement post trial of treatment (5/8).

Table 77. Characteristics of patients who had EPOER-diagnosed asthma, but without positive spirometry-based tests

Patient number	Pre (post)-ICS FEV1/FVC ratio [% predicted LLN]	BDR	Pre- ICS Methacholine challenge PD$_{20}$ (or % FEV1 fall on max dose)	Post- ICS Methacholine challenge PD$_{20}$ (or % FEV1 fall on max dose)	Mannitol challenge % FEV1 fall on max dose	Pre (post)- ICS Blood eosinophils (x10^9 cells/L)	Pre (post) -ICS FeNO (ppb)	Sensitisation on skin prick test	PEFv*	Notes
1 (RAD027)	78% (80%) [72%]	5%	19% fall at maximum dose	12% fall at maximum dose	-13%	0.07 (0.04)	50 (19)	Y	9%	
2 (RAD074)	84% (87%) [74%]	4%	0.516mg	0% fall at max dose	-9%	0.08 (0.10)	9 (12)	N	6%	ACQ improved by 0.8 points post ICS.
3 (RAD075)	79% (83%) [74%]	7%	0.229mg	15% fall at maximum dose	Not done	0.11 (not done)	15 (11)	Y	11%	
4 (RAD038)	79% (84%) [75%]	3%	0.272mg	Not done	Not done	0.3 (not done)	94 (36)	Y	13%	ACQ improved by 1.4 points post ICS.
5 (RAD103)	77% (not done) [76%]	3%	19% fall at maximum dose	Not done	Not done	0.5 (not done)	58 (not done)	Y	5%	ACQ improved by 1.4 points post ICS.
6 (RAD050)	83% (83%) [76%]	3%	10% fall at maximum dose	6% fall at maximum dose	Not done	0.83 (0.31)	22 (15)	Y	11%	ACQ improved by 1.6

										points post ICS.
7 (RAD037)	72% (not done) [71%]	11%	Not done	Not done	Not done	0.77 (not done)	65 (not done)	Y		borderline FEV1/FVC, BDR, markedly raised blood eosinophils and FeNO
8 (RAD064)	71% (77%) [71%]	9%	3% fall at maximum dose	2% fall at maximum dose	Not done	0.32 (not done)	28 (21)	Y	5%	clear symptomatic benefit from salbutamol, borderline FEV1/FVC, BDR and blood eosinophils;

Abbreviations: ICS- inhaled corticosteroids; BDR- bronchodilator reversibility, FeNO- fractional exhaled nitric oxide; FEV_1- forced expiratory volume within 1 second; FVC- forced vital capacity. LLN- lower limit of normal, ACQ: Asthma control questionnaire. *Peak flow variability was calculated as **Eq 1:** daily amplitude percentage mean = [($PEF_{highest} - PEF_{lowest}$) / PEF_{mean}] x 100, **Eq 2:** PEFv = Σ daily amplitude percentage mean/number of days

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5.4.2. What is the diagnostic performance of individual tests (aerosol generating versus non aerosol generating)?

The sensitivity and specificity of each test individually to differentiate symptomatic patients with asthma from those without asthma (defined by EPOER) is presented in table 77.

Table 78. Sensitivity, specificity, positive and negative predictive values of individual tests of asthma diagnosis

		N	Sensitivity Number % (95% CI)	Specificity Number % (95% CI)	Negative predictive value	Positive predictive value
Individual components:						
Non-aerosol generating procedures and home PEF	Auscultated wheeze	60	7/36 19% (8-36)	24/24 100% (86-100)	45%	100%
	Eos (>0.40 x 10^9 cells/L)	58	11/35 31% (17-49)	23/23 100% (85-100)	49%	100%
	PEFv (> 20%)	47	3/30 10% (2-27)	17/17 100% (80-100)	43%	100%
	PEFv(alt) (at least 3 days of > 20% diurnal variability)	47	10/30 33% (17-53)	17/17 100% (80-100)	50%	100%
	FeNO (≥ 40ppb)	60	20/36 56% (38-72)	21/24 88% (68-97)	57%	87%
In-clinic aerosol generating procedures	FEV_1/FVC (<70% or LLN)	60	14/36 39% (23-57)	24/24 100% (86-100)	52%	100%
	BDR (≥ 12% and 200ml)	60	16/36 44% (28-62)	24/24 100% (86-100)	55%	100%
	BCTmeth (y/n)	47	15/26 58% (37-77)	21/21 100% (84-100)	61%	100%

Abbreviation: Eos, Eosinophil levels, PEFv, Peak expiratory flow variability, PEFv(alt), Peak expiratory flow variability alternative, FeNO, Fractional exhaled nitric oxide, FEV_1, Forced Expiratory Volume in one second, FVC, Forced vital capacity, BDR, Bronchodilator reversibility, BCTmeth, Methacholine Bronchial challenge test, Wh, wheeze.

Using standard thresholds for a positive test (defined by NICE)(38) all in-clinic AGPs performed with specificity 100%, and sensitivity ranging 39% to 58%. All of the proposed Non AGPs (including home PEF) had specificity 100%; with the exception of FeNO (specificity 88%, sensitivity 56%), and sensitivity ranging 10% to 33%. The presence of wheeze alone was highly specific for asthma (100%) but identified fewer than 20% of asthma patients. Blood eosinophilia (>$0.4x10^9$cells/L) had high specificity (100%) and identified approximately a third of asthma patients. FeNO of ≥40ppb(38) had higher sensitivity and identified more than half of asthma patients, but was less specific (88%). Peak expiratory Flow variation (PEFv >20%); grouped with non AGPs because this could be performed in a non-clinical setting (i.e., at home), only identifies one in ten patients with asthma. Using the alternative method (PEFv(alt), three days >20% diurnal variability), improved the performance of this test to confidently identify one in three patients with asthma.

When comparing AGP spirometry-based tests, FEV_1/FVC ratio of <70% or <LLN and BDR of ≥12% and 200ml in FEV1 were both highly specific for asthma, with moderate sensitivities (39-44%). However, confirmation of BHR to methacholine provided the best diagnostic power as a single test with a sensitivity of 58%.

5.4.2.1. Non AGP predictors of asthma

Roc Curves: Non-AGP Predictors of asthma (univariate analysis)

Receiver operating characteristic (ROC) curves were computed for all non-AGP tests. Area under the curve (AUC) was reported in to help answer the question 'which tests are best at correctly predicting 'asthma' from 'non asthma' in all symptomatic patients?' An AUC ≥0.7 was considered as acceptable(200) (Table 78, Figure 60).

Table 79.   Area under receiver operating characteristic curves for non AGP tests

Non-aerosol generating procedures and home PEF	Asthma N = 36	Not asthma N =24	P value	AUC(95%CI)
FeNO, median (IQR) ppb	61 (21 – 95)	16 (11 – 23)	**0.001**	0.758 (0.631-0.885)
PEFv, median (IQR) %	10.9 (6.7-13.0) N = 30	6.1 (3.4-11.6) N = 17	**0.027**	0.696 (0.533-0.859)
PEFv alternative, n (%) at least 3 days of >20% diurnal variability	10 (33.3) N = 30	0 (0.0) N = 17	**0.008** *ⱡ*	0.651 (0.496-0.806)
Eos, median (IQR) x10⁹cells/L	0.29 (0.15- 0.51) N = 35	0.10 (0.06-0.16) N = 23	**<0.001**	0.829 (0.726-0.931
Sensitised, n (%) ≥1 SPT allergen positive	27 (75.0%)	13 (54.2%)	0.094	0.604 (0.455-0.753)
Sensitised, median (IQR) number	2.00 (0.25-3.00)	1.00 (0.00-2.00)	0.066	0.636 (0.491-0.781)
Auscultated wheeze, n (%)	7 (19.4%)	0 (0.0%)	**0.035** *ⱡ*	n/a

P values refer to the difference between the 'asthma' and 'not asthma' groups, Mann Whitney U for non-normally distributed data, chi squared (or Fisher's exact test [ⱡ]) for categorical data.

Abbreviations: FeNO, fractional exhaled nitric oxide; PEFv, peak expiratory flow variability; Eos, eosinophil levels; SPT, skin prick test.

All non-AGP tests; with the exception of 'sensitised on skin prick testing,' were significant predictors of asthma. ROC curves are shown for all non-AGP tests. FeNO and blood eosinophils performed best.

Figure 60. ROC curve for non-AGP predictors of asthma
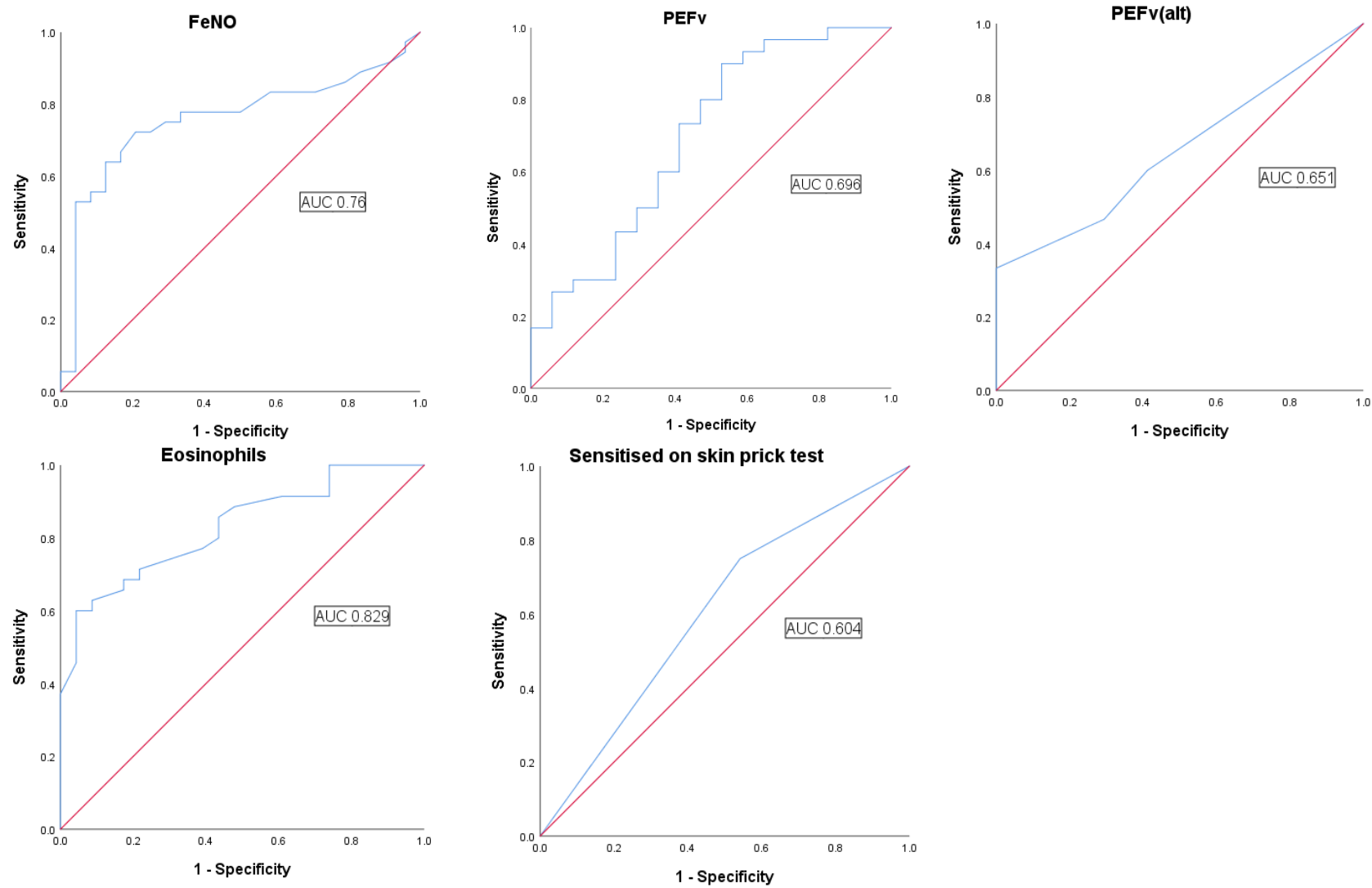
Roc curves: comparison with AGP predictors of asthma (univariate analysis)

Receiver operating characteristic (ROC) curves were then computed for conventional AGP tests in order to compare them with non-AGP tests (table 79, figure 61).

Table 80. Area under receiver operating characteristic curves for AGP tests

	Asthma N = 36	Not asthma N =24	P value	AUC(95%CI)
In-clinic aerosol generating procedures				
FEV_1/FVC Ratio, mean (SD) %	74.1 (9.0)	82.5 (3.6)	**<0.001**	0.803 (0.692-0.914)
BDR, median (IQR) %	9.0 (5.3-14.8)	4.0 (1.3 – 7.0)	**<0.001**	0.828 (0.726-0.929)
BCTmeth, n (%) PD_{20} ≤0.2mg (equivalent of PC_{20} ≤8mg/ml)	15 (57.7%) N = 26	0 (0.0%) N = 21	**<0.001**	0.904 (0.810-0.998)
P values refer to the difference between the 'asthma' and 'not asthma' groups, t-test for normally-distributed data; Mann Whitney U for non-normally distributed data, chi squared (or Fisher's exact test [Ĩ]) for categorical data.				
Abbreviations: FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; BDR, bronchodilator reversibility; BCTmeth, methacholine bronchial challenge test;				

Figure 61. ROC curve for non-AGP predictors of asthma

BCTmeth

AUC 0.904
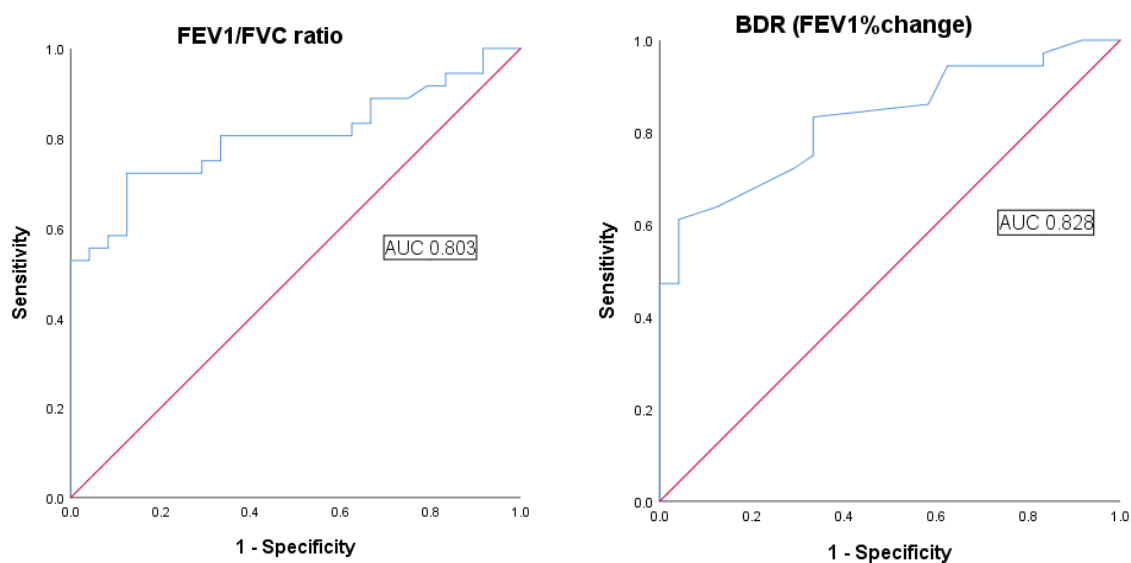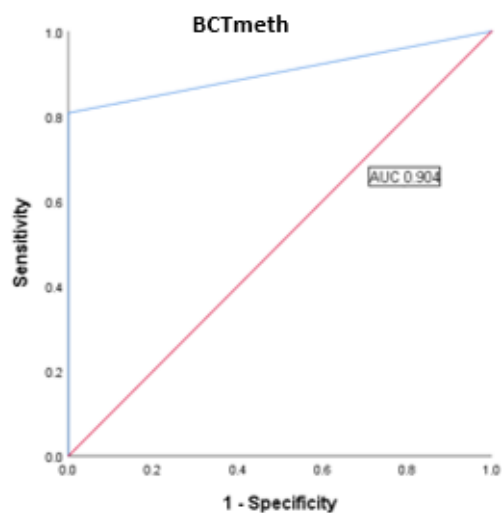
A FEV$_1$/FVC ratio (%), BDR (%) and BCTmeth (mg) provided better discriminative ability (AUROCC=0.80, 0.83 and 0.904 respectively) than the non-AGPs and home peak flow monitoring. For the diagnosis of asthma, blood eosinophils had AUROCC of 0.83 and FeNO 0.76. Of the calculations of peak flow variability, PEFv had AUROCC of 0.70 and PEFv(alt) 0.65 (figure 62).

Figure 62. Box and whisker plots demonstrating the medians, upper and lower quartiles and maximum and minimum of i) potential non-AGP or home predictors of asthma ii) AGP in clinic predictors of asthma, in symptomatic untreated patients (dashed line shows the current suggested diagnostic cut-offs).

ii) Non-AGP and at home predictors of asthma
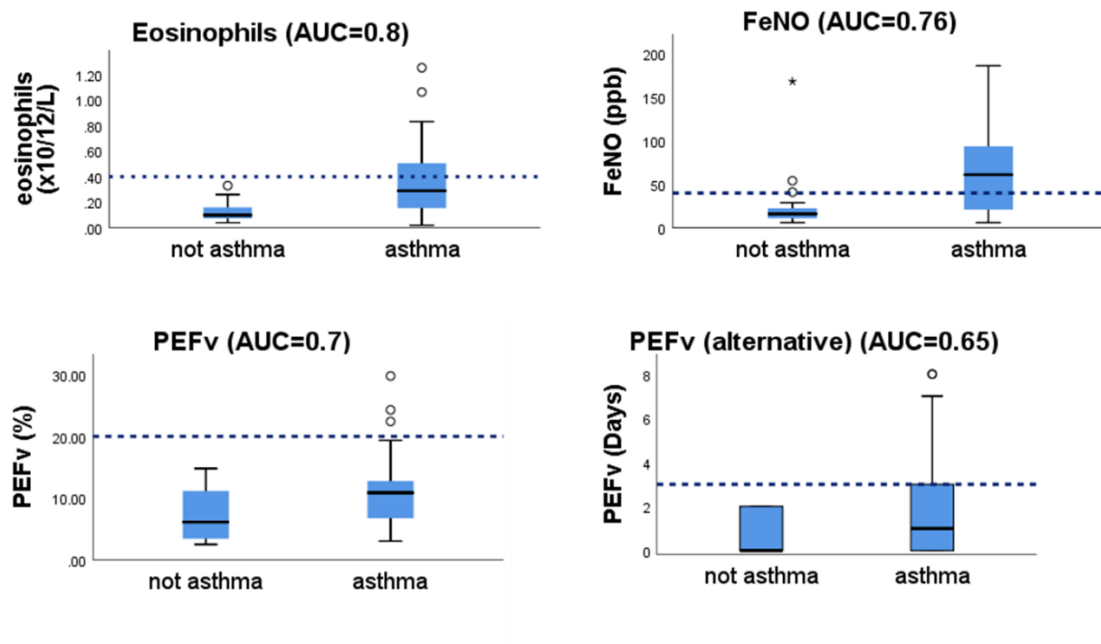
iii) AGP predictors of asthma
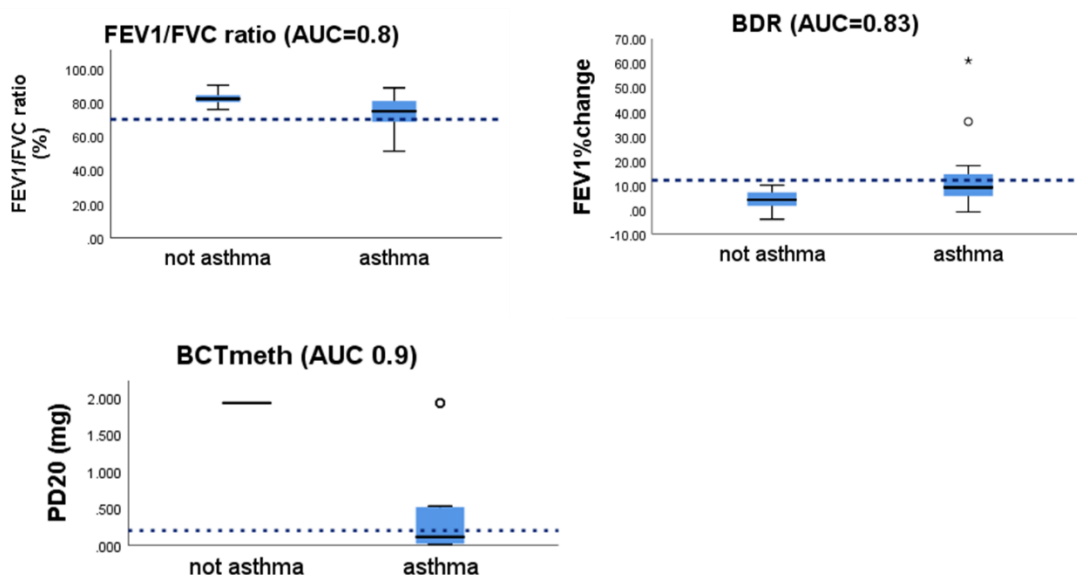
5.4.2.2. Exploring PEFv at different thresholds for positive

We compare PEFv with different cut-off thresholds for a positive result. At present different guidelines have used different thresholds for positive(38, 71) .

Peak expiratory flow variation:

Whilst a PEFv of more than 10%(71) gave a much higher sensitivity than other cut-offs (57%), its utility for "ruling in" asthma was poor with a specificity of only 76% (table 80). On the other hand, applying the cut-off for PEFv of >20%(38) gave high specificity (100%) but low sensitivity (7%) (table 80). Sensitivity only modestly improved to 17% with a specificity of 100% when a cut off of >15% was used. Applying the diagnostic criterion of more than 20% diurnal variation on at least 3 days [PEFv(alt)](98) resulted in equally high specificity (100%) but moderately improved sensitivity (33%) (table 78 above)

Whilst the amplitude % mean is the most recommended method in calculating PEFv, we further explored using amplitude % highest as it is a simpler calculation.(38) Amplitude % highest provided similar discriminative ability to Amplitude % mean (AUC = 0.74 and 0.70 respectively) (table 80, figure 63).

Table 81.   Diagnostic performance of peak flow variability calculated as amplitude percentage highest value.

PEFv calculated as: amplitude percentage mean/(maximum reading)]/number of days*; N=47				
Cut off	Specificity	Sensitivity	PPV	NPV
>10% (n=21)	76.5%	56.7%	81.0%	50.0%
>15% (n=5)	100%	16.7%	100%	40.5%
>20% (n=2)	100%	6.7%	100%	37.8%
PEFv was calculated using the 'daily amplitude percentage highest' equation (equation 1), from this calculation the mean PEFv was calculated (equation 2). *Eq 1: daily amplitude percentage highest = [($PEF_{highest}$ − PEF_{lowest}) / $PEF_{highest}$] x 100; Eq 2: PEFv = Σ daily amplitude percentage highest/number of days*				

Figure 63. Assessing the discriminative ability of PEFv (using amplitude % highest calculation) in diagnosing asthma using ROC curve.
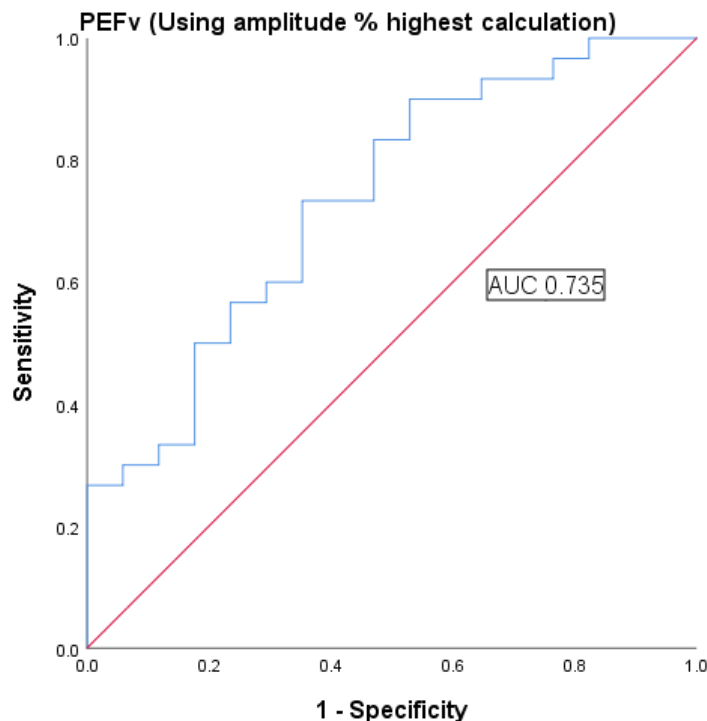
22% (n =13) patients failed to perform home peak flow monitoring at all and only 15% of those who performed peak flow monitoring completed for 14 days. Nevertheless, data from the first 5 days only (available in 94% of those who performed peak flow monitoring) provided a similar diagnostic value to longer period monitoring (median [IQR]: 10 [8-12] days) (table 81).

Table 82.   Diagnostic performance of peak flow variability calculated as amplitude percentage mean using data from the first 5 days only.

PEFv calculated as: (Sum of daily amplitude/mean)/number of days (first 5 days); N=44				
Cut off	Specificity	Sensitivity	PPV	NPV
>10% (n=17)	76.5%	46.4%	75.0%	44.4%
>15% (n=7)	100%	25.0%	100%	43.2%
>20% (n=4)	100%	14.3%	100%	40.0%

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Skin prick testing:

At present skin prick testing is not a recommended first line test for asthma diagnosis. We explored the predictive power of skin prick testing to diagnose asthma (table 82, figure 64).

Table 83.   Diagnostic performance of number of positive skin prick test to common inhaled allergens in asthma

Number of positive SPTs	Specificity	Sensitivity	PPV	NPV
≥1 (n=40)	45.8%	75.0%	67.5%	55.0%
≥2 (n=32)	62.5%	63.9%	71.9%	53.6%
≥3 (n=14)	83.3%	27.8%	71.4%	43.5%
≥4 (n=4)	95.8%	8.3%	75.0%	41.1%
≥5 (n=3)	95.8%	5.6%	66.7%	40.4%

Figure 64. Assessing the discriminative ability of the number of positive skin prick test for common inhaled allergens in diagnosing asthma using ROC curve.

Number of positive skin prick tests for common aeroallergens

AUC 0.636

Diagonal segments are produced by ties.
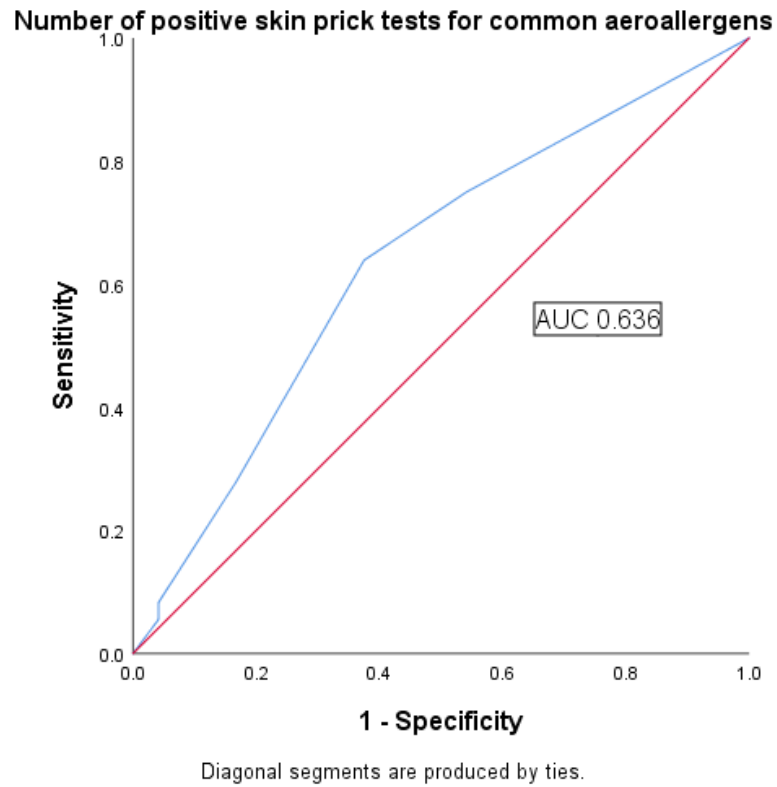
The number of positive skin prick test to common inhaled allergens had AUROCC of 0.64.

This test did not perform as well as the other suggested non-AGP tests.

5.4.3. What is the diagnostic performance of multi-test algorithms using non Aerosol Generating Procedures?

The discriminative ability of several diagnostic algorithms based on different combinations of non-AGP tests (wheeze on auscultation, FeNO and blood eosinophils) and home peak flow monitoring was investigated (table 83). Fifteen of the 60 participants (seven with asthma) did not complete all three tests and therefore could not be included.

Table 84. Proposed non-AGP diagnostic algorithms

		N	Sensitivity Number % (95% CI)	Specificity Number % (95% CI)	Negative predictive value	Positive predictive value
Algorithms:						
Non-aerosol generating procedures and home PEF	At least one positive feature of: • Wheeze (present) • Eos (> 0.40 x 10^9 cells/L) • PEFv (> 20%) • FeNO (≥ 40ppb)	45	20/29 69% (49-85)	14/16 88% (62-98)	65%	89%
	At least two positive features of: • Wheeze (present) • Eos (> 0.40 x 10^9 cells/L) • PEFv (> 20%) • FeNO (≥ 40ppb)	45	9/29 31% (15-51)	16/16 100% (79-100)	49%	100%
	At least one positive feature of: • Wheeze (present) • Eos (> 0.40 x 10^9 cells/L) • PEFv (> 20%)	45	11/29 38% (21-58)	16/16 100% (79-100)	52%	100%
	At least one positive feature of: • Wheeze (present) • Eos (> 0.40 x 10^9 cells/L) • PEFv (alt) (at least 3 days of > 20% variability) • FeNO (≥ 40ppb)	45	23/29 79% (60-92)	14/16 88% (62-98)	74%	90%
	At least two positive features of: • Wheeze (present) • Eos (> 0.40 x 10^9 cells/L)	45	13/29 45% (26-64)	16/16 100% (79-100)	55%	100%

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	• PEFv (alt) (at least 3 days of > 20% variability) • FeNO (≥ 40ppb)					
	At least one positive feature of (recommended): • Wheeze (present) • Eos (> 0.40 x 10^9 cells/L) • PEFv (alt) (at least 3 days of > 20% variability)	45	16/29 55% (36-74)	16/16 100% (79-100)	60%	100%

Abbreviation: Eos, eosinophil levels, PEFv, peak expiratory flow variability, PEFv(alt), peak expiratory flow variability alternative, FeNO, fractional exhaled nitric oxide, FEV₁, forced expiratory volume in one second, FVC, forced vital capacity, BDR, bronchodilator reversibility, BCTmeth, methacholine bronchial challenge test.

A three-variable model using 1) presence of wheeze on auscultation, 2) blood eosinophil count >0.4x10^{9}cells/L and 3) PEFv (alt) of more than 20% for at least 3 days performed best at "ruling in" asthma. The presence of at least one feature provided 100% specificity and identified more than half of patients with asthma (approximately a third of all patients). Four (27%) of those who could not be included due to incomplete data had raised eosinophils and/or wheeze, all of whom had EPOER-confirmed asthma (57% of all EPOER-confirmed asthma who had incomplete data).

5.4.4. How does the proposed algorithm for diagnosing asthma without AGPs compare to current standard UK (NICE) and International (GINA) asthma guidelines?

A summary of the performance of our best performing multivariable algorithm compared with established algorithms from current guidelines (e.g., NICE, and GINA) is presented above in table 84.

Table 85. **Non-AGP Algorithm compared to current recommended asthma diagnostic algorithms**

	N	Sensitivity Number % (95% CI)	Specificity Number % (95% CI)	NPV	PPV
Algorithms					
Non AGP algorithm **One of: Wh-Eos-PEFv(alt)**	45	**14/29** **55% (36-74)**	**16/16** **100% (79-100)**	**60**	**100**
NICE algorithm	46	14/29 48% (29-67)	17/17 100% (80-100)	56	100
GINA algorithm	60	17/36 47% (30-65)	24/24 100% (80-100)	56	100
Abbreviation: Eos, Eosinophil levels, PEFv(alt), Peak expiratory flow variability alternative, Wh, wheeze.					

This recommended three-variable diagnostic model provided comparable discriminative ability to the current NICE and GINA diagnostic algorithm, useful in "ruling-in" the disease.

5.5. Discussion

Asthma diagnosis during a pandemic (adapted from published paper)(2)

5.5.1. Is it possible to diagnose asthma when Aerosol Generating Procedures are not available?

A pragmatic diagnostic algorithm based on non-AGPs and home peak flow monitoring has been developed, which is highly specific for asthma, and can be completed in primary care in adults with symptoms of asthma. In this group, the presence of any of: audible wheeze on physical examination by a doctor, raised blood eosinophils or peak flow variability were highly specific for the diagnosis. Based on these parameters, it was possible to confirm asthma diagnosis in almost a third of symptomatic patients (accounting for half of all asthma cases). Although highly specific, this algorithm has limited sensitivity, meaning that two-third of patients would still require further investigations involving spirometry-based tests to confirm or refute the diagnosis. Nevertheless, use of this algorithm could reduce pressure on respiratory physiology services (a particular problem during the COVID-19 pandemic, and in many resource-limited settings) and allow commencement of treatment promptly in up to half of those with asthma. However, it is important to note that in patients who fail to respond to ICS treatment, further evaluation using spirometry-based tests should be considered.

5.5.2. How does our Non-AGP algorithm compare with current recommended asthma diagnostic algorithms?

The algorithm showed comparable discriminative ability to the current NICE (England) and GINA asthma guidance. This proposed algorithm enables a confident diagnosis without the need for spirometry-based tests in some patients. Such tests were not readily available during the start of the COVID-19 pandemic, potentially resulting in a delay in diagnosis and treatment. Although, it should be noted that research performed by Sheikh et al (2022)

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after the main waves of the pandemic indicates that the use of standard viral filters are sufficient to allow guidelines to remove lung function testing from the list of AGPs.{Sheikh, 2022 #4} However, the limited access to high-quality spirometry-based tests including bronchial provocation challenges as well as FeNO pre-dates the pandemic in many primary care settings, where the majority (up to 85% in the UK) of asthma patients are solely managed.(201) Therefore this diagnostic algorithm has potential application beyond the current era in order to minimise the number of patients requiring full testing according to guidelines.

Whilst the diagnostic tests used in this algorithm may be more widely accessible, one of the key hurdles may be the quality of peak flow monitoring and compliance. Even in the research setting, 22% did not complete peak flow monitoring for two weeks. Adding to the challenge, accurate peak flow calculation is often complex and time consuming. The current study has demonstrated that data from 5-days of readings had comparable discriminative ability to 2-weeks of measurements. Thus, a much shorter duration may be sufficient for home peak flow monitoring, and this has the potential to significantly improve patients' compliance (from 15% to 94% in our study). Amplitude % highest calculation for PEFv showed similar diagnostic power to amplitude % mean and maybe a less time-consuming alternative calculation (by eliminating the need of calculating daily mean). The optimal algorithm used PEFv(alt) calculation where the number of days with >20% variability is recorded. This method has been suggested in the literature and may be more accurate than calculating average diurnal variations.(38, 98) In addition, this approach avoids summing up the 2-week daily variabilities and calculating the mean, which may save a substantial amount of time and reduce potential calculation errors in the clinical setting.

Whilst bronchodilator reversibility is a recommended diagnostic test in adult asthma, there is limited evidence to support the cut-off values used, particularly in symptomatic patients at diagnosis.(202) In this study, BDR of FEV_1 ≥12% and 200mls was highly specific in all patients; irrespective of baseline spirometry, but only positive in 44% of treatment-naïve patients. Despite its high specificity, NICE and GINA guidelines both recommend its use only in patients with obstructed spirometry. In the current study, a minority of patients (n =5) had a positive BDR despite a non-obstructive spirometry (i.e., with an FEV_1:FVC ratio greater than the lower limit of normal), all of whom had EPOER-diagnosed asthma (accounting for

14% of all asthmatics). This highlights that the requirement of obstructive spirometry before performing BDR may further reduce test sensitivity.

It is important to note that in the current study patients with significant respiratory comorbidities and smoking history were excluded. Further, all patients were ICS-naïve and had no recent course of oral corticosteroids or antibiotics when diagnostic tests were performed. Thus, this diagnostic algorithm should only be applied in similar patients who have intermediate/high clinical probability of asthma following the initial consultation, are diagnosis-naïve, untreated, with minimal smoking history and absent major respiratory comorbidities. For symptomatic patients with a low clinical probability where an alternative diagnosis is more likely, or for patients who are already on ICS, this approach would not be appropriate. In particular, corticosteroids reduce diurnal variation in peak flow and blood eosinophils,(203, 204) potentially reducing the sensitivity of this diagnostic algorithm. Moreover, the presence of other respiratory comorbidities (such as those with coexisting chronic obstructive pulmonary disease [COPD] or cardiac failure) or substantial smoking history (hence increasing the probability of COPD) may result in higher false positive rates. Therefore, this algorithm is not designed to differentiate asthma from COPD in patients where both diagnoses are being considered.

Notably, in this current study, the majority (83.3%) of EPOER-defined asthmatics demonstrated features of allergic sensitisation, high FeNO or blood eosinophilia. As blood eosinophilia is one of the key components in the algorithm, the current algorithm may have lower sensitivity when applied in patients with a Th2-low asthma phenotype. Nevertheless, the current algorithm is useful to rule-in asthma (with high specificity) for approximately a third of patients, and for those in whom asthma cannot be diagnosed based on blood eosinophils, auscultation and PEFv measurement (regardless of asthma phenotype), further detailed spirometry-based testing will be needed. It is also noteworthy, that the diagnostic value of this algorithm was tested in patients who presented to their GP due to symptoms suggestive of asthma and therefore suitable for use in primary care patients. However, for asymptomatic or very mildly symptomatic patients (i.e. for asthma screening) who otherwise would not present to GP, the diagnostic efficiency of this algorithm is unlikely to be adequate for identifying asthma. In addition, the algorithm was not tested in children or

those who have previously failed ICS treatment and should not be extrapolated to these groups.

This current study is limited by its small sample size. The diagnostic efficiencies demonstrate wide 95% confidence intervals, indicating significant uncertainties in the evaluated sensitivity and specificity. Therefore, it is imperative to externally validate these findings in large groups of patients with similar clinical history before implementation.

6. Final conclusions and future work

6.1. Final conclusions

Despite being the most common chronic respiratory disease affecting adults and children, asthma diagnosis is under researched with minimal evidence base to support current national asthma diagnostic guidelines. Recent studies demonstrate asthma is misdiagnosed with both over-diagnosis and under-diagnoses being highlighted as areas of concern.

This thesis provides a substantial contribution to the field of respiratory medicine by investigating four major questions in relation to asthma diagnosis. Firstly, we are the first to report on the current performance of the NICE recommended diagnostic algorithm (NG80). Second, using currently available respiratory tests, we report best diagnostic tests and combination of tests (algorithm) to accurately diagnose asthma in symptomatic patients, using stringent criteria as a gold standard for confirming asthma. Third, we investigate Airways Oscillometry, a test not currently used in diagnostic guidelines. To our knowledge we are the first prospective study to report upon diagnostic potential of AO in symptomatic steroid naïve adults referred from primary care with suspected asthma. Finally, following the recent SARS-CoV-2 pandemic, we report best approach to diagnose asthma during a pandemic when in-clinic AGPs are not available.

6.1.1. How well does the NICE recommended asthma diagnostic algorithm (NG80) perform?

Feasibility to perform the NICE (NG80) algorithm was limited in both adults (one in six not completed) and children (two in five not completed), predominantly due to compliance to perform PEFv testing (a test required by most pathways in the algorithm). When attempted, technically acceptable measurements were obtained for all tests in adults and children over eight years old. A small number of children under eight years old were unable to perform FeNO and/or BCTmeth to a technically acceptable standard (the latter not currently featured in the NG80 for children).

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When the algorithm was completed, three out of five adults, and one out of two children received the correct diagnostic outcome, and no false positive outcomes were assigned. However only one-third adults and children with EPOER asthma, received the correct diagnostic outcome. A third of adults, and one in seven children with EPOER asthma were not identified and did not receive a trial of treatment.

The performance of the NG80 algorithm was not prospectively tested or validated prior to implementation in 2017. To our knowledge, this is the first study to prospectively test the NG80 algorithms, however we acknowledge our small sample size, which is largely due to the complexity of recruiting into this specific population (i.e., symptomatic steroid naïve patients presenting predominantly to primary care services). A large amount of work went into collaborating with local general practitioners and designing and implementing electronic 'pop up' prompts within electronic patient records.

Our results to date indicate that the NG80 algorithms accurately rule in asthma (i.e., outcome 'diagnose with asthma') in both adults and children. In addition, adults labelled with possible asthma (i.e., suspect asthma and trial of treatment) could confidently have asthma diagnosed without need for post-trial of treatment investigations. A large proportion of patients receiving the outcome "consider alternate diagnosis or second opinion" did have EPOER confirmed asthma. Further research is imperative in this group to explore the best approach to management of these patients. From our data we speculate that performance of the 'paediatric' NG80 algorithm may benefit from the inclusion of a BCTmeth. All children with EPOER asthma and 'false negative' NG80 outcome (e.g., "consider alternative diagnosis and referral for specialist assessment," no immediate treatment commenced) had positive BCTmeth. We also highlight that in both adult and paediatric algorithms, the same pathway (e.g., spirometry negative, FeNO negative, and PEFv negative) resulted in an outcome of "consider alternative diagnosis/second opinion" which may result in misdiagnosis or treatment delays in asthma patients. We speculate that rewording this outcome to "clinical review/further investigation required" where a more individualised review could take place and take into account all tests (i.e., borderline results) not accounted for using a dichotomised algorithm, could improve the algorithm. This

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approach has not been tested. Further collaboration with primary care and further studies are essential to understand how to best manage these patients.

The overall performance of the NG80 algorithm in its current form is concerning due to the large number of "false negatives" in both adult and paediatric versions. Our study reveals an urgent need to develop a new diagnostic pathway that performs with greater sensitivity and/or lower false negative outcomes. In the interim, we conclude patients who are given an asthma diagnosis (i.e., 'diagnose with asthma') following the algorithm can be confident that they have asthma, however many of those remaining will also have asthma, and so we propose that this should be made clearer to GPs and further testing and review of tests completed would be required so that a diagnosis of asthma is not missed.

6.1.2. What is the best approach to diagnosing asthma using standard tests?

No single test reliably differentiated EPOER confirmed asthma from 'not asthma' in symptomatic patients, reflecting the heterogeneous nature of this disease. We show that other tests described in the literature but not recommended in current NICE guidelines, (blood eosinophils, PEF(alternative), MMEF%predicted, and MMEF%change) performed well in asthma diagnosis. The latter two, outperform their equivalent measurements from the NG80 guideline (FEV$_1$/FVC ratio and FEV$_1$%change respectively) and should be considered as an alternative measurement in asthma diagnosis. Auscultated wheeze was also a very specific sign for asthma diagnosis in adults. Using only standard asthma tests described in the literature and currently available in the UK, we report the best algorithm to diagnose asthma in adults (any one test positive of: wheeze auscultated, FEV$_1$/FVC <LLN, BDR ≥12 and 200ml, PEFv (alternative) >20%, Eosinophils >0.4x10^9/L, BCTmeth PD$_{20}$ ≤0.2). Tests can be completed in any order, once one test is positive the diagnosis is confirmed. We found a similar algorithm performed best in children (Any two tests positive; wheeze auscultated, FEV$_1$/FVC <LLN, BDR ≥12, PEFv (alternative) >20%, Eosinophils >0.4x10^9/L, FeNO ≥35ppb, BCTmeth PD$_{20}$ ≤0.2). Any patients not diagnosed as asthma from our recommended algorithm would require clinical consultation to review all individual results. Both of the proposed algorithms outperformed the current NG80 algorithm as a 'rule in' algorithm for

asthma diagnosis. However, they would need to be fully tested in a prospective validation cohort.

6.1.3. Can Airways Oscillometry be used to diagnose asthma in adults?

We show AO is a reliable test with good concordance between visits. AO BDR (X5ex%change and Fres%change) was the only measurement that was significantly different in those diagnosed with asthma patients compared to symptomatic not asthma patients. Thresholds for confirming asthma diagnosis were reported (X5%change >25%, Fres%change <-18%). Using these cut-offs AO was more sensitive at diagnosing asthma when compared to recommended cut-offs using standard tests (sensitivity range 60-69% versus 10-56%). However, specificity was compromised. These measurements show potential in the future of asthma diagnosis in adults. We show AO BDR could be useful as a complementary test in addition to current standard asthma tests opposed to a replacement test.

6.1.4. What is the best approach to diagnosing asthma when aerosol generating procedures are not available?

We developed an algorithm for asthma diagnosis in adults, to be used when access to AGPs is not possible or when AGPs are contra-indicated. Our simple algorithm requires one of the following tests to be positive; auscultated wheeze, blood eosinophils $>0.4 \times 10_9$/L, PEF(alternative) >20%. Tests can be completed in any order, a positive tests diagnoses asthma. Patients with all negative tests require personalised consultation with the clinician having access to all results opposed to dichotomised outcomes. Again, this would require validation in a prospective cohort and would only be relevant is those with appropriate symptoms and a minimal smoking history.

6.2. Strengths and limitations of the research

Results from this thesis will require validation to confirm our findings before we would recommend, a change to national asthma guidelines, implementation of new algorithms, or a recommendation supporting transition Airways Oscillometry into clinical practice.

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Strengths of study design

The strengths of this research is that the RADicA study is a large prospective observational study investigating steroid naïve patients presenting with respiratory symptoms (wheeze, cough, breathlessness, chest tightness) associated with the disease asthma. This replicates the population of patients in whom the diagnostic algorithm would be used. The literature shows that few studies have previously managed to capture this population. In our study asthma diagnosis was confirmed ("asthma" or "not asthma") using a rigorous process in which a team of asthma specialists reviewed all subjective and objective clinical information on each patient pre- and post- treatment over a six to eight weeks. Where information was missing and/or if a diagnosis wasn't clear patients were classified as "insufficient evidence" or "possible asthma."

Limitations of study design

Whilst our 'gold standard' for confirming asthma was based upon a rigorous process that did not rely or put weight upon any one objective or subjective measure, we acknowledge that the lack of a placebo arm for inhaled corticosteroid in our patient group means that using post treatment subjective or objective response (i.e., improvement in ACQ) to guide the expert panels diagnostic decisions may be subject to the 'placebo' effect or the Hawthorne effect. Post treatment subjective response formed a small part of the data reviewed by the diagnostic panel, however to reduce the risk of potential bias future studies should consider adding a placebo arm to the study groups. Other limitations we acknowledge in our study is that the results can only be applied to a similar patient cohort. Exclusions to our study included; smoking history (>10 pack years), history of corticosteroid use, and other respiratory co-morbidity. In patients that have significant smoking history (>10 pack years), confirmed or suspected alternative respiratory comorbidity, or those already established on regular asthma treatment, these conclusions cannot be extrapolated. The work presented on Airways Oscillometry and asthma diagnosis without the use of AGPs were performed only upon adults. We do not recommend extrapolating outcomes from this thesis outside the population demographics we tested. We also appreciate, when separating adults and children, our recommendations are based upon a smaller sample size. Larger validation studies would be required for both sub-groups. In the RADicA study both

patients and healthy controls were predominantly Caucasian, this work should therefore be interpreted with caution in other ethnic backgrounds. We acknowledge that 'referral bias' may impact our study population making it difficult to generalise our findings. It is possible that GPs only referred certain types of patients (i.e., those with less classical asthma that are harder for general practitioners to diagnose in community) or that only certain types of General practices referred into RADicA. Our cohort may not fully represent all potential presentations of asthma. Finally, predictors of asthma in our univariate and multivariate analysis were described using measures of diagnostic accuracy (i.e., sensitivity, specificity, PPV, and NPV), we acknowledge that due to the heterogeneity of asthma (i.e., different phenotypes and endotypes) it is likely that our results are oversimplified and that no single outcome is adequate for all subgroups of asthma. However, for this interim analysis the sample size is too small to perform cluster analysis and differentiate between different subgroups. This is a pragmatic approach to diagnosing asthma now. This will be further considered when we analyse the final RADicA data and if numbers are sufficient we will perform a cluster analysis to further explore subgroups of asthma.

6.3. Future work

This thesis presents an interim analysis from the RADicA study which is currently still recruiting. The interim analysis demonstrates that current national guidance on diagnosing asthma may lead to misdiagnosis of asthma or treatment delays due to false negative outcomes using the current NG80 algorithms in both adults and children. The thesis highlights important information that should be made available to clinicians that are using the NG80 guidance whilst awaiting further validation studies. The key message is that in those patients in whom the NG80 outcome is 'consider alternate diagnosis/second opinion' many will have asthma and will require further investigation and review in a timely manner. The interim analysis presented also highlights that alternative approaches to asthma diagnosis are required, we recommend a practical and pragmatic approach that could be implemented in primary care, subject to further validation studies. Further analysis using the RADicA final dataset will improve the strength of our findings. All aspects of this thesis require external validation in the population we describe. In addition, it will be necessary to test our findings outside of our selected population demographics to see how they perform.

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It is the aim that the output from this thesis along with the final analysis with the completed dataset and further validation studies will contribute to guideline changing evidence that will optimise asthma diagnosis in the UK.

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8. Appendix

8.1. Appendix A: Summary tables of studies in literature review (tables a-j)

a) **Spirometry**

Studies assessing the use of Spirometry in asthma				
Author	Study design/Aim/	Index Test / Test Standard	Outcome / summary	Other info
Adults: asthma diagnosis. Index Test: FEV1/FVC ratio				
Smith et al (2004) (92)	Prospective observational cohort. Compare FeNO with Spirometry and other conventional tests in asthma diagnosis pre & post po steroids course N 47 (n17 asthma, n30 not asthma by test standard). Age 9-75yr. incl: symptoms of asthma >6weeks, referred from GP. Excl: recent steroids (inh/po/iv)(<4weeks steroid free). Smokers excluded; 3/17 vs 2/30 were ex-smokers in asthma and non-asthma group respectively. Mean pack yr 11.3 vs 12.5 respectively.	**Index**: Spirometry: ratio <70% **Other Tests**: FENO (>20ppb), SpE >3%, PEFv: amplitude %mean≥20% **Test Standard:** Objective symptoms (ATS criteria) plus BHR(4.5% saline)PD15 and/or BDR≥12%	-FEV1/FVC ratio <70%: S.35%, sp.100%, PPV.100%, NPV.73% -mean FEV1/FVC ratio lower in asthma group (77% vs 84%) p<0.05	note; only 35% of asthmatic patients had obstructive spirometry by definition. Very poor sensitivity. Authors report when adjusted ratio <80% no significant change in outcome. Authors found 76% of the asthma patients were **atopic** compared to 43% in non-asthma group. (SPT)
Children: asthma diagnosis. Index Test: FEV1/FVC ratio				
Smith et al(2004)(92)	See subsection above. No raw data on number of children in study.			
Additional papers (March 2017 – June2018): FEV1/FVC ratio				
Murray et al(2017)(43)	Observational study. Data collected from prospective population-based cohort. Aim: assess diagnostic value of spirometry in symptomatic asthma patients not on regular ICS and assess NICE guide diagnostic algorithm.. N 630 (n 74 asthma on test standard, n 403 not asthma, n 153 possible asthma). Age: 13-16yr (mean 15.5yr) Excl: regular ICS	**Index**; FEV1/FVC ratio <70% **Other tests:** FeNO ≥35, BDR≥12% **Test Standard:** Subjective physician diagnosis AND current wheeze(within 12mn) AND requiring asthma medication (within 12mn)(non-asthma controls were negative to all three)	FEV1/FVC<70% to predict asthma: S 3% sp 99% PPV 29% NPP 85% (adjusted to ratio<83.8: S 54% sp 81% PPV 35% NPV 91%) Summary: - only 2.7% asthma patients had FEV1/FVC<70%. (increased to 14% on reassessing using ratio below lower limit of normal(LLN).) - FEV1/FVC ratio was independently associated with asthma (p=0.0075) - Current FEV1/FVC cut off values poor sensitivity in paediatric population	Note: authors suggest combination testing with: FEV1/FVC<83.8% and BDR≥3.48% and FeNO≥24ppb is better than current NICE algorithm for asthma diagnosis in children. However only 62% of their cohort completed all three tests so could not be used in 38% of children.

Studies assessing the use of FEV1/FVC Ratio or FEV1 in asthma				
Author	Study design/Aim/	Index Test / Test Standard	Outcome / summary	Other info
Adults: asthma diagnosis. Index Test: **FEV1 OR FEV1/FVC ratio**				
Schneider et al (2009)(205)	Cross-sectional observational study Aim- evaluate predictive value of spirometry in asthma & COPD N=219 (n 90 asthma, n 50 COPD, n 79 no evidence of any OAD). M:F 42:58. Average age 43yr incl: Respiratory symptoms not on any treatment for OAD	**Index:** FEV1<80% **&/or** FEV1/VC≤70% **Test Standard:** Objective symptoms plus Bodybox (WBP): BDR (defined as: FEV1≥12%**&**>200ml (used if FEV1<80) (applied to n 14) **OR** BHRmeth PC20≤16) (applied to n 76)	Diagnosing airway obstruction in Asthma using FEV1/VC<70% or FEV1<80: S 29% sp 90% PPV 77% NPV 53% Summary: -authors suggest spirometry as screening 'rule in' asthma test but poor rule out test - Spirometry was normal in 70% of patients presenting with symptoms of asthma - "prevalence of asthma was overestimated with 58% of healthy subjects suspected of having asthma" by general practitioner and "7.8% of patients with asthma were considered to be healthy"	Note: in the cohort of 216 patients receiving spirometry *in general practice* only 39% were performed in full adherence with ERS spirometry guidelines. Note: 8.8% received false positive diagnosis of asthma, of these spirometry was adherent to ERS guideline standards in only 25%.

Studies assessing the use of FEV1 in asthma				
Author	Study design/Aim/	Index Test / Test Standard	Outcome / summary	Other info
Adults: asthma diagnosis. Index Test: **FEV1**				
Fortuna et al 2007(93)	Prospective observational study. Compare diagnostic accuracy of spirometry (FEV1 **or** ratio), FENO, SpE N=50 (n 22 asthma on test standard). Age 18-68yr. Incl: symptoms consistent with asthma. Excl: recent po/ICS, recent LRTI, systemic manifestations of atopy	**Index**: Spiro (FEV1<80% **&/OR** ratio <75%) (no raw data to differentiate the two) **Other tests**: FeNO ≥20, SpE ≥3% **Test standard: objective** Symptoms plus BHRMeth (PD20≤16mg/ml)	FEV1<80%: S 22% sp 100% PPV 100% NPV 56% Spiro: only 77% asthmatic patients had normal spirometry . Summary: authors comment that all of these patients had positive FeNO (>20ppb) suggesting FeNO has a higher sensitivity than spirometry.	Note: Only provided raw data on FEV1 in paper FeNO more sensitive than FEV1 but FeNO was also positive in 35% non-asthmatics. -FeNO plus SpE was better than FeNO in isolation
Popovic et al 2003(94) Only abstract obtainabl e	Observational study. Aim: determine most useful tests in diagnosis of asthma in patients with dyspnoea. N=195 with dyspnoea (141 diagnosed with asthma in test standard). Control grp n-18	**Index Test**: Spirometry (FEV1) **Other tests:** BHR, SPT, IgE, Sp.E, blood eosinophils (Bl.E) **Test Standard:** no raw data in abstract	FEV1<%predicted:: PPV 79%, diagnostic accuracy <50% Summary: unable to diagnose asthma based upon lung function. Note: study only comment on FEV1 not ratios. Thus not possible to comment on ratio Which is the NICE recommended value in spirometry	BHR was highest rated test in asthma diagnosis. Followed by SPT. No other test could be used in isolation to confirm a diagnosis of asthma
Smith et al (2004)(92)	Prospective observational cohort. Compare FeNO with Spirometry and other conventional tests in asthma diagnosis pre & post po steroids course N 47 (n17 asthma, n30 not asthma by test standard). Age 9-75yr. incl: symptoms of asthma >6weeks, referred from GP. Excl: recent steroids (<4weeks steroid free) Smokers excluded; 3/17 vs 2/30 were ex-smokers in asthma and non-asthma group respectively. Mean pack yr 11.3 vs 12.5 respectively.	**Index**: FEV1 <80% Other Tests: FENO (>20ppb), SpE >3%, PEFv: amplitude %mean≥20% **Test Standard**: Objective Symptoms (ATS criteria) underline plus BHR(4.5% saline)PD15 and/or BDR≥12%	FEV1<80%: S.29% sp.100% PPV.100% NPV.71% FEV1 improvement (>15%) post steroid: S.12%, sp.100% PPV.100% NPV.66%	comment; Very poor sensitivity. *note: authors found 76% of their asthma patients were **atopic** compared to 43% in non-asthma group. (SPT)
Children: asthma diagnosis. Index Test: **FEV1**				
Sivan et al2009(13 7)	Observational cohort Aim: evaluate FeNO compared to spirometry in asthma diagnosis. N 150 (on test standard and at follow up: n 69 steroid naïve asthma, n 44 no	**Index:** FEV1<80 **or Ratio <75%** **Other tests:** FENO (18ppb), SpE≥ 3% **Test Standard**: Subjective clinical	FEV1 S 52% sp 72% PPV 75% NPV 48% (not data on FEV1/FVC ratio)	Note: "FeNO and SpE better than 'spirometry"

	asthma, n 37 with asthma on put on controller inhalers) Age: 5-18yrs. Incl: symptoms >12 weeks, ability to complete all 3 tests (spirometry/ FeNO/ sputum). Excl: LRTI, systemic manifestation of atopy	impression (considering symptoms, exacerbations, FEV1 variability≥15%, or provocation test if available)		
Smith et al(2004)(92)	See subsection above. No raw data on number of children in study.			

b) Peak expiratory flow variability

Author	Study design	Index Test / Test Standard	Outcome / summary	Other info
Adults: asthma diagnosis. Index Test: **PEFv**				
Den Otter et al (1997)(97)	Cross sectional observational Aim; compare PEFv to standard BHR test Incl; signs or symptoms of asthma N 323, Age:25-70yr. population: random selection of population selected for asthma screening. Incl: ≥1symptoms of asthma. Excl: unable to perform PEF	**Index Test**: PEFv ≥15% [PEFv= (PEF highest – PEF lowest÷PEFmean)x100] (3wk bd monitoring using best of 3attemps) **Test Standard:** Objective BDR ≥9% OR BHR to histamine challenge (PC20≤8)	Correlation between PEFv and BHRc (PC20) was -0.27 (p<0.0001) Summary: 38% of pts with negative PEFv had asthma diagnosed at BHR challenge. The authors conclude that PEFv is not sufficient to make a diagnosis of asthma.	
Thiadens et al (1998) (98)	Observational study Aim: Look at value of diurnal peak flow variability (DPV) in diagnosis of asthma N 182 (on test standard: n 69 asthma, n 12 COPD) Age 18-75yr. Incl: attended GP with ≥2wk cough Excl: prior diagnosis asthma or COPD	**Index test**: PEFv (PEFv >15% or >20% for ≥4 or ≥3days in 2wk period). PEFv= [(PEFhighest – PEF lowest÷PEF highest)x100] **Test Standard:** Objective >3wks symptoms in past year plus BHRmeth PD20 ≤15µmol **or** BDR >9%	>40% of patients categorised as asthma had no evidence of PEFv therefore PEFv missed >40% of patients with asthma.. Summary: -authors show models incorporating DPV 15%≥4days + female sex + pack yr history can predicting patients with asthma or COPD. -number of days with variability correlated c diagnosis of asthma. calculating %amplitude was most effective method	Note: -low cut offs will potentially over diagnose asthma group, perhaps classify COPD as asthma. -included incomplete PEF diaries if ≥6measurements recorded)
Ulrik et al (2005)(99)	Population survey – random sample from civil registration list. N 609(13% classified as asthma from self-reported symptom questionnaire) 288M:321F Age 13-23	**Index test**: PEFv ≥20% measured as: PEF best of 3 recorded bd for 2wks calculating Amplitude%mean = [(PEFhighest-PEFlowest)÷PEFmean] **Other test**: BHRHistamine PC20 (fev1<16), BDR **Test Standard:** Subjective Self-reported asthma symptom questionnaire	Summary: PEFv only identified 45% of asthma cases. Therefore missed >50% of patients with asthma. AHR identified 93% of patients with current asthma	Note: ; authors use BHR cut-off(PD20 <16), this may account for the high sensitivity of this test. Also PEFv was high which could result in the lower sensitivity seen
Smith et al (2004)(92)	Prospective observational cohort.	**Index:** PEFv ≥20%. (Best of 3 PEF recorded for 1wk). PEFv =	PEFv>20%: s 0% sp100% PPV n/a NPV 70%	

	Compare FeNO with Spirometry and other conventional tests in asthma diagnosis. N 47 (n17 asthma, n30 not asthma by standard test). Age 9-75yr. incl: symptoms of asthma >6weeks, referred from GP. Excl: recent steroids (<4weeks steroid free)	{PEFhigh – PEFlow ÷PEFmean)x100)] **Other Tests**: FENO (>20ppb), SpE >3%, **Test Standard**: Objective symptoms (ATS criteria) plus BHR(4.5% saline)PD15 and/or BDR≥12%		
Children: asthma diagnosis. Index Test: **PEFv**				
Brouwer(2010)(96)	Observational study. N61 (21 asthma, 40 other non-asthma) 27M:34F, Mean Age 10 (6-16) Incl: GP referral for respiratory symptoms but not standard asthma Excl: classical asthma symptoms	**Index:** PEFv > 12% variation expressed as amplitude as % of days mean, best of 3 recorded for 2weeks. PEFv= [(PEFhighest – PEF lowest ÷ PEFmean)x100] **Other Tests:** FeNO, BHR, FEV1 **Test Standard:** Subjective Physician impression after review of history/exam/lung function data	Mean diff in PEFv in asthma vs non asthma 4.4% Summary: authors conclude PEFv limited use in diagnosis of asthma in their cohort of children.	Note: the study excludes children with classical asthma symptoms, therefore results are not necessarily transferable.
Smith et al (2004)(92)	See adult PEFv subsection above			
Ulrik et al (2005)(99)	See adult PEFv subsection above			

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c) Bronchodilator reversibility

Author	Study design	Index Test / Test Standard	Outcome / summary	Other info
Adults: Asthma diagnosis: Index BDR				
Brand et al(1992)(103)	Observational study using data from multicentre trial Aim: assess best measure of BDR to establish between respiratory lung diseases. N 274 Age 18-60yr Inclu: evidence of obstructive airway *disease AND FEV1>1.2L but 1.6-4.5sd below predicted AND BHR to histamine positive (PC20<8mg/ml)* *Excl: normal baseline spirometry*	**Index Test**: BDR measuring: ΔFEV1%pred (>9) ΔFEV1% initial (>15) **Test standard:** Subjective Clinical history (history consistent with asthma in absence of chronic cough or sputum production.)Note: all patient had abnormal BHR and spirometry to enter study.	ΔFEV1%pred: s 73% sp 56% likelihood ratio 1.7. ΔFEV1%initial: s 68% sp52% likelihood ratio 1.45 Authors: found "FEV1%pred post BD appears to be the most useful method in separating asthma and COPD. - asthma group had larger mean bronchodilator response than non-asthma groups	- standard test to confirm asthma or assign other group was based upon a clinical history. -Very specific group, too specific a group to look at credibility of BDR in general population -ΔFEV1%pred found to be better than ΔFEV1%initial but hard to compare as used different cut off values.
Chhabra(2005)(101)	Prospective observational study. Aim: assess the diagnostic value of BDR to differentiate asthma from COPD N 354 (200 asthma, 154 COPD) Mean age (asthma group): 35yrs Incl: FEV1/FVC ≤70%, Excl; smokers (in asthma group), recent exacerbation	Index test: BDR (salbutamol). Measuring: 1)absolute change in FEV1 & FVC (ΔFEV1 and ΔFVC)of ≥0.2L 2) change as % of baseline (ΔFEV1%baseline) **Test standard:** subjective Clinical diagnosis to categorise asthma or COPD based upon symptom and smoking history.	Asthma grp: ΔFEV1 & FVC: S 73% sp80% NPV 69% PPV 82% Summary: -authors found no expression of response gave a clear cut separation between the two diseases." -"absolute change in FEV1 had best result" -FEV1≥0.2L was best for diagnosing asthma	note; - excluded people with normal spirometry therefore the results on BDR wouldn't be transferable an unscreened population and may explain why higher sensitivity and specificity was seen in this study. -the *study included pts on steroids which may increase false negative and explain low NPV of 69%* -grouping patients to Asthma vs COPD category was based on clinical history alone .

Quadrelli et al (1996)(104)	Prospective observational study Aim; to define the most useful way to define BDR to distinguish between COPD and asthma N 200 (142 asthma, 58 COPD) Asthma grp; mean age 55yrs+/-19yrs (100%female). COPD group; mean age 67yrs (79%men) Inclu: obstructive spirometry on past records AND current ratio FEV1/FVC ratio 1.64SEE below predicted. Study criteria: all asthma were non-smokers with symptoms or physiology consistent with asthma and on an ICS and all COPD were "heavy" smokers/ex-smokers and NOT on an ICS.	**Index test:** 1.Absolute change (ΔFEV1)≥200ml 2. %initial ≥15% 3. %pred ≥9% 4.%of max poss response **Test Standard:** Subjective Clinician diagnosed asthma (not necessarily using any objective tests- could be symptoms with symptom free periods.	-In Asthma group (sub group analysis on those with FEV1<55%): Δ%pred has S 67% sp 70.%. - In patients with FEV1<55%: Δ%FEV1initial and Δ%FEV1pred was higher in asthma patients(p<0.05 and p<0.001 respectively) - Δ%FEV1initial show worst results of positive and negative predictive values Summary: -the authors conclude that in clinical practiceΔ FEV1%initial is a poor diagnostic tool and a negative test doesn't exclude the presence of asthma	**Note:** population of asthma patients all on ICS treatment compared to COPD patients not on ICS. Also 43% of asthma group has FEV1 <55% which is not a classical finding. Analysis is primarily reported on this group. This data isn't transferable to general asthma population.
Children: Asthma diagnosis: Index BDR				
Nil				
Additional papers: March 2017 – June2018				
Murray et al(2017)(43)	Observational study. Data collected from prospective population-based cohort. Aim: assess diagnostic value of BDR in symptomatic asthma patients not on regular ICS and assess NICE guide diagnostic algorithm.. N 630 (n 74 asthma on test standard, n 403 not asthma, n 153 possible asthma). Age: 13-16yr (mean 15.5yr) Excl: regular ICS	Index; BDR ≥12% FeNO ≥35ppb **Other Tests:** FeNO≥35ppb, FEV1/FVC ratio <70% **Standard Test:** Subjective physician diagnosis AND current wheeze(within 12mn) AND requiring asthma medication (within 12mn)(non-asthma controls were negative to all three)	In whole cohort: BDR ≥12 to predict asthma: S 16% sp 93% PPV 32% NPV 86% (note re adjusted for BDR≥3.48 S77% sp 45% PPV 21% NPV 91%) In symptomatic only: BDR ≥12 to predict asthma: S 9% sp 93% PPV 32% NPV 86% (note re adjusted for BDR≥3.2 S79% sp 42% PPV 46% NPV 77%) Summary: - FeNO and FEV1/FVC were independently associated with asthma (p <0.0001 and p=0.0075), however BDR was not.	Note: authors suggest combination testing with: FEV1/FVC<83.8% and BDR≥3.48 and FeNO≥24ppb is better than current NICE algorithm for asthma diagnosis in children. However only 62% of their cohort completed all three tests so could not be used in 38% of children.

d) Bronchial challenge testing

Studies assessing the use of BHRc in asthma				
Author	Study design	Index Test / Test Standard	Outcome / summary	Other info
Adults: asthma diagnosis: Index BHRc				
Nieminen [1992](108)	Observational (Population study based in Finland.) Aim: assess role of BHR in asthma. N 791 (n319 asthma on test standard) + 30 H.C (healthy controls) Age 43yr (mean). Incl; respiratory symptoms to OPC Controls: healthy, non-smokers	Index: BHRmeth PD20 <2600microgram cumulated dose Other tests: PRFv, BDR Standard: Subjective "clinician diagnosed based on clinical evaluation" – taking into account PEFv/FEV1/allergen provocation or exercise testing	BHRmeth to predict asthma: s 89%, sp 76% PPV 91%, NPV 91%, accuracy 81% Summary: - 89% of asthma group had positive BHRc -In non-asthma group 82 had allergic rhinitis and 49 chronic bronchitis, of these 27% and 22% had positive BHR respectively. -degree of hyperresponsiveness in asthma is greater than other groups but overlap does exist with other conditions including: bronchitis, COPD, allergic rhinitis	Note: Specificity only 76% which could result in false positives, also 11% asthma group had normal BHR therefore test would have been falsely negative. - some patients on ICS or oral steroids, this may impact the results. - study included smokers (179 current smokers and 138 exsmokers).
Hedman et al(1998) (109)	Observational study Aim: validate a rapid dosimetric methacholine test in asthma diagnosis N 230 Age 44yr (mean) Incl; symptomatic Exclu: previous asthma diagnosis or on ICS	Index: BHRmeth Other tests: SPT, spirometry, E' Standard: Objective Physician diagnosed PLUS FEV1 or PEF variation of>15% after medication or >20%spontaneous variation of PEF PLUS >15% dec in FEV1 after allergen provocation or exercise test.	-BHR to predict asthma (using cut off <6900micrograms): S75% sp 82% NPV 91% Summary: - 40% of patients with positive BHR did not have asthma. -PD20FEV1 separated asthma from non-asthma best -best cut off was <6900mcg, using this value 77% of asthma patients had positive BHR	Note: The study use a lower dose threshold for a positive test, this may account for why 40% of patients with positive test did not have asthma.
Koskela et al [2003] (112)	Prospective study. Aim; compare BHRc with mannitol, histamine and cold air to demonstrate AHR in asthma diagnosis. N 37 asthma + N10 healthy controls. Age 44-54 Criteria: steroid naïve with recent asthma diagnosis	Index: 1)Mannitol PD15FEV1 at any dose (upto max cumulative dose of 635mg) 2) cold air – pos ≤9%fall in FEV1 3)Histamine PD15≤.4mg & ≤1mg Standard: Objective physician plus one objective test out of a]>15% drop in	Mannitol: positive in 51% asthmatics Cold air challenge: positive in 24% asthmatics Histamine PD15≤0.4: positive in 81% asthmatics Histamine PD15≤1: positive in 49% Summary: - Authors report only 51% of asthma pts had a positive BHR challenge to mannitol and that it is less reliable than histamine	Note: conclusions are made when comparing to a higher provocation dose of histamine, and also using PD15hist opposed to PD20. In a different population this may lead to false positives. -in addition the cohort used were 44-54yrs, >50% were previous or current smokers and most common symptom was cough and least common was wheeze. This is

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Author	Study design	Index Test / Test Standard	Outcome / summary	Other info
		FEV1 on exercise challenge test b]>15% increase FEV1 c BDR c}PEFv>20% on 2+days d]>15% incr PEF after BDR		atypical of the demographics of new presenting asthmatics and it is therefore not possible to transfer this knowledge to the general population
Popovic et al 2003(94) Only abstract obtainable	Observational study. Aim: determine most useful test in asthma diagnosis in patients with dyspnoea. N 195 (141 asthma on test standard)+ N18 controls	Index Test: Spirometry (FEV1) Other: BHR, SPT, IgE, Sp.E, blood eosinophils (Bl.E)	BHR- S 97% sp 85% PPV 94% *NPV 92% (highest of all the tests)*, *diagnostic accuracy (S+sp)=93% (Highest)* Highest diagnostic accuracy to predict asthma is BHR. (diagnostic accuracy <50% in all tests except BHR & SPT)	
Anderson et al (2009)(113)	Aim: compare BHRmann & BHRmeth in EIB and asthma diagnosis N 509. Age 6-50yrs (25% ≤18yrs old) excl; "Extremely likely" & "extremely unlikely" asthma (decided by physician impression at screening)/ patients with symptoms from aeroallergen	Index: BHRmeth PC20≤16mg/ml (and ≤1mg/ml) and BHRmann PC15 <635mg (or 10% fall between doses) Other tests: BDR Standard: Subjective Clinician diagnosis based on clinical assessment /spirometry/SPT	BHRmann to predict asthma: S 69% sp 62% PPV 79% NPV48% BHRmeth to predict asthma: S 80 sp 65% PPV 78% NPV 46% Summary: -in mild asthma BHRmann & BHRmeth performed worse than previously documented in the literature. - agreement between BHRmann & meth is only 69%	Note: -analysed adult and paediatric data together - higher dose of methacholine used compared to other studies, may account for low specificity. - excluded likely asthma and unlikely asthma groups which would impact on the overall sensitivity of the test.
Child: Asthma diagnosis: Index BHRc				
Anderson et al (2009)(113)	See above in Adult section			

Studies comparing Methacholine and Mannitol in asthma diagnosis				
Author	Study design	Index Test / Test Standard	Outcome / summary	Other info
Adults: asthma diagnosis: Index BHRc				
Cancelliere et al (2013). (114)	Aim: compare diagnostic efficiency & tolerability of mannitol and methacholine BHRc.	Index: BHRmann & BHR meth Other: FeNO, SPT	- both techniques showed agreement of 89% (n9 had positive BHRmeth & n10 had positive BHRmann) -methacholine better tolerated using cough severity score	Note: authors recommend methacholine above mannitol as similar performance but better tolerated.

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	N 28 (15-54yr) symptomatic, normal spirometry/neg BDR.	Standard: not defined in paper		
Sverrild et al (2010)(117)	Cross-sectional population study. Aim: Compare BHRmeth, BHRmann & FeNO in a non-selected population. N 238 adults	Index: BHRmann <635mg, and BHR meth ≤16mg/ml Other: FeNO, Standard: Subjective +/- Objective clinical plus either FeNO>30 or self/family history atopic conditions FEV1/FVC <75%.	- BHRmann higher specificity (98.4% vs 80.2%) and PPV (90.4 vs 48.6%) compared to BHRmeth. Sensitivity was slightly higher in BHRmeth 68.6% vs 58.8% in BHRmann. -BHRmann was associated with raised FeNO.	Note: BHRmeth was done immediately after BHRmann on the same day once FEV1 had improved to 95%baseline. -ICS were stopped 12hours before challenge therefore not steroid naïve patients. -Definition for asthma used one or more of FeNO>30, atopy, and possible obstructed spirometry (depending on LLN) – this may be why mannitol performs better as selected certain phenotypes of asthma.
Porpodis et al (2017)(116)	Aim: compare BHrmann & meth and correlate with clinical status, atopy and inflammation. N 88 (age 12-75)	index: BHRmeth ≤16mg/ml, and BHRmann <635mg standard: Objective positive BDR test	- similar ability of both tests to predict asthma. BHRmeth PPV/NPV 93%/42%, and BHRmann PPV/NPV 98%/45% -BHRmann slightly more specific in this cohort. -22% asthma patients negative on both BHR tests. -	Note: - standard for asthma diagnosis BDR which will potentially rule out milder less obstructed patients with asthma.
Anderson et al (2009)(113)	See table 9a			
Kim et al(2014)(115)	Aim: prospective multi-centre study. Criteria: using anti-asthma treatment for 6months before enrolment but held prior to test. Exclusion: >10pack year or current smoker.	index: BHRmeth ≤16mg/ml, and BHRmann <635mg standard: Subjective clinician diagnosed.	- BHRmeth S44% Sp 98.1&, PPV 95.7, NPV 65.4 - BHRmann S48% Sp 92.6&, PPV 85.7%, NPV 65.8% - similar performance – BHRmeth slightly more specific.	Note: - Tests separated by minimum 24hrs. -
Child: Asthma diagnosis: Index BHRc				
Anderson et al (2009)(113)	See table 9a			
Cancelliere et al	See subsection above			

(2013).(114)				
Porpodis et al (2017)(116)	See subsection above			

e) **FeNO**

Author	Study design	Index Test / Test Standard	Outcome / summary	Other info
Adults: FeNO Vs Comparison Tests				
Fortuna et al 2007(93)	Prospective observational study. Comparing diagnostic accuracy of spirometry, FeNO and SpE. N=50 (22 confirmed c asthma with methacholine). Age 18-68yr. Referral as symptoms consistent with asthma. Excl= on po/ICS/recent LRTI	Index Test: FeNO ≥20 Other; Spiro (FEV1<80% &/OR ratio <75%), E≥3% Test Standard: Objective BHRMeth (PD20≤16mg/ml)	FeNO – S 77% sp 64% PPV 62% NPV 78% Summary: -Diagnostic accuracy (=s+sp) best when compared to spirometry (FEV1) or SpE. - In the cohort 77% of asthma patients had normal spirometry, all of these patients had positive FeNO (>20ppb) - a positive FeNO and SpE in combination had best accuracy.	Note: -the cut off for +ve FeNO is low compared to other studies. At this level it is a good screening test but specificity is low. -FeNO positive 77% of asthmatic group however also positive in 35% of non-asthma group.
Smith et al (2004)(92)	Prospective observational cohort. Aim: Compare FeNO with Spirometry and other conventional tests in asthma diagnosis pre & post po steroids course N 47 (n17 asthma, n30 not asthma by standard test). Age 9-75yr. incl: symptoms of asthma >6weeks, referred from GP. Excl: recent steroids (<4weeks steroid free)	Index: FENO (>20ppb), Other Tests: FEV1/FVC ratio <70%, SpE >3%, PEFv: amplitude %mean≥20% Test Standard: Objective symptoms (ATS criteria) plus BHR(4.5% saline)PD15 and/or BDR≥12%	FeNO S 88% sp 79% PPV 70% NPV92%	Note: 76% of asthma group were **atopic** compared to 43% in non-asthma group. (SPT). This may have an effect on FeNO results. Smokers excluded; 3/17 vs 2/30 were ex-smokers in asthma and non-asthma group respectively. Mean pack yr 11.3 vs 12.5 respectively.

| Chatkin et al (1999)(120) | Cross-sectional observational study. Aim: determine role of FeNO in patients with chronic cough.

N 105 (n 38 chronic cough [of which: using asthma 'test standard' n 8 asthma and n 30 not asthma], n 44 previously diagnosed atopic asthma, n 23non-atopic control)

Inclusion: all patients in cough group had cough >3week, FEV1>80%
Exclu: active smoking, recent LRTI, steroids in last 6wk) | Index: FENO ≥30ppb positive

Standard: Objective If in chronic cough (n 38 grp): BDR ≥12% or if neg then methacholine challenge ≤8mg/ml. note: test standard not defined in previously diagnosed atopic asthma group | FENO≥30 to predict asthma in pts with chronic cough: S 75%, sp 87%, PPV 60%, NPV 93%

Negative FeNO rules out asthma: sp 87% NPV 93%

Summary:
-a negative FENO may be more useful than a positive test. (rule out test)
-Median FENO in asthmatic pts with cough 75ppb, compared to 28ppb in healthy controls.
-Non asthmatic coughers had lower FENO (median 16) compared to asthmatic coughers. | Note: results are only transferable to a steroid naïve non-smoking population. |
|---|---|---|---|---|
| **Adults: FeNO Vs Clinician (with at least one test)** | | | | |
| Cordeiro et al (2011)(124) | Prospective, Observational cross-sectional study
Aim: to see if FeNO can assist in differentiating asthma diagnosis in patients with atopy.
N 114 (n 42 asthma and n 72 non asthma on test standard)
Age 7-87yrs

Inclu: Symptomatic steroid naive patients | Index: FeNO

Standard; Objective BDR (≥12% and >200ml) or if neg BHRhistamine PC20 | FeNO >27 predicted asthma: s 78%, sp 92%, PPV 86%,NPV 87%
Positive FeNO +BDR best predictor: S 87% sp 90%
Summary:
- FeNO higher in asthma group vs non-asthma group (p <0.001) 44ppb Vs 17ppb respectively.
- FeNO positive correlation with respiratory symptoms (p<0.01) and airflow reversibility (p<0.01) | **Notes:**
- "atopy not a signif predictor of FeNO" in this cohort FeNO could predict asthma in atopic patients |
| Fukuhara et al (2011)(121)
Abstract only | Observational.
Aim: assess if respiratory symptoms and FeNO can predict asthma diagnosis

N 61 (N 41 asthma by test standard)
Incl: respiratory symptoms | Index: symptoms with FENO≥40ppb

Standard: Objective symptoms with two of: BDR/SpE/BHR | Feno+symptoms predict asthma: S 78% sp 89%
Summary:
- FeNO + symptoms picked up 80% of asthma cases in this cohort | Note: using only FeNO in patients with respiratory symptoms missed 20% of asthma cases. (1 in 5 patients misdiagnosed) |

Heffler et al (2006)(126)	Prospective study Aim: assess if FeNO can identify asthma in patients with rhinitis and asthma type symptoms. N 48 (N 18 asthma and N 30 non asthma on test standard) + 30 healthy controls. Age 17-75yrs Criteria: patients referred to allergyOPC for persisting rhinitis plus lower airway symptoms for >8/52. Incl; all had rhinitis Excl: smokers and steroids <8wks	Index: FENO >36ppb Other: SPT (14 aeroallergen panel. Atopic IF any ≥3mm wheal) Standard: Objective BDR≥12% or BHRmeth PD20<8mg/ml	FENO >36 to diagnose asthma: S 78% sp 60% PPV 54%, NPV82% Summary: -FeNO signif higher in rhinitis patients with asthma Vs non-asthma rhinitis Vs controls (60ppb vs 30ppb, vs 12ppb) - if FeNO levels <25ppb likelihood of asthma is virtually absent	Note: -more potential as a rule out asthma test (only transferable to steroid naïve non-smoking) -both asthma and non-asthma had similar proportion of atopic patients (77% vs 70%)
Kowal et al (2009)(122)	Observational study. Aim: look at FeNO in pts with chronic cough as a predictor of asthma N 540 (n 178 asthma on test standard)+100 non-atopic controls Average age: 26yr (range 18-45) Criteria: referral to asthma OPC with chronic cough. Incl: chronic dry cough >8wks, non-smoker, normal spirometry&CXR. Excl: recent LRTI, asthma medication, ACE-i	Index: FENO>40ppb Other: BHRhist (performed in all patients (excluding Healthy Control grp) – but not used as test standard), Bl.E Standard: Objective assessed at 6months with documented diurnal PEFv or BDR ≥12% (GINA criteria)	FeNO to predict asthma: S 88% sp 82%, NPV 94%. Positive likelihood ratio 5.08. Summary: - median FeNO higher in patients with positive BHR vs neg BHR (p<0.0001) -high NPV of FeNO indicates its potential role in selecting patients unlikely to have asthma. Preferential to BHR as first line in screening chronic cough patients for asthma.	Note: -Asthma group: 7% asthma patients had negative BHR test. Of these FeNO was higher compared to non-asthma group (p<0.0001) suggesting superiority in sensitivity --approx 30% patients with chronic cough had asthma - specific group of patients – this result may not transfer to general population
Sato et al (2008) (123)	Observational study. Aim; assess FeNO in cough pts. N 71 (on test standard: n 48 asthma, N 8 eosinophilic bronchitis, N 15 other) Age: 20-78yr Criteria: referred to respiratory OPC with chronic cough or wheeze for >3/52 Excl: previous or current ICS or po steroid, abnormal CXR or CT, Study includes smokers	Index: FeNO >38 Other tests: BHRmeth (PD20 <12.5units and/or spirometry and/or BDR (≥12% & 200ml). SpE ≥3%, BlE. IgE. Standard: no raw data classified on review of test results –exact criteria not defined in paper	FENO>38 predicted asthma: S79% sp 91% Summary: - FeNO signif higher in bronchial asthma (BA) (93ppb) than eosinophilic bronchitis (EB) (16ppb, p<0.01), Cough-variant asthma (46ppb, p=0.01) or non-asthma (NA) (21ppb) groups.	Note: contrary to other studies FeNO levels were higher in smoking asthmatics vs non-smoking asthmatics. -FeNO correlated with :non-sp IgE and dust-mite IgE, FEV1/FVC ratio (neg correlation), BHR, SpE, and Bl.E -Atopy defined as: non-sp IgE≥250IU/ml or any antigen-sp IgE (≥0.69UA/ml)

Voutilainen et al (2013)(206)	Observational study Aim: evaluate FeNO as predictor of asthma in athletes N 174 (N 87 athletes (n 54 confirmed asthma on test standard) + N 87 sedentary controls (n 30 confirmed asthma on test standard)) all had symptoms of asthma. Age: range 14-31	Index: FENO >30 Other: EVH (in athlete grp) Standard: Objective non very clear "symptoms <u>and</u> an objective test of airway obstruction i.e.: Grp 1 athletes: BHRmeth <u>PD20</u> <u>≤0.4</u>,, Gr2 controls: BHRHist <u>PD15<0.4</u>, or BDR≥12%, PEFv≥20%	FENO>30 not associated with asthma in athletes but was in non-athletes	<u>Note:</u> used diff diagnostic criteria in the 2grps: BHRHist PD15 in control compared with BHRmeth PD20 in athletes. So not direct comparison of the two groups.
**Adult: FeNO (Asthma vs Non Asthma)**
Berylne et al (2000)(133)	Descriptive Cross-sectional study. Aim: relationship of FeNO amongst different patient populations to assist diagnosis. N 131 (5grps: healthy non atopic/healthy atopic/steroid naïve asthma/asthma on ICS/EB) Median Age across groups: 33-46yrs (no data on total population) Excl: smoking in last 12mnth	Index: FeNO Other: SpE>3% Standard: Objective asthma symptoms plus BDR ≥**15** (if FEV1/VC ratio ≥70%), otherwise BHRmeth PD20<8	FeNO >17ppb to predictor of steroid naïve asthma: S 81% sp 90%. Summary: -FeNO levels not statistically different in atopic and non-atopic healthy controls. -Signif correlation between FeNO and SpE -Raised FeNO not specific for eosinophilic inflammation	Note: Higher BDR cut off may have miss categorised some asthma pts into a non-asthma group
Deykin et al (2002)(125)	Observational study Aim: evaluate ability of FeNO to discriminate asthma from healthy controls using different flow rates. N 62 (34 asthma+28 controls) Age: 29yrs (asthma) vs 27yrs (control) Excl: smokers	Index: FENO >30 Standard: Objective BHRmeth PC20<8 or BDR≥12% (controls had BHRmeth PC20 >10)	FeNO to predicted asthma: S 70% sp 75% PPV 74% NPV 71%. In control group: PPV 12% NPV 98% (latter shows FeNO is a good test for excluding asthma) Summary: -FeNO 57ppb vs 26ppb in healthy controls	
Kostikas et al (2008)(128)	Observational study. Aim: evaluate portable FeNO device as a screening test for asthma. N=149 (63asthma/57 Allergic rhinitis/29 non-sp resp symptoms) +70control. Inclusion: smokers and non-smokers accepted. all had symptoms of asthma picked up on general population questionnaire. Excl: ICS/ RTI	Index: FeNO>19ppb Standard: Objective poorly defined with minimal raw data. Diagnosis clinical plus BDR >12%&200ml or BHRmeth or clinical spirometric response to 4wk ICS (response not defined!!)	FeNO to predict asthma: S 52% sp 85%. Median FeNO: asthma 20ppb, Allergic Rhinitis 17ppb, control 11ppb Summary: -"diagnostic performance of FeNO was better in non-smokers" - higher FeNO in Asthma group compared to those with non-sp respiratory symptoms (p<0.0001) -Asthmatic non-smokers presented with a higher FeNO compared to asthmatic smokers (p =0.005)	<u>Note:</u> FeNO could not predict between asthma and allergic rhinitis group, however this study uses very low cut off for FENO comparative to other studies, higher value may help distinguish groups

Louhelainen et al(2008) (136)	Aim: review FENO as marker of oxidative stress/ airway inflammation. N= 65 (23 asthma children, 13 control child, 14 asthma adult, 15 control adult) Range 7-72yrs. Inclu: symptomatic	Index: FeNO Other tests: SpE, isoprostane Standard: Objective Asthma: any of: post exercise PEF or FEV1 ≥15% dip, BDR ≥12, BHR c histamine PD15 <0.4	Adults: asthma vs control (81 vs 16ppb) p=0.025. Children: 35 vs 11 (p<0.001) Summary: -Correlation between FENO and eosinophils	Note: atopic status tested by SPT to ≥1 aeroallergens(wheal ≥3mm)
Shimoda et al (2013)(2 07)	Observational study Aim: compare FeNO and hs-CRP to differentiate bronchial asthma (BA), cough variant (CVA), and controls (C). N=272 (n 90 CVA, n 92 BA, n 90 C) Inclus: new diagnosis Excl: steroids/ recent anti-allergy/ HTN/DM/Cholesterol. Note: smokers included in study	Index: FeNO Other; SpE, Hr-CRP Standard: Objective BA: symptoms +BDR ≥15% or BHR PC20<8000microra m/ml c acetylcholine CVA: cough but NO wheeze/dyspnoea symps +BDR or BHR as above	FENO ≥20ppb: distinguishes BA Vs control S72%,sp83% FENO≥28ppb: distinguishes BA vs CVA S69% sp 73% Summary: -FeNO higher in BA than CVA group 92+/-85ppb VS 35+/-43ppb (p<0.001) -FeNO higher in CVA than C grp 35ppb vs 18ppb (P<0.001) -FeNO higher in BA than C grp: 92 vs 18ppb (p<0.001) -"signif correlation of FeNO with severity, atopy, and sputum E' in BA group." -"FeNO signif lower in mild BA than in moderate BA patients	**Note:** patients with "too severe cough" had been excluded, this may explain why FeNO lower than BA grp (perhaps a less severe cohort of patients)
Shome et al (2006)(2 08)	Observational study. Aim: review FeNO in asthma patients and to assess relation to severity of asthma N 19 (+17controls) Excl: other significant co-morbidity (i.e. COPD/CF/CCF/recent illness <6wks). Smoking history.	Index: FeNO Other tests: blood lymphocytes/IL-4/IL-3 Standard: Objective clinical + spirometry AND BDR ≥12%	Summary: - FeNO significantly greater in mod/severe asthma Vs healthy control but not significantly different in mild asthma vs healthy controls -After asthma treatment commenced, FeNO reduced within first week and reached near normal levels <4wks.	Note: -FeNO may under detect mild asthma . - No useful in patients on treatment - post treatment reduction in FeNO did not correlate with improvements in spirometry.
Zietkow ski et al(2006) (209)	Observational. Cohort. Aim: review FeNO in steroid naïve patients against other diagnostic tests in asthma. N 101 asthma (split into allergic asthma n 56 and non-allergic n450 plus N 39 controls Incl; stale asthma Excl; any prior history of ICS, smoking, nitrate rich diet	Index: FENO 20ppb Other tests: bl.E, IgE Standard: Objective FEV1 (if <80) add BDR . If FEV1>70 they had BHRhist PC20	Summary: - FeNO raised in asthmatics (atopic and non-atopic) compared to controls. - FeNO higher in allergic asthma than non-allergic. -"no correlation between FeNO and baseline FEV1" -"signif correlation between FeNO and Bl.E" - "signif relationship between FeNO level and reversible airway obstruction.	Note: allergic asthma group had more severe asthma than non-allergic group, this may account for the higher FeNO instead of it being due to allergic component.!
Children: Asthma diagnosis: Index FeNO				

Cardinale et al (2005)(210)	Observational study AIM: assess relationship between FeNO, IgE and SPT in asthma. N 109 asthma (split to n 51 Asthma+rhinitis, n 58 asthma only), N 41 rhinitis, N 25 healthy non atopic controls Age 7-15 (mean 10) Excl: smoke exposure/ FEV1<80	Index: FeNO Standard: Subjective Asthma: clinical +/- other i.e. BDR (raw data not shown)	Median FeNO:; asthma 22ppb / rhinitis 15pbb / HC 5.9 ppb Summary: - Positive FENO correlation with IgE and skin prick test (p<0.0001) – (more closely with IgE) -FeNO significantly lower in allergic rhinitis group compared to allergic asthma group.	Note: low FeNO levels in asthma group compared to other studies, note exclusion of all patients with FEV1<80. Perhaps excluded mod/severe asthma
Ciprandi et al(2013) (211)	Cross-sectional study. Aim; assess ability of FeNO to predict BDR (a marker of asthma) n- 330 (n180 asthma , n 150 allergic rhinitis). Mean age 12yr. M:F 184:164 Criteria: prior documented asthma or allergic rhinitis diagnosis in primary care paediatrician. Excl: neg skin prick test, recent infection, steroids	Index: FENO >34ppb Other: spirometry (FEV1,FVC, FEF 25-75%) Standard: Objective BDR. ≥12% Diagnosis of asthma at entry into study: classified by physician, diagnosed using GINA criteria (no raw data in paper)	FeNO to predict BDR: S 80% sp 77% PPV26% NPV97% Summary: -Median FENO: asthma 34ppb vs allergic rhinitis 27ppb (p<0.001) -Median spiro value (FEV1/FVC&FEF25-75%) higher in allergic rhinitis (95%, 88%, 73% respectively) than asthma group (92%, 81%, 69%) -FeNO correlated with ΔFEV1 post BDR test (r=0.69, p<0.001) - both FeNO and perennial sensitisation predicted BDR	Note: paper only assesses FeNO to predict asthma in those with BDR. Not all patients with asthma show significant BDR, therefore ability of FeNO to predict asthma in this cohort would need a separate study. Also the study shows BDR is also positive in >1/3rd of allergic rhinitis patients, so not a specific marker of asthma.
Sivan et al (2009)(137)	Observational cohort Aim: assess FeNO in asthma diagnosis. N 150 (at follow up: N 69 steroid naïve asthma, N 44 not asthma, N 37 asthma on ICS) . Age 5-18 Criteria: symptomatic >12wks, referral as ?asthma to OPC and able to complete all 3 tests; (spiro/FeNO /induced sputum. Excl: conditions that affect FeNO or SpE (recent LRTI, systemic signs atopy i.e. anaphylaxis/angioedema /urticarial) [note: allergic rhinitis and eczema not excluded as common in asthma)	Index: FENO >18ppb Other tests:FEV1<80, FEV1/FVC ratio <75%, SpE>3% Standard: Subjective diagnosis at 18month+ of follow up. Clinical decision utilising: Clinical history/exam,&/or BDR ≥15% &/or provocation test if available.	FeNO: S 82% sp 84% PPV 89% NPV 75% Summary: - FeNO + SpE best diagnostic predictor. -FeNO higher in steroid-naïve asthma (p<.0001) -FeNO higher in symptomatic asthma vs symptomatic no asthma group -FeNO good predictor of SpE	
Louhelainen et al (2008 (136)	See adult subsection above			
Smith et al (2004)(92)	See adult subsection above			

Cordeiro et al(2011) (124)	See adult subsection above			
Additional papers: March 2017 – June2018				
Feng-Jia(2017)(212)	Retrospective Observational study. Aim: assess FeNO to differentiate chronic cough and cough variant asthma (CVA) and assess optimal FeNO cut off. N 450 chronic dry cough patients (n 150 diagnosed as CVA by test standard) Criteria: Age 18-75yrs, chronic dry cough >8wks, no wheeze or SOB, normal CXR, no po steroids >8wks, non-smoking >6months. No prior lung diagnosis (i.e. asthma, COPD)	Index: FeNO Other tests: spirometry (MEF, MEF25-75. FVC), BHRhist PC20≤8mg/ml Standard: Objective CVA if: BHR AND no other respiratory symptoms i.e. wheeze/sob	Best FeNO to distinguish between groups. FeNO>25ppb: S 81 sp 84. Summary: -Median FeNO: 42ppb (CVA grp) Vs 16ppb (chronic cough no asthma grp) (p<0.05)	**Note: small airway function (MEF, MEF25,50,75) lower in CVA vs non CVA croup (p<0.05). interestingly measure of larger airways FEV1 not statistically different between groups.**
Murray et al(2017) (43)	Observational study. Data collected from prospective population-based cohort. Aim: assess diagnostic value of FeNO in symptomatic asthma patients not on regular ICS and assess NICE guide diagnostic algorithm.. N 630 (n 74 asthma on test standard, n 403 not asthma, n 153 possible asthma). Age: 13-16yr (mean 15.5yr) Excl: regular ICS	Index; FeNO ≥35ppb Other: FEV1/FVC ratio <70%, BDR≥12% Standard: objective physician diagnosis AND current wheeze(within 12mn) AND requiring asthma medication (within 12mn)(non-asthma controls were negative to all three)	-FeNO≥35ppb to predict asthma: S 52% sp 83% PPV 35% NPV 91% -Re adjusted for ≥24ppb: S 63% sp 73% PPV 29% NPV 92%) - FeNO was independently associated with asthma (p <0.0001)	Note: authors suggest combination testing with: FEV1/FVC<83.8% and BDR≥3.48% and FeNO≥24ppb is better than current NICE algorithm for asthma diagnosis in children. However only 62% of their cohort completed all three tests so could not be used in 38% of children.

f) Sputum Eosinophils

Author	Study design	Index Test / Test Standard	Outcome / summary	Other info
Adults: asthma diagnosis: Index SpE				
Fortuna et al 2007(93)	Prospective observational study. Aim: Comparing diagnostic accuracy of spiro, FeNO & SpE. N 50 (n 22 asthma on test standard). Age 18-68yr. Incl: asthma symptoms Excl: steroid (ISC/PO)	Index: E≥3% Other:FEV1<80% &/OR ratio <75%, FeNO ≥20 Standard: objective BHRmeth (PD20 ≤16mg/ml)	SpE: S 41 sp 75 PPV 56 NPV61 Summary: - SpE had the worse PPV of all tests	Note: -The addition of SpE to FeNO increased specificity and diagnostic accuracy - The addition of spirometry to SpE did not improve diagnostic accuracy
Smith et al (2004)(92)	Prospective observational cohort. Aim: Compare tests in asthma diagnosis pre & post po steroids course. N 47 (n17 asthma, n30 not asthma by test standard). Age 9-75yr. Incl: symptoms of asthma >6weeks, referred from GP. Excl: recent steroids (<4weeks steroid free)	Index: SpE>3% Other Tests: FEV1/FVC<70%, FeNO (>20ppb), PEFv: amplitude %mean≥20% Test Standard: objective symptoms (ATS criteria) plus BHR(4.5% saline)PD15 and/or BDR≥12%	SpE >3%: 40/47 samples achieved. S.86% sp.88% PPV.80% NPV.92% Summary: - FeNO and SpE were superior to standard asthma tests (all other conventional tests had sensitivities ≤35%	note: authors found 76% of their asthma patients were atopic compared to 43% in non-asthma group. (SPT)
Louhelainen et al (08)(136)	Observational study Aim: review SpE, sputum 8-Isoprostane and FeNO in patients with new asthma Vs controls. N 65 (n 23 asthma [paediatric], n 13 control [paediatric], n 14 asthma [adult], n 15 control [adult]) plus n11 COPD included as a positive disease control	Index: SpE Other tests:FeNO, isoprostane, Standard: objective asthma symptoms plus any of: 1.post exercise drop in PEF or FEV1 ≥15%, BDR ≥12%, BHRhist PD15 <0.4	Summary: -Asthma group had raised SpE compares to control: children 2.4% vs 1.4% (not statistically significant) Adults 10.4% vs 0.2% (p=0.005) -correlation between FENO and eosinophils (p <0.0001)	Note: atopic status tested by SPT to ≥1 aeroallergens(wheal ≥3mm)

	grp. Age: 7-72yrs.			

Children: asthma diagnosis: Index SpE

Sivan et al2009(137)	Observational cohort Aim: evaluate FeNO and other in asthma diagnosis. N=150 (at diagnosis at end of study >>n 69 steroid naïve asthma, n 44 no asthma, n 37 with asthma and put on controller inhalers). Age: 5-18yr. Incl: asthma symptoms. Excl: not able to perform all 3 tests.	Index: SpE 3% Other: Spiro (FEV1<80 or Ratio <75%), FeNO >18ppb. Standard: subjective Clinical history/exam, or variability FEV1>15%, or provocation test if available	SpE >2.7%:S 81% sp 92% PPV 89%NPV 85% Summary: -FeNO plus SpE better than spirometry -FeNO and SpE higher in steroid-naïve asthma (p<.0001) -mean FeNO and SpE are significantly higher in untreated asthma group than non-asthma group.	Note: -FeNO and SpE performed better than FEV1. No data on FEV1/FVC – which is the recommended spirometry measure.
Louhelainen et al(2008) (136)	See adult section above			
Smith et al (2004)(92)	See adult section above			

g) Skin prick testing

Author	Study design	Index Test / Test Standard	Outcome / summary	Other info
Adults: SPT				
Soriano et al(1999)(139)	Cross-sectional study (random sample of population) Aim; determine risk of asthma attributable to atopy. N 1816 Age 20-44yr	Index: SPT to 5 aeroallergens Other: Sp IgEs >0.35kU/l Standard: objective BHRmeth PD20 <8umol AND symptomatic	Summary: -"Percentage of the population attributable risk (PAR) of atopy in explaining asthma is 41.97%" - atopy diagnosed by SPT OR Sp IgE testing was shown to be an independent predictor of bronchial hyperresponsiveness. -dust mite and timothy grass had highest association with asthma.	
Tschopp et al(1998) (138)	Observational study. Random selection of population. Aim; assess diagnostic efficiency of IgE, SPT, & "Phadiatop" in diagnosing allergic asthma" N 8329. Age 18-60yrs Incl: results available to all 3 tests	Index: total IgE ≥100kU/l and Phadiatop (tests specific IgE to common aeroallergens) Other: -SPT (≥1 out of 8panel: ≥3mm wheal) Test Standard: subjective Asthma diagnosis categorised into 3 groups: a)doctor diagnosed asthma **(DA):** PMH asthma & doctor confirmed previously b) Current asthma **(CA):** DA+ symptoms in last 12mnths c) Current Allergic Asthma **(CAA):** CA with symptoms associated with specific trigger	SPT to predict CAA: S 65.4% sp 77.8% PPV 5.2% NPV 99.2%, diagnostic efficiency 77.6% Summary: - SPT 65% ability to diagnose asthma -SPT best PPV for CAA - SPT significantly better than IgE to diagnose CAA (s 85.9% vs 81.4%) - SPT and Total IgE better diagnostic accuracy in CAA compared with specific IgE - no difference in specificity between IgE and SPT to predict CA	Note: PPV low compared to other studies, this is perhaps due to low prevalence of allergic diseases in cohort compared to other studies that look at patients selected from an allergy clinic.
Popovic et al(2002)(94) Only Abstract reviewed	Retrospective observational study. Aim: determine most useful tests in asthma diagnosis. Incl: patients with dyspnoea. N 195 (n 141 asthma on test standard)+ N18 controls	Index: SPT Other: BHR, IgE, spiro, SpE, Bl.E	SPT to predict asthma: S 62%, PPV 81%, diagnostic accuracy 62%. Summary: - second most efficient test after BHR -diagnostic accuracy 62%. All other tests <50% (except BHR) -authors conclude that it is not possible to use skin sensitisation to aeroallergens to diagnose asthma.	
Children: SPT				

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Drkulec et al (2013)(141)	Observational study Aim: sensitisation markers to differentiate allergic asthma from non-specific chronic cough N 131 (allergic asthma grp n 71 Vs no-sp symptoms grp n60) Median 7.5yrs (1-15yrs). Criteria: attended allergy&pulmonology clinic with respiratory symptoms.	Index: SPT Other: total IgE, Sp IgE to three aeroallergens (dust mite/ragwort/timothy grass) Standard: subjective reported symptoms of wheeze (≥3 episodes) OR BHRc (cough grp diagnosed based on: cough >6wks plus <3 episodes of wheeze.	Summary: -Total IgE and specific IgEs higher in allergic asthma group. -Sp IgEs had a better diagnostic value than total IgE -SPT S 78.8%. (sensitivity to differentiate between groups is 91.3%) -In both sp IGE and SPT the best aeroallergen for predicting asthma was dust mite	Note: Asthma test standard not very comprehensive.

h) Immunoglobulin E (IgE)

Author	Study design	Index Test / Test Standard	Outcome / summary	Other info
Adults				
Abraham et al(2007) (140)	Cross-sectional observational. Aim: assess association of asthma with seroatopy N 702 (n 140 doctor diagnosed asthma pre study) Age 21-49yr. Criteria: women in second/third trimester	Index: Seroatopy (defined as IgE specific to any of the 9 allergens tested: ≥35kU/L) Standard: : subjective Previous diagnosis of asthma	Summary: -Those with asthma were more at risk of seroatopy than those without asthma (OR 2.12	
Linneberg et al(2006) (142)	Observational study. Aim: review allergy screen assay compared to SPT in diagnosis of allergic respiratory disease N 709 Criteria: subjects originally from random population sample (categorised into symptom grp and control group based on allergy questionnaire).	Index: **Allergy screen (AS) assay** "ADVIA Centaur" – panel includes 19inhalent allergens Other: SPT-10 allergen panel) Standard: : objective ΔAllergic Asthma based on allergy questionnaire PLUS positive SPT to allergen which related to patient symptoms also	Summary: -AS to predict allergic asthma (defined by positive assay to any of; dust mite/animal /pollen): S93% sp 67% PPV 27% NPV98% accuracy 70%. - Allergy Assay compared to SPT: S 86% sp 96% PPV 94% NPV 89% - IgE assay has good concordance with SPT (sensitivity range: 85% and increasing to 100% in patients with ≥4 positive SPT results). - AS is of value in demonstrating allergic asthma and may be a more accessible diagnostic tool than SPT.	Note: -Test standard not very comprehensive.
Plaschke et al (1999)(143)	Cross sectional observational study. (random sample of the population.) Aim; assess association between atopic sensitization and asthma/BHR N 1859 Age 20-44yrs	Index: IgE total & specific Other: SPT, BHRmeth PD20 ≤2mg *Standard:* : subjective history of physician diagnosed asthma PLUS recent symptoms	Summary: - ≥1 positive sp IgE was x2.5 more common in asthma group - positive SPT and sp IgE were more prevalent in subjects with asthma than in subjects without asthma for all allergens - SPT and specific IgEs had comparable results -"asthma was found twice as frequently in subjects with atopy "	Note: -use high cut off on BHR, this could result in false positives in asthma group.
Soriano et al(139)	Cross-sectional study, random sample of population Aim; determine attributable risk of asthma due to atopy. N1816 Age 20-44yr	Index: Sp IgEs >0.35kU/l and/or SPT (panel of 5 aeroallergen) Standard: : objective BHRmeth PD20 <8umol AND symptomatic	Summary: -"Percentage of the population attributable risk (PAR) of atopy in explaining asthma is 41.97%" - atopy was diagnosed by SPT **OR** Sp IgE testing and was shown to be an independent predictor of bronchial hyperresponsiveness. -dust mite and timothy grass had highest association with asthma.	

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Tschoppl et al(1998) (138)	Observational study. Random selection of population. Aim; assess diagnostic efficiency of IgE, SPT, & "Phadiatop" in diagnosing allergic asthma" N 8329. Age 18-60yrs Incl: results available to all 3 tests	Index: total IgE ≥100kU/l and Phadiatop (tests specific IgE to common aeroallergens) Other: -SPT (≥1 out of 8panel: ≥3mm wheal) Test Standard: : subjective Asthma diagnosis categorised into 3 groups: a)doctor diagnosed asthma (**DA**): PMH asthma & doctor confirmed previously b) Current asthma (**CA**): DA+ symptoms in last 12mnths c) Current Allergic Asthma (**CAA**): CA with symptoms associated with specific trigger	Sp IgE: S 72.5% sp 71.9% PPV 4.6% NPV 99.3%, diagnostic efficiency 71.9% Total IgE: S 56.9% sp 77.9% PPV4.6% NPV 99%, diagnostic efficiency 77.6% Summary: - SPT significantly higher than IgE to diagnose allergic asthma (s 85.9% vs 81.4%) - SPT and Total IgE better diagnostic accuracy in CAA compared with specific IgE - no difference in specificity between IgE and SPT to predict asthma	Note: PPV low compared to other studies, this is perhaps due to low prevalence of allergic diseases in cohort compared to other studies that look at patients selected from an allergy clinic.
Cordeiro et al (2011)(1 24)	Prospective, Observational cross-sectional study Aim: assess FeNO and IgE in asthma diagnosis. N 114 (n 42 asthma and n 72 non asthma on test standard) Age 7-87yrs Inclu: Symptomatic steroid naive patients	Index: IgE Other: FeNO Standard; : objective BDR ≥12% or if neg BHR histamine PC20	Summary: -"FeNO positively correlates with symptoms, airflow reversibility, and total IgE - Asthma group had significantly higher IgE compared to non-asthma group, 239kU/L vs 47kU/L respectively (p<0.001)	
Obaidi et al (2008)(1 44)	Cross-sectional study Aim: clarify relationship between IgE and Asthma N 562 Age: 17-52yrs. From asthma/allergy OPC	Index: Total IgE standard: : subjective physician diagnosed using NHLBI/WHO criteria.	Summary: -mean IgE 554IU/ml vs 69IU/ml in controls - authors conclude IgE may be able to predict asthma from non-asthma - serum IgE was normal in 5.9**%** of asthmatic patients. - inverse correlation between IgE levels and FEV1 (P< 0.0001).	
Popovic et al 2003(94) Only abstract obtaina ble	Retrospective observational study. Aim: determine most useful tests in asthma diagnosis. Incl: patients with dyspnoea. N 195 (n 141 asthma on test standard)+ N18 controls	Index Test: Total IgE Other: BHR, SPT, IgE, Sp.E, blood eosinophils (Bl.E)	Total IgE to predict asthma:- S <50% / PPV 72% / Summary: - diagnostic accuracy <50% in all tests (except BHR & SPT) -authors conclude that it is not possible to IgE to diagnose asthma.	
Children: Asthma diagnosis. Index IgE				

See Cordeiro et al (2011)(124)				
Drkulec et al (2013)(141)	Observational study Aim: sensitisation markers to differentiate allergic asthma from non-specific chronic cough N 131 (allergic asthma grp n 71 Vs no-sp symptoms grp n60) Median 7.5yrs (1-15yrs). Criteria: attended allergy&pulmonology clinic with respiratory symptoms.	Index: Total IgE, Sp IgE to three aeroallergens Other: SPT Standard: : subjective reported symptoms of wheeze (≥3 episodes) OR BHRc (cough grp diagnosed based on: cough >6wks plus <3 episodes of wheeze.	Total IgE cut off to differentiate asthma from chronic 116.6kIU/L s 96.8 Sp 77.8% Summary: -Total IgE and specific IgEs higher in allergic asthma group. -Sp IgEs had a better diagnostic value than total IgE -SPT S 78.8%. (sensitivity to differentiate between groups is 91.3%) -In both sp IGE and SPT the best aeroallergen for predicting asthma was dust mite	Note: Asthma test standard not very comprehensive.

i) Airways Oscillometry - IOS

Author	Study design	Index Test / Test Standard	Outcome / summary	Other info
Adults: asthma diagnosis. Index Test: IOS				
Nil				
Adults: General studies including IOS and asthma patients				
Manoharan(2016)(152)	Single centre randomised open label crossover study. Aim: compare repeated dose effect of small and large particle inhalers using IOS. N 16. Mean age: 43yrs Incl: persisting asthma AND mean total airway resistance at 5Hz (R5) >150% predicted despite ICS, plus FEV1>60 Excl: smokers or >10pc yr hx, exacerbation <3mnths,	Index: Primary outcome: %Δ R5-R20 from baseline (used to represent peripheral airway changes) Secondary outcome: change from baseline in other IOS variables (R5, R20, X5, RF) Asthma Standard: subjective PMH of asthma	Summary: - Small particle inhaler was associated with improvements in IOS in all measurements (i.e. R5-20,R5,R20,X5 etc.) - Significant %change from baseline in R5 and R5-R20 post BD (p0.003 and p0.004 respectively)	Note: - significant change in IOS in small particle inhaler was not demonstrated with FEV1/ FEF25-75). This indirectly implies that IOS is superior at assessing smaller distal airway pathophysiology compared with spirometry
Short(2012)(153) <mark>Abstract only</mark>	Aim: compare IOS vs 'spiro' (FEV1 or FEF25-75) at assessing bronchoconstriction and bronchodilation N 13. Mean age: 34yrs. Incl: mild/mod stable asthma Excl: high dose ICS (>1000ug/day)	Index: IOS (R5, R5-R20, AX,RF) Standard: N/A spiro (FEV1 and FEF25-75)	Summary: - IOS more sensitive than spirometry to detect bronchoconstriction and dilatation in asthma patients. - all IOS indices significantly worse after bronchoconstriction challenge with beta-blocker. - Largest change observed in R5 and RF (mean %change 30.8% and 39.4 respectively)	Note: -IOS could have a role in asthma diagnosis. -this study uses FEV1 and FEF25-75 and not FEV1/FVC ratio (NICE guidelines recommend ratio for asthma diagnosis). Further studies needed to validate IOS against current standard (FEV1/FVC)
Yamagguchi(2009)(154)	Open-label randomised study. Aim: assess fine particle steroids effect on small airways in asthma using IOS, spirometry, and BHR. N 38. Mean 44yr Criteria: consecutive patients referred to asthma OPC with steroid naïve mild/mod asthma. Excl: >5pack yr or smoking in last 12months. Abnormal CXR.	Index: IOS (R5, R20, R5-R20, AX). AX was measured as a sensitive measure of small airway obstruction Spiro (FEV1, FEF25-75, PEF, RV, RV/TLC), BHRmeth Standard: ?Subjective ATS statement on asthma and COPD diagnosis produced in 1989.	Summary: - good correlation between FEF25-75 (a marker of peripheral airways obstruction) and R5-R20 were demonstrated supporting use of IOS as a measure of the small airways disease. - IOS (R5-R20) detected improvements in small airway obstruction post treatment.	Note: whilst study doesn't aim to assess IOS as a diagnostic tool, it is evident that IOS can detect both peripheral and proximal airflow obstruction and it could therefore be a useful tool in asthma diagnosis.
Children: asthma diagnosis. Index Test: IOS				

Shin et al (2012)(213)	Multicentre cohort study Aim: compare spirometry to IOS as a measure of BD response in asthma. N 30 + 29 controls. Age 2-6yr.	Index: IOS: Respiratory resistance at 5Hz (Rrs5) and percent change in respiratory resistance at 5Hz of initial (ΔRrs5%int) ≥29% and respiratory reactance at 5Hz (Xrs5) Other; Spiro- FEV1 and FEF25-75 Standard: Subjective prior history of asthma and under follow up	ΔRrs5int ≥29% - S33% sp100% ΔRrs5int≥15.6% S87% sp62% Summary: -ΔRrs5%init: using percent change of initial measurement was found to differentiate asthma from control group best compared with using absolute difference or percentage of predicted. - FEV1 performed better that IOS however when used as an adjunct to FEV1 sensitivity was higher than FEV1 alone for detecting asthma in children.	Note: -Using ΔRrs5int≥15.6% cut off: sensitivity and specificity was 87% and 62% suggesting IOS may be more sensitive as a rule out test when negative.. More work looking at cut off values for a 'positive' result is needed to satisfy the balance between test sensitivity and specificity.
Children: General studies including IOS and asthma patients				
Schulze (2016)(145)	Non-blind cross-sectional study Aim: comparison of IOS against standard tests in predicting asthma exacerbations. N 69. Age 4-7yr. recruited from allergy/pulmonology OPC. Criteria: ≥3 episodes of wheeze lasting more than 3days or ≥1 prolonged wheezy episode requiring ICS or LTRA in the last 12months. Excl: infection <4wks.	Index: IOS %predicted (presented as absolute values adjusted for height & weight) Test standard: FEV1, FEV1/FVC ratio, BHR Standard for asthma: subjective Physician diagnosed.	Ability to predicted exacerbation: Rrs5:S 68% sp 83%, accuracy 78% (p<0.001), Xrs5: S 29% sp 100% Rrs5-20: S 90% sp 57% Summary: - IOS predicted the probability of asthma exacerbation in children and was superior to FEV1 and methacholine challenge.	Note: physician diagnosed asthma – but all children also had Methacholine PD20 in the study.
Mondal (2016)(148)	Aim: interpret BHRmeth using IOS instead of spirometry to assess different inhaler effectiveness N 10. Age 4-11 (mean 8yr) Criteria: only proceeded to BHRmeth if BDR negative (<20%incr in R5 c saline) Incl; regular ICS continued (8/10 were on ICS) Excl; unstable or severe asthma or admission <12mnths. also SABA, LABA,LT, antiHistamines stopped at specific time pre visits.	Index: IOS: R5. IOS positive if: MC40R5 <8mg/ml (provocation conc ≤8mg/ml causing incr airway resistance (Rrs) >40% (test was stopped at this dose and recorded neg if not achieved cut-off at visit 1, in visits 2/3 with test inhalers concn attempted upto 64mg/ml or cut off. Standard: subjective Physician diagnosed asthma	Mean baseline IOS (R5 = 6.9cmH2O/L/sec) – no healthy control to compare to. Summary: -Significant BDR detected by R5 -"baseline total airway resistance (R5) remained constant each study day for each subject, indicating reproducibility of IOS"	Note: - study relevant because visit 1 looked at IOS pre and post BD (to assess BDR).

Nieto(2006)(149)	Aim: measure lung function response to montelukast by IOS. N 46 (23treated 23 not treated) Age: children 5+ yrs Incl: mild asthma, baseline Bronchodilating response of at least: >12%FEV1,or>12%PEF, >25% FEF25-75, >25% in Xrs5	Index: IOS %predicted: Rrs5, Rrs20, Rrs5-Rrs20, Xrs5 Standard: n/a Spiro (FEV1, FEF25-75, orPEF)	Summary: -Rrs5: 21% change from baseline in montelukast group supporting the use of montelukast. - IOS is a sensitive test for demonstrating changes in lung physiology in asthma patients.	
Ortiz(2002)(151)	Placebo-controlled, randomised, crossover Aim: assess IOS ability to measure BDR in children N 10. Age 2-5yrs Criteria: PMHx reactive airways disease receiving bronchodilators Excl: regular ICS	Index: IOS: X5 Standard: subjective Physician diagnosed asthma	X5 values significantly different (p<0.01) after salbuterol bronchodilation compared to placebo in asthmatic children. Summary: -IOS may have a role in BDR testing in those unable to perform spirometry	
Kim(2013)(214)	Aim: assess IOS and FeNO in eosinophilic bronchitis (EB) and asthma patients. N 341 (n232 asthma, n109EB) +N 115 healthy controls. Mean age: 9yrs Excl: recent LRTI <6wks.	Index: IOS Other: SpE, bl.E, IgE (total and sp), spirometry, FeNO Standard: objective symptoms plus: BDR ≥12% OR BHRmeth PC20≤16	Summary: -R5%predicted highest in asthma group (102.5%) vs EB 90.9% or vs HC 88.2%) (p<0.05) - X5%predicted highest in asthma group (133%) vs EB (114%) or vs HC 106%) (p<0.05) - Both R5 and X5 are significantly higher in asthma compared to EB. (p<0.0001)	Note: BDR measured with IOS and FeNO are both also raised in EB therefore these tests should be interpreted with caution in asthma diagnosis as they are not specific to asthma.
Olaguibel(2005)(150) abstract only	Aim: assess IOS repeatability and differences between atopic and non-atopic asthma N 33. Age 3-6yr	Index: IOS : Rrs5, Rrs20, Xrs5 Standard: objective Spiro (FEV1) Whole body plethysmography (sRaw)	Summary: - IOS is a reproducible and sensitive marker of lung function	Note: IOS well tolerated by young children
Cross-referenced				
Gonem et al(2013) (155)	Aim; assess small airway obstruction markers in predicting asthma severity, control and predicting exacerbations. N 74 asthma (+ n18 HC) Excl; smokers (incl anyone smoked in last 12mnths or >10pack yr)	Index: MBW SF-6 (Wash in method) Other Tests: IOS, spiro, body box Standard: subjective physician diagnosed –"using BTS criteria."	Summary: - markers of airway resistance: total (R5) and mean of large and small airways (R20) were higher in severe asthma group compared with the mild-moderate group (0.47 vs. 0.37, P < 0.05 for R5; 0.39 vs. 0.31, P < 0.01 for R20). -small airway obstruction markers (Sacin, R5-20 and AX) did not differentiate severity of asthma.	Note: Sequence of tests: Bronchodilator administered > IOS >>MBW >> lung volumes (Body box) >> spirometry MBW: 12breaths/min, constant tidal volumes 1L, triplicate test)
Abbreviation key: <u>R5</u> (total airway resistance at 5Hz), <u>R5-20</u> (peripheral airway resistance as the difference between 5 and 20hz), <u>R20</u> (central airway resistance at 20Hz), <u>RF</u> (resonant frequency), <u>X5</u> (total airway reactance at 5hz) <u>AX</u> (reactance area under the curve)				

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j)	**Airways Oscillometry - FOT**			
Author	Study design	Index Test / Test Standard	Outcome / summary	Other info
Adults: asthma diagnosis. Index Test: **FOT**				
Heijkens kjold rentzho g et al (2017)(62)	Aim: determine ability of FOT to diagnose asthma and its association with spirometry. Asthma N234 healthy control n60 Age 13-39years sub analysis of those ≥18yrs. No sub analysis of paediatric cohort	Index; FOT (ResTech, milan, Italy): R5, R19, X5,Fres, R5-R19, Used linear regression with each variable to compare mean across two groups as no defined reference values Standard; subjective clinician diagnosed asthma.	Higher R5 (3.31 [1.95,5.62]) and R19 (2.54 [1.65,3.91]) associated with asthma diagnosis. -higher R5, R19, R5-19, and fres was related to higher likelihood of having asthma -lower X5 was related to higher likelihood of having asthma -AUC>0.70 in the ROC analysis of asthma vs control was only seen in R5 in univariate analysis.	Note- -sub analysis on those ≥18yrs -all asthma group were on inhaled corticosteroids &/0r LTRA -Some children in analysis but not enough to analyse children separate from adults.
Children: asthma diagnosis. Index Test: **FOT**				
Evans et al(156)	Aim: determine which FOT outcomes best differentiate asthma from control population. Standardised FOT using Calogero et al reference population to produce z score reported as mean+/-s.d Asthma n99, control n200 . Children 3-7yrs	Index: FOT i22m, chess medical, Belgium: Xrs6,8,10, AX, Rrs6,8,10, Fres, Fdep, standard: subjective clinical doctor diagnosed asthma	-Fres best able to discriminate healthy control from asthma -Fres, AX and Xrs6 able to distinguish asthma from healthy control -Rrs6 and Rrs4-24 unable to distinguish asthma from control -Rrs6 not able to distinguish asthma from control -no signif difference between asthma and CF in any FOT parameter to determine between the two -children born pre-term had higher resistance and more negative reactance values	Note; -all asthma group were identified in acute setting or specialist OP clinics and all on inhaled steroid. This may mask some of the ability of FOT to distinguish between asthma and healthy group
Starcze wska-Dymek et al. (2018)(158)	Aim: 1/ review Rrs and Xrs in asthma compared to healthy controls, Children: n53 uncontrolled asthma, n 53 controlled asthma, n 45 healthy control. (age 2-6 years)	Index: Rrs8 and Xrs8, Resmon pro device (Italy). standard: subjective Martinez criteria	Both Xrs and Rrs differentiated between uncontrolled asthma, controlled asthma and healthy groups (p <0.05)	
Starcze wska-Dymek et al. (2019)(159)	Same cohort as above(158)	Same as cohort above	71.6% asthma patients had a positive test opposed to 7.7% healthy controls using the Calogero reference ranges	Used reference ranges defined by
Heijkens kjold rentzho g et al.(62)	See section above (children not analysed separate from adults)			-

Abbreviation key: R5 (total airway resistance at 5Hz), **R5-20** (peripheral airway resistance as the difference between 5 and 20hz), **R20** (central airway resistance at 20Hz), **RF** (resonant frequency), **X5** (total airway reactance at 5hz) **AX** (reactance area under the curve)

8.2. Appendix B: RADicA protocol (summarised version)

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

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:

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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.

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.

Primary Endpoints:
<u>Primary objective 1a</u>**.** Determine the optimum diagnostic pathway for asthma based on conventional tests of large airway function and novel tests of small airway function.

<u>Asthma definition i:</u> 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".

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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.

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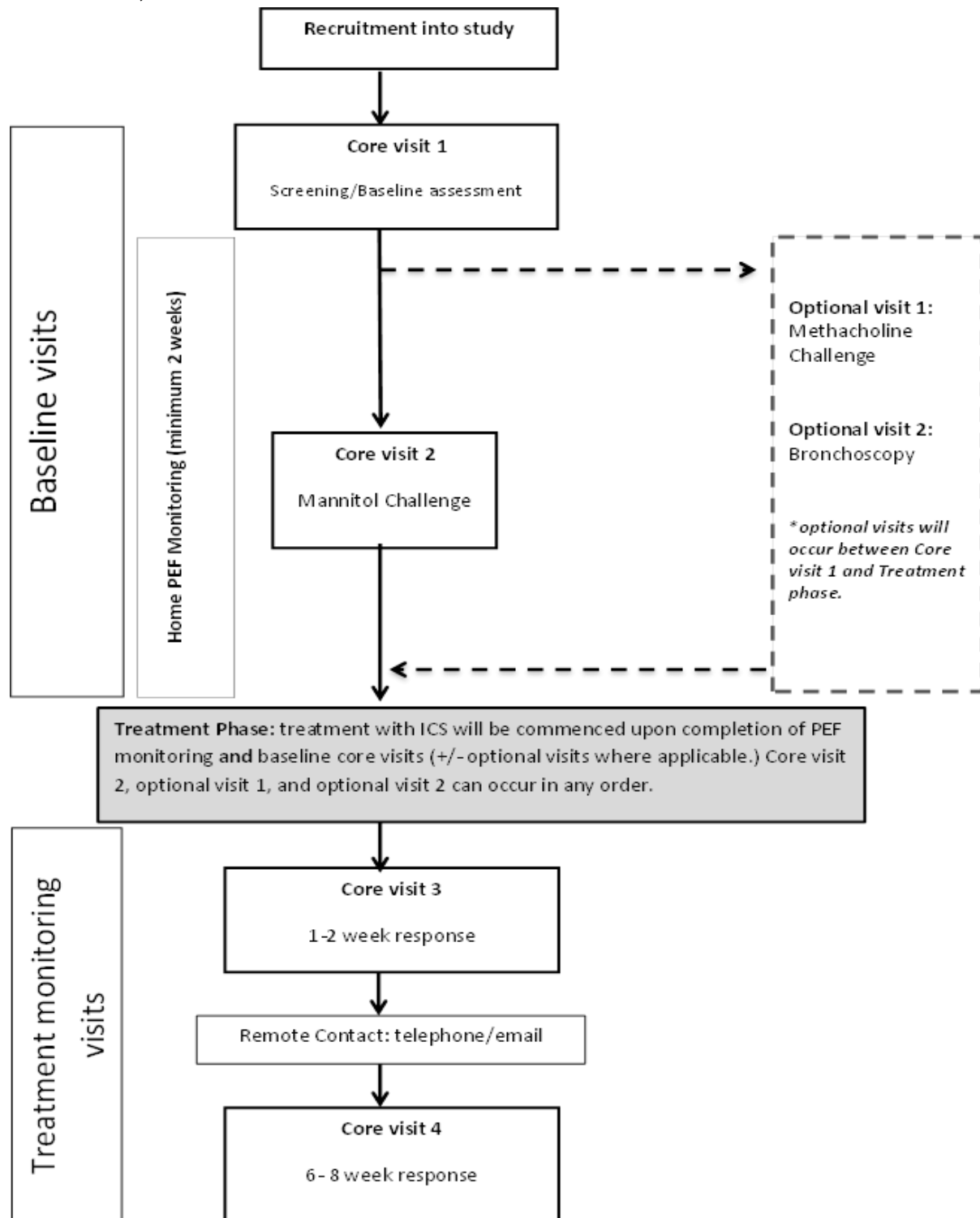
Visits	CV 1	(CV2/OV1/OV2)	Commence treatment period	CV 3	CV 4
Shortest study pathway	D 0	(Range D1- D14)	D 14	D 21	D 56
Longest study pathway	D 0	(Range D1 – D42)	D 42	D 56	D 98

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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.

Eligibility check and booking 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).

Core Visit 1

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.

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.

Core visit 2- Mannitol 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 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 core visit 2 will also be asked to refrain from any antihistamines for 72hrs before the appointment.

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-mannitol)

4.   PExA (pre-mannitol)

5.   MBW (pre-mannitol)

6.   Spirometry (pre-mannitol)

7.	Mannitol provocation challenge

8.	IOS (post-mannitol)

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 mannitol 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.

Optional visit 1- Methacholine 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 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.

1.	Symptoms (ACQ)

2.	IOS (pre-methacholine)

3.	Spirometry (pre-methacholine)

4.	Methacholine provocation challenge

5.	IOS (post-methacholine)

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.

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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.

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 Characteristics26.

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 Accuhaler26. 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)

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).

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.

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.   Mannitol 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.

Visit Schedule:

Visit name	Core Visit 1 (Screening/ baseline/ dispense treatment)	Core Visit 2 (Mannitol challenge)	Optional Visit 1 (Methacholine challenge)	Optional Visit 2 (Bronchoscopy)	Treatment Phase starts: (commence ICS)	Core Visit 3 (1-2week response)	Remote contact	Core Visit 4 (6-8 week response)
Demographics / Clinical history	✔							
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					✔			

Healthy Controls:

	Visits	V 1	V2
Recruitment into study			
	Shortest study pathway	D 0	D7
Visit 1 — Baseline assessment (D 0)			
	Longest study pathway	D 0	D84
Visit 2 — Reproducibility assessment (D7-D84)			
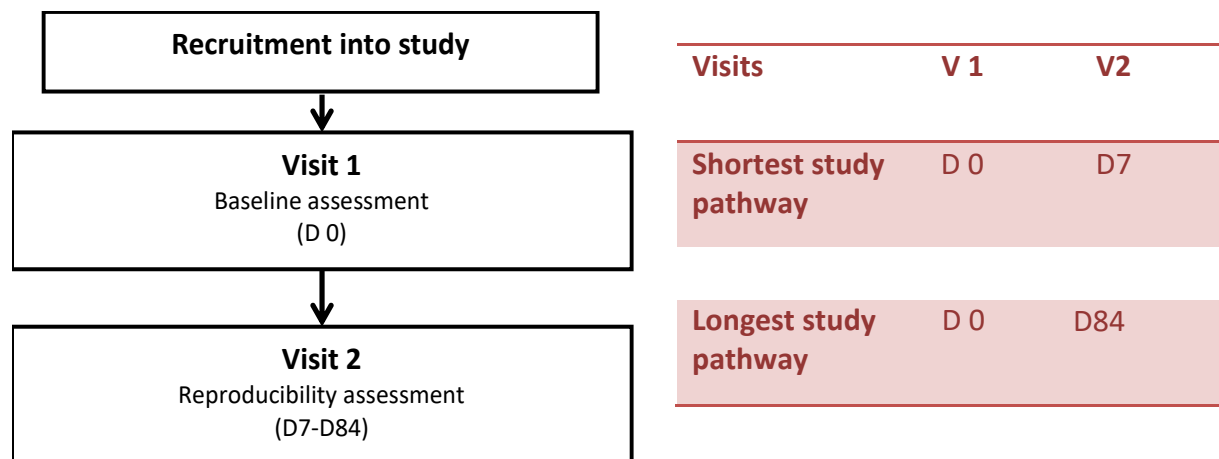
Recruitment:

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.

Eligibility check and booking:

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

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 treatment of asthma past or present
ii.   Significant respiratory, cardiac or other medical co-morbidity
iii. Any chest infection in the last 1 year needing antibiotics
iv.   Pregnant women
v.   >10 pk yrs smoking history
vi.   Recent antibiotic treatment for any cause within previous 4 weeks
vii. Active symptoms of rhinitis (with 2 weeks)

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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)

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

Visit schedule: Healthy volunteers

	Visit 1	Visit 2
Visit name	baseline assessment	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	✔	✔

Trial Outcome Measures:

	Test	Outcome measures	Established threshold for positive results
Symptoms	Asthma Control Questionnaire (ACQ)	ACQ-5	Change of 0.5(172)
Tests included in NICE algorithm(38)	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% <u>and</u> 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

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Other	Blood - eosinophils	Blood eosinophil count	$> 0.4 \times 10^9$/L(173)
	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 neutrophil %	≥2% eosinophils(134)
	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	

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:

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.

Nasopharyngeal Swabs

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

VOCs

VOCs will be analysed using mass spectrometry at the Manchester Institute for Biotechnology as previously described(215)

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.

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.

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.

Statistical Analysis Plan:

<u>Primary objective 1a</u>. 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 mannitol and/or methacholine)

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

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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. Appendix C: RADicA tests not described in main thesis

Procedure of Measurements in RADicA (not analysed within the Thesis)

Nasopharyngeal Swabs

Nasopharyngeal samples were taken from both nostrils in order to store samples for later virology profiling. Samplings were performed for each participant pre and post inhaled corticosteroid treatment (i.e., CV1 and CV4). After sampling, the collection tubes were immediately taken to the lab, logged into SLaB and stored at -80°C. In the event of any delay samples were put on ice. The Nasopharyngeal swabs will be analysed for metagenomics/viromics at the University of Manchester at a later date.

Multiple Breath Washout (MBW)

Lung clearance index (LCI) measurements were recorded using the modified Innocor LCI system using the mini-RVU (respiratory valve unit) via an open circuit (Innocor software version 8.1).(216) Participants were seated in an upright and comfortable position wearing a nose clip, they were asked to breathe through a mouth piece at a comfortable and steady rate. Whilst patients set their own tidal volumes, the technician closely monitored this to ensure a regular stable pattern with a reasonable tidal volume (approximately 10ml/kg) and expiration to the same relaxed functional residual capacity (FRC). Patients were guided with their breathing if it was seen to be unsteady. During the wash-in, a mixture of a blood soluble gas (N_2O) and an inert insoluble gas (0.2% sulphur hexafluoride (SF_6)) was supplied via the mouthpiece, until the concentration in their exhaled breath reached steady state (the wash-in phase). Wash-in was achieved when the difference between inspired and expired SF6 was <0.2%. Participants were then switched to breathing room air and encouraged to maintain the same steady respiratory pattern. A fan was turned on so that a stream of air was directed over the end of the flowmeter during washout. During the wash-out the concentration of SF6 in exhaled breath was recorded, a measure of 1/40 of the

original concentration (0.005%) marked the end of the wash-out. The test was repeated for a minimum of three times where possible.

multiple breath washout system

Multiple breath washout outcome measures:

Several parameters were derived from the raw MBW data using custom software, including LCI, Scond and Sacin. LCI is a measure of the number of 'lung turnovers' to reduce the inert gas concentration to $1/40^{th}$ of the initial concentration, calculated as the cumulative volume at the point end-tidal SF6 concentration falls below $1/40^{th}$ original concentration divided by the FRC. Scond is thought to represent ventilation inhomogeneity arising from conductive airway disease, while Sacin represents ventilation inhomogeneity arising from acinar airspace disease.

Experimental Biomarkers of small airway inflammation:

Particles in Expired Air (PExA)

Breath sampling of particles in expired air was measured using PExA 2.0. The PExA device was turned on and allowed to warm up to temperature range 36-37.5 degree centigrade. The participant was sat in a comfortable position with head positioned slightly back to prevent saliva from building up in the mouthpiece. A nose clip was applied. The participant was instructed to take at least three breaths before the first exhalation manoeuvre. The

participant was then instructed to breathe out as much as possible into the PExA device through the mouthpiece, then hold their breath for 5 seconds before a quick deep inspiration and immediate exhalation. Particles were collected during the final exhalation. The manoeuvre was repeated until 125ng were achieved (or 30 breaths if this occurred first). In younger children a smaller collection of 60ng (or 15-20 breathes) were accepted if they were finding it difficult to continue. Breath samples were collected onto a filter paper which was subsequently removed from the PEXA machine and stored in a collection tube, where greater than 100ng was achieved the filter paper was split into two in order to enable more extensive analysis. The collection tube(s) was then labelled and frozen for later analysis.

PExA

Particle bin	Acc. Particle	Acc. Mass [	Rel. Count	Rel. Mass ["
Particle 1	57395	3.428004086	0.1731791	0.027191
Particle 2	112109	11.90316512	0.3463661	0.094416
Particle 3	97210	21.90536215	0.3003351	0.173753
Particle 4	35943	17.4433007	0.1110482	0.13836
Particle 5	10052	9.440192424	0.0310562	0.07488
Particle 6	6833	16.66274296	0.0211103	0.132169
Particle 7	2178	18.14091906	0.0067289	0.143894
Particle 8	1352	27.14794611	0.0041762	0.215337
Sum	323672	126.07163		
Acc Volume	65.3 [l]			
Exhale Coun	20 [n]			

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Volatile Organic Compounds (VOC)

Breath sampling of expired volatile organic compounds was performed using the RECIVA breath sampler device (ReCIVA sampling Protocol v1.3). Exhaled volatile organic compounds (VOCs) contained in exhaled human breath were collected into the ReCIVA breath sampler (Owlstone, Cambridge, UK). Prior to patient sampling, background samples from room air and a 'glass head' in order to record VOCs from the mask, were completed. Temperature and humidity within the testing room was also recorded. The participant was asked a series of questions regarding recent exposures and last food and drink. The participant was then instructed to wear the face mask ensuring a good seal with no air leak. They were asked to breathe tidally during the sampling period and focus on breathing with an open mouth opposed to nose breathing (6-10 minutes). Samples were collected onto 10cm long steel tubes packed with adsorbent material (Tenax GR) that trapped the VOCs. The tubes were then stored at 4°C until analysis (within two weeks). Samples were analysed at the Manchester Institute of Biotechnology using our existing methodology(217).

Summary of test outcomes

Tests performed during RADicA (not used in thesis analysis)

Tests performed but not analysed in this thesis			
Tests of small airway function	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
	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 neutrophil %	≥2% eosinophils(134)

	*only performed if spontaneous sample obtained.		
	Sputum - other* only performed if spontaneous sample obtained.	Cell culture, immune cell count, measures of immune cell activation (Mbd2, CCL17, CCR4) YKL-40 (CHI3L1), AMCase (CHIA), Chitotriosidase (CHIT1)	Experimental (funding dependent)
	Serum specific IgE	allergen specific IgE to common inhalants (mite, cat, dog, grass, tree)	Atopic if 1 or more positive
	Nasopharyngeal swabs	Virology profile	Experimental

8.4. Appendix D: General Practices referring into RADicA

Barlow Medical Centre, lmslow Road
Manchester M20 2RN

Barrington Medical Centre
68 Barrington Road, Altrincham WA14 1JB

Benchill Medical Practice
171 Brownley Road, Manchester M22 9UH

Bodey Medical Centre
28 Ladybarn Lane, Manchester M14 6WP

Bowland Medical Practice
52 Bowland Road, Manchester M23 1JX

Brooklands Medical Practice
94-596 Altrincham Road, Manchester M23 9JH

Brownley Green Health Centre
171 Brownley Road, Manchester M22 9UH

Cheadle Medical Practice
1-5 Ashfield Crescent, Cheadle SK8 1BH

Chorlton Family Practice
1 Nicolas Road, Manchester M21 9NJ

City Health Centre
Second Floor (Boots) 32 Market Street, Manchester M1 1PL

Cornishway Group Practice
Forum Health, Manchester M22 5RX

Didsbury Medical Centre
45 Wilmslow Road, Manchester M20 6BA

Fallowfield Medical Centre
75 Ladybarn Lane, Manchester M14 6YL

Gatley Medical Centre
Old Hall Road, Cheadle SK8 4DG

Heaton Moor Medical Centre
2 Heaton Moor Road, Stockport SK4 4NX

Kingsway Medical Practice
720 Burnage Lane, Manchester M19 1UG

Ladybarn Group Practice
54 Briarfield Road, Manchester M20 4SS

Lostock Medical Centre
431 Barton Road, Manchester M32 9PA

The Maples Medical Centre
2 Scout Drive, Manchester M23 2SY

Northenden Group Practice
89 Palatine Road, Manchester M22 4DH

Northern Moor Medical Practice
216 Wythenshawe Road, Manchester M23 0PH

Park Medical Practice
119 Park Road, Altrincham WA15 6QQ

Peel Hall Medical Practice
Forum Health Simonsway, Manchester M22 5RX

Peterloo Medical Centre
133 Manchester Old Road, Manchester M24 4DZ

Spring House Surgery
555 Chorley Old Road, Bolton BL1 6AF

The Alexandra Practice
65 Wilbraham Road M16 8NG

The Range Medical Centre
Withington Road, Manchester M16 8EE

Tregenna Group Practice
399 Portway, Manchester M22 0EP

Unsworth Medical Centre
Parr Lane, Bury BL9 8JR

Valentine Medical Centre
2 Smethurst Street, Manchester M9 8PP

Wellfield Health Centre
116 Oldham Road, Rochdale OL11 1AD

Washway Road Medical Centre
67 Washway Road, Sale M33 7SS

West Timperley Medical Centre
21 Dawson Road Broadheath, Altrincham WA14 5PF

The Wilbraham Surgery
515 Wilbraham Road, Manchester M21 0UF

8.5. Appendix E: Data on patients excluded from analysis

Summary tables are displayed for all patients excluded from the analysis

1) **Description of patients with EPOER insufficient evidence (see Table a below)**

2) **Description of patients with EPOER possible asthma (see table b below)**

Table 86.

Table a) Description of patients with EPOER insufficient evidence

ID	Status: completed study/withdrawn (reason)	Data Missing - (Reason for missing data)	Patient Data		
			Test	result	result
RAD003 Child 11 year	Withdrawn CV1/2/3, OV1 (DNA CV4)	1. treatment response - (Non-compliant with ICS)	FEV1/FVC BDR FeNO PEFv PEFv (alt) Wheeze heard BCTmeth BCTmann Eos SPT	90.41 1% 15ppb 21.3% 3 Day (>20%) No PD20 1.92 11.1%drop 0.56 x10⁹/L 0/8	Neg Neg Neg Pos Pos Neg Neg Pos Neg
RAD004 Child 10 year	Withdrawn CV1/2, OV1 (DNA CV3)	1. PEFv monitoring - (Non-compliant) 2. Treatment response - (DNA)	FEV1/FVC BDR FeNO PEFv PEFv (alt) Wheeze heard BCTmeth BCTmann Eos spt	84% 8% 16 / 26 - - no 0.544 5.2% drop 0.83 3/8	Neg Neg Neg - - Neg Neg Neg Pos Pos
RAD023 Child 5 year	Withdrawn CV1 (DNA CV2)	1. PEFv monitoring - (DNA) 2. SPT - (DNA) 3. BCT - (DNA) 4. Treatment response - (DNA)	FEV1/FVC BDR FeNO PEFv PEFv (alt) Wheeze heard BCTmeth Eos spt	85.72 -4% 8ppb - - -No - 0.66 -	Neg Neg Neg - - Neg - Pos -
RAD025 Child 8 year	Completed study CV1/2/3/4	1. PEFv monitoring - (non-compliant) 2. BCT - (unable to perform) 3. Treatment response - (Non-compliant – parent reports child not taking ICS MDI)	FEV1/FVC BDR FeNO PEFv PEFv (alt) Wheeze heard BCTmeth	92.49 -5% 6ppb - - No -	Neg Neg Neg - - Neg -

			Eos	0.33	Neg
			spt	0/8	Neg
RAD039 Adult 26 year	Withdrawn (DNA CV1) – left part way through CV1 for CXR to rule out pneumothorax – didn't return	1. FEV1/FVC - (DNA) 2. BDR - (DNA) 3. PEFv monitoring - (DNA) 4.BCT - (DNA) 5. Treatment response - (DNA)	FEV1/FVC BDR FeNO PEFv PEFv (alt) Wheeze heard BCTmeth Eos spt	- - 17ppb - - No - 0.16 x10^9/L 1/8 (hdm)	- - Neg - - Neg - Neg Pos
RAD047 Child 14 year	Withdrawn CV1/2 (contra-indication – unrelated to study)	1. BCT - salbutamol prior to visit 2. Treatment response - Withdrawn so no data	FEV1/FVC BDR FeNO PEFv PEFv (alt) Wheeze heard BCTmeth Eos spt	87.71 5 36 5.5% 0 day No - 0.21x10^9/L 2/8 (grass/hdm)	Neg Neg Pos Neg Neg Neg - Neg Pos
RAD080 Child 7 year	Withdrawn CV1 (DNA CV2)	1. PEFv monitoring - (DNA) 2. BCT - (DNA) 3. Treatment response - (DNA)	FEV1/FVC BDR FeNO PEFv PEFv (alt) Wheeze heard BCTmeth Eos spt	79.34 12% 16 - - No - 0.34 x10^9/L 0/8	Neg Pos Neg - - Neg - Neg Neg
RAD087 Adult 30 year	Withdrawn CV1/2 (DNA CV3)	1.Treatment response - (DNA)	FEV1/FVC BDR FeNO PEFv PEFv (alt) Wheeze heard BCTmeth Eos Spt	78 % 7% 89 / 141 5.7 0 day No PD20 0.613 0.12 0/8	Neg Neg Pos Neg Neg Neg Neg Neg Neg
RAD092 Adult 30 year	Withdrawn CV1 (DNA CV2)	1. PEFv monitoring - (DNA) 2. BCT - (DNA) 3. Eos - (non-compliant) 4. Treatment response - (DNA)	FEV1/FVC BDR FeNO PEFv PEFv (alt)	68% 2% 19ppb - -	Pos Neg Neg - -

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ID / Age	Status	Items (reasons)	Test	Value	Result
			Wheeze heard	No	Neg
			BCTmeth	-	-
			Eos	-	-
			spt	0/8	Neg
RAD093 Child 6 year	Completed study CV1/2/3 (CV4 COVID Telephone visit)	1. FeNO - (Unable to perform) 2. PEFv monitoring - (non-compliant) 3. BCT - (unable to perform) 4. Eos - (non-compliant) 5. Treatment response visit - COVID telephone	FEV1/FVC	82.32	Neg
			BDR	3%	Neg
			FeNO	-	-
			PEFv	-	-
			PEFv (alt)	-	-
			Wheeze heard	No	Neg
			BCTmeth	-	-
			Eos	-	-
			spt	- 1 /2(dog)	-
RAD098 Child 9 year	Completed CV1/2/3 (CV4 COVID Telephone visit)	1.PEFv monitoring - (unable to perform) 2. Treatment response - (COVID telephone visit)	FEV1/FVC	91.83	Neg
			BDR	-12%	Neg
			FeNO	18ppb	Neg
			PEFv	-	-
			PEFv (alt)	-	-
			Wheeze heard	No	Neg
			BCTmeth	PD20 0.024	Pos
			Eos	0.12 x10^9/L	Neg
			spt	2/8 (grass/hdm)	Pos
RAD104 Child 15 year	Withdrawn CV1/2 (ICS started on arrival for CV2- early trial treatment as clinical deterioration) (DNA CV4)	1. BCT - (cough; unwell can't exclude COVID) 2. Treatment response - (DNA)	FEV1/FVC	97.11	Neg
			BDR	1%	Neg
			FeNO	12ppb	Neg
			PEFv	9.45%	Neg
			PEFv (alt)	0 Days	Neg
			Wheeze heard	No	Neg
			BCTmeth	-	-
			Eos	0.27 x10^9/L	Neg
			SPT	2/8(grass/birch)	Pos
RAD106 Adult 34 year	Withdrawn CV1/2, and OV1 (patient withdrew after OV1)	1. Treatment response - (Withdrawn)	FEV1/FVC	63%	Pos
			BDR	9%	Neg
			FeNO	29 / 38ppb	Neg
			PEFv	4%	Neg
			PEFv (alt)	0 days	Neg
			Wheeze heard	No	Neg
			BCTmeth	PD20 >0.9mg	Neg
			Eos	0.04 x10^9/L	Neg
			spt	0/8	Neg
RAD113 child	Completed CV1	1. BCT - (not offered due to pandemic)	FEV1/FVC	80.98	Neg
			BDR	2%	Neg

6 year	(CV4 COVID telephone visit)	2. Treatment response - (COVID telephone visit)	FeNO	15ppb	Neg
			PEFv	10.47%	Neg
			PEFv (alt)	1 Day	Neg
			Wheeze heard	No	Neg
			BCTmeth	-	-
			Eos	-	-
			spt	1/8 (cat)	Pos
RAD116 Child 14 year	Completed CV1 (CV4 COVID telephone visit)	1. BCT - (not offered due to pandemic) 2. Treatment response - (COVID telephone visit)	FEV1/FVC	88.87	Neg
			BDR	2%	Neg
			FeNO	11ppb	Neg
			PEFv	4.89%	Neg
			PEFv (alt)	0 Days	Neg
			Wheeze heard	No	Neg
			BCTmeth	-	-
			Eos	0.06×10^9/L	Neg
			spt	3/8(hdm/gr/bir)	Pos

Table b) Description of patients with EPOER possible asthma

	Status:	EPOER Notes	Patient Data		
			Test	result	result
RAD035 Child 6 year	Completed Study	Young child, no objective evidence except some variability noted on PEFv test (5 days over 20%). Good clinical history. Panel feel high probability asthma, however panel feel not enough evidence to confirm definite asthma.	FEV1/FVC	86.37	Neg
			BDR	8%	Neg
			FeNO	7ppb	Neg
			PEFv	16.18%	Neg
			PEFv (alt)	5 day (>20%)	Pos
			Wheeze heard	No	Neg
			BCTmeth	PD20 >0.9mg	Neg
			Eos	0.14 x10^9/L	Neg
			Spt	0/8	
RAD049 Adult 26 year	Completed Study	Objective tests mostly negative, except for 3 days PEFv >20%. Patient had minimal symptoms at time of presentation. History suggestive of seasonal asthma but panel feel not enough evidence to confirm definite asthma.	FEV1/FVC	78%	Neg
			BDR	9%	Neg
			FeNO	9ppb	Neg
			PEFv	14%	Neg
			PEFv (alt)	3 days >20%	Pos
			Wheeze heard	No	Neg
			BCTmeth	PD20 >0.9mg	Neg
			Eos	0.14 x10^9/L	Neg
RAD085 Child 7 year	Completed study	Young child, no objective evidence to suggest asthma but mother and child report good subjective response to treatment. In addition the clinical history was suggestive of asthma. Panel feel low probability asthma, however cant definitely exclude diagnosis.	FEV1/FVC	88.16	Neg
			BDR	8%	Neg
			FeNO	11ppb	Neg
			PEFv	5.60%	Neg
			PEFv (alt)	0 Days	Neg
			Wheeze heard	No	Neg
			BCTmeth	-	
			Eos	0.12 x10^9/L	Neg
			SPT	0/8	Neg
RAD088 Child 6 year	Completed study	Young child, evidence of atopy and borderline PEFv. Other tests in range. Mother and child report good subjective response to treatment. ACQ improved on treatment. Panel unable to agree definite diagnosis therefore possible asthma.	FEV1/FVC	88.63	Neg
			BDR	7%	Neg
			FeNO	7ppb	Neg
			PEFv	19.31%	Neg
			PEFv (alt)	3 Days >20%	Pos
			Wheeze heard	No	Neg
			BCTmeth	-	-
			Eos	0.47 x10^9/L	Pos
			Spt	5/8	Pos